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Substrate Dependency and Effect of Xylanase Supplementation on Enzymatic Hydrolysis of Ammonia-Treated Biomass

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Abstract Pretreatment based on aqueous ammonia was investigated under two different modes of operation: soaking in aqueous ammonia and ammonia recycle percolation. These processes were applied to three different feedstocks with varied composition: corn stover, high lignin (HL), and low lignin (LL) hybrid poplars. One of the important features of ammonia-based pretreatment is that most of the hemicellulose is retained after treatment, which simplifies the overall bioconversion process and enhances the conversion efficiency. The pretreatment processes were optimized for these feedstocks, taking carbohydrate retention as well as sugar yield in consideration. The data indicate that hybrid poplar is more difficult to treat than corn stover, thus, requires more severe conditions. On the other hand, hybrid poplar has a beneficial property that it retains most of the hemicellulose after pretreatment. To enhance the digestibility of ammonia-treated poplars, xylanase was supplemented during enzymatic hydrolysis. Because of high retention of hemicellulose in treated hybrid poplar, xylanase supplementation significantly improved xylan as well as glucan digestibility. Of the three feedstocks, best results and highest improvement by xylanase addition was observed with LL hybrid poplar, showing 90% of overall sugar yield.

Keywords Hybrid poplar · Corn stover · Xylanase · Aqueous ammonia · Pretreatment

Introduction

Various pretreatment technologies are being used to improve the digestibility of carbohydrates in biomass by making the cellulosic part more accessible by enzymes [1]. Among the major findings of recent pretreatment research is that a given pretreatment

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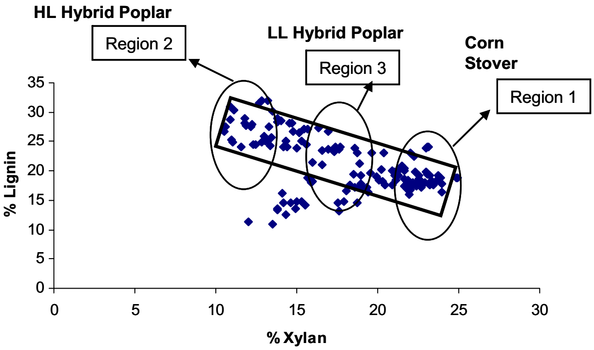
reagent exhibits selectivity toward a certain type of reaction attacking specific chemical bonds in lignin–hemicellulose–cellulose matrix. Pretreatment methods operated at low pH including steam explosion [2, 3], hot water treatment [4–6], controlled pH treatment, and dilute acid treatment [7, 8] remove substantial amount of hemicellulose. On the other hand, pretreatment methods applying high pH such as ammonia fiber explosion [9–11], lime pretreatment [12, 13], and ammonia recycle percolation (ARP) [14, 15] show little effect on hemicellulose but high interaction with lignin. In low pH pretreatments, the liquid contains hemicellulose sugars and other degradation byproducts. These byproducts are known to be inhibitory in subsequent bioconversion process. The examples of the inhibitory compounds are phenolics derived from lignin degradation, furan derivatives (hydroxymethylfurfural and furfural) from sugar decomposition, and aliphatic acids [16, 17]. Sugar–lignin condensation reactions in pretreatment liquid further reduces the sugar yield [18–22]. Glucose/xylose co-fermenting microorganisms were found to be very sensitive to these inhibitory chemicals [23, 24]. Various methods have been used to detoxify these toxins [17], but it bears substantial additional cost. From this viewpoint, hemicellulose retention is a positive feature in pretreatment because it eliminates the need of converting sugars in pretreatment liquid [25].

In most of the pretreatment studies, process conditions of pretreatment are optimized in terms of glucan digestibility. One of the factors that limit the digestibility of pretreated biomass is insufficient xylanase activity in the “cellulase” used in the hydrolysis [45]. This is particularly true for substrates with high xylan content. According to Berlin et al., the hydrolysis rate of different organosolv-treated hardwood did not show any correlation with the filter paper activity of different enzyme preparations, yet showed significant correlation with the endogenous xylanase activity [26]. Covalent and non-covalent association of xylan with cellulose and lignin is an essential factor for holding the structural integrity of cell wall [27]. Removal of xylan either by pretreatment or by xylanase would enhance enzymatic hydrolysis of cellulose. Xylanase not only degrade xylan but also assists in delignification as observed in bio-bleaching of pulp [28].

In Fig.1, lignin vs xylan content of various biomass feedstocks is shown. This data has been taken from the DOE website (http://www.eere.energy.gov/biomass/progs/search1.cgi). The data indicate that there is an inverse relationship between the lignin and xylan content of biomass. These feedstocks were divided into three different categories: Regions 1, 2, and 3 represent the feedstocks with high xylan and low lignin, low xylan and high lignin, and moderate xylan and moderate lignin, respectively. In this study, feedstocks representing the three regions were selected: corn stover, high-lignin (HL) hybrid poplar, and LL hybrid

Fig. 1 Relation between lignin and xylan content in different biomass



poplar. Recent work in our laboratory has proven that SAA and ARP are very efficient for delignification of corn stover yet retaining high amount of hemicellulose [29–31].

The objective of this study is to apply pretreatments based on aqueous ammonia on feedstocks of varying physical and structural properties and seek insights on how the pretreatment effects are influenced by definable parameters. The optimum conditions were defined for different feedstocks, and the investigation was focused on the pretreatment region near optimum condition. Special attention was paid to the role of xylan and its removal by external xylanase in the enzymatic hydrolysis of biomass.

Materials and Methods

Substrates and Reagents

Corn stover was supplied by NREL. It was ground and screened, and the fraction collected between 9 and 35mesh was used in all experiments. Two different batches of hybrid poplar chips (1/4 in.) were supplied by NREL. The moisture content of both the hybrid poplar batches was approximately 50%. Avicel PH-101 was purchased from Sigma (cat. no. 11365 and lot no. 1094627). Ammonium hydroxide of 30 wt% was purchased from Fisher-Scientific.

Enzymes

Cellulase enzyme (Spezyme-CP) and xylanase (multifect xylanase) were kind gifts of Genencor International Inc. (Paulo Alto, CA, USA). Two different batches of Spezyme-CP were used in this study: Spezyme CP-A (lot no. 301-00348-257) and Spezyme CP-B (lot no. 301-04075-054). The nominal filter paper activities for Spezyme CP-A and Spezyme CP-B were 31.2 FPU/ml (determined by NREL) and 59 FPU/ml (determined by Genencor), respectively. Spezyme CP-B was used in ARP-treated samples, and Spezyme CP-A was used in SAA-treated samples. The protein content of multifect xylanase was 42 mg of protein/ml. β-Glucosidase was purchased from Sigma (Novozyme 188 from Novo Inc., Sigma no. C-6150 and lot no. 11K1088). The specified activity as determined by the authors was 750 CBU/ml.

Experimental Setup and Operation of ARP

The ARP experimental system employs a packed-bed column reactor through which the pretreatment liquid flows. Details of the reactor setup are described elsewhere [30]. ARP experiments were conducted under the following optimum set of conditions chosen for different feedstocks:

Corn Stover The temperature of 170 °C was used with 20 min of reaction time (10 min with ammonia solution followed by 10 min with deionized, DI, water) and 5ml/min of liquid flow rate. The overall solid to liquid (15% ammonia) ratio was 1:3.33 (*w/w*). The conditions for corn stover were chosen on the basis of our previous study [30].

LL and HL Hybrid Poplar Substrate was first soaked overnight in 15% ammonia solution with solid to liquid ratio of 1:3.67 (*w/w*) before ARP experiment. The temperature of the reactor containing the soaked biomass was raised to 185 °C, and DI water was passed for 27.5 min with 2 ml/min flow rate. The temperature of the reactor during the reaction remained constant. Unless stated otherwise, all the ARP pretreatment experiments were conducted under these conditions.

It was found that overnight soaking of HL hybrid poplar before ARP treatment does not affect the hydrolysis of treated biomass much. Therefore, in other experiments where effect of different process variable in ARP process was studied (Tables 2, 3, and 4), HL hybrid poplar was soaked for only 10 min before starting the pretreatment. For HL hybrid poplar, the upper limit of temperature was set at 195 °C, above which, severe decomposition of carbohydrates were observed, and the system pressure rose to 450 psig.

Experimental Setup and Operation of SAA

Batch reactors were used for the SAA pretreatment. For low temperature operation up to 80 °C, biomass was soaked with 15% ammonia in screw-capped laboratory glass bottle and kept in an oven. For runs above 80 °C, stainless steel reactors (1.375 in. ID×6 in. l) were used. For corn stover, 60 °C temperature was used with 12 h soaking time and 1:8 solid to liquid ratio. For HL and LL hybrid poplar, the temperature of 150 °C was employed with 24 h soaking time and 1:10 solid to liquid ratio.

Enzymatic Digestibility

Enzymatic digestibility tests were done with 1.0% *w*/*w* glucan loading. Thus, total amount of solids in the reactor varied according to the glucan content in biomass. The reaction of enzymatic digestibility was carried out in 250 ml Erlenmeyer flask with total liquid volume of 100 ml. This test was carried out according to the NREL Chemical Analysis and Test procedures [32].

Cellulase loading of 15 FPU/g glucan and β-glucosidase loading of 30 CBU/g glucan were used in all of the enzymatic digestibility experiments. Unless noted otherwise, xylanase loading was 31.5 mg protein/g glucan in the hydrolysis experiments with xylanase supplementation. The glucan or xylan digestibility was defined as the percentage of theoretical glucan or xylan released after 72 h of incubation with enzyme.

Analytical Procedures

Composition analysis of the treated/untreated biomass was done according to the NREL Laboratory Analytical Procedures: “Preparation of samples for compositional analysis” and “determination of structural carbohydrates and lignin in biomass” (draft version) [32]. The moisture content in biomass was measured by an infrared moisture balance (Denver Instrument, IR-30). Sugar content in compositional analysis and enzymatic digestibility was determined by HPLC using a Bio-Rad Aminex HPX-87P.

Crystallinity Index

Crystallinity of treated and untreated hybrid poplar feedstock was measured by X-ray diffractometer (Rigaku DMAX). Cu–Kα radiation was generated at 40 kV and 40 mA. Samples were scanned from $2\theta=10$–40 with 0.01 increment. The following formula has been used for the calculation of crystallinity index of samples [33]:

$$\mathrm{CrI} = (I_{002} - I_{\mathrm{am}} / I_{002}) \times 100,$$

where I_{002} is the peak intensity corresponding to 002 lattice plane of cellulose molecule observed at 2θ equal to 22.5°, and I_{am} (at $2\theta=19°$) is the peak intensity corresponding to amorphous cellulose.

Results and Discussion

Observation of Feedstocks

The composition of corn stover, LL hybrid poplar, and HL hybrid poplar is shown in Table 1. Moving from corn stover to HL hybrid poplar, lignin content is increasing, and xylan content is decreasing. Among these three feedstocks, LL hybrid poplar is highest in carbohydrates.

The surface of HL hybrid poplar and LL hybrid poplar look very much similar, but the surface of corn stover shows more open structure, which would allow more adsorption capacity for pretreatment reagent into the solid, hence, higher reactivity. Diffusivity of the pretreatment reagent in agriculture residues was found to be much higher than the hardwood [34]. The surface characteristics of biomass are one of the important factors affecting the global reactivity of pretreatment reagent with biomass.

Effect of ARP Pretreatment

Table 1 shows the effect of ARP treatment on the composition and the digestibility of different feedstocks. The optimum conditions described earlier in "Materials and Method" were applied in these tests. The extent of delignification and xylan removal in corn stover is much higher than hybrid poplar. With corn stover, more than 50% xylan is lost to liquid. The glucan digestibility of ARP-treated corn stover reached 90%. Xylan removal in LL and HL hybrid poplar were 22 and 33%, respectively. The glucan digestibilities of ARP-treated LL and HL hybrid poplar were 66 and 49%, respectively.

Effects of ARP Process Variables on Hybrid Poplar

Effects of ARP temperature on pretreatment of HL hybrid poplar are shown in Table 2. Delignification or xylan removal was not significantly affected by the ARP temperature, staying relatively constant over the range of 170–195 °C. However, there was a significant effect on glucan digestibility, increasing from 27 to 52% as the temperature was raised from 170 to 195 °C. This indicates that the accessibility of enzymes to cellulose can be increased

Table 1 Effect of ARP treatment on different feedstocks.

	Composition (%)						Digestibility of treated biomass (%)	
	Glucan		Xylan		Lignin			
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Glucan	Xylan
Corn stover	36.1	35.6	21.4	10.3	17.2	5.1	88.1	77.9
LL hybrid poplar	44.9	44.5	17.9	13.5	21.4	14.6	66.1	66.6
HL hybrid poplar	43.8	40.8	14.9	9.6	29.1	17.6	48.9	51.1

Table 2 Effect of temperature on the HL hybrid poplar in ARP treatment.

Temperature (°C)	Composition of treated biomass (%)			Digestibility (%)	
	Glucan	Xylan	Lignin	Glucan	Xylan
170	42.06	11.56	17.33	27.24	30.24
175	42.15	12.41	17.62	38.47	41.53
180	41.14	10.18	16.31	38.32	44.85
185	40.84	9.88	16.66	47.86	50.00
195	41.09	9.82	15.90	50.67	43.14

Liquid (DI water) flow rate, 2 ml/min; reaction time, 27.5 min

without significant change in composition. It is most likely caused by breakage of certain bonds and reconfiguration of components in lignin–hemicellulose matrix. A possibility exists that the ARP can be operated in such a way that digestibility is enhanced without further loss of carbohydrates. This claim may be limited to highly recalcitrant substrates such as hybrid poplar. Retention of carbohydrate in the solid is a desirable trait in pretreatment technology, and hybrid poplar appears to be a substrate well suited for this purpose within the context of ARP.

The effect of reaction time on the performance of ARP pretreatment with the HL hybrid poplar is shown in Table 3. Lignin removal of hybrid poplar increases with reaction time. Xylan removal, however, was only slightly affected by ARP reaction time. After a certain point, the xylan content remained constant, indicating that there is a portion of xylan that is resilient and difficult to remove. The 72-h glucan and xylan digestibilities correlate more closely with xylan removal than lignin removal.

These results are in line with the findings of Kim et al. [30] that aqueous ammonia is highly effective in delignifying lignocellulosic biomass. Reactivity of ammonia with hemicellulose appears to decline after the removal of certain fraction of hemicellulose from biomass. The reasons for this are found in previous work concerning mechanistic studies. Alkaline degradation of carbohydrate during the Kraft pulping occurs from the reducing end and the reaction of alkali with the carbohydrate chain stops when the reducing end (–OH) is replaced by the alkali stable end (–COONa) [35]. The same mechanism may apply to ammonia treatment. It has also been reported that the reducing ends of xylans consist of a combination of xylose and galacturonic acid with the sequence of β-D-Xylp-1–4–β-D-Xylp-1–3–α-L-Rhap-1–2–α-D-GalpU-1–4–β-D-Xyl. When xylose is removed from the reducing end, galacturonic acid makes the xylan molecule more stable toward alkali degradation. [36, 37]. Vian et al. [38] and Reis et al. [39] have provided evidences for tight

Table 3 Effect of reaction time on the HL hybrid poplar in ARP treatment.

Reaction time (min)	Composition of treated biomass (%)			Digestibility (%)	
	Glucan	Xylan	Lignin	Glucan	Xylan
27.5	41.13	10.68	14.51	42.34	42.23
55.0	40.33	9.88	12.41	50.75	44.77
82.5	40.01	9.83	11.05	52.38	44.43

Liquid (15% ammonia solution) flow rate, 2 ml/min; temperature, 185 °C association of glucuronoxylans with cellulose microfibril. Mora et al. [40] have proven that there is a strong interaction and retention of heteroxylans with cellulose microfibril even after a strong alkaline treatment such as Kraft pulping. These findings suggest that the hemicellulose bound with the cellulose microfibril is difficult to remove. Among the three feedstocks, xylan loss during the ARP pretreatment is found to be inversely proportional to the cellulose content in the biomass. One plausible explanation for this is that the hemicellulose–cellulose linkage makes the hemicellulose stable, thus, making it less amenable for ammonia-induced degradation. Corn stover, having less cellulose content, possesses fewer cellulose–hemicellulose linkages than poplar. This may explain why corn stover loses more xylan than hybrid poplar during ARP pretreatment.

Sugars in ARP liquid exist only in oligomer form, i.e., xylo-oligosaccharides (XOS). After acid hydrolysis of the ARP liquor, large amount of xylose monomer appears. It proves that hemicellulose sugars in the ARP liquor is either in the form of XOS or lignin carbohydrate complex. Hemicellulose removal in ARP is proportional to the extent of lignin removal. The reason for this is unclear at this time.

Table 4 shows the effect of ammonia flow rate on the composition and digestibility. It is reaffirmed here that the digestibility correlates better with xylan removal than lignin removal, especially for hybrid poplar. This also indicates that delignification alone is not sufficient to attain high digestibility and that xylan removal is also required.

Effect of Process Variable on Crystallinity

As indicated in Fig. 2a and b, crystallinity of HL hybrid poplar increases after the ARP pretreatment. These results are in accordance with the findings of Kim et al. [30] that the total content of crystalline cellulose increases because of the removal of the amorphous part (lignin and hemicellulose) and that contributes to the increase of crystallinity. Cao and Huimin [33] also observed that the crystallinity of the treated pulp increases because of the removal of the amorphous part by enzymatic action. Figure 2a shows that the crystallinity increases with the reaction time, as removal of lignin and hemicellulose also increases. Further increase of ARP temperature from 175 to 195°C caused a slight decrease in crystallinity (Fig. 2b). In this case, the decrease of crystallinity is caused by the structural change in cellulose rather than in composition. With ARP operated below 185 °C, the basic structure of cellulose is not altered regardless of reaction time; only the removal of hemicellulose and lignin occurs.

SAA Pretreatment

Our previous work on the SAA using corn stover provided satisfactory digestibility [29]. Corn stover treated by SAA under moderate process conditions (60 °C, 1:8 S/L ratio, and

Table 4 Effect of reaction time on the HL hybrid poplar in ARP treatment.

Liquid (15% ammonia solution) flow rate (ml/min)	Composition of treated biomass (%)			Digestibility (%)	
	Glucan	Xylan	Lignin	Glucan	Xylan
2.0	41.13	10.68	14.93	42.34	42.23
5.0	40.88	11.00	12.78	44.84	39.79
7.5	40.94	9.76	11.38	48.94	45.49

Reaction time, 27.5min; temperature, 185 °C

12 h) gave approximately 90% glucan digestibility. In the case of hybrid poplar, higher severity conditions (150 °C, 24 h) were required to attain the acceptable level of digestibility. Even at the temperature as high as 150 °C, glucan digestibility of SAA-treated LL hybrid poplar and HL hybrid poplar was only 73 and 60%, respectively, with 15 FPU cellulase loading.

Composition and digestibility of the SAA-treated feedstocks are summarized in Table 5. SAA of corn stover removes 65% of lignin, yet retains 85% of xylan in solid. In case of hybrid poplar, negligible amount of xylan is lost to liquid, and delignification is much less than corn stover. The effectiveness of pretreatment is assessed with the two criteria; first, the retention of carbohydrates in solid and, second, the enzymatic digestibility of pretreated biomass. According to the first criterion, SAA is quite effective, retaining above 90% of xylan in hybrid poplar. The enzymatic digestibility of SAA-treated hybrid poplar, however, is not in acceptable range. With xylanase supplementation, the digestibility rose to 94 and 86% for LL and HL hybrid poplar, respectively (Table 6), meeting the second criterion as well.

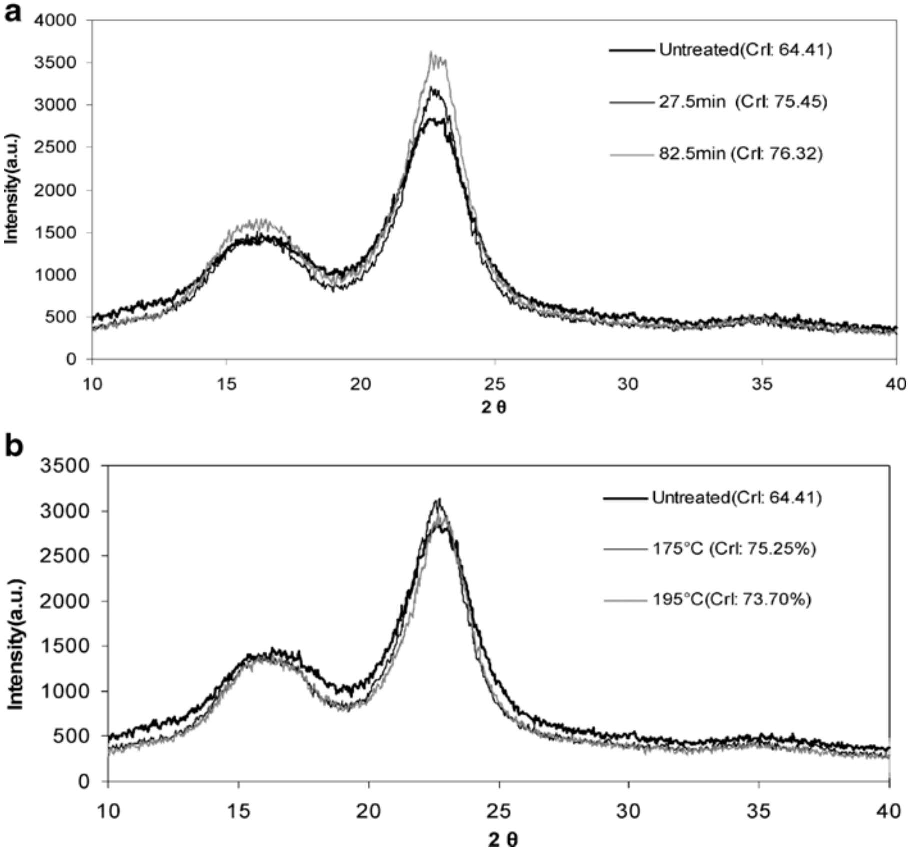


Fig. 2 **a** XRD plots of ARP-treated HL hybrid poplar samples treated with various reaction time; **b** XRD plots of ARP-treated HL hybrid poplar samples treated at different temperature

Table 5 Effect of SAA treatment on different feedstocks.

	Composition (%)						Digestibility of treated biomass (%)	
	Glucan		Xylan		Lignin			
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Glucan	Xylan
Corn stover	36.1	35.3	21.4	18.4	17.2	6.1	91.2	80.6
LL hybrid poplar	44.9	44.9	17.9	16.7	21.4	19.3	72.7	67.2
HL hybrid poplar	43.8	40.5	14.9	13.6	29.1	20.0	60.0	54.7

Effect of Xylanase Supplementation on the ARP- and SAA-Treated Biomass

The effects of xylanase supplementation on glucan/xylan digestibility of ARP- and SAA-treated feedstocks are summarized in Table 6. In all cases, digestibility increased significantly after xylanase supplementation. The increase was highest with LL hybrid poplar and lowest with corn stover. Glucan and xylan digestibility of ARP-treated LL hybrid poplar increased to more than 95% after xylanase supplementation. In Fig. 3, the digestibilities of pure cellulose (Avicel) with and without xylanase addition are shown. The fact that there is no difference between them proves that the xylanase enzyme does not have any cellulase activity. This further proves that xylanase addition increases the accessibility of cellulase enzymes to cellulose chains by removing the hemicellulose barrier.

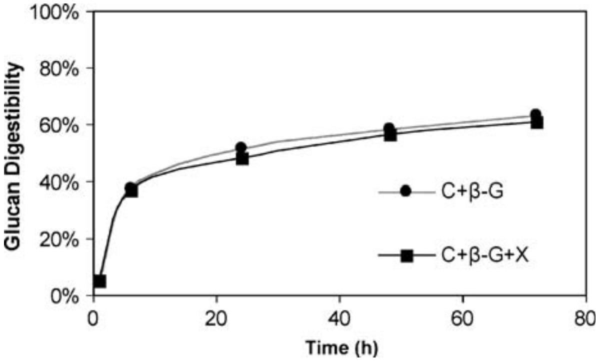
ARP of corn stover removes 70% of lignin and 50% of hemicellulose. Therefore, a large part of the hindrance for cellulase action has been removed, and high digestibility is attained without xylanase addition. On the other hand, ARP of LL hybrid poplar removes only 32% of lignin and 25% of xylan; consequently, glucan digestibility of only 65% is obtained (Table 1). When supplemented with xylanase, a drastic improvement of the digestibility was seen with LL hybrid poplar reaching 97% (Table 6). It has been reported that cellulase availability to cellulose is reduced because of unproductive binding of cellulase to lignin [41, 42]. Hydrophobic interaction of surfactants with lignin reduces the unproductive binding of cellulase with lignin. It decreases the effect of hydrophobic environment caused by lignin, thus, increasing the access of water and cellulase molecule to

Table 6 Effect of xylanase addition on the digestibility of ARP and SAA treated biomass.

	ARP treatment				SAA treatment			
	Glucan		Xylan		Glucan		Xylan	
	Digestibility (%)	Increase (%)	Digestibility (%)	Increase (%)	Digestibility (%)	Increase (%)	Digestibility (%)	Increase (%)
Corn stover	92.37	4.79	86.27	10.74	–	–	–	–
LL hybrid poplar	97.14	46.91	95.54	43.41	93.80	29.11	88.52	31.80
HL hybrid poplar	69.65	42.49	73.35	43.60	86.46	44.20	76.94	40.61

Enzymes used: cellulase + β-glucosidase + xylanase; percent increase shows the increase in digestibility because of xylanase addition

Fig. 3 Effect of xylanase addition on digestibility of Avicel (C + β-G: cellulose + β-glucosidase, C + β-G + X: cellulose + β-glucosidase + xylanase)



cellulose [43]. Despite these evidences, lignin may not be the overriding factor in cellulase action. Increase of digestibility to near quantitative level under the presence of high amount of lignin, as seen here, with LL hybrid poplar indicates that high level of lignin removal is not a necessary condition to achieve high digestibility. In the case of LL poplar, the main barrier to hydrolysis appears to be hemicellulose rather than lignin. This is not to say that lignin is not a major factor controlling digestibility. Lignin needs to be removed but only to a certain extent to achieve accessibility to carbohydrates. Kanda et al. reported that xylan molecule also binds with endo-glucanase-active site, and the Km value of endo-glucanase with xylan is greater than with cellulose by a factor of 1.6, but the *V*max value for xylan is only 18% than for the cellulose substrate [44, 45]. This suggests that the xylan acts as a competitive inhibitor to endo-glucanase.

ARP of HL hybrid poplar removes 40% lignin and 32% of xylan, which is higher than LL hybrid poplar. Yet, the lignin content in the ARP-treated HL hybrid poplar is higher than treated LL hybrid poplar (Table 1). With similar amount of xylanase supplementation, high level of digestibility was not achieved with treated HL hybrid poplar. In the case of HL hybrid poplar, lignin is still a deciding factor for enzyme accessibility to carbohydrate. Glucan digestibility increases in proportion with the amount of xylanase addition for both corn stover and LL hybrid poplar as shown in Fig. 4. For HL hybrid poplar, the effect of xylanase addition is significant only with higher xylanase loading. Unproductive binding with lignin may be the reason because HL hybrid poplar contains higher amount of residual lignin.

Fig. 4 Glucan digestibility of ARP-treated feedstocks with different xylanase loading

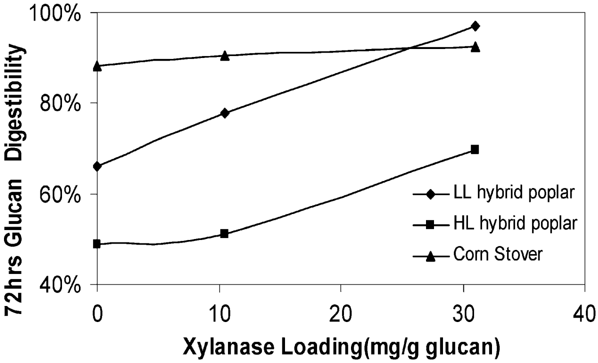


Table 7 Overall sugar yield for different feedstocks with ARP and SAA treatment.

	ARP treatment		SAA treatment	
	Without xylanase %	With xylanase %	Without xylanase %	With xylanase %
Corn stover	67.63	72.64	81.78	-
LL hybrid poplar	61.21	89.43	69.85	90.66
HL hybrid poplar	42.37	60.47	54.04	77.47

As is the case with the ARP, the glucan/xylan digestibilities with xylanase addition are higher for LL hybrid poplar than HL hybrid poplar after the SAA treatment too.

Conclusion

In ARP pretreatment of hybrid poplar, delignification is increased with the treatment severity, but xylan removal occurs only to a certain extent. Increase of temperature from 175 to 195 °C in ARP does not affect xylan/lignin removal in hybrid poplar, yet the glucan digestibility of treated solid is increased. This is caused by an increase of cellulase accessibility to cellulose created by breakage of certain bonds and reconfiguration of the components in hemicellulose–lignin matrix. Crystallinity index of hybrid poplar increases after the ARP treatment. It is primarily caused by the removal of amorphous components, i.e., hemicellulose and lignin.

Xylanase supplementation in enzymatic hydrolysis is effective for the substrates with high xylan content. Xylanase supplementation not only increases the xylan digestibility of treated biomass but also the glucan digestibility. Xylanase reduces the hindrance caused by resilient hemicellulose layer on the cellulose microfibril, thus, improving cellulase accessibility to the cellulosic part.

Addition of external xylanase improves the digestibility of ARP-treated hybrid poplar. The digestibility is higher with LL hybrid poplar than with HL hybrid poplar. Higher amount of residual lignin in HL hybrid poplar is a plausible cause of it. Lignin is the primary barrier in enzymatic hydrolysis of biomass. A certain degree of lignin removal is, therefore, necessary to facilitate enzyme access to carbohydrates in treated biomass.

In SAA treatment, higher amount of hemicellulose is retained than in ARP because of lower severity of SAA. The SAA is more effective for LL hybrid poplar than HL hybrid poplar.

Performance of ARP and SAA treatments are summarized in Table 7. In both ARP and SAA, LL hybrid poplar showed the best results. The overall sugar yield from LL hybrid poplar reached 90% (56 g of fermentable sugar/100 g of feedstock). It was achieved with xylanase supplementation. High retention of carbohydrate in the solid after pretreatment and added xylanase activity in enzymatic hydrolysis are two contributing factors for high overall sugar yield. High reactivity of aqueous ammonia with lignin over carbohydrate is the primary reason for retention of hemicellulose in ARP and SAA.

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