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neutralization and corrosiveness remain significant processing issues. In this context, the development of a low-temperature pretreatment with easy solvent and/or catalyst recovery remains an important challenge for biomass conversion research.

Recently, Luterbacher et al. reported a novel non-enzymatic saccharification method using γ-valerolactone (GVL, a biomass-derived molecule [19]) as a solvent.[20] The process demonstrated that sugars yields of 70-90% could be obtained from different feedstocks without using enzymes. In experiments conducted in a flow-through reactor with a progressive temperature increase from 160 to 220°C, minimal residues were observed at the end of operation, indicating that all biomass components including lignin were depolymerized and solubilized in the 80-90% GVL and 20-10% water solvent system. Moreover, more sugars were recovered from the GVL-water system than when using an aqueous solution at the same reaction conditions.[20] The higher sugar yields associated with GVL/water were attributed in part to the excellent lignin dissolution ability of GVL, which could continuously remove lignin and expose fresh surface for acid-catalyzed hydrolysis. Further investigation showed that the GVL/water solvent system also affected the activation energies of glycosidic bond hydrolysis and sugar degradation to different degrees.[21,22] The hydrolysis of glycosidic bonds and the dehydration of sugars (glucose and xylose) had similar activation energies ranging from 130 to 140 kJ $mol^{-1}$ in the aqueous phase. However, the activation energy of glycosidic bond hydrolysis significantly decreased to 90 kJ $mol^{-1}$ in the GVL/water solvent with no obvious change observed for the activation energies of glucose and xylose dehydration. This decrease in the activation energy of the hydrolysis of glycosidic bonds made its rate much faster than the dehydration rate, which benefitted the selective production of sugars in GVL/water.[21,22] Nevertheless, around 20-30% of the sugars in biomass still degraded or formed byproducts due to the use of high temperatures (160-220°C), which, depending on the price of enzymes, could make the development of a more selective enzymatic process interesting.[20]

According to Arrhenius's law, reaction temperature affects the reaction with the highest activation energy more than the reaction with a lower activation energy. Therefore, the rate of glycosidic bond hydrolysis ($E_a$=90 kJ$mol^{-1}$) will be influenced to a lesser extent than the rate of sugar dehydration ($E_a$=130-140 kJ$mol^{-1}$) in GVL/water. In other words, the rate of sugar dehydration should significantly slow down compared to the rate of glycosidic bond hydrolysis when the reaction temperature decreases.[23] The ability of GVL to solubilize lignin could further enable the mild pretreatment of biomass at moderate temperatures. Furthermore, cellulose's crystalline structure is difficult to disrupt with low acid concentrations at moderate temperatures,[24] which could maximize its selective conversion by enzymes. Here we report that the use of a GVL-based pretreatment process at mild temperatures (120°C) can lead to high sugar yields after enzymatic hydrolysis. Notably, the use of GVL leads to markedly increased digestibilities compared to typical organosolv solvents such as tetrahydrofuran (THF) or ethanol used at the same conditions. Furthermore, GVL can be easily separated and recycled from the pretreatment liquor by $CO_2$ extraction. A proposed GVL pretreatment process integrated with enzymatic hydrolysis is shown in Figure S1.

After testing three different pretreatment temperatures, we found that wood particles (3-10 mm) were significantly deconstructed after treatment for 1 h at 120°C in 80% GVL 20% water (5:1 w/v solvent:solids slurry). Partial deconstruction was observed at 110 °C and no obvious deconstruction was observed at 100 °C. Therefore, subsequent experiments were conducted at 120°C.

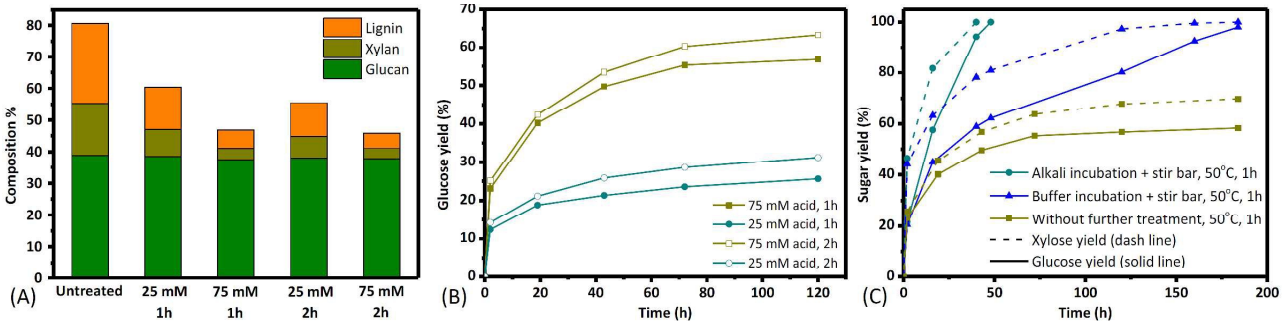



**Figure 1.** Pretreatment of hardwood particles at 120°C with 80 wt% GVL and 20 wt% water at different acid concentrations (25 mM $H_2SO_4$ and 75 mM $H_2SO_4$) and different pretreatment times (1 h and 2 h) (untreated particles showed a very low yield of less than 5% due to the big particle size, which was not presented in the figure). (a) Composition analysis of pretreated substrates, (b) Enzymatic digestibilities of pretreated substrates, (c) The effects of mechanical agitation during hydrolysis and alkali incubation on the enzymatic digestibilities of the substrate (75 mM $H_2SO_4$ for 1 h).

To further optimize pretreatment conditions, combinations of two different acid concentrations (25 mM and 75 mM $H_2SO_4$) and two different pretreatment times (1 h and 2 h) were tested. The composition analyses of pretreated substrates are shown in Figure 1a and Table S2. Over 95% of original cellulose remained in the pretreated substrates for all acid concentrations and residence times (Figure 1a). These results indicate that at 120°C, the crystalline structure of cellulose was not affected by the low-concentration acid solution. The breaking of hydrogen bonds within cellulose's crystalline structure requires the uses of high temperature and/or concentrated acids.[24,25] However, this is not true for amorphous components such as lignin and xylan. At the high acid concentrations (75 mM $H_2SO_4$), the removal of lignin (77.0% in 1 h and 81.8% in 2 h) and xylan (78.5% in 1 h and 80.9% in 2 h) was higher than at the low acid concentration (25 mM $H_2SO_4$; lignin: 47.3% in 1 h and 58.1% in 2 h; xylan: 48.4 % in 1 h and 58.2% in 2 h) (Figure 1a). These differences in lignin and xylan removal indicate that $H_2SO_4$ acts as a catalyst for both lignin depolymerization and xylan hydrolysis. In addition, the wood particles pretreated at the high acid concentration (75 mM $H_2SO_4$)

were significantly deconstructed while those pretreated at the lower acid concentration still kept the morphology of the original wood particles (Figure S2). Most of the dissolved xylan was recovered in the form of oligomers in the pretreatment liquor (Table S2), indicating that the hydrolysis of xylosidic bond and glycosidic bond was very slow under these reaction conditions. These slow hydrolysis rates facilitated the reduced degradation of sugars due to the limited formation of monosugars. At these pretreatment conditions, we did not detect the formation of furfural or HMF which usually form at higher temperatures and/or higher acid loadings.[15] Our reaction conditions (75 mM $H_2SO_4$ (0.75 w% sulfuric acid solution) at 120 °C for 1 h) are even milder than the conditions of the standard sugar composition analysis of biomass 4 wt% sulfuric acid at 120 °C for 1 h).[26] Therefore, a negligible formation of sugar degradation products was expected. Enzymatic hydrolysis was performed on the resulting solids to evaluate their enzymatic digestibilities (Figure 1b). At an enzyme loading of 15 FPU (filter paper units) per gram glucan, the substrates treated with the high acid concentration of 75 mM led to much higher glucose yields (55% for 1 h pretreatment time and 63% for 2 h pretreatment time) than those treated with the low acid concentration of 25 mM in 120 h (24% for 1 h pretreatment time and 30% for 2 h pretreatment time). This doubling in glucose yields is likely attributable to the significant difference in lignin and xylan removal for these two different acid concentrations. At the high acid concentration (75 mM), the higher removal of lignin and xylan generated more enzymes-accessible cellulose surface, thereby leading to higher enzymatic hydrolysis rates and higher glucose yields. At the same acid concentration, extending pretreatment time from 1 h to 2 h only led to slightly higher glucose yields due to the limited increase in lignin and xylan removal. These results are consistent with previous reports that the removal of lignin and xylan could significantly improve the enzymatic hydrolysis of cellulose.[27]

However, the low glucose yields (55% and 63%) were unexpected in light of the high delignification that was reached (77.0% and 81.8% of the original lignin removed). Previous reports showed that in typical organosolv pretreatments such as ethanol pretreatment and THF pretreatment, the resulting substrates could produce glucose yields of nearly 100% at comparable delignification levels.[27,28] A similar phenomenon had also been reported by Pan et al.[29] They observed that the use of acetic acid-treated wood led to a low enzymatic conversion of 10 % at a lignin removal of over 60%. Their investigation clarified that the grafting of hydrophobic acetate on the cellulose surface blocked the adsorption of cellulase, thereby reducing the enzymatic hydrolysis rate and the final glucose yield. Further incubation of these materials with dilute alkali solution at 50°C to remove acetyl groups could significantly improve the conversion of cellulose to glucose.[29] Based on this work, we propose that GVL as a carboxylate ester also had the ability to graft on the cellulose surface through transesterification. However, given that our glucose yields are still above 60% this grafting is likely much more limited compared to what occurs during acetic acid pretreatment. Transesterification of plant oil with ethanol for the production of biodiesel has been widely researched and reported.[30,31] Recently, Kakuchi et al. reported a method to modify cellulose with isopropenyl acetate through transesterification.[32] These reports showed that transesterification reactions generally occurred under acidic conditions with the limited presence of water.[31,32] With the high concentration of GVL (80/20, w/w) and limited water under acidic conditions, the transesterification of GVL with the hydroxyl groups in cellulose and lignin is possible. To verify this, the GVL-treated substrate was further incubated with a dilute alkali solution (1 wt% NaOH based on the dry substrate) at 50°C in an incubator for 1 h. This incubation was followed by neutralization with acetic acid to form a pH=5 acetate buffer solution before the addition of enzymes. The substrate pretreated with 75 mM $H_2SO_4$ for 1 h was selected for these investigations. The purpose of this incubation was to hydrolyze the possible ester groups on the surface of the substrate. As is discussed further below, the small amount of alkali (1 wt% based on the dry substrate) had little effect on digestibility in the absence of grafted groups on cellulose. Previous research has also shown that dilute alkali does not affect the crystallinity of cellulose.[33] Furthermore, In order to simplify processing, the same incubation temperature of 50 °C as used in the enzymatic hydrolysis was employed so that no additional temperature adjustment was needed. The NaOH added in the incubation was also used to form the buffer solution, which is required to eliminate pH effects. In addition, a stir bar was added to provide better mixing and dispersion of substrates. Upon the addition of this stir bar, the enzymatic hydrolysis rate and glucose yield increased significantly and reached 98% after 200 h. The reason behind this enhancement could be attributed to the further mechanical defibrillation of cellulose. In our experiment, the biomass structure was still visible likely due to the low pretreatment temperature and large particle size. Further defibrillation could help speed up the mass transfer between the enzymes and the substrate. Zhu et al. has shown that post-pretreatment disk-milling for size reduction could save up to 80% of the energy required to mill untreated biomass before pretreatment.[34]

The alkali incubation of GVL-treated substrates significantly improved enzymatic hydrolysis rates (Figure 1c). Almost identical results were obtained with an incubation using $Ca(OH)_2$ (a less expensive base; see supplemental information). Hydrolysis with the alkali-incubated substrate led to glucose yields of 100% in 40 h compared to 200 h for the control experiment (incubation of substrate with pH=5 acetate for 1 h before the addition of enzymes). Interestingly, the alkali incubation just increased the enzymatic hydrolysis rate but did not affect the final glucose yield, which was almost indistinguishable from the hydrolysis of the alkali-treated and control substrate (100 and 98%). This likely indicates that almost all cellulose was accessible to enzymes after the removal of lignin and xylan. Alkali incubation probably decreased the non-effective adsorption of enzyme by removing the grafted groups from the surface. Since surface characterization methods such as XPS and FT-IR could not distinguish between the functional groups of GVL and those of residual lignin and cellulose, HPLC was used to detect the surface-bound GVL that was re-solubilized after alkali incubation. Our hypothesis was that GVL and/or hydroxy pentanoic acid (i.e. ring-opened GVL) would be detected in a solution of washed and alkali treated pretreated biomass if GVL was grafted on the surface. Using this method, we detected 20.7 mg GVL per kg of the pretreated substrate. At the same time, no GVL was detected for a control experiment consisting of washed GVL-immersed wood particles (see the supplementary material for detailed procedure). Though these results are strongly indicative of GVL grafting on the biomass surface, it is possible that the absence of GVL in the control experiment could be due to the lower surface area in the untreated wood. Nevertheless, the combination of the detection of GVL in solution and the strong effect of alkali incubation strongly points towards chemical grafting. Current studies based on dynamic nuclear polarization NMR are underway to attempt to detect the GVL grafted on the surface which would provide definite proof of its presence.

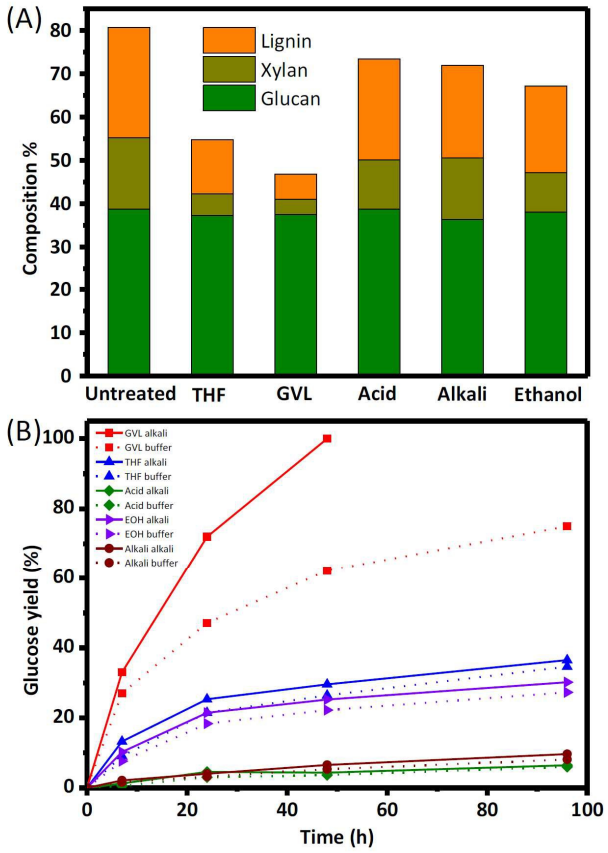



**Figure 2.** Comparison of different pretreatment methods at 120 °C for 1 h. In the case of THF and GVL, 80% solvent and 20% water was used. With ethanol, 50% solvent and 50% water was used. Pure water was used in the acid and alkali conditions. Except for the alkali condition (250 mM NaOH) all solutions contained 75 mM $H_2SO_4$. (a) Composition analysis of pretreated substrates, (b) Enzymatic digestibilities of pretreated substrates.

It is expected that the transesterification rate will increase with increasing temperature, higher acid concentration and higher ester concentration. If GVL increasingly reacts with biomass, more GVL should be grafted on the biomass surface and the enzymatic hydrolysis should be further reduced. To verify this, we increased the pretreatment temperature from 120 to 140°C and the resulting substrate had lower enzymatic digestibilities despite having had more lignin and xylan removed (Figure S3). When the substrate pretreated at 140°C was incubated with dilute alkali (1 wt% based on the dry substrate) at 50°C for 1 h, the enzymatic hydrolysis rate of the incubated substrate was much higher and indistinguishable from that of the substrate treated at 120°C followed by alkali incubation. This similar hydrolysis rate indicates that similar cellulose morphologies might have formed in the two substrates pretreated at different temperatures. The respective similarity and difference in hydrolysis rates with and without alkali treatment is consistent with GVL grafting on the cellulose surface and with the assumption that more severe pretreatment conditions may lead to more of this grafting.

GVL was very efficient at removing lignin and xylan and retaining cellulose, thereby enhancing enzymatic hydrolysis rates and the glucose yields (Figure 2a). An overall mass balance showed that almost no xylose or glucose degraded in the pretreatment liquors (Table S2). After complete enzymatic conversion (Figure 1c), total glucose and xylose yields of 99% and 96% were obtained when combining the sugars obtained during hydrolysis and in the pretreatment liquor. In order to demonstrate the unique performance of GVL's mild-temperature pretreatment, several alternate organosolv pretreatment methods were selected for comparison at 120 °C. GVL demonstrated improved xylan and lignin removal than, in order, THF, ethanol, dilute acid in water and dilute alkali in water. Unlike results with higher temperature pretreatments (150-180 °C),[3,4,28,35] dilute acid, dilute alkali and ethanol used at 120°C showed poor abilities in removing xylan and especially lignin. Although THF showed good performance in the removal of lignin and xylan at a temperature of 150°C,[16] it did not perform as well at 120°C. Compared to THF, GVL dacilitated significantly enhanced lignin removal at this mild temperature. Compared to ethanol and water, the superior delignification ability of GVL and THF could be attributed to their polarity, which can facilitate lignin dissolution, and their ability to destabilize acidic protons, which can increase their catalytic activity and thereby depolymerization rates. Proton destabilization by GVL and THF has been extensively studied in recent work by Mellmer et al.[21,22]

Remarkably, the GVL-treated substrates had markedly higher digestibilities than those treated with all other solvents (Figure 2b). The GVL-treated substrates had digestibilities that were twice as high as THF or ethanol-treated samples and about 15 times higher than samples treated in water. These enhancements jumped to 3 and 20 times, respectively, when an alkali incubation was performed. Since THF and ethanol were unlikely to graft on the cellulose surface, the additional alkali incubation of the corresponding pretreated substrates had negligible effects on enzymatic hydrolysis rates and final glucose yields. The glucose yields of the resultant substrates did follow the same trend as that of the fraction of lignin removed by GVL>THF>ethanol>acid>alkali. The removal of hemicellulose followed that of lignin for most cases except for the alkali treatment. Therefore, the glucose yields may be controlled by the synergistic removal of lignin and hemicellulose.[36] However, given the large difference between delignification for ethanol and THF-treated substrates and the comparatively small difference in digestibilities, there are likely other factors influencing the enzymatic process. Notably, the increased delignification and hydrolysis rates could be explained in part by GVL grafting, which could help disrupt the structure and hydrogen bonding of lignin and/or cellulose. Further investigation into this phenomenon could help clarify these different solvent effects.

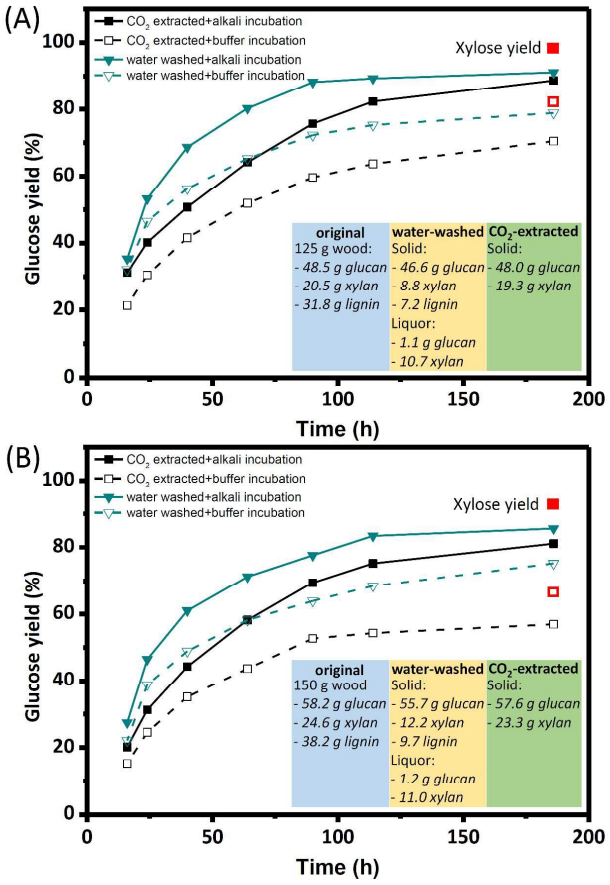
In the last section, we have demonstrated that this mild GVL-based pretreatment method produced highly digestible pretreated substrates and led to negligible sugar degradation. However, the success of this technique depends on successful GVL recovery and reuse. In previous reports, liquid $CO_2$ has been used to extract GVL from a homogeneous GVL-water-sugar-lignin stream. In past experiments, subsequent extraction steps produced an aqueous phase containing 90% of the sugars with most of the lignin precipitating.[20] In this experiment, we used a modified liquid $CO_2$



extraction technique to evaluate the recovery of GVL from the pretreatment slurry. The technique used here involved pumping liquid $CO_2$ through the slurry continuously at high pressure and continuously recovering GVL in a connected low-pressure vessel (Figure S6). To increase the amount of pretreated material, a high-solids 1 L Parr reactor was used for pretreatment. The slurry resulting from biomass pretreated with a solvent/biomass ratio of 5:1 (w/w) using the 1 L Parr reactor contained GVL, water, solids, lignin and sugars and was directly extracted with liquid $CO_2$. Over 99.5 % of GVL could be recovered (Figure S5 and Table S3) and almost all sugars, lignin and solid material were left in the aqueous phase. Only a negligible amount (0.3-1% of the solubilized sugars) was extracted into $CO_2$-GVL phase (Figure 3 and Table S3). The improved recovery of sugars compared to the previously used discontinuous system (0.3-1% vs. 10-20%) was likely the result of reduced back mixing of the GVL-$CO_2$ and aqueous phases in the continuous setup. [20,37]

To produce a high-concentration sugar stream, the digestibilities of the water-washed and $CO_2$-extracted substrates were compared during high-solids (30% w/v) enzymatic hydrolysis. At a substrate concentration of 30% w/v, no visible free liquid was observed at the beginning of hydrolysis but the slurry was fully liquefied after 8 h (Figure S4). The $CO_2$-extracted substrate showed slower enzymatic hydrolysis rates than the water-washed substrate (Figure 3a). This was probably caused by the presence of lignin and additional sugars in the $CO_2$-extracted substrate. During the $CO_2$ extraction process, most of the dissolved lignin precipitated out and remained in the slurry along with cellulose for the subsequent enzymatic hydrolysis. In contrast, in the water-washed substrate, most of the dissolved lignin was removed with the pretreatment liquor during filtration. Lignin and, to a lesser extent, additional sugars are known to reduce enzymatic hydrolysis rates due to their ability to competitively bind cellulases. [38,39] However, this precipitated lignin did not significantly impact the final cellulose conversion, which was similar for water-washed and $CO_2$-extracted pretreated hardwood. Hydrolysis of the water-washed substrate led to a final glucose yield of 90% in 72 h with a glucose concentration of 124 g/L and the $CO_2$-extracted substrate led to a comparable glucose yield of 87% in 100 h, with a glucose concentration of 124 g/L. Additionally, since only 0.3 % of dissolved xylose was extracted into the $CO_2$ phase, almost all xylan was left in the $CO_2$-extracted substrate. This additional xylan further increased its sugar concentration. A total xylose yield of 97% was obtained with the $CO_2$-extracted substrate, which gave a xylose concentration of 58 g/L and led to a total sugar concentration of 182 g/L. In summary, $CO_2$ extraction left all sugars in the substrate and led to a higher final sugar concentration, simplifying the GVL recovery process. In contrast, water washing could separate xylan from glucan but would require further steps to recover GVL from the diluted liquor. In order to further decrease the pretreatment cost by lowering solvent usage, a liquid/biomass ratio of 4:1(w/w) during pretreatment was also tested. It was found that decreasing the liquid/biomass ratio from 5:1 to 4:1 did not significantly affect the efficacy of the system. High consistency (30 w/v%) enzymatic hydrolysis showed a glucose yield of 85% for water-washed substrate and 80% for the $CO_2$-extracted substrate. Hydrolysis of $CO_2$-extracted substrate led to a slightly lower final sugar concentration of 167 g/L (glucose: 114 g/L and xylose: 53 g/L).




**igure 3.** High solids enzymatic hydrolysis of water-washed substrates and $CO_2$-extracted pretreated hardwood. Final xylose yields are shown in red (the detailed xylose yield time-course curves are shown in Fig. S5). (a) Liquid/biomass ratio of 5:1 (w/w) (625 g GVL/$H_2O$ (80:20, w/w), 125 g air-dried wood particles) (b) Liquid/biomass ratio of 4:1 (w/w) (600 g GVL/$H_2O$ (80:20, w/w), 150 g air-dried wood particles)**.** The insets show the composition analyses of the original wood particles, water-washed substrate and $CO_2$-extracted substrate at the corresponding reaction conditions.

Compared to the nonenzymatic process reported previously, the combination of this mild pretreatment with enzymatic hydrolysis led to higher total sugar yields (85-100% for enzymatic hydrolysis versus 70-90% for nonenzymatic hydrolysis) and higher sugar concentrations (167-182 g/L versus up to 127 g/L). Besides, the high biomass concentration (up to 20 w%) and reduced temperature (120°C) in this mild pretreatment, solvent usage was reduced at least three-fold compared to the nonenzymatic case. All these elements will decrease energetic and processing costs. In summary, the pretreatment process likely requires significantly lower energy input and gave higher sugar yields and sugar concentrations, but required the usage of enzymes. Therefore, the ultimate choice between processing options will depend on a careful techno-economic analysis and the current and future cost of enzymes as is the case for other pretreatment techniques.

**Conclusion**

In this paper, we demonstrated that the GVL/water solvent system could be used to perform a mild biomass pretreatment process at 120°C using 75 mM of $H_2SO_4$. When using GVL/water as a solvent, improved lignin and xylan removal from hardwood was observed compared to other solvents including ethanol, THF, water (all with dilute acid) and a dilute alkali control. Differences in glucose yields obtained during enzymatic hydrolysis were even more pronounced between these solvents. The use of GVL led to glucose yields of about 100%, which was three times higher than when using THF or ethanol and 20 times higher than for the pure water. The mild pretreatment temperature and low acid concentration facilitated the retaining of cellulose and decreased the degradation of sugars leading to both glucose and xylose being recovered at a yields close to 100% when both pretreatment and hydrolysis liquors were analyzed. We also demonstrated that GVL could be recovered through liquid $CO_2$ extraction leaving over 99% of the sugars with the aqueous and solid fraction of the pretreatment slurry. High solids enzymatic hydrolysis (30% w/v) led to yields of 90 and 97% for glucose and xylose, respectively and a sugar concentration of 182 g/L. The high concentration of this sugar stream could significantly decrease downstream processing costs. In future work, we will continue applying this method to various kinds of feedstock, including softwood, agriculture residues and energy crops. In addition, the effect of GVL on the surface morphology of pretreated substrates will be further investigated. We suspect that these effects could be at the origin of the impressive delignification properties of GVL and could help disrupt cellulose crystallinity. A better understanding of these reactions could lead to additional insights and improvements in the use of green solvents for biomass conversion.

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