Application of High Throughput Pretreatment and Co-Hydrolysis System to Thermochemical Pretreatment. Part 2: Dilute Alkali

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ABSTRACT: High throughput pretreatment (HTPH) and enzymatic hydrolysis systems are now vital for screening large numbers of biomass samples to investigate biomass recalcitrance over various pretreatment and enzymatic hydrolysis conditions. Although hydrothermal pretreatment is currently being employed in most high throughput applications, thermochemical pretreatment at low and high pH conditions can offer additional insights to better understand the roles of hemicellulose and lignin, respectively, in defining biomass recalcitrance. Thus, after successfully applying the HTPH approach to dilute acid pretreatment [Gao et al. (2012) Biotechnol. Bioeng. 110(3): 754–762], extension to dilute alkali pretreatment was also achieved using a similar single-step neutralization and buffering concept. In the latter approach, poplar and switchgrass were pretreated with 1 wt% sodium hydroxide at 120°C for different reaction times. Following pretreatment, an H_2Cit^-/$HCit^{2-}$ buffer with a pH of 4.5 was used to condition the pretreatment slurry to a pH range of 4.69–4.89, followed by enzymatic hydrolysis for 72 h of the entire mixture. Sugar yields showed different trends for poplar and switchgrass with increases in pretreatment times, demonstrating the method provided a clearly discernible screening tool at alkali conditions. This method was then applied to selected *Populus tremuloides* samples to follow ring-by-ring sugar release patterns. Observed variations were compared to results from hydrothermal pretreatments, providing new insights in understanding the influence of biomass structural differences on recalcitrance.

Introduction

Biomass recalcitrance is collective resistance of plant cell wall structural polymers, including lignin, hemicellulose, and cellulose, to chemical or biological deconstruction (Himmel, 2008; Lynd et al., 1999). Pretreatment of lignocellulosic biomass is a critical prerequisite to reduce biomass recalcitrance and achieve high enough sugar yields by enzymes and microorganisms to be economically viable (Lynd et al., 1991; Wyman, 1994, 2007). Researchers are also working to reduce biomass recalcitrance through two other major approaches: genetic modification of plant cell walls to reduce their recalcitrance and consolidating processing of enzymes and microorganisms to overcome biomass recalcitrance. To connect these three approaches, interactions and impacts among cell wall modification, pretreatment conditions, and biological deconstruction are very important to understand. However, a large number of factors must be considered in this integration: (1) numerous energy crop species and genetic modification options provide thousands of biomass samples that need to be tested; (2) various pretreatment pH, temperature, and reaction times have to be considered; and (3) numerous enzyme and/or microorganism combinations and formulations need to be evaluated. In response to this challenge, high throughput pretreatment and

enzymatic hydrolysis (HTPH) systems have been developed and applied to much more efficiently evaluate the vast number of combinations of variables that can affect sugar release from biomass in a fast and automatable manner (DeMartini and Wyman, 2012).

Although hydrothermal pretreatment is currently applied in most current HTPH systems, operation with chemicals at high temperature is also desirable to evaluate whether dilute alkali and/or dilute acid pretreatment alter biomass differently and expand the range of pretreatment conditions that can be applied to large numbers of biomass materials and enzyme/organism combinations. For example, alkaline conditions are more effective in removing lignin from the cell wall polysaccharide matrix while acidic conditions usually facilitate hemicellulose removal (Gupta and Lee, 2010b; Kumar et al., 2009; Mosier et al., 2005; Ragauskas et al., 2006). To date, several alkaline pretreatments have been developed including those based on sodium hydroxide, wet alkaline oxidation, aqueous ammonia, lime, and ammonia fiber expansion (AFEX) (Alizadeh et al., 2005; Holtzapple et al., 1991; Kaar and Holtzapple, 2000; Kim et al., 2003; Sierra et al., 2012; Xu et al., 2010). Sodium hydroxide is perhaps the most widely used base, with Table I summarizing typical conditions that have been reported for this approach (Farid et al., 1983; Gupta and Lee 2010a,b; McIntosh and Vancov, 2010; Silverstein et al., 2007; Wang et al., 2010; Xu et al., 2010; Zhao et al., 2009). Compared to hydrothermal and dilute acid pretreatments, alkaline pretreatments tend to employ lower temperatures but relatively longer reaction times.

Because most HTPH systems based on a "co-hydrolysis" approach in which the whole slurry from pretreatment is subjected to enzymatic hydrolysis without an intermediate step for liquid/solid separation, the high pH slurry (usually over 12) from sodium hydroxide pretreatment needs to be neutralized prior to hydrolysis to maintain enzyme activity. One approach is to neutralize the slurry with acid; however, neutralization by acid titration is time and labor intensive and impractical for high throughput applications. To avoid this problem, a very low sodium hydroxide concentration (0.025 wt%) was employed (Santoro et al., 2010), but the concentration was so dilute that the results did not reflect the true benefits of alkaline pretreatment. Therefore, in this study, an $H_2Cit^-/HCit^{2-}$ buffer with pH 4.5 was developed that successfully adjusted the pH value of biomass slurries

from about 12 for 1 wt% sodium hydroxide pretreatment to a range appropriate for enzymatic hydrolysis. Then, this one step neutralization and buffering method was applied to whole slurries produced by sodium hydroxide pretreatment of poplar and switchgrass prior to 72 h co-hydrolysis. Relatively high sugar yields from poplar and switchgrass over a range of reaction times demonstrated that 1 wt% sodium hydroxide can be effectively used in HTPH systems, thereby offering a much less labor-intensive and timely route to evaluate the effectiveness of alkaline pretreatments for releasing sugar from biomass. Finally, the dilute alkali HPTH system was applied to selected Aspen (*Populus tremuloides*) cross-section samples to investigate ring-by-ring differences in recalcitrance, and sugar release was compared to prior results from hydrothermal and dilute acid pretreatments.

Materials and Methods

Plant Material

Poplar (*Populus trichocarpa*) was grown at Oak Ridge National Laboratory (ORNL) and provided through BioEnergy Science Center (BESC), Oak Ridge, TN. The logs were debarked, split, and chipped (Yard Machine 10HP, MTD Products Inc., Cleveland, OH) at the National Renewable Energy Laboratory (NREL) in Golden, CO. Switchgrass (*Panicum virgatum*) was grown at Pierre, South Dakota, dried, and shipped to the University of California at Riverside (UCR). Both poplar and switchgrass samples were knife milled (Model 4, Wiley Mill, Thomas Scientific, Swedesboro, NJ), and fractions between 20-mesh (<0.85 mm) and 80-mesh (>0.180 mm) (RX-29, W.S. Tyler, Mentor, OH) were collected for subsequent experiments. The moisture content of biomass samples was determined by an automatic infrared moisture analyzer (Model No. HB43-S, Mettler-Toledo, LLC, Columbus, OH). As determined according to the NREL two-step strong acid hydrolysis procedure (Sluiter et al., 2008), poplar was found to contain 46.5% glucan and 20.3% xylan; and switchgrass contained 32.4% glucan and 21.2% xylan.

Trembling Aspen (*P. tremuloides*) samples were prepared by fractioning a 20–30 years old cross-section, which was obtained from Benchmark International in Alberta, Canada, into its individual annual rings, as discussed in detail elsewhere (DeMartini and Wyman, 2011a). Samples were labeled as 1–26 from pith to bark, according to the relative

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Table I. Typical conditions reported to give high sugar yields from sodium hydroxide pretreatment.

Biomass	Pretreatment conditions	Reference
Cotton	1, 2, 5, and 10 wt% NaOH; 100°C; 60 min	Farid et al. (1983)
Cotton stalks	2 wt% NaOH, 121°C, 90 min	Silverstein et al. (2007)
Sugarcane bagasse	10 wt% NaOH, 90°C, 90 min	Zhao et al. (2009)
Switchgrass	1 and 5 wt% NaOH; 60 and 80°C; 24 h	Gupta and Lee (2010a)
Corn stover, poplar	1, 1.5, and 5 wt% NaOH; 25, 60, and 120°C; 24 h	Gupta and Lee (2010b)
Switchgrass	0.5, 1, and 2 wt% NaOH; 121°C; 1 h	Xu et al. (2010)
Bermuda grass	1 wt% NaOH, 121°C, 30 min	Wang et al. (2010)
Wheat straw	2 wt% NaOH, 121°C, 30 min	McIntosh and Vancov (2010)

year in which that ring was formed and knife milled through a 20-mesh screen (<0.85 mm). Samples corresponding to Year 2, Year 15, Year 20, as well as bark were selected for this study.

Pretreatment in Tube Reactors

Poplar and switchgrass were first subjected to pretreatment with three sodium hydroxide concentration (1, 2, and 5 wt%) and two pretreatment temperatures (60 and 120°C) to determine the pH range of the pretreated biomass slurry. Before pretreatment, 0.1 g of biomass material was soaked overnight in a 0.9 mL of sodium hydroxide solution at room temperature to allow full penetration. Then, 0.1 g biomass on a dry basis was loaded into 14 mL Hastelloy tube reactors (150 mm length, 12.5 mm OD, 0.8255 mm wall thickness) with stainless steel end caps (Swaglok, San Diego, CA). The 60°C pretreatment was conducted in a water bath, while the 120°C pretreatment was conducted in an autoclave chamber (Model HA300MII, Hirayama Manufacturing Corporation, Saitama, Japan), for a total reaction time of 24 h. After pretreatment, the reactors were quenched in cold water prior to opening. The pretreated slurry was next mixed with 9 mL of deionized (DI) water to reach a 1 wt% solids concentration, and the resulting slurry was centrifuged (Allegra X-15R; Beckman Coulter, Fullerton, CA) in a 15 mL centrifuge tube (Corning Life Science, Tewksbury, MA) for 10 min at 4,200 g, and the clear hydrolyzate was then transferred into 2 mL high recovery glass vials (Agilent, Santa Clara, CA) for pH measurement. The pH values were determined by a Core Module robotics platform (Freeslate; Sunnyvale, CA) using a MI-414 Micro-combination pH electrode (Microelectrodes, Bedford, NH), with details described elsewhere (Gao et al., 2012).

Buffer Preparation and Effectiveness Test

One molar citrate buffer (pH 4.5) was prepared by titration of 50 wt% sodium hydroxide solution (Cat No. 72064, Sigma–Aldrich, St. Louis, MO) into sodium citrate monobasic (Cat No. 71498, Sigma–Aldrich) water solution, while monitoring the pH (Model Seven Easy, Mettler Toledo). To test the buffering ability of this citrate buffer, slurries were produced by pretreatment of poplar and switchgrass in a 1 wt% sodium hydroxide at 120°C for 10 min, 70 min, 3 h, and 24 h in tube reactors that were heated in a custom-built steam chamber (Studer et al., 2010). After pretreatment, the pretreatment slurry was mixed with a 9 mL of DI water to reach a 1 wt% solid concentration. The slurry was then centrifuged as described above. After that, 1.425 mL of clear hydrolyzate was transferred into a 2 mL high recovery glass vial, and then 75 μL of the prepared 1 M citrate buffer was added to adjust the pH of the pretreatment slurry to the proper pH range while keeping the final buffer concentration at 0.05 M. The corresponding pH value was also measured with the micro pH electrode coupled to the robotic platform.

Sodium Hydroxide Pretreatment and Enzymatic Co-Hydrolysis HTPH System

Sodium hydroxide pretreatment and enzymatic co-hydrolysis was performed in a HTPH system (DeMartini and Wyman, 2011b; Gao et al., 2012; Studer et al., 2010, 2011), using a customized 96-well plate reactor. Dry biomass (4.5 mg) was added to each well by an automated solid and liquid dispensing robotics platform (Core Module II, Freeslate Inc.) followed by 40.5 μL of 1 wt% sodium hydroxide solution. The well plates were then clamped together and allowed to soak overnight at room temperature. After that, the plate reactors were placed in a custom-built steam chamber for pretreatment, as described in detail elsewhere (Studer et al., 2010), at 120°C for different pretreatment times. Because the objective was to evaluate the effectiveness of pretreatment on sugar release from the combined operations of pretreatment and enzymatic hydrolysis, low cellulose concentrations and high enzyme loadings were employed in enzymatic hydrolysis to minimize sugar inhibition from obscuring determination of pretreatment effectiveness. Accordingly, following pretreatment, 405 μL of DI water was added to each vial to bring the solids loading for enzymatic co-hydrolysis to 1 wt%, followed by addition of 30.5 μL of prepared citrate buffer (1 mol/L, pH 4.5), sodium azide (10 g/L), and dilute enzyme mixture, resulting in final buffer and sodium azide concentrations of 0.05 mol/L and 0.2 mol/L, respectively. Cellulase (Spezyme® CP, protein concentration 116 mg/mL, Lot #3016295230) and xylanase (Multifect® xylanase, protein concentration 42 mg/mL, Lot #4900667792) enzymes from Genencor (DuPont™ Genencor® Science, Palo Alto, CA) were added at a protein ratio of 3:1 and a high protein loading of 100 mg/g structural carbohydrates in the raw materials. The well plates were then incubated at 50°C in a Multitron shaker (Multitron Infors-HT, ATR Biotech, MD) at 150 rpm for 72 h. Following 72 h of incubation, the plates were centrifuged at 2,700 rpm for 30 min, and the hydrolyzate was transferred into HPLC vials for analysis. All enzymatic hydrolysis experiments were performed in quadruplicate. Sugar concentrations were determined by a Waters Alliance e2695 HPLC with a 2414 refractive index (RI) detector (Waters Corporation, Milford, MA) and a BioRad Aminex HPX-87H column (Bio-Rad Life Science, Hercules, CA). Reported sugar yields reflect the amount of sugars released as a percent of the maximum possible sugar in raw biomass.

Results and Discussion

pH Range of Sodium Hydroxide Pretreatment Slurry

During sodium hydroxide pretreatment of lignocellulosic biomass, hydroxyl groups are consumed in several types of reactions, such as C–O–C bond cleavage within lignin polymers as well as between lignin and hemicellulose, deprotonation of phenol units, and removal of acetyl groups from hemicellulose, reducing the pH of the pretreatment

slurry (Sierra et al., 2012). In addition, some inorganic salts in biomass can also "neutralize" hydroxyl groups. Thus, the pH change at typical sodium hydroxide pretreatment conditions must be accounted for to select proper sodium hydroxide concentrations for HPTH applications. In light of this, poplar and switchgrass were first pretreated in tube reactors using 10 wt% solids loading for three sodium hydroxide concentrations (1, 2, and 5 wt%) and two temperatures (60 and 120°C). The corresponding pretreatment slurries were collected, and the pH values determined, as reported in Table II. Overall, the pH values of hydrolyzates from 120°C pretreatment were lower than those from 60°C pretreatment; suggesting pretreatment at 120°C consumed more hydroxyl groups. At 120°C, perhaps the most widely used temperature for sodium hydroxide pretreatment, the hydrolyzate pH values following pretreatment of poplar and switchgrass for 24 h were 8.79, 11.92, 12.61, and 8.97, 11.72, 12.63, respectively, under corresponding tested sodium hydroxide concentrations of 1, 2, and 5 wt%. Considering that the low total citric ion concentration in the citrate buffer of 0.05 mol/L appropriate for enzymatic hydrolysis limits the buffering ability, pretreatment hydrolyzate with relatively low pH is more promising to achieve simple one step neutralization and buffering by the prepared citrate buffer. In addition, because co-hydrolysis is performed in the HPTH system, conditions with high sodium hydroxide concentration should be avoided to minimize enzyme inhibition. Thus, pretreatment with 1 wt% sodium hydroxide at 120°C was selected for subsequent experiments.

Preparation and Verification of the New Citrate Buffer

For hydrothermal pretreatment with the HTPH system, sodium citrate buffer with pH of 4.8 was used to control the pH of hydrolyzate for enzymatic hydrolysis (DeMartini and Wyman, 2012; Selig et al., 2008; Studer et al., 2010, 2011). However, its buffering capacity is insufficient to neutralize the extra hydroxyl groups in the hydrolyzate following sodium hydroxide pretreatment and maintain a pH appropriate to maximize enzyme activity. For a citrate buffer with a pH around 4.5–5, $H_2Cit^-/HCit^{2-}$ are the major conjugate acid base pairs with a pK_a of 4.77. Approximate pH calculations based on buffering chemistry (data not shown) indicated that a slight reduction in the pH of the citrate buffer could provide greater buffering capacity for high pH pretreatment hydrolyzates. Thus, an alternative citrate buffer (1 mol/L) was prepared by quantitative titration of aqueous sodium hydroxide into sodium citrate monobasic solution to obtain a pH of 4.5. To verify its buffering ability, a 10% solids loading of both poplar and switchgrass was pretreated with 1.0 wt% sodium hydroxide in tube reactors at 120°C. The pH values measured before and after adding this new pH 4.5 buffer to hydrolyzates from 10 min, 70 min, 3 h, and 24 h pretreatments are shown in Table III. The hydrolyzate pH dropped continually with pretreatment time; with the result that pH following the 24 h pretreatment was significantly lower than that from the 10 min pretreatment, suggesting that hydroxyl groups were continuously consumed over the pretreatment time.

After adding citrate buffer, the hydrolyzate pH values were in the range of 4.69–4.87 and 4.72–4.89 for poplar and switchgrass, respectively. These results demonstrated that the prepared citrate buffer with a pH of 4.5 had sufficient buffering capacity to be effective for pretreatment with 1 wt% sodium hydroxide over a wide range of pretreatment times. In this way, neutralization of the slurry from high pH alkaline pretreatment and buffering of the hydrolyzate for enzymatic hydrolysis were accomplished simultaneously for application to the HPTH system.

Application of HTPH to Sodium Hydroxide Pretreatment

After demonstrating that the pH 4.5 citrate buffer effectively adjusted and controlled the pH of hydrolyzates resulting from 1% sodium hydroxide pretreatment in tube reactors for 10 min to 24 h, 1 wt% sodium hydroxide was applied to the HTPH system at similar 10 wt% solids loading. In this case, both poplar and switchgrass were pretreated in the 96 well plate HTPH system at 120°C for 10 min, 20 min, 40 min, 70 min, 3 h, and 24 h. After pretreatment, a mixture of DI water, pH 4.5 citrate buffer, sodium azide, and enzymes were added to each well, as described previously. Seventy-two hours co-hydrolysis was then performed at an enzyme loading of 75 mg cellulase +25 mg xylanase/g structural carbohydrates in the original untreated biomass. Figure 1

Table II. pH values of hydrolyzates produced by sodium hydroxide pretreatment of switchgrass and poplar following dilution to prepare for enzymatic co-hydrolysis.

NaOH concentration (wt%)	Poplar		Switchgrass	
	60°C	120°C	60°C	120°C
1	11.66	8.97	10.63	8.97
2	12.28	11.92	12.09	11.72
5	12.76	12.61	12.70	12.63

Twenty-four hour pretreatment with 10 wt% solid loading.

Prior to pH measurement, hydrolyzate was diluted with DI water to 1 wt% solid loading.

Table III. pH of hydrolyzates produced by sodium hydroxide pretreatment of switchgrass and poplar following dilution to prepare for enzymatic co-hydrolysis before and after addition of new citrate buffer.

Pretreatment time	Poplar		Switchgrass	
	Before	After	Before	After
10 min	11.09	4.87	11.16	4.89
70 min	10.64	4.82	10.73	4.83
3 h	9.92	4.77	10.10	4.76
24 h	8.97	4.69	8.97	4.72

1 wt% sodium hydroxide concentration and 10 wt% solid loading for pretreatment at 120°C.

Prior to pH measurement and buffer addition, hydrolyzate was diluted with DI water to 1 wt% solid loading.
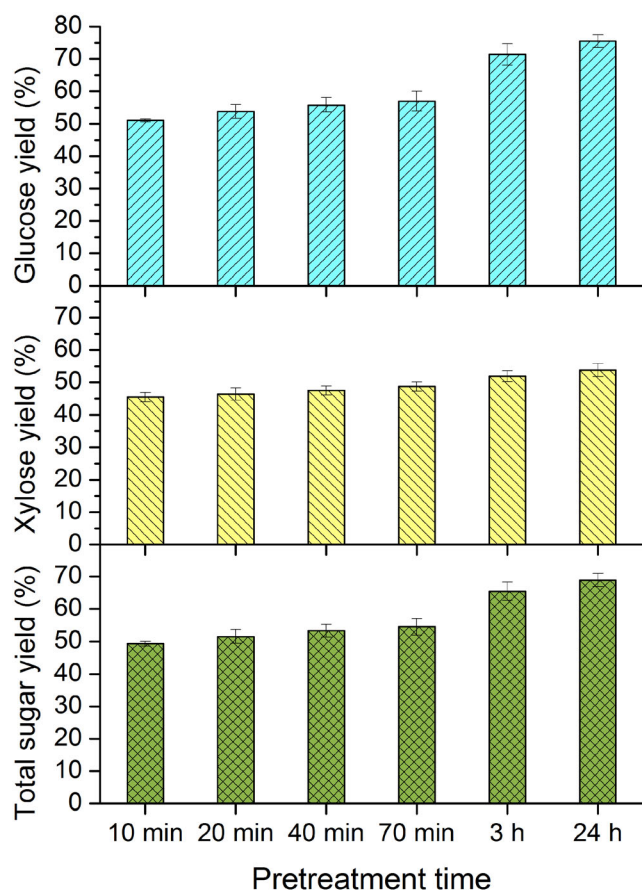
Figure 1. Glucose, xylose, and total sugar (glucose + xylose) yields from sodium hydroxide pretreatment and co-hydrolysis of poplar. Pretreatment was performed at 120°C at a 1 wt% sodium hydroxide concentration, followed by enzymatic hydrolysis of the entire pretreated slurry at 50°C for 72 h using 75 mg cellulase +25 mg xylanase/g glucan + xylan in the unpretreated raw material. The error bars represent the standard deviation of four replicates.

Figure 2. Glucose, xylose, and total sugar (glucose + xylose) yields from sodium hydroxide pretreatment and co-hydrolysis of switchgrass. Pretreatment was performed at 120°C with a 1 wt% sodium hydroxide concentration, followed by enzymatic hydrolysis of the entire pretreated slurry at 50°C for 72 h using 75 mg cellulase +25 mg xylanase/g glucan + xylan in the unpretreated raw material. The error bars represent the standard deviation of four replicates.

shows the glucose, xylose, and total sugar (glucose + xylose) yields from combined pretreatment and co-hydrolysis of poplar. Overall, sugar yields increased slightly with pretreatment time. In contrast to results from hydrothermal HTPH (DeMartini and Wyman, 2011b; Studer et al., 2010, 2011) and dilute acid HTPH (Gao et al., 2012), which were conducted at 180 and 160°C, respectively, sugar yields from 120°C sodium hydroxide pretreatment changed more slowly with pretreatment time. Glucose and xylose yields for high pH pretreatment of poplar ranged between 51.1–75.5% and 45.4–53.8%, respectively, corresponding to a range of glucose plus xylose yields of 49.4–68.8%. Results for switchgrass, however, showed a different trend than for poplar, as shown in Figure 2. The maximum glucose yield of 85.1% appeared following pretreatment for 3 h, while the highest xylose yield of 71.1% was observed for pretreatment for 70 min. However, sugar yield results did drop significantly at 24 h, indicating degradation reactions at longer pretreatment times.
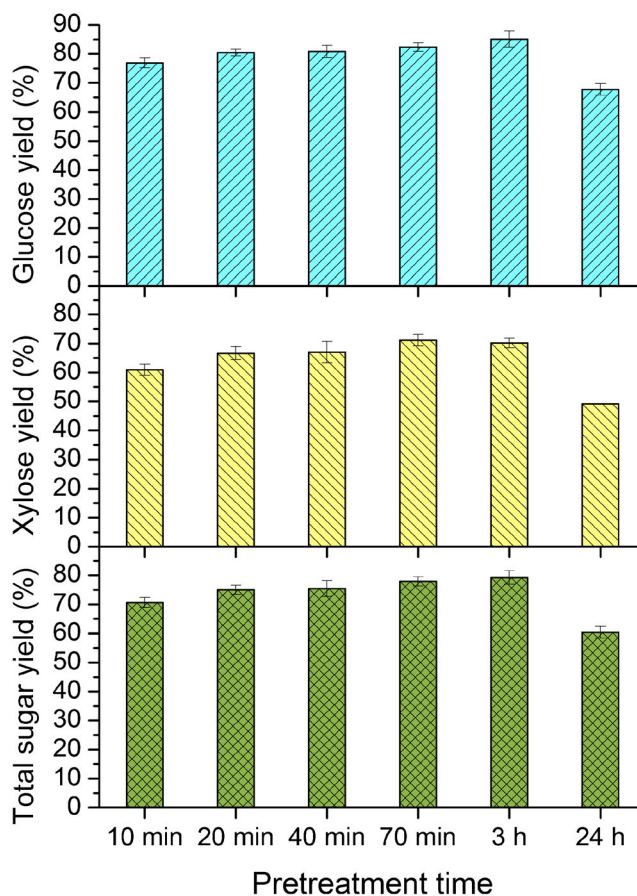
To confirm the effects of sodium hydroxide on sugar release from the HTPH system, pretreatment without sodium hydroxide were also conducted for pretreatment times of 10, 20, 40, and 70 min at 120°C, followed by enzymatic co-hydrolysis. As shown in Figure 3, glucose and xylose yields were very low without addition of sodium hydroxide, demonstrating the effectiveness of the sodium hydroxide pretreatment conditions applied to obtain the high sugar yields in Figures 1 and 2 in the HTPH co-hydrolysis system. In addition, the different trends in sugar yields from poplar and switchgrass also showed that the HTPH system can effectively screen for dilute alkali pretreatment conditions that realize high sugar yields from different biomass types.

Application of Dilute Alkali HTPH to Aspen Wood Rings

An important application of the HTPH system is to screen large number of biomass samples to identify differences in recalcitrance as measured by sugar yields following
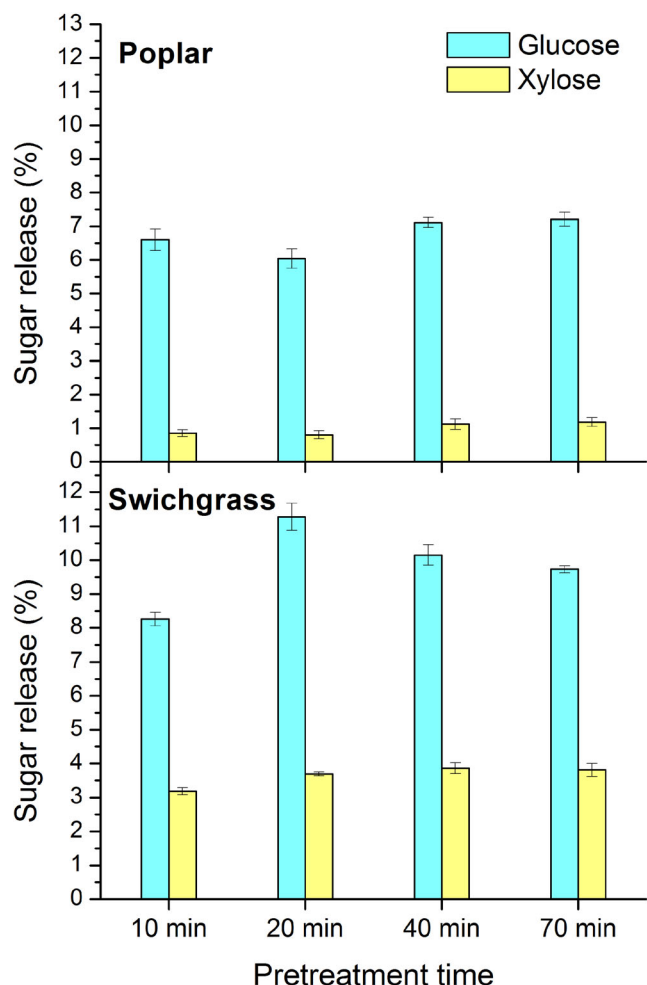
Figure 3. Glucose and xylose yields from hydrothermal (water only) pretreatment and co-hydrolysis of poplar (upper) and switchgrass (bottom). Pretreatment was performed at 120°C, followed by enzymatic hydrolysis at 50°C for 72 h using 75 mg cellulase +25 mg xylanase/g glucan + xylan in the unpretreated raw material. The error bars represent the standard deviation of four replicates.

application of different biomass–pretreatment–enzyme combinations. Thus, four Aspen samples that were fractionated from different annual rings (DeMartini and Wyman, 2011a) were selected to investigate their sugar release performance for the sodium hydroxide HTPH system, with their compositions summarized in Table IV. In this case, a short

Table IV. Chemical compositions of selected rings of Aspen wood.

	Glucan	Xylan	Lignin
Bark	16.4	8.8	36.7
Year 2	33.9	16.1	33.3
Year 15	48.2	17.7	22.4
Year 20	42.5	18.5	22.5

Full dataset reported elsewhere (DeMartini and Wyman, 2011a).

pretreatment time of 10 min was applied to look for biomass that could release sugars at milder conditions where degradation would be less and containment costs lower. Also, shorter pretreatment time reduces release of degradation products and inhibitors in pretreatment that interfere with co-hydrolysis.

Figure 4 shows how 72 h glucose, xylose, and total sugar yields varied for pretreatment with 1% sodium hydroxide followed by co-hydrolysis of different Aspen samples. Sugar yields from hydrothermal HTPH experiments (DeMartini and Wyman, 2011a), which used the same protein loading for co-hydrolysis, are also shown for comparison. These results clearly show that sodium hydroxide gave different sugar yields than hydrothermal pretreatment from Aspen wood rings. For example, although hydrothermal pretreatment resulted in sample 2 (juvenile wood), which had high lignin content, releasing less glucose than samples 15 and 20, sodium hydroxide pretreatment gave the opposite results. Xylose yields from application of the HTPH system at hydrothermal conditions to samples 2, 15, and 20 were quite high at 97.2%, 91.8%, and 95.4%, respectively, but the sodium hydroxide HTPH system resulted in the xylose yield from sample 2 being about 15% higher than that from samples 15 and 20. These differences indicate that sodium hydroxide is more effective in achieving higher sugar yields for biomass with high lignin content, consistent with expectations (Sierra et al., 2012).

Application of the HTPH system to the bark sample provided some additional interesting observations. Because the bark contained higher lignin but significantly less
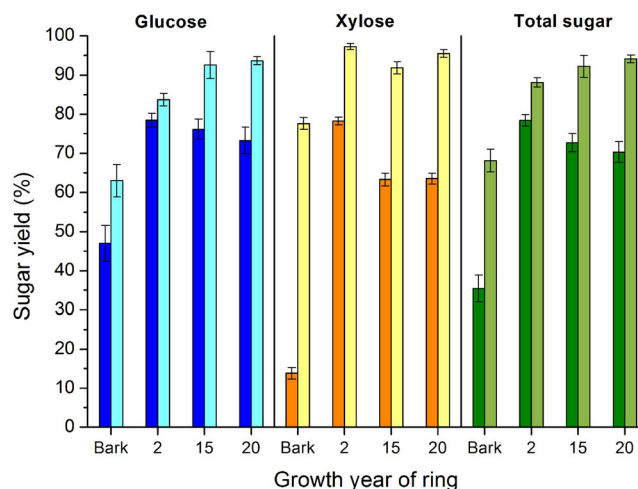

Figure 4. Glucose, xylose, and total sugar (glucose + xylose) yields from pretreatment of aspen wood samples 2, 15, and 20 and bark with 1 wt% NaOH at 120°C for 10 min (darker bars on the left of each pair) and hydrothermal pretreatment with just water at 160°C for 70 min (right lighter colored bar of each pair). The co-hydrolysis enzyme loading for both was 75 mg + 25 mg of cellulase + xylanase/g glucan + xylan in the unpretreated raw material. The error bars represent the standard deviation of three replicates for the experiments in the well-plate. Data for hydrothermal pretreatment are from DeMartini and Wyman (2011a).

carbohydrates than the woody samples, we might expect higher yields with sodium hydroxide than from hydrothermal pretreatment based on the trends above. However, although hydrothermal HTPH achieved reasonable glucose (63.0%) and xylose (77.6%) yields from bark, glucose and xylose yields were only 47.2% and 13.8%, respectively, from sodium hydroxide HTPH. These results support other observations that lignin content alone does not control recalcitrance, but that other differences in cell wall structure are also important (Chundawat et al., 2011).

Overall, sugar yields from the four Aspen samples demonstrated that sodium hydroxide HTPH was capable of discerning differences in recalcitrance among samples. In addition, the different sugar release performance between hydrothermal HTPH and sodium hydroxide HTPH reveal that application of dilute alkali HTPH system can offer new insights in screening biomass recalcitrance.

Conclusions

Pretreatment with 1 wt% sodium hydroxide at 120°C of 10 wt % solids loadings of poplar and switchgrass was successfully combined with enzymatic co-hydrolysis in the HTPH system. The one step buffering and neutralizing method developed with a pH 4.5 citrate buffer for a dilute acid HTPH system (Gao et al., 2012) effectively neutralized and adjusted the pH of sodium hydroxide pretreatment slurries to a range of 4.69–4.89 prior to whole slurry enzymatic co-hydrolysis. Sugar yields showed different trends for poplar and switchgrass with increasing pretreatment times, demonstrating the method was capable of clearly discerning differences in the susceptibility of different feedstocks to alkali pretreatment. The variations observed in sugar yields from Aspen wood ring and bark samples for hydrothermal and sodium hydroxide pretreatments show that HTPH pretreatment at alkali conditions can effectively screen for materials that deserve more detailed study to gain better insights into understanding the influence of biomass structural differences on recalcitrance.

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