

## Enhancement of Enzymatic Saccharification of Poplar by Green Liquor Pretreatment

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Green liquor ( $\text{Na}_2\text{S} + \text{Na}_2\text{CO}_3$ , GL) pretreatment is an effective pathway for improving the enzymatic digestibility of lignocellulosic biomass for the production of bioethanol. In this work, GL was employed as a pretreatment to enhance the enzymatic saccharification of poplar. During pretreatment, the increase of H-factor and TTA charge resulted in enhanced delignification and increased degradation of polysaccharides. The sugar yield of enzymatic hydrolysis increased rapidly with increasing TTA charge in GL pretreatment, while the effect of different H-factors (from 400 to 800) on sugar yield was unnoticeable. The pretreated solid recovery was 75.5% at a lignin removal rate of 29.2% under optimized conditions of total titratable alkali (TTA) charge 20%, sulfidity 25%, and H-factor 400. The sugar yield of glucan, xylan, and total sugar of GL-pretreated poplar in enzymatic hydrolysis reached up to 89.9%, 65.5%, and 82.8%, respectively, at a cellulase loading of 40 FPU/g-cellulose.

*Keywords:* Poplar; Green liquor pretreatment; H-factor; Enzymatic hydrolysis; Sugar

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### INTRODUCTION

The economic and environmental benefits that can be derived from bioethanol have led to extensive research in the bioconversion of lignocellulosic feedstocks into ethanol (Christersson 2008). Bioethanol is a renewable energy source produced through the fermentation of sugars and can reduce greenhouse gas emissions (Yang *et al.* 2013). Although current research and development attention have focused mainly on agricultural residues and dedicated energy crops, wood biomass (hardwood and softwood) is still an important feedstock for bioethanol production (Zhu and Pan 2010). Large quantities of wood biomass are also sustainably available in various regions of the world.

The major differences between wood biomass and agricultural biomass are based on both physical properties and chemical components. Wood biomass is physically larger, structurally stronger, and denser than agricultural biomass. Its higher density significantly reduces transportation cost. Wood biomass has a higher lignin content than agricultural biomass. The lignin is one of the major hurdles for cellulase access to carbohydrate fraction in woody biomass and thus reduces the effectiveness of enzymatic hydrolysis. Therefore, more effort is required to overcome the recalcitrance of wood biomass through pretreatment for an efficient enzymatic saccharification.

Poplar has been widely planted in temperate zones because of its rapid growth and ease of stock establishment through stem- or root-cuttings (Kang *et al.* 1996). Since the introduction of clones in the 1970s, poplar has been incorporated into many managed

systems for the production of timber and fiber throughout south temperate central China, which has an area of roughly 600,000 km<sup>2</sup> (Fang *et al.* 1999). Poplar has many characteristics that make it suitable for plantation culture, which enables the production of large quantities of wood in short periods of time.

Lignocellulosic biomass is primarily composed of a complex network of intertwined polymers including cellulose, hemicelluloses, and lignin. Cellulose and hemicelluloses can be broken down into their component monomeric sugars, which are then fermented into ethanol (Wyman 2007). However, cellulose has a high crystallinity and is closely associated with hemicelluloses and lignin. Lignin is generally accepted as an obstacle restricting polysaccharide degradation, thereby limiting the enzymatic hydrolysis conversion. Therefore, the removal of lignin and hemicellulose are of great importance to bioethanol production from lignocellulosic biomass. Pretreatment processes change the structural features and chemical compositions of lignocellulosic biomass. The purpose of pretreatment is to improve its ability to subsequently form sugars by enzymatic hydrolysis and avoid the degradation or loss of carbohydrates (Sun and Cheng 2002). The most investigated pretreatment processes for hardwood include steam explosion, dilute acid, sodium hydroxide, lime, sulfite, ammonia, organosolv, and even ionic liquid pretreatment technologies, many of which have achieved some level of success. However, some pretreatment processes lead to high costs of equipment and chemical reagent.

Green liquor (GL) is a mixture of sodium sulfide and sodium carbonate, which can be completely recovered after combustion of the spent cooking liquor (black liquor) in the recovery boiler in a kraft pulp mill. The use of GL for pretreatment can be easily implemented in a repurposed kraft pulp mill (Jin *et al.* 2010). GL pretreatment removes lignin selectively and retains the majority of carbohydrates in the pretreated solid. This process uses proven technology and has several additional advantages (Jin *et al.* 2010), such as high sugar yields. The advantage of alkali pretreatment technology lies in the fact that it would create a washed clean substrate that is highly digestible and rich in cellulose and xylan (Chen *et al.* 2013). At the same time, GL pretreatment retains a great part of polysaccharides in the substrate for enzymatic hydrolysis. It prevents the collection of fermentable sugars from pretreatment steps such as dilute acid pretreatment and SO<sub>2</sub> steam explosion pretreatment (Tian *et al.* 2011; Wyman *et al.* 2009) and retains a higher sugar concentration for fermentation to ethanol. With GL pretreatment, the additional step of combining the fermentable sugars from the pretreatment step into the fermentation can be avoided. In this study, the effects of the different GL pretreatment conditions on the chemical compositions of poplar, as well as the sugar conversion of enzymatic hydrolysis were investigated.

## EXPERIMENTAL

### Materials

The poplar wood used for this project was collected in Jiangsu, China. Air-dried raw materials were cut into chips with the size of 3 to 5 cm in length, 2 to 3 cm in width, and 1 to 2 mm in thickness. Air dried wood chips were sealed in plastic bags and stored in a refrigerator at 4 °C. The main chemical composition of the material is listed in Table 1. Prior to composition analysis, the biomass was ground using a Wiley mill, and particles between the size of 40 and 80 mesh (0.42 mm and 0.177 mm) were collected.

**Table 1.** Main Chemical Composition of Poplar (on Dry Basis)

Benzene-ethanol Extractives (%)	Lignin (%)			Sugar (%)			
	KL	ASL	Total	Glucan	Xylan	Mannan	Total
1.5±0.1	23.3±0.1	2.6±0.0	25.9±0.1	44.1±0.2	15.4±0.2	0.6±0.0	60.1±0.4

### Green Liquor Pretreatment

The GL pretreatment process was carried out in an electrically heated oil bath containing 10 bombs of 1-L capacity and rotating at a speed of 6 rpm. GL was prepared by mixing Na<sub>2</sub>S and Na<sub>2</sub>CO<sub>3</sub> with a sulfidity (the percentage of Na<sub>2</sub>S as Na<sub>2</sub>O to the sum of Na<sub>2</sub>S and Na<sub>2</sub>CO<sub>3</sub> as Na<sub>2</sub>O) of 25%. The total titratable alkali (TTA) charge (as Na<sub>2</sub>O) on oven dried (od) biomass ranged from 4% to 24%. The ratio of pretreatment liquor to biomass was 4:1 (v/w). The raw materials were first impregnated with the pretreatment liquor at 80 °C for 30 min; immediately after impregnation, the temperature was raised to the target temperature (160 °C, 170 °C) at a rate of 2 °C/min. The H-factor was 400 (160 °C) and 800 (170 °C), as calculated by Eq. (1) (Vroom 1957),

$$\text{H-factor} = \int_0^t K_{r(T)} dt \quad (1)$$

where  $t$  is the cooking or pretreatment time (h),  $T$  is the temperature, and  $K_{r(T)}$  is equal to the ratio of the relative reaction rate constant at certain temperatures and at 100 °C.

The pretreatment process was terminated by cooling the bombs to room temperature in cold water. The pretreatment spent liquor was collected for pH measurement. The pretreated solid was collected and washed with deionized water to remove residual chemicals and dissolved chemical components.

### Enzymatic Hydrolysis

A laboratory refiner (KRK, Jinlin, China) with a disk diameter of 300 mm was used to defiberize the pretreated samples at 3,000 rpm to prepare substrates for enzymatic hydrolysis. Enzymatic hydrolysis of the substrates was carried out in a 150-mL Erlenmeyer flask at a consistency of 5% (w/w) in sodium acetate buffer (pH 4.8) at 50±2 °C using a shaking incubator at 180 rpm for 48 h. The series enzyme Cellic<sup>®</sup> Ctec2, which was provided by Novozymes (Novo Nordisk A/S, Demark), was used for enzymatic hydrolysis. The enzyme loadings (based on cellulase activity) were 10, 20, and 40 filter paper unit (FPU) per gram of cellulose in the pretreated solid, respectively. Enzymatic hydrolysis residue and hydrolysate were separated by centrifugation. Hydrolysate was sampled for monomeric sugar (glucose, xylose, and mannose) analysis. All determinations were conducted in duplicate.

### Analytical Methods

Lignin and carbohydrate composition of the raw material and pretreated solid were analyzed using Laboratory Analytical Procedures from the National Renewable Energy Laboratory (NREL) (Sluiter *et al.* 2008). The Klason lignin (KL) content was taken as the ash free residue after acid hydrolysis. The hydrolysate from acid hydrolysis was retained for the analysis of sugars and acid-soluble lignin (ASL). Sugars were analyzed by HPLC (Yang *et al.* 2012). ASL was determined by absorbance at 205 nm in a UV-Vis spectrometer (TU-1810, Puxi, Beijing, China).

Filter paper activity of cellulase was determined following the standard method

recommended by the Commission of Biotechnology, IUPAC (Ghose 1987). The monomeric sugars in enzymatic hydrolysate were determined following the method described by Yang *et al.* (2012). The average of duplicate runs was used in reporting. The data for glucose, xylose, and mannose contents were corrected to glucan, xylan, and mannan for sugar yield calculation. All experiments were replicated twice.

## RESULTS AND DISCUSSION

### Effects of GL Pretreatment on Solid Recovery and Spent Liquor pH

Poplar wood chips were pretreated by GL at a different TTA charges (4% to 24%) and H-factors (400 and 800). The GL was prepared in the laboratory by mixing sodium carbonate and sodium sulfide with a sulfidity of 25%. Table 2 shows the effects of TTA charge and H-factor on GL-pretreated solid recovery and spent liquor pH. The pretreated solid recovery was calculated using Eq. 2.

$$\text{Solid recovery (\%)} = \frac{\text{Dry pretreated solid (g)}}{\text{Dry raw material (g)}} \times 100\% \quad (2)$$

**Table 2.** Effects of TTA Charge and H-factor on Pretreated Solid Recovery and Spent Liquor pH in GL Pretreatment

H-factor	TTA (%)	4	8	12	16	20	24
400	Solid recovery (%)	87.1±0.2	84.8±1.6	80.1±0.4	77.7±1.3	75.5±0.2	73.0±0.7
	Spent liquor pH	6.41±0.1	7.71±0.0	8.80±0.1	9.18±0.0	9.44±0.0	9.62±0.0
800	Solid recovery (%)	83.0±1.9	80.0±1.4	77.2±0.2	74.3±0.8	71.3±0.3	70.4±0.9
	Spent liquor pH	6.22±0.0	7.37±0.2	8.75±0.0	9.23±0.0	9.53±0.0	9.71±0.0

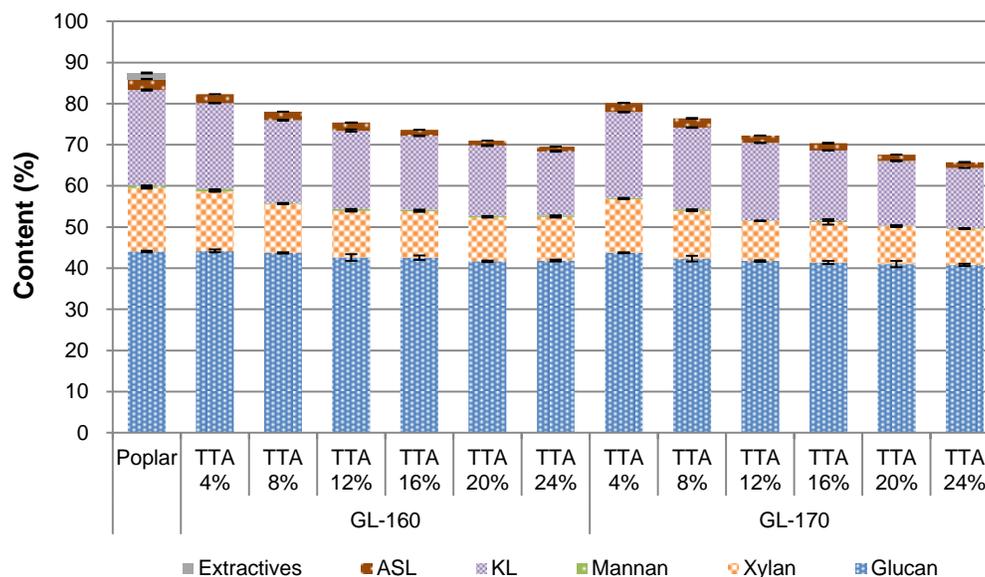
The pretreated solid recovery under the described conditions was in the range of 70% to 90%. The H-factor can combine temperature and time into a single variable (Vroom 1957). The TTA charge and H-factor play predominant roles in the GL-pretreated solid recovery. Pretreated solid recovery dropped with increasing H-factor (400 to 800) and TTA charge (4% to 24%) in GL pretreatment. This is mainly caused by the gradually degradation of lignin and carbohydrates in poplar.

This low final pH in spent liquor is an important feature of GL pretreatment. The higher H-factor contributes to the alkaline degradation of raw materials. More uronic acid substitutions and acetyl groups in hemicellulose can be hydrolyzed to form uronic acid and acetic acid with higher alkaline degradation, as well as cellulose peeling form saccharic acid, which results in more acidic substances.

The highest pH of spent liquor was 9.7, and it is significantly lower than that of traditional alkaline cooking (13 to 14). Low pH could effectively reduce alkaline degradation and secondary peeling reaction of polysaccharides during pretreatment, thus higher pretreatment solid recovery can be obtained (Jin *et al.* 2010). At the same time, a certain degree of swelling obtained for the fibers of poplar wood will be beneficial to the following enzymatic saccharification. However, a low pH was not able to lead to a high delignification because of the limited cleavage of non-phenolic  $\beta$ -aryl ether linkages (Santos *et al.* 2013).

## Effects of GL Pretreatment on Chemical Composition of Poplar

The conversion of polysaccharides from virgin lignocellulosic biomass into monomeric sugars is relatively low because of its native recalcitrance, which is attributed to lignin content, as well as its structure, hemicellulose, cellulose crystallinity, and other factors. Pretreatment of lignocellulosic materials is required to overcome recalcitrance. The purpose of the pretreatment process is to change the properties of raw materials, remove or dissolve a part of lignin and hemicellulose, and reduce cellulose crystallinity (Kumar *et al.* 2009). The purpose of the pretreatment process is also to make cellulose more accessible to the enzymes and convert the carbohydrate polymers into fermentable sugars by altering the physical features and chemical structure of the lignocellulosic materials (Hu and Ragauskas 2012). The effects of the pretreatment procedure on the pretreated solid recovery and chemical compositions of poplar are important indicators for evaluating its effectiveness. Figure 1 shows the effects of TTA charge and H-factor on the pretreated solid recovery and the retention of various components in GL-pretreated solid based on starting materials. The chemical components of pretreated poplar declined with increasing pretreatment H-factor and TTA charge without exception.



**Fig. 1.** Effects of TTA charge and H-factor on the retention of various components in GL-pretreated solids based on the original weight of poplar

At the same H-factor, the contents of KL and ASL decreased with rising TTA charge. For example, when the H-factor was 400, the KL decreased from 21.0% to 17.1% (based on original biomass) and the ASL decreased from 2.2% to 1.2% (based on original biomass), with TTA charge rising from 4% to 20%. There was a significant decrease of lignin with increasing TTA charge, but the H-factor (from 400 to 800) had only a slight effect on lignin removal. For example, when the H-factor was 400, the total lignin decreased from 19.4% to 18.3%, with TTA charge rising from 16% to 20%. But when the TTA charge was 16%, the decrease of total lignin (from 19.4% to 19.0% based on original biomass) was not noticeable, with the H-factor rising from 400 to 800.

Lignin is one of the major barriers for enzymatic hydrolysis, so delignification usually improves enzymatic hydrolysis, especially for alkali pretreatment (Chang and Holtzapple 2000). The removal of amorphous lignin and hemicellulose lead to an

increase in accessible pore volume, while crystallinity increases with delignification (Yu *et al.* 2011). The correlation between lignin removal and solid recovery of GL-pretreated poplar is presented in Fig. 2. The pretreated solid recovery decreased with the increasing of delignification. The pretreated solid recovery decreased clearly with the rising H-factor at the equal degree of lignin removal. This means that increasing the pretreatment H-factor inhibited delignification selectivity.

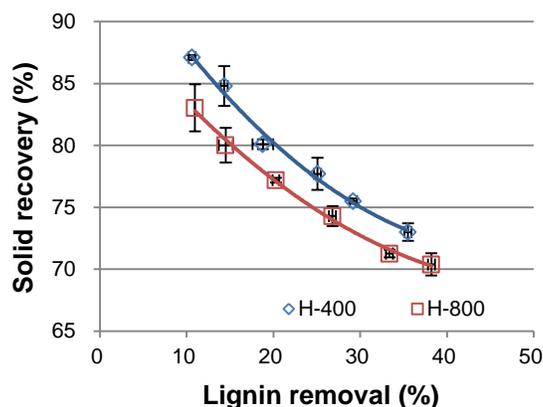


Fig. 2. Residual lignin in GL-pretreated solid as a function of solid recovery

Table 3. Lignin and Polysaccharide Losses of Poplar in GL Pretreatment (Based on the Original Weight of Poplar)

H-factor	TTA (%)	Lignin (%)			Sugar (%)			
		KL	ASL	Total	Glucan	Xylan	Mannan	Total
400	4	2.2±0.1	0.5±0.0	2.7±0.1	0.4±0.3	0.9±0.1	0.1±0.1	1.4±0.5
	8	3.1±0.1	0.6±0.0	3.7±0.1	0.4±0.1	3.5±0.1	0.1±0.0	4.0±0.2
	12	4.2±0.2	0.7±0.1	4.9±0.3	1.5±0.8	4.1±0.1	0.1±0.0	5.7±0.9
	16	5.3±0.1	1.2±0.0	6.5±0.1	1.5±0.5	4.1±0.1	0.2±0.0	5.7±0.6
	20	6.1±0.1	1.4±0.0	7.5±0.1	2.4±0.2	4.8±0.0	0.2±0.0	7.4±0.2
	24	7.6±0.1	1.5±0.0	9.1±0.1	2.3±0.2	4.8±0.1	0.2±0.0	7.3±0.3
800	4	2.4±0.1	0.4±0.0	2.8±0.1	0.3±0.1	2.3±0.0	0.3±0.0	2.9±0.1
	8	3.3±0.1	0.4±0.1	3.7±0.2	1.8±0.7	3.8±0.1	0.2±0.0	5.8±0.8
	12	4.3±0.1	0.9±0.0	5.2±0.1	2.3±0.2	5.7±0.0	0.4±0.0	8.4±0.2
	16	6.0±0.0	0.9±0.1	6.9±0.1	2.7±0.4	5.5±0.7	0.4±0.0	8.6±1.1
	20	7.5±0.1	1.2±0.0	8.7±0.1	3.1±0.8	6.2±0.2	0.4±0.0	9.7±1.0
	24	8.6±0.1	1.3±0.1	9.9±0.2	3.3±0.3	6.6±0.1	0.5±0.0	10.4±0.4

Table 3 shows the lignin and polysaccharide losses of poplar pretreated with GL at different TTA charges and H-factors based on the dry weight of raw materials. Less than 40% of lignin was removed even under the severest condition in this work. It is well known that cleavage of  $\beta$ -O-4 bond connecting non-phenolic units is responsible for delignification not only in kraft but also alkaline-based cookings (Sjöström 1993). Conditions of GL pretreatment in this work (initial pH around 12, temperature 160 °C) are milder than those of common kraft cooking (initial pH ~14, temperature 160 to 170 °C). Because majority of  $\beta$ -O-4 bond cleaves only at high temperature and pH, this cleavage reaction would not sufficiently occur under the conditions of GL pretreatment. The removal of each polysaccharide increased with increasing TTA charge and H-factor in GL pretreatment. The losses of polysaccharide increased with the rising H-factor (from 400 at 160 °C to 800 at 170 °C), as polysaccharides become more degradable under a

higher temperature. Compared with cellulose, hemicellulose is easier to be degraded for its amorphous structure.

An efficient pretreatment should minimize sugar loss during pretreatment and ensure that the sequential enzymatic hydrolysis yields maximal sugar productivity (Jørgensen *et al.* 2007). The appropriate delignification and relatively high retention of carbohydrates should be considered when developing an ideal pretreatment process. The recovery of glucan suffers less loss than xylan with GL pretreatment. For example, after GL pretreatment with a TTA charge 16%, a sulfidity of 25%, and an H-factor of 400, the degradation of glucan was less than 1.5%, while that of xylan was more than 4.1%. The data shows that glucan is more stable than xylan with GL pretreatment. Compared with hemicellulose, cellulose is significantly more stable, with a large percentage of crystalline region and a high degree of polymerization. During GL pretreatment, hemicellulose can be hydrolyzed to form uronic acid and acetic acid with higher alkaline degradation, as well as cellulose peeling form saccharic acid. The degradation removal ratio of carbohydrates was 17% under the severest conditions of pretreatment (at H-factor 800 and TTA charge 24%).

Compared with dilute acid and alkali (Ucar 1990) pretreatment of poplar, more lignin is removed with GL pretreatment. However, compared with nonwoody biomass such like corn stover (Gu *et al.* 2012) and rice straw (Gu *et al.* 2013), the lignin of poplar is more difficult to remove with GL pretreatment. In the severest GL pretreatment conditions, a higher ratio of hemicellulose retention and more delignification can be obtained compared with poplar pretreated with dilute acid (Tian *et al.* 2011). Because cellulose is surrounded by a protective layer of lignin and hemicellulose, the lignin and hemicellulose layers may have a greater chance of being attacked during pretreatment (Bak *et al.* 2009). Higher pretreatment H-factors and TTA charges resulted in an increase of delignification. However, these conditions also caused the degradation of carbohydrates, which is adverse to the subsequent enzymatic hydrolysis.

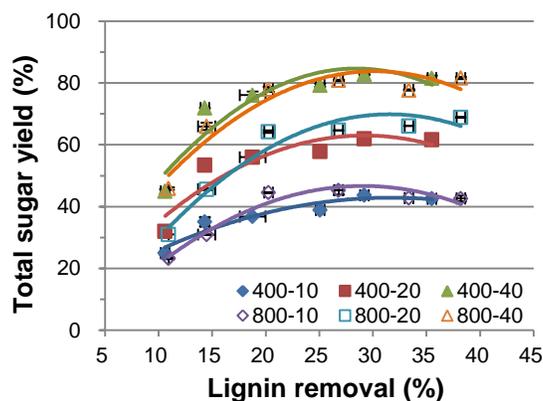
### Effects of GL Pretreatment on the Enzymatic Digestibility of Poplar

Enzymatic hydrolysis involves cleaving the polymers of cellulose and hemicellulose by using enzymes in bioethanol production from lignocellulosic biomass. In this work, the pulp was hydrolyzed by using the enzyme Cellic<sup>®</sup> CTec2, the details of which were described earlier. Enzyme activity was calculated by filter paper activity and enzyme dosages ranged from 10 to 40 FPU/g-cellulose. However, porosity (accessible surface area) of biomass, crystallinity of cellulose fiber, lignin content, as well as cellobiose and glucose all affect enzyme accessibility and the efficiency of enzymatic hydrolysis (Binod *et al.* 2010). In this study, the GL pretreatment and KRK refining process all contribute to enhancing enzymatic hydrolysis. The efficiency of enzymatic hydrolysis was evaluated by sugar (glucan, xylan, total sugar) yield, which was calculated using Eq. (3).

$$\text{Sugar yield (\%)} = \frac{\text{Sugar in enzymatic hydrolyate (g)}}{\text{Sugar in raw material (g)}} \times 100\% \quad (3)$$

As mentioned above, lignin is one of the major barriers to enzymatic hydrolysis. The effect of lignin removal on the total sugar yield of GL-pretreated substrate after 48 h of enzymatic hydrolysis at different enzyme loadings is illustrated in Fig. 3. Lignin removal also reflected the extent of pretreatment, and the ratio of delignification during

pretreatment is a crucial factor that greatly affects enzymatic hydrolysis. The removal of lignin has been reported to effectively improve cellulose accessibility by creating pores and breaking the lignin-carbohydrate complex (Yu *et al.* 2011). It is observed in Fig. 3 that the total sugar yield reached a maximum at a lignin removal of 30% and then dropped down due to the increment of polysaccharides loss in the GL pretreatment at severe conditions. Therefore, it is important to control a suitable lignin removal level with reasonable pretreatment. In fact, it is not necessary to remove all lignin from the material to achieve the maximal enzymatic digestibility of carbohydrates. Generally, it is believed that a lignin removal of 20% to 65% is sufficient to increase the accessibility of the cellulose to enzymes (Yu *et al.* 2011). In this work, the total sugar yield reached a plateau when about 30% of lignin was removed from poplar pretreated with GL.

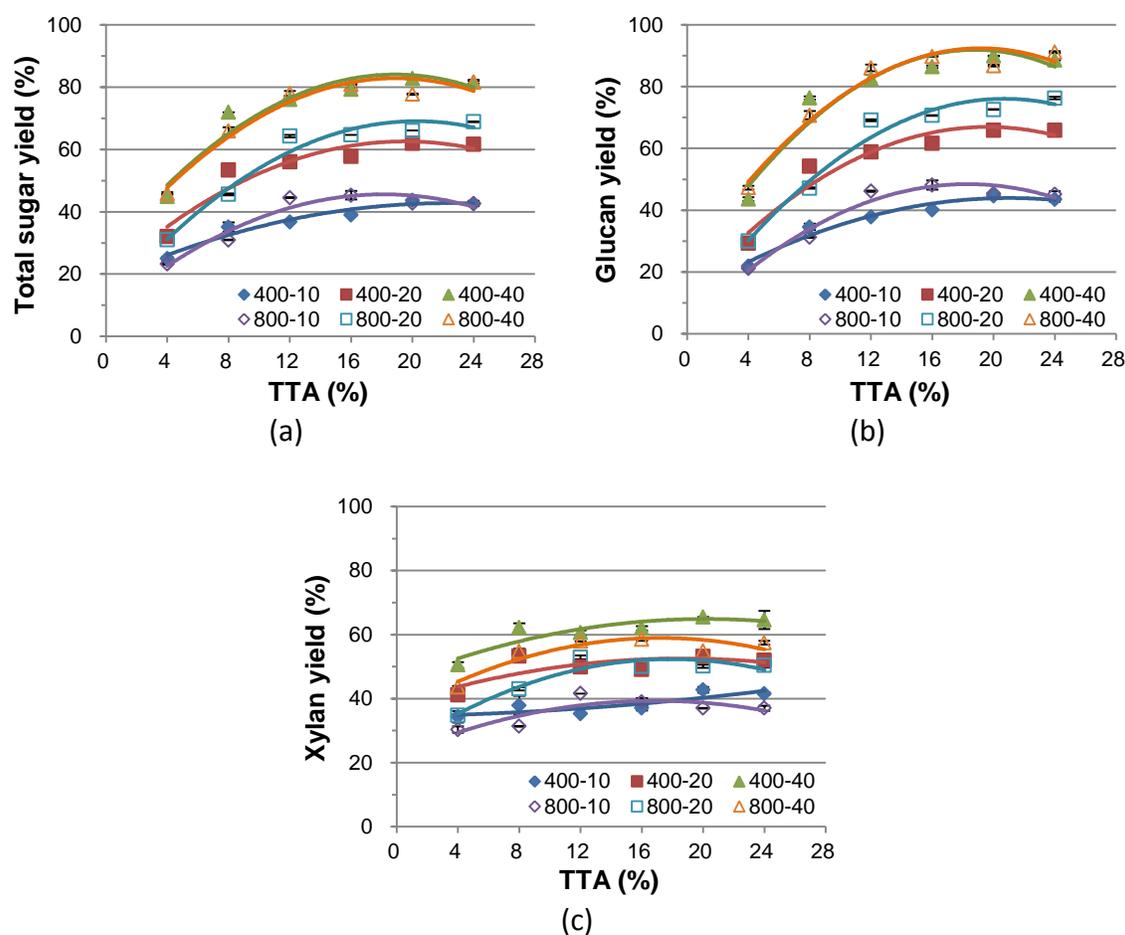


**Fig. 3.** Effect of lignin removal on total sugar yield of GL-pretreated poplar. 400 and 800 in the figure are pretreatment H-factors; 10, 20, and 40 are cellulase loadings (FPU/g-cellulose) in the process of enzymatic hydrolysis

The enzymatic hydrolysis sugar yields of all GL-pretreated samples under different H-factors and TTA charges increased with increasing enzyme dosage. At the same H-factor, the increment of glucan was greater than that of xylan with the increasing TTA charge. For example, under the same conditions of an H-factor of 400 and an enzyme loading of 40 FPU/g-cellulose, the glucan yield increased from 44% to 93.4%, while the yield of xylan increased from 53.8% to 93.7%, with the TTA charge rising from 4% to 24%. This is because the degradation of xylan was greater than that of glucan. Under the same condition of TTA charge 16%, the enzyme dosage increased from 10 to 40 FPU/g-cellulose, the increase of total sugar yield at an H-factor of 400 (from 38.9% to 79.3%) was slightly greater than that at an H-factor of 800 (from 45.3% to 80.9%). This means that the pretreatment H-factor (from 400 to 800) displayed little effect on the total sugar yield under equal increase intervals of enzyme dosage at the same TTA charge. This is mainly because the effect of H-factor on lignin removal was not as significant as alkali charge (Chen *et al.* 2013). Although the H-factor (from 400 to 800) has a great effect on the degradation of hemicellulose, which can increase enzymatic hydrolysis efficiency, polysaccharide degradation was negatively correlated to the enhancement of enzymatic hydrolysis.

The sugar yield of enzymatic hydrolysis was limited at a low TTA charge (4% to 8%) in pretreatment, as only a little part of hemicellulose and lignin were removed, which did not effectively improve the final sugar yield. As shown in Fig. 4 (a), total sugar yield increased rapidly with the TTA increasing from 4% to 16%, while it leveled off and then

slightly decreased with the continuous increasing TTA charge. This is mainly because of the fact that the removal of lignin and hemicellulose were enhanced with the rising TTA charge, which results in loosening the structure of poplar, as well as the swelling of the pulp fibers, which thus improve the sugar yields (Yu *et al.* 2011). However, high TTA charges (20% to 24%) at the same pretreatment H-factor also cause the degradation of polysaccharides. Therefore, continuously increasing TTA charge does not lead to an ever-improving efficiency of enzymatic hydrolysis. It can be seen in Fig. 4 (a) that the highest total sugar yields were achieved for the samples pretreated by a TTA charge of 16% to 20%. The degradation of polysaccharides under severe pretreatment conditions resulted in a low carbohydrate recovery, which led to the reduction of the final sugar yield of enzymatic hydrolysis. Therefore, the appropriate TTA charge and pretreatment H-factor should be considered to avoid the excessive degradation of carbohydrates.



**Fig. 4.** Total sugar yield (a), glucan yield (b), and xylan yield (c) of GL-pretreated poplar at enzyme loadings of 10, 20, and 40 FPU/g-cellulose

Figure 4 (b) shows that, with the increase of TTA charge in pretreatment, the change of glucan yield was similar to that of total sugar yield. However, the change of xylan yield was not so significant, and it started to reach a plateau at a TTA charge of 12%, as shown in Fig. 4 (c). The pretreatment H-factor plays an important role in improving the xylan yield of enzymatic hydrolysis. The H-factor had more effect on xylan yield than on glucan yield, and the increment of xylan yield was significantly lower

than that of glucan. This is mainly because the degradation of hemicellulose is more serious than that of cellulose, as described before. As for pretreated poplar with the same TTA charge, the total sugar yield under the same enzyme loading was slightly increased corresponding to the rising H-factor from 400 to 800. These results suggest that the TTA charge of GL pretreatment played an important role in improving the sugar yield of enzymatic hydrolysis, while the effect of H-factor (from 400 to 800) on final sugar yield was unnoticeable.

The highest glucan yields of GL-pretreated poplar were 70.7% and 89.9%, respectively, for enzyme loadings of 20 and 40 FPU/g-cellulose. Compared with that of GL-pretreated corn stover, poplar wood exhibited a higher sugar yield, though pretreatment on poplar required more severe conditions than corn stover. For example, after enzymatic hydrolysis at a cellulase loading of 20 FPU/g-substrate, the total sugar yield (70%) of corn stover (Gu *et al.* 2012) with the optimal GL pretreatment conditions (TTA charge 8%, sulfidity 40%, and a temperature of 140 °C for 1 h) was lower than that of poplar (82.8%).

In this work, the highest sugar yields for glucan, and xylan, and total sugar were 89.9%, 65.5%, and 82.8%, respectively, for the poplar pretreated at a TTA charge of 20%, 25% sulfidity, and an H-factor of 400. At this point, the pretreated solid recovery was 75.5% and the ratio of delignification was 29.2%. This result is close to the GL-pretreated mixed hardwoods (Jin *et al.* 2010). The enzymatic hydrolysis glucan yield of birch pretreated by 7% w/w NaOH at 80 °C for 2 h was about 80% at 20 FPU/g-woods (around 40 FPU/g-cellulose) (Mirahmadi *et al.* 2010). Gupta and Lee (2010) reported that the glucan yield of hybrid poplar pretreated with sodium hydroxide (1.5% w/w NaOH at 80 °C) was 56.1% under an enzyme loading of 15 FPU/g-glucan. The total sugar yield of GL-pretreated poplar is similar to that of other alkali-pretreated hardwoods. In summary, GL pretreatment results in a high sugar yield after enzymatic digestion and can effectively improve the enzymatic saccharification of poplar.

## CONCLUSIONS

1. GL pretreatment provides an obvious change in poplar wood composition, mostly by reducing lignin and hemicellulose. The extent of lignin removal, sugar retention, and enhancement of the enzymatic digestibility of poplar highly depended on pretreatment variables such as H-factor and TTA charge.
2. Under optimized pretreatment conditions (TTA charge 20%, temperature 160 °C, and H-factor 400), the highest sugar yields for the pretreated poplar were obtained at an enzyme loading of 40 FPU/g-cellulose; the yields were 89.9%, 65.5%, and 82.8%, respectively, for glucan, xylan, and total sugar. At this point, a pretreated solid recovery of 75.5% with 29.2% lignin removal was achieved.
3. GL pretreatment is an effective pathway to improve the enzymatic conversion of poplar polysaccharides to monomeric sugars. This work represents an attractive pretreatment process for the production of bioethanol from lignocellulosic materials.

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