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Enhancing Hemicellulose Recovery and the Enzymatic Hydrolysis of Cellulose by Adding Lignosulfonates during the Two-Stage Steam Pretreatment of Poplar

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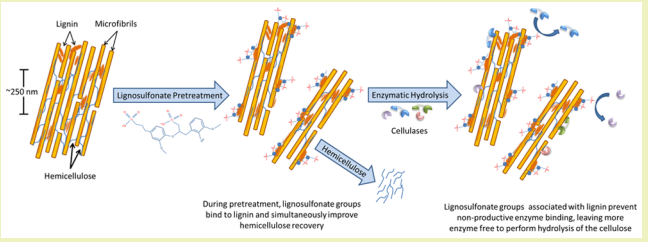
ABSTRACT: To enhance overall sugar recovery, poplar was steam pretreated in two stages. First, under mild pretreatment conditions (160 °C, 15 min) using various acid catalysts (H_2SO_4, SO_2, oxalic, citric) to assess optimum hemicellulose recovery, followed by an uncatalyzed, second pretreatment (200 °C, 5 min), to facilitate enzymatic hydrolysis of the cellulose. The first stage solubilized and recovered about 50% of the xylan, as compared to the 30% obtained in the more severe, single-stage pretreatment. As surfactants are known to increase hemicellulose accessibility during pretreatments, lignosulfonates were added to birch xylan and poplar, increasing xylose yields and retaining 60 mmol/kg strong acid groups, even after the second pretreatment stage and extensive washing, suggesting that lignosulfonates adsorbed to the substrate and enhanced cellulose accessibility. This was confirmed by the increased water retention values and Direct Orange dye adsorption. A two-stage steam pretreatment, incorporating lignosulfonate addition, increased cellulose hydrolysis from 75 to 92%.



KEYWORDS: *Pretreatment, Poplar, Lignin, Lignosulfonate, Cellulase, Hydrolysis*

INTRODUCTION

The inherent recalcitrance of lignocellulosic substrates means that some form of pretreatment is required to fractionate effectively the cellulose, hemicelluloses and lignin into usable forms while enhancing the enzymatic hydrolysis of the cellulosic component.[1] Steam pretreatment typically involves the use of high temperatures/pressures and some chemical loading, so as to generate a substrate that can be hydrolyzed at low enzyme loadings.[2] However, these conditions can be so severe that the hemicellulose and cellulose-derived sugars are degraded to 5-hydroxymethylfurfural and furfural, compromising yields and inhibiting subsequent fermentation.[3,4] It has been shown that the addition of an acid catalyst improves the solubility of hemicellulosic sugars into the liquor stream, while allowing pretreatment to be carried out at lower temperatures and shorter residence times.[5] Various acid catalysts have been explored for their effectiveness including sulfur dioxide, sulfuric acid[6] and, more recently, organic dicarboxylic acids, which are thought to mimic the active site of carbohydrate degrading enzyme.[7] Although the targeted removal of hemicellulose from the substrate increases the accessibility of the cellulases to the cellulose, increasing the residence time, temperature and acid catalyst loading to enhance hemicellulose solubilization comes at the expense of compromising sugar yields. One approach to overcome this conundrum has been to use a two-stage steam pretreatment approach where the more labile hemicellulosic sugars are first extracted after an initial, mild pretreatment stage, followed by a second pretreatment at elevated severity to enhance the enzymatic hydrolysis of the cellulosic component.[8]

Unlike softwoods, where accessibility to the cellulose after pretreatment is predominantly influenced by the type and location of the lignin component, typically requiring higher enzyme loadings of up to 40 FPU/g glucan,[11] the location and type of hemicellulose has been shown to have more of an influence on restricting accessibility to the cellulosic component of pretreated agricultural and hardwood substrates. Thus, the selective hemicellulose removal mediated by the two-stage pretreatment approach meant that lower enzyme loadings could be used to achieve effective cellulose hydrolysis.[12,13]

Poplar has been advocated as a promising biorefinery feedstock, as wood residues are available from its current use for lumber and pulp production, it exhibits rapid growth, has a sequenced genome, is drought and pest resistance and is able to grow on marginal land.[4] As poplar hemicellulose is composed primarily of xylan, hemicellulose recovery can be readily estimated by measuring overall xylose yields.[14] Previous work has shown that acid-catalyzed, lower temperature steam pretreatment combined with extended residence times increased the solubilization and recovery of hemicellulose.[15] Although most previous work has used stronger acids such as

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H_2SO_4 or SO_2, it is likely that milder acids such as oxalic, with and without the addition of salts, might also improve hemicellulose and cellulose sugar recovery[16] particularly if a two-stage approach without the addition of supplemental acid in the second stage is used.

As well as assessing whether milder catalysts could be used to enhance hemicellulose recovery while ensuring good cellulose hydrolysis at low enzyme loadings, we also looked at other ways of enhancing the overall sugar recovery. As other work had shown that the addition of surfactants could also enhance hemicellulose recovery,[17] we wanted to assess whether lignosulfonates, which are amphiphilic lignin byproducts from the sulfite pulping process, could also function in a similar fashion. The influence of lignosulfonate addition was also of interest since we[18] and others[19] had previously shown that lignosulfonates could improve enzymatic hydrolysis of the cellulosic component of pretreated substrates. It was anticipated that this type of two-stage pretreatment approach would result in good overall sugar yields by maximizing hemicellulose recovery while providing a cellulosic substrate that could be readily hydrolyzed at low enzyme loadings.

MATERIALS AND METHODS

Biomass Substrate. Poplar was obtained from the BC Ministry of Forests, Canada, chipped and screened using a plate screen with the fraction between 2.5 × 2.5 cm and 5.0 × 5.0 cm, and collected as the raw material for pretreatment. The moisture content of the chips was 7%. The chemical composition of the poplar was glucan (49.3%), xylan (17.6%), mannan (2.8%), arabinan (0.7%), galactan (0.4%) and lignin (25.4%) measured as described below. Birchwood xylan was purchased from Sigma-Aldrich.

Steam Pretreatment of Poplar and Birch Xylan. Prior to steam pretreatment, 200 g of dry poplar chips was placed in plastic bags, mixed with water containing the given catalyst at a solid:liquid ratio of 1:1 and left at room temperature overnight. The impregnated biomass samples were subsequently loaded into a 2 L Stake Tech II steam gun (Stake Tech II batch reactor, SunOpta (formerly Stake Technologies) of Norval, ON, Canada) and pretreated in a first pretreatment stage at 160 °C and a residence time of 15 min. Due to the limited capacity of the steam pretreatment reactor, each of the five batches (approximately 200g dry wt each) of pretreated biomass samples were combined into one consolidated sample for subsequent post-treatment and enzymatic hydrolysis. This consolidation also minimizes any batch-to-batch variation that might occur between the steam pretreated samples as has been shown previously.[20,21]

The resulting slurry was collected and the water-soluble fraction was separated from the solid fraction with vacuum filtration with the filtrate recycled through the filter cake two times using Fisher Scientific coarse grade filter paper with pore size p8, which retains particles >20 um and has a flow rate of 175 mL/min, as described by the manufacturer. To minimize any loss of fine particles during filtration, the filtrate was recycled through the filter pad twice. After filtration, the water-insoluble fraction was subjected to a second-stage pretreatment at 190 °C for 5 min without the addition of an acid catalyst, and subsequently filtered as described above. When specified, 25 g (OD basis) of each substrate was suspended in 5 L of distilled water and stirred with a mechanical stirrer at 200 rpm using a Teflon impeller for 5 min, then filtered through a Buchner funnel. This process was repeated five times to ensure thorough washing of the substrate for a total of 25 L of water on 25 g of each substrate. For those experiments where acid catalysts were added to birch xylan, an autoclave was used instead of the steam gun. Based on the ratio of the weight of H_2SO_4 (6.0 g) to the amount of xylan in the poplar biomass (approximately 30 g of xylan in 200 g of poplar biomass) that was used for the steam pretreatment, birch xylan (3.0 g) was suspended in 20 mL of distilled water and reacted with 0.6 g of H_2SO_4 and an equimolar amount of the other catalysts in a 100 mL septa bottle. The bottles were sealed and heated at 121 °C for 90 min in an autoclave. The septa bottles were subsequently cooled, the solid particles settled by gravity after sitting for 30 min, and the supernatants subsequently analyzed for sugar monomers after mild acid hydrolysis as described below.

Analytical Methods. The chemical composition of the substrates was determined by the Klason protocol according to TAPPI Standard Method T-222. The concentration of monomeric sugars was determined by high performance liquid chromatography (HPLC) analysis as previously described by Bura et al.[12] To assess the accessibility of cellulose, direct orange (Pontamine Fast Orange 6RN, lot no. 814071) dye was obtained from Pylam Products Co. Inc. (Garden City, NY). Direct Orange staining was performed according to the modified method by Chandra et al. (2012). The water retention value (WRV) was determined and calculated according to TAPPI Useful Method-256. The carboxylic acid group content of the pretreated substrates and biomass were measured using the conductometric method according to Katz et al.[22] The concentration of Furfural, 5-hydroxymethyl furfural and acetic acid present in the water-soluble fractions were determined according to the NREL method of Sluiter et al.[23]

Enzymatic Hydrolysis. Enzymatic hydrolysis of the pretreated poplar was performed using Spezyme-CP (Genencor-Danisco, Palo Alto, CA), which had an activity of 59 FPU/mL and a protein content of 123 mg/mL, and a commercial β-glucosidase preparation (Novozym 188, Novozymes, Bagsværd, Denmark; 160 CBU/mL and 120 mg protein/mL). Standard conditions of enzymatic hydrolysis were cellulase (5 or 10 FPU/g cellulose), β-glucosidase (10 or 20 CBU/g cellulose), solids consistency 2% and 10% using either washed or unwashed substrate as specified, 50 mM acetate buffer at pH 4.8, temperature 50 °C, incubation time 72 h, stirring speed 150 rpm. 1 mL of each hydrolysate was sampled after 72 h; the enzyme activity was stopped by incubating the samples on a hotplate at 100 °C for 10 min (time to temperature was 10 min) and it was subsequently analyzed for sugar release using HPLC.

Error Analysis. The coefficient of variance (COV) was calculated for sugar analyses and enzymatic hydrolysis of the water-soluble and -insoluble fractions respectively, by repeating (5 times) the analysis of the sample pretreated with 3% H_2SO_4 in two pretreatment stages, giving COV values of 5.1% (sugar analysis in liquor) and 2.3% (enzymatic hydrolysis). Coefficient of variance (COV) values for the Direct Orange assay and water retention value were obtained using the standard deviation of the Direct Orange adsorptions and water retention values of the sample pretreated with 3% H_2SO_4 after two pretreatment stages. This was repeated five times to obtain COV values of 4.3% and 13.7% for the DO dye adsorption and water retention values, respectively.

RESULTS AND DISCUSSION

As discussed earlier, a two-stage pretreatment approach has been successfully used with softwoods to optimize hemicellulose recovery in the first stage while enhancing the substrate conditions for subsequent enzyme-mediated cellulose hydrolysis after the second stage.[7−9] Although hemicellulose solubilization increases the cellulose hydrolysis yields of pretreated softwood, the lignin component of softwoods has been shown to be much more influential in limiting cellulose hydrolysis,[11] thus somewhat limiting the benefits of a two-stage pretreatment approach in maximizing sugar recovery from softwoods. However, because of the more influential role that hemicellulose plays in influencing the effectiveness of cellulose hydrolysis in hardwood and agricultural residues,[13,14] a two-stage steam pretreatment strategy may be even more effective with these types of lignocellulosic substrates. Most of the previous work on softwoods has utilized stronger acids such as H_2SO_4 or SO_2 as the catalysts during steam pretreatment.[7,8] Due to the more labile nature of hardwood-derived hemicelluloses, we initially assessed how effective organic acids, such

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as oxalic and maleic acids that possess multiple carboxylic acid groups and have been shown to enhance the solubilization of hemicellulose at lower steam/dilute acid pretreatment conditions,[24] might be in catalyzing pretreatment. As previous work had also indicated the addition of a detergent could enhance the solubilization of the hemicellulose during pretreatment,[17] we wanted to assess whether lignosulfonates could also function in a similar fashion.

Before comparing the effectiveness of the various acid catalysts on poplar wood chips, we first wanted to assess how they would react with isolated birchwood xylan at mild pretreatment conditions in an autoclave. When the hydrolyzates were separated and analyzed for their oligomeric and monomeric xylose content it was apparent that the oxalic acid−$MgCl_2$ catalyst combination was the most effective in solubilizing and recovering the birch xylan, with the addition of $MgCl_2$ to the oxalic acid more than doubling xylose production (Figure 1). This confirmed earlier work showing

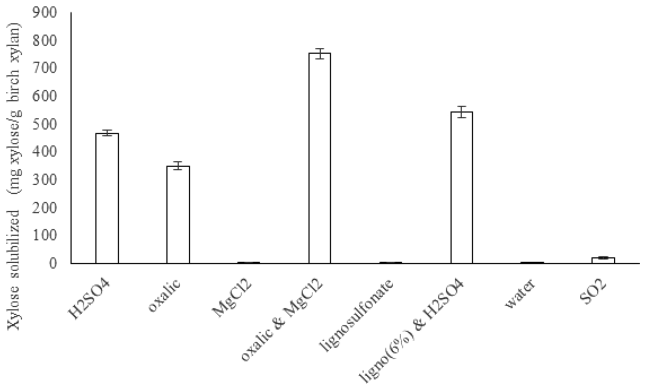

Figure 1. Solubilisation and recovery of birchwood xylan after autoclave pretreatment with different supplemented and unsupplemented acid catalysts.

that the addition of salts such as NaCl and $MgCl_2$ to the oxalic acid enhanced the hydrolysis of cellulose to monomeric sugars.[16] It was also evident that the supplementation of H_2SO_4 with lignosulfonates increased xylan solubilization (Figure 1). As mentioned earlier, the addition of surfactants such as Tween 80 was thought to result in an increase in accessibility of the hemicelluloses to the acid, improving sugar solubilization and recovery.[25] At the lower temperatures used when treating the birchwood xylan in the autoclave in comparison to the more severe conditions used in a typical steam pretreatment, the addition of SO_2 only resulted in the release of 20 mg xylose per gram of xylan (Figure1). This implied that the milder autoclave conditions were not favorable for the conversion of SO_2 to H_2SO_4, which has been shown to be the intermediate step by which SO_2 catalyzes hemicellulose solubilization.[6,26] On the basis of this initial work, we next compared the effectiveness of the various catalyst combinations to see if the two most successful combinations, sulfuric acid plus lignosulfonate, and oxalic acid plus $MgCl_2$, were as effective at catalyzing the solubilization and recovery of the hemicellulose present in poplar wood chips during an initial, mild pretreatment.

As 3% H_2SO_4 was the acid catalyst concentration that had been used for much of the previous steam pretreatment work,[27] this was the concentration to which the other catalysts were dosed to an equimolar basis. A temperature of 160 °C and a residence time of 15 min was also chosen because we[15] and others[28] had shown that extended (>5 min) residence times, combined with lower temperatures, resulted in better hemicellulose solubilization and recovery. As previous work had also shown that H_2SO_4 was more effective than SO_2 in solubilizing and recovering hemicellulose from steam pretreated willow, it was anticipated that H_2SO_4 might be more effective in solubilizing the hemicellulose when lower temperatures were used.[10,26]

As was observed previously with the birchwood xylan, the oxalic acid plus $MgCl_2$ and the 6% lignosulfonate plus H_2SO_4 treatments were again the combinations that resulted in the highest hemicellulose-derived sugar recovery from the poplar wood chips (Figure 2a). However, the higher sugar yields

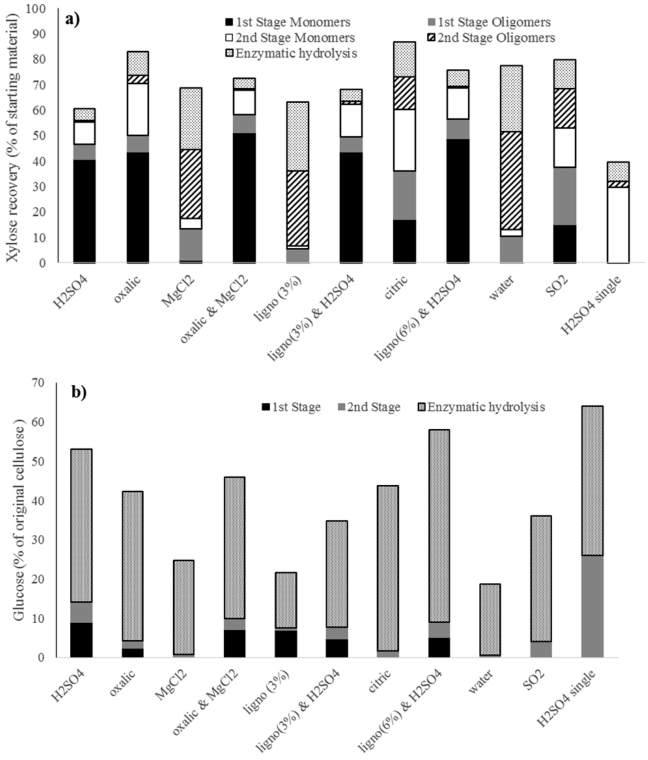

Figure 2. (a) Xylose and xylo-oligomers recovered after each pretreatment stage and, (b) cellulose hydrolyzed after second-stage pretreatment (190 °C, 5 min) at an enzyme loading of 3 FPU/g of substrate.

observed after the oxalic acid plus $MgCl_2$ treatment over the 6% lignosulfonate plus H_2SO_4 of the Birchwood xylan was not reproduced after the steam pretreatment of the poplar wood chips (Figure 2a). Although the pH of the H_2SO_4 and H_2SO_4 plus 6% lignosulfonate-derived liquors were in the same range of 1.8−1.9, the samples supplemented with lignosulfonates solubilized approximately 10% more of the xylan, similar to the beneficial effects that lignosulfonate addition had on the acid hydrolysis of the purified xylan (Figure 2a).

It was evident that the SO_2 and citric acid provided milder treatments in the first stage than did the H_2SO_4 and oxalic acid treatments as the water-soluble streams contained a greater amount oligomeric xylose (Figure 2a). The slower conversion of SO_2 to H_2SO_4 at the 160 °C steam pretreatment temperature compared to the higher temperatures typically used for single-stage pretreatments, resulted in the pH of the two-stage SO_2 pretreatment liquor being slightly higher, at 2.8,

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Table 1. Chemical Composition, Substrate Characterization and Enzymatic Hydrolysis of Two-Stage Pretreated Poplar Substrates

	chemical composition (%)						enzymatic hydrolysis[a]						
							5 FPU/g cellulose		10 FPU/g cellulose				
pretreatment catalyst/additions	Ara	Gal	Glc	Xyl	Man	AIL[b]	2% solids	10% Solids	2% solids	10% solids	direct orange dye adsorbed (mg/g)	water retention value	strong acid groups (mmol/kg)
H_2SO_4	0	0	59	0.4	0	36	47	42	85	75	82	1.8	bdl[c]
oxalic acid	0	0	60	1.4	0.5	32	43	37	62	63	85	2.2	bdl
oxalic acid MgCl2	0	0	59	0.6	0.6	35	45	43	77	66	81	2.2	
H_2SO_4+3% lignosulfonates	0	0	59	0.4	0.6	36	52	51	92	90	92	2.3	50
citric acid	0	0	58	2.2	0.5	31	43	40	67	58	87	2.4	bdl
H_2SO_4+6% lignosulfonates	0	0	59	0.4	0.5	36	63	58	95	92	93	2.8	60
H_2SO_4 single	0	0	42	0.7	0.5	49	68	61	100	98	92	1.8	bdl
water	0	0	53	9.9	1.8	26	9	16	34	38	72	2.5	bdl
SO_2	0	0	59	2.3	1.0	30	31	36	63	62	87	2.2	10
birch xylan								bdl	bdl	bdl	bdl	5.6	bdl

[a]Enzymatic hydrolysis yields are defined as the conversion of cellulose in the substrate to glucose after enzymatic hydrolysis at a pH of 4.8 in sodium acetate buffer at the specified enzyme and solids loading. [b]AIL: acid insoluble lignin. [c]bdl: below detectable limit.

compared to a pH of 1.8 for the H_2SO_4 two-stage treated liquor. As the pK_a of the citric acid's three carboxylic acid group protons range from approximately 3.1 to 6.4, it is possible that, during pretreatment, a smaller proportion of the citric acid's protons are liberated when compared to those of oxalic acid which have pK_a values of 1.3 and 4.4. It is likely that the oxalic acid acts as a stronger acid during pretreatment, solubilizing a greater amount of the hemicellulose, as the pH of its liquor was 2.4 compared to a pH of 3.0 for the citric acid. As the water insoluble substrates were not washed after the initial pretreatment stage, it was also likely that a small amount of residual sugars and acid catalyst remained in the cellulose rich substrate, possibly contributing to the sugar yields observed after the second-stage pretreatment stage and subsequent enzymatic hydrolysis.

Once we had assessed how the various combinations might enhance hemicellulose recovery after an initial, milder pretreatment, the cellulose rich water-insoluble fractions were then pretreated for a second time at a temperature of 190 °C for 5 min, conditions that had previously been used to enhance effective enzymatic hydrolysis of the cellulose.[12] It was apparent that the cellulosic substrates that had been originally pretreated in the absence of an acid catalyst were more readily hydrolyzed after the second pretreatment step. This was likely due to the greater amount of residual hemicellulose remaining in these substrates, which was liberated by the higher pretreatment temperatures used in the second step due to autocatalysis via deacetylation of the hemicellulose.[29]

The xylan-derived sugars that were solubilized during the steam pretreatment of chips that were soaked in either water or $MgCl_2$ and the lignosulfonate control samples were predominantly oligomeric in nature. This was likely due to the weak acidic autocatalyzed reactions occurring at these conditions. Unlike the autocatalyzed reactions, the use of citric acid solubilized xylan as mostly monomers, suggesting that much of the citric acid was retained in the substrate, consequently enhancing xylan depolymerization during both pretreatment stages. Similarly, the SO_2 catalyzed samples resulted in about equal amounts of xylose solubilized in both the first and second pretreatment stages, supporting earlier observations that minimal amounts of retained sulfur dioxide could be converted to H_2SO_4 when high temperature (190−210 °C) steam pretreatment conditions were used.[6,26] When the maximum overall combined xylose and glucose yields from both the initial and second pretreatments are considered, those treatments employing citric acid and lignosulfonate (6%)/H_2SO_4 were the most effective acid catalysts, as 70−90% of the original xylose could be recovered while 45−60% of the cellulose present in the original substrate could be hydrolyzed to glucose using an enzyme loading 3 FPU/g (Figure 2a,b). There was good correlation between the xylose recovered and the furfural detected from each of the substrates after more severe pretreatments. The single stage, H_2SO_4 catalyzed pretreatment resulted in the greatest amount of furfural generation (1 mg/mL) while the water pretreated substrates (autocatalyzed) resulted in combined furfural concentration of 0.1 mg/L over the two pretreatment stages. The concentration of hydroxymethylfurfural and acetic acid ranged from 0 to 0.8 mg/mL and 0.57−3.1 mg/L, respectively, after the various pretreatments.

When the water-insoluble substrates after the second pretreatment were analyzed (Table 1), it was apparent that virtually all of the hemicellulose in each of the samples had been solubilized, with the exception of the uncatalyzed substrate, which contained low levels of xylan. The single-stage H_2SO_4 treatment was enriched in lignin likely due to the solubilization of the cellulose component of the biomass during the H_2SO_4 pretreatment while, with the exception of the two-stage H_2O and the single-stage H_2SO_4 pretreatment, the glucan content of the water-insoluble samples ranged from 58 to 60%. The single-stage H_2SO_4 pretreatment resulted in the recovery of only 78% of the original glucose (water-soluble plus water-insoluble fraction), indicating the severe nature of this condition that likely resulted in sugar degradation, compared to the greater than 90% overall glucose recovery for the two-stage pretreatments.

Although the two-stage pretreatments recovered a greater amount of hemicellulose compared to a single pretreatment stage, initial work showed that the resulting water-insoluble substrates were less amenable to enzymatic hydrolysis after the

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washing step. For example, a cellulose conversion of only 47% was obtained at 2% solids during hydrolysis of the substrate treated with 3% H_2SO_4 in the two-stage approach compared to 68% in the case of the substrate pretreated in a single pretreatment stage using 3% H_2SO_4 (Table 1). The only two-stage pretreated substrate that resulted in a comparable cellulose hydrolysis yield to the single-stage H_2SO_4 pretreatment was the two-stage treatments supplemented with 3 and 6% lignosulfonates, as hydrolysis of these substrates resulted in a nearly complete conversion of the cellulose at an enzyme loading of 10 FPU/g glucan (Table 1). In the work presented here, although the samples were washed extensively prior to enzymatic hydrolysis, even after extensive washing the sulfonate groups were still retained, as evidenced by the presence of strong acid groups (Table 1). In addition to their role in possibly blocking nonproductive adsorption of enzymes to lignin through a surfactant effect,[30] it was possible that lignosulfonate addition may be influencing structural changes to the biomass through the incorporation of sulfonate groups. In previous work, we had used two techniques, Simons stain[31] using Direct Orange dye, and an assessment of the substrates water retention values (WRV)[32] to determine how the gross and more detailed cellulose accessibility of a substrate might have changed during pretreatment and subsequent enzymatic hydrolysis.

Previous work has shown that the relative adsorption of the Direct Orange dye provides an accurate way of estimating the cellulose accessibility of a pretreated substrate to cellulase,[31] whereas an assessment of a substrates water retention value (WRV) gives more of an estimate of the substrates porosity and tends to provide a cruder estimate of potential enzyme accessibility to the substrate.[32] It was apparent that the Simons stain values were in good agreement with ease of substrate hydrolysis as the two substrates that showed the highest enzymatic hydrolysis yields after the two-stage pretreatment (H_2SO_4 plus 3% lignosulfonate and H_2SO_4 plus 6% lignosulfonate) also absorbed the greatest amount of the Direct Orange dye. Although this likely reflected an increase in cellulose accessibility, mediated by the H_2SO_4 plus lignosulfonate treatments, it was also possible that the polymeric 5-nitro-*o*-toluenesulfonic acid structure of the dye is interacting to some extent with the lignosulfonate moieties bound to the substrate surface. Compared to the >100 kDa Direct Orange dye, water is a very small probe, and as such has greater access to the substrate than either the dye or the enzymes. Water can also access and interact with the hydrophilic portions of the substrate, thereby potentially resulting in higher water retention values when hydrophilic noncellulosic components such as hemicellulose, are present.[34,35] When the water retention value of purified birch xylan powder was determined, its relatively high value illustrated the hydrophilic nature of the hemicellulose component (Table 1). Unlike the poplar biomass, the purified xylan does not have a fiber structure but its relatively high water retention value illustrated both the hydrophilic nature of the hemicellulose component and some of the challenges that can be encountered when using methods such as the water retention value to determine cellulose accessibility, particularly when hemicellulose is present.

Thus, although the substrates pretreated with single- and two-stage H_2SO_4 were relatively susceptible to enzymatic hydrolysis and had correspondingly high adsorption of the Simons staining dye, these samples exhibited the lowest water retention value measurements, likely due to the low hemicellulose content of these substrates (Table 1). In contrast, the samples pretreated with water, SO_2 and citric acid, which contained a greater amount of hemicellulose and were less susceptible to enzymatic hydrolysis, showed higher water retention values. Good correlation between the Simons stain values and the enzymatic hydrolysis yields obtained using 10% solids was observed, with r^2 values of 0.78 and 0.72 at enzyme loadings of 5 and 10 FPU/g respectively obtained, compared to the r^2 values of 0.045 and 0.026 obtained when comparing the enzymatic hydrolysis yields to the water retention values (WRV). This further illustrated the limitations of using the WRV for measuring cellulose accessibility when there are significant differences in the hemicellulose content of the biomass samples. Similar to the Direct Orange values, the water retention values for the two-stage pretreated substrates catalyzed by H_2SO_4 supplemented with lignosulfonates were high, even though these samples contained the lowest amount of hemicellulose. Due to their hydrophilic nature, lignosulfonate adsorption to the pretreated substrates likely results in an increase in their water retention values, a reduction in the nonproductive binding of enzymes to lignin and a consequential increase in the ease of enzymatic hydrolysis of the cellulose.[19]

CONCLUSIONS

The two-stage steam pretreatment of poplar pulp chips using an initial, mild acid-catalyzed first stage followed by a subsequent high temperature second stage resulted in enhanced hemicellulose recovery and cellulose hydrolysis compared to a single-stage treatment at elevated temperatures. Simons stain, using Direct Orange dye adsorption, could effectively assess the pretreated substrates cellulose accessibility. The addition of lignosulfonates to the initial pretreatment stage enhanced both hemicellulose recovery and the subsequent enzymatic hydrolysis of the cellulose. A two-stage steam pretreatment process, incorporating the addition of lignosulfonates, increased cellulose hydrolysis and overall sugar recovery (from both the hemicellulose and cellulose).

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Notes

The authors declare no competing financial interest.

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