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Green Liquor Pretreatment of Mixed Hardwood for Ethanol Production in a Repurposed Kraft Pulp Mill

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Abstract: The development of a new, relatively simple process, which uses green liquor (sodium carbonate and sodium sulfide) as a pretreatment for the production of ethanol is described in this article. The pulps produced by this process can be enzymatically hydrolyzed to monomeric sugars with a high overall sugar recovery. The use of green liquor for pretreatment ensures that the chemicals used during pretreatment can be recovered efficiently using proven technology and can be easily implemented in a repurposed kraft pulp mill. The yield of pulps produced by the green liquor pretreatment process is about 80% with nearly 100% cellulose and 75% xylan in retention in mixed southern hardwood. The low pH prevents the random hydrolysis of polysaccharide and secondary peeling reactions from occurring during the pretreatment, resulting in higher retention of the polysaccharides in pulp. About 35% of the lignin is removed during the green liquor pretreatment process, which is sufficient for efficient enzymatic hydrolysis. The amount of sugar produced in enzymatic hydrolysis increased with both the green liquor and enzyme charge. The increase in enzymatic hydrolysis efficiency was small as the total titrateable alkali was increased beyond 12–16%. With green liquor pretreatment at 16% Total Titratable Alkali (TTA), the overall sugar recovery for hardwood was shown to be around 77% at a cellulase charge of 20 FPU/gm of substrate. A sugar recovery of 80% could be achieved at higher enzyme charges. These levels of sugar recovery are competitive with other pretreatments for hardwood. This novel pretreatment process can be used to repurpose kraft mills, which are being closed due to a decrease in the demand for paper in North America, for production of ethanol.

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INTRODUCTION

It is well known that the supply of fossil fuels is finite, and the combustion of these fuels is a major source of greenhouse gases. Lignocellulosic biomass is an alternative energy source that can be used to produce electricity, heat, and biofuels, with the potential for greatly reducing greenhouse gas emissions. Lignocellulosic biomass, which can be converted to glucose and other monomeric sugars for fermentation by hydrolytic enzymes,^[1-7] has the potential to contribute substantially to the production of bioethanol for transportation. Enzymatic hydrolysis produces higher yields than acid-catalyzed hydrolysis and has become more attractive recently with the reduction of enzyme manufacturing cost. The enzymatic process is regarded as the most attractive way to degrade wood polysaccharides to monomeric sugars.^[8-10] However, cellulose and hemicelluloses in lignocellulosic biomass are intrinsically resistant to enzyme attack, as native cellulose is well protected by a matrix of lignin. As a result, a pretreatment stage is necessary for lignocellulosic biomass to expose the carbohydrates by removing some of the lignin and/or hemicellulose in a cost-effective manner to make the cellulose more accessible to enzymes. Many pretreatment methods have been developed, but pretreatment remains a major technological challenge for process commercialization.^[11] Physical methods include chipping, milling, and grinding the lignocellulosic biomass into a fine powder to increase the surface area of the cellulose, but most physical methods are too expensive to be used in a commercial process.^[12,13] Chemical pretreatments under alkaline or acid condition are commonly used to increase the accessibility of the cellulose.^[14-17] They are considered the most practical method for a full-scale process. Biological pretreatment performed by applying lignin-degrading microorganisms,^[9,18] is considered to be environmentally friendly and energy saving as it is performed without chemicals at ambient temperature. However, it is not a viable solution for industrial use because the reaction time is too long and some of the wood components, such as hemicellulose and cellulose are lost along with the lignin.

In spite of various processes that have been developed very few have been commercially implemented due to either the high capital cost requirement or the high production cost. While many projects at both pilot demonstration and commercial scale have been proposed, funded, and some under construction, generally the capital efficiency (measured as \$US per annual liter of production), yield of ethanol (measured as liters ethanol per Bone Dry tonne per liter [l/BDt]), and biomass cost (\$US per BDt) have to date limited commercial implementation. The best option for implementing lignocellulosic ethanol would be to repurpose a kraft mill for the production of ethanol. In concept, repurposing features a number of significant advantages over other approaches to the production of bioethanol:

1. A supply chain to grow, harvest, and deliver biomass is already in place, thus avoiding new demand and creating price pressure on the raw material.
2. Since pulp is not produced for papermaking, undebarked chips and significant levels of harvest residues may be used as raw material, offsetting some of the traditional cost of biomass from pulpwood.
3. When kraft mills are permanently closed, significant equipment assets are left behind, such as woodyard, digesters, evaporators, power and waste treatment plants, which can be directly repurposed to ethanol production.
4. Moreover, kraft mill closures typically require environmental remediation and demolition costs in excess of \$US 10 Million.
5. Much of the process equipment in a repurposed kraft mill has little technology risk, since most equipment has been operating in place for many years.
6. Along with proven equipment, a trained and likely to be highly motivated workforce may be available to operate in the new facility. Local, state/provincial, and federal incentives are likely available to offset a significant portion of the new fixed investment.
7. Repurposing offers an uneconomical kraft mill the opportunity to be competitive in the production of cellulosic ethanol, this preserving jobs and permitting the owners to enter a new business—as opposed to simply writing off assets—to attract investment.

There are many pretreatments that are compatible with the assets available in a repurposed mill. In this article, we describe the development of a relatively simple process, which uses green liquor as a pretreatment for the production of pulp that can be enzymatically hydrolyzed to monomeric sugars with a high overall sugar recovery. Green liquor, which is a mixture of sodium carbonate and sodium hydroxide, is a part of the kraft chemical recovery process and is produced by dissolving the smelt from the recovery boiler. Use of green liquor for pretreatment ensures that the chemicals used during pretreatment can be recovered efficiently using proven technology. The process for using and producing green liquor in a kraft pulp mill is shown in Figure 1.

MATERIALS AND METHODS

Materials

Mixed hardwood chip were provided by a mill in the Southeastern United States and was a mixture of mostly oak and sweet gum. The wood chips were screened to remove the fraction greater than 38 mm and less than 6 mm in length. Barks and knots were manually removed. Air dried wood chips was stored in plastic bags. The chemical compositions of mixed hardwood were determined according to TAPPI standards.

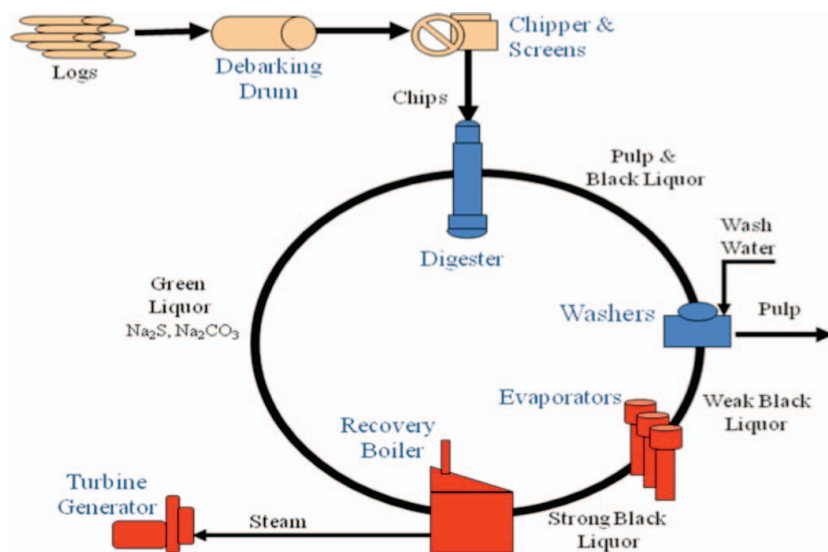


Figure 1. Production of green liquor in a kraft recovery cycle. Figure available online in color.

Green Liquor Cooking and Substrate Preparation

A lab-scale M/K pulping digester was used with a capacity of 800 g of oven dry wood. Wood chips were directly subjected to pretreatment using the mixture of sodium carbonate and sodium sulfide with a sulfidity of 25%. The ratio of pretreatment liquor to wood chip (od) was 4 (v/w). The Total Titratable Alkali (TTA) charge as Na_2O on OD wood chips was varied from 4% to 20% (w/w). The TTA charge takes into account both the sodium carbonate and the sodium sulfide. The wood chips were first impregnated with the pretreatment liquor at 80°C for 30 min. Immediately after impregnation, the temperature was raised to 160°C and maintained for a certain period until a H-factor of 400 was reached. At the end of the pretreatment, the solid was collected and washed by hot water overnight to completely remove residual chemicals and dissolved wood compounds. The moisture of the cooked chip was measured to calculate the green liquor pretreatment yield. Washed wood chips were refined by a Bauer 148–2 disk refiner twice with the disk gap of 0.25 mm and 0.05 mm for size reduction.

Enzymatic Hydrolysis

Enzymatic hydrolyses of the substrates were carried out in a 250 ml Erlenmeyer flask at a consistency of 5% (w/w) with 38 mL sodium acetate buffer (pH 4.8)

at 50°C using a shaking incubator at 180 rpm. An enzyme cocktail of cellulase, xylanase, and β -glucosidase (1 FPU:1.2 FXU:1 CBU) was used for enzymatic hydrolysis. The activity loading was based on a cellulase charge between 5 to 40 FPU/g substrate. Excessive β -glucosidase was used to prevent cellobiose accumulation.^[19] Commercial enzymes used for the enzymatic hydrolysis were NS 50013 (cellulase), NS 50044 (xylanase), and NS 50010 (β -glucosidase) and these were provided by Novozymes for this study.

Sodium azide was charged at 0.3%, based on total weight of the substrate slurry as an antibiotic. Enzymatic hydrolysis residue and hydrolysate was separated by centrifugation. The residues were washed by deionized water three times to remove the protein and other dissolved substances. Frozen and vacuum dried residues were weighed to calculate substrate weight loss. Hydrolysates were sampled for monomeric sugar (glucose, xylose, and mannose) content. Each data point was the average of duplicate experiments.

Analytical Methods

Enzyme Activity Measurement

Cellulase activity of cellulase 50013 was determined by the filter paper method.^[20] Whatmann No. 1 filter paper was used as a standard substrate. Manufacturer specified activity of Novozyme 50044 and 50010 was directly used to calculate loading.

Measurement of Sugars

Enzymatic hydrolysate was boiled in water for 5 min. The hydrolysate was diluted 250 times with the addition of fucose as internal standard. Monosaccharides were determined using a Dionex ICS-3000 ion chromatography with pulsed amperometric detection (HPAEC-PAD) method. Degassed super-purified deionized water was used as an eluent at a flow rate of 1.1 mL/min. Aliquots (20 μ L) were injected after passing through a 0.22 μ m nylon syringe filter. Optimization of baseline stability and detector sensitivity was achieved by post-column addition of 400 mmol/L NaOH. The column was reconditioned using 200 mmol/L NaOH after three analyses. Monosaccharides were quantified with reference to standards using the same analytical procedure. The average of duplicate runs was used. Data of glucose, xylose, and mannose contents were corrected to glucan, xylan, and mannan for sugar yield calculation.

Lignin Content Measurement

Klason lignin content was determined using the acid hydrolysis method. The sample was autoclaved at 121°C for 1.0 h in 3% H₂SO₄ hydrolysis. The

hydrolysate from this determination was retained for analysis of sugars and acid-soluble lignin. Sugars were determined as described earlier, except that sugar standards were autoclaved at 121°C for 1.0 h prior to analysis to compensate for destruction during heating.^[21] Acid-soluble lignin was determined by absorbance at 205 nm in an HP 8453E UV-VIS spectrometer. The solid materials were milled using a Wiley mill before acid hydrolysis (acid-insoluble lignin procedure).

RESULTS AND DISCUSSION

Effects of Chemical Charge on Yield and Chemical Composition of Pulp

Mixed hardwoods were cooked using simulated green liquor with Total Titratable Alkali (TTA) charge varying from 4–20%. The simulated green liquor was prepared in laboratory by mixing sodium carbonate and sodium sulfide with a sulfidity of 25%. The pulping conditions were described earlier.

Table 1 shows the total yield and spent liquor pH after the green liquor pretreatment. The yield drops rapidly when TTA charge increases to 8% and levels off at charges greater than 12%. The pH value of the pretreatment spent liquor ranges from 8 to 9.8. These pH values are considerably lower than that of a normal kraft pulping, which has a pH range of 13–14. This low final pH is an important feature of the green liquor pretreatment. The low pH prevents the random hydrolysis of polysaccharide and secondary peeling reactions from occurring during the pretreatment, resulting in higher retention of polysaccharide in pulp.

Lignin and polysaccharide contents of wood and various GL pulps are shown in Table 2. With increasing TTA in green liquor, the lignin decreases and carbohydrates, mainly glucan, increases. The sums of sugar and lignin of GL pulps are very close to 100%, suggesting that other minor components in wood such as O-acetyl group, extractives, galactans, and some 4-O-methylglucuronic acid group were removed during the GL pretreatment. The glucuronic acid groups are attached to xylan as side chains (1:10 glucuronic acid:xylose ratio in hardwood) and play a key role in preventing xylan from peeling reactions during alkaline pulping.^[22] It is expected that some 4-O-methylglucuronic acid groups will remain with xylan either as it is or as its degradation product, hexenuronic acid groups.

Table 1. GL pretreatment yield and spent liquor pH

TTA, %	4	8	12	16	20
Cooking yield, %	89.2	82.2	79.7	78.4	77.0
Spent liquor pH	8.03	9.05	9.38	9.59	9.78

Table 2. Chemical composition of wood and GL pulps

	Benzen- ethanol extractives	Sugar			Lignin		Total sugar	Total lignin	Sum
		Glucan	Xylan	Mannan	Klason	Acid soluble			
Wood	2.0	47.8	16.3	2.2	22.7	4.0	66.4	26.7	94.9
GL-04	—	54.5	16.2	2.0	23.0	3.9	72.7	26.9	99.6
GL-08	—	56.2	15.0	1.1	22.5	3.6	72.3	26.1	98.4
GL-12	—	58.9	15.7	0.7	20.6	3.3	75.3	23.9	99.2
GL-16	—	61.0	15.0	0.6	20.0	2.9	76.6	22.9	99.1
GL-20	—	60.8	15.5	0.5	19.3	2.9	76.9	22.2	98.9

Table 3 shows the chemical composition of GL pulps based on the original weight of wood. Mannan is easily degraded under alkaline condition. Around 60% of the mannan is dissolved when green liquor charge reaches 8%. At 20% TTA, mannan retention on the basis of wood in green liquor cooked pulps is about 0.4% out of the original 2.2% in wood. Thus, more than 80% of mannan is dissolved. Glucose to mannose ratio in hardwood glucomannan is 1:1.5–2,^[23] which means an additional 0.9–1.2% glucan is liberated from glucomannan in GL pretreatment. Since the total loss of glucan in green liquor pulping at 20% TTA was only around 1%, glucomannan accounts for all the loss in glucan during GL pretreatment. This also implies that cellulose is stable under the green liquor pretreatment conditions. Unlike mannan, xylan content decreases initially with an increase in TTA charge of up to 8%. However, beyond 8% TTA charge, the xylan becomes stable and the loss remains around 25% even at a TTA charge of 20%.

The pattern of carbohydrate reactions under the green liquor pretreatment conditions can be summarized as follows. Cellulose is basically stable. After

Table 3. Chemical composition of wood and GL pulps based on wood

	Benzene- ethanol extractives,%	Sugar,%			Lignin,%		Total sugar, %	Total lignin, %	Sum, %
		Glucan	Xylan	Mannan	Klason	Acid soluble			
Wood	2.0	47.8	16.3	2.2	22.7	4.0	66.4	26.7	94.9
GL-04	—	48.6	14.5	1.8	20.5	3.5	64.9	24.0	88.8
GL-08	—	46.2	12.3	0.9	18.5	3.0	59.4	21.5	80.9
GL-12	—	46.9	12.5	0.6	16.4	2.6	60.0	19.1	79.1
GL-16	—	47.8	11.8	0.5	15.7	2.3	60.1	18.0	77.7
GL-20	—	46.8	11.9	0.4	14.9	2.2	59.2	17.1	76.2

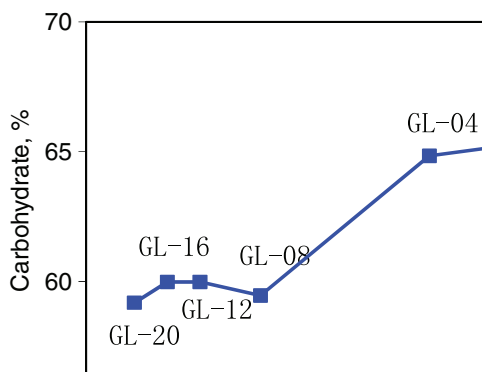


Figure 2. Carbohydrate content as a function of green pulp yield. Figure available online in color.

an initial rapid degradation, both xylan and mannan became stable even at high TTA charges. This relationship is well demonstrated in Figure 2 where carbohydrate content is plot against pulp yield. It is well known that under kraft pulping conditions, carbohydrates are degraded by both primary peeling reactions and the random hydrolysis followed by the secondary peeling reactions. The present results here strongly suggest that under green liquor pretreatment conditions, only the primary peeling reactions are operative. The pH of the green liquor appears to be too low to cause random hydrolysis, which is known to require the ionization of the hydroxyl group at carbon 2 of any polysaccharides in wood.^[24] Thus, primary peeling reaction accounts for the initial rapid loss of both xylan and mannan. Then the stopping reaction kicks in and the carbohydrates become stable. More xylan is retained because it has 4-o-methylglucuronic acid group attached as side chain at C-2 position of xylan and hence slows down the primary peeling reaction in favor of the stopping reaction.^[22]

Residual lignin content at different pretreatment yields is shown in Figure 3. Unlike carbohydrate in Figure 2, the residual lignin content drop rapidly as the yield decreased from 85–77%. At pulp yield of 77–78%, the lignin content decreased from the original 26.7% to 17–18%, a delignification of about one third of the original lignin in wood. This level of delignification is apparently sufficient to afford efficient enzymatic hydrolysis of the remaining carbohydrates in pulp as discussed in the next section.

Alkaline pretreatments for ethanol production have not been very popular, because typically polysaccharides are degraded under such conditions, and converted to non-fermentable degradation products. As summarized in Figure 4, the green liquor pretreatment is unique in that the pulp retains high amount of carbohydrates in the wood (about 90%) while enabling sufficient delignification to allow efficient enzymatic hydrolysis. This will not be achievable with kraft

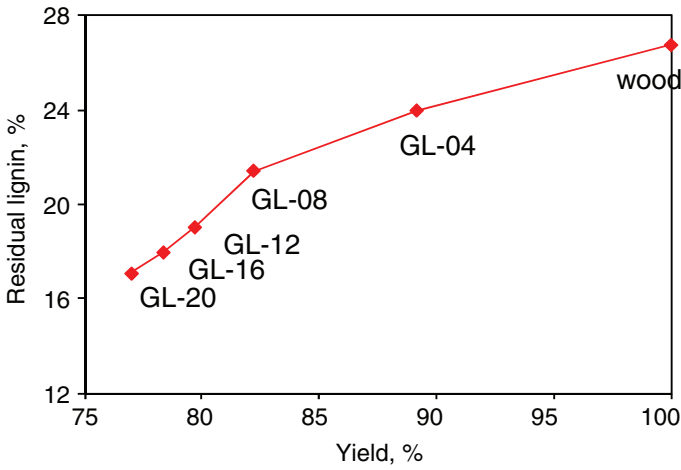


Figure 3. Residual lignin content as a function of green liquor pulp yield. Figure available online in color.

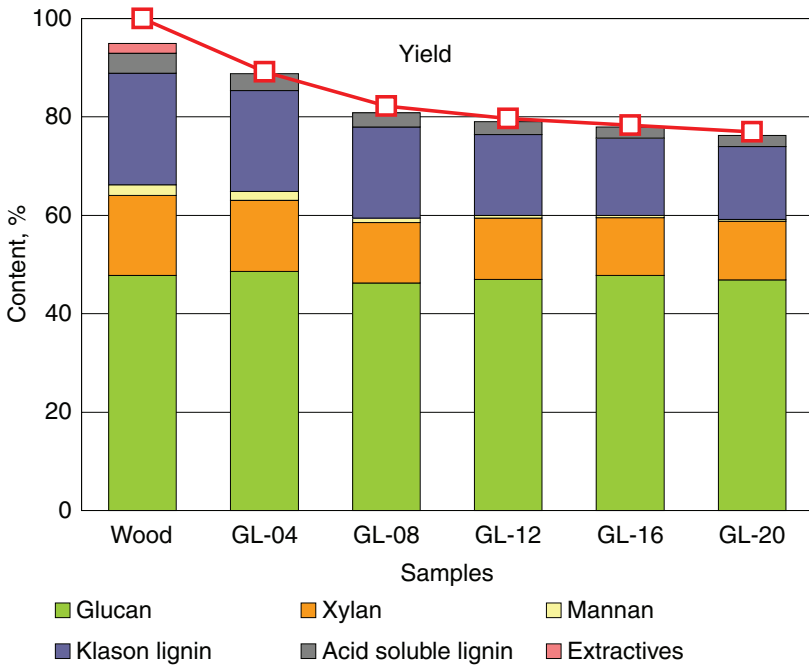


Figure 4. Pulp yield and retention of various components during green liquor pretreatment. Figure available online in color.

white liquor, which will delignify efficiently but will not retain hemicelluloses to the same extent as green liquor. The use of NaCO_3 or $\text{NaCO}_3 + \text{NaOH}$ may achieve the same retention of carbohydrate, but, in the absence of Na_2S , the high lignin content of the resulting pulp would not be hydrolyzed efficiently by enzymes. Thus, the presence of Na_2S at low pH in the green liquor result in high carbohydrate retention and high enzymatic hydrolysis efficiency, making the green liquor based pretreatment an ideal process for the production of ethanol from hardwood.

Effects of Green Liquor Pretreatment on Enzymatic Hydrolysis

Green liquor pretreated mixed hardwood pulps with TTA charges of 4–20% were hydrolyzed using the enzyme mixture described earlier at 50°C for 48 h. The activity loading is based on a cellulase charge of 5 to 40 FPU/g of substrate. Weight losses of hydrolyzed GL pulp was determined to evaluate the efficiency of enzymatic hydrolysis, and are shown in Figures 5 and 6 based on pulp and on wood. The weight loss results shown in Figure 6 takes into account the yield loss during the pretreatment and represent the weight loss on the basis of the original wood. Table 4 and Figures 7 and 8 show the results of the sugar produced by enzymatic hydrolysis for different green liquor pretreatments and cellulase charges on the basis of both pulp and wood. There is good agreement between the results from the weight loss and the sugars in the enzymatic hydrolysis. The total sugar yield increases with enzyme loading when enzyme loading is changed from 5 FPU/g to 20 FPU/g. The sugar

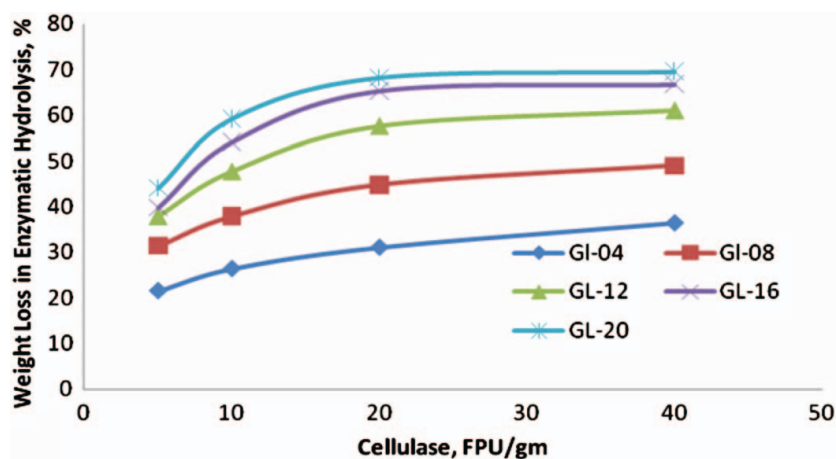
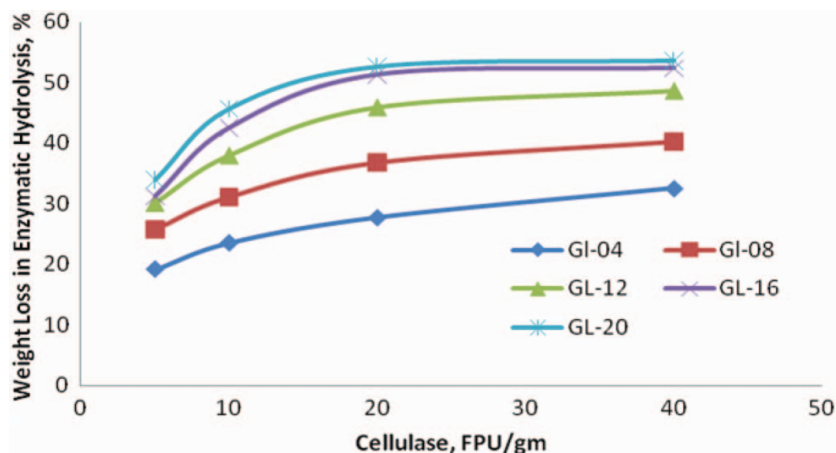


Figure 5. Enzymatic hydrolysis weight loss as a function of enzyme charge for green liquor pulps pretreated with varying TTA. Weight loss% based on pulp. Figure available online in color.

Table 4. Effect of green liquor pretreatment and cellulase charge on sugars from enzymatic hydrolysis

TTA, %	Cellulase, FPU/g	Glucan, % on pulp	Glucan, % on wood	Xylan, % on pulp	Xylan, % on wood	Total Sugar, % on pulp	Total Sugar, % on wood
GL-04	5	14.8	13.2	6.5	5.8	21.3	19.0
	10	19.4	17.3	7.6	6.8	27.1	24.1
	20	24.1	21.5	8.5	7.6	32.6	29.1
	40	29.9	26.7	9.8	8.7	39.7	35.4
GL-08	5	22.5	18.5	8.5	7.0	30.9	25.4
	10	29.2	24.0	9.8	8.1	39.1	32.1
	20	36.1	29.6	11.0	9.0	47.0	38.6
	40	41.0	33.7	11.9	9.7	52.8	43.4
GL-12	5	28.1	22.4	9.5	7.6	37.6	29.98
	10	37.9	30.2	11.3	9.0	49.2	39.2
	20	47.0	37.5	12.7	10.1	59.7	47.5
	40	51.6	41.1	13.6	10.8	65.2	52.0
GL-16	5	30.3	23.7	9.8	7.7	40.1	31.5
	10	41.9	32.9	11.9	9.3	53.9	42.2
	20	51.3	40.2	13.3	10.5	64.7	50.7
	40	53.1	41.6	14.0	11.0	67.2	52.6
GL-20	5	32.1	24.7	10.4	8.0	42.5	32.7
	10	44.7	34.4	12.7	9.8	57.4	44.2
	20	51.8	39.9	13.9	10.7	65.8	50.6
	40	54.0	41.6	14.7	11.3	68.7	52.9

**Figure 6.** Enzymatic hydrolysis weight loss as a function of enzyme charge for green liquor pulps pretreated with varying TTA. Weight loss% based on wood. Figure available online in color.

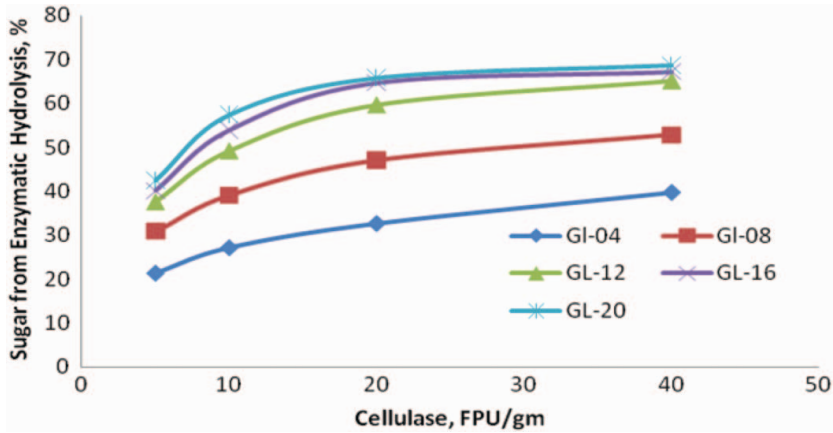


Figure 7. Sugar from enzymatic hydrolysis as a function of enzyme charge for green liquor pulps pretreated with varying TTA. Sugar% based on pulp. Figure available online in color.

recovery changes little when the enzyme loading is increased from 20 FPU/g to 40 FPU/g, especially for pulps with a high TTA charge. As the TTA charge in green liquor pretreatment is increased the enzymatic hydrolysis efficiency increases significantly up to 12%. Beyond 12% TTA charge the improvement in enzymatic hydrolysis significantly lower and the difference between 16 and 20% TTA charge is minimal. At 16% TTA charge with 20 FPU/g cellulose

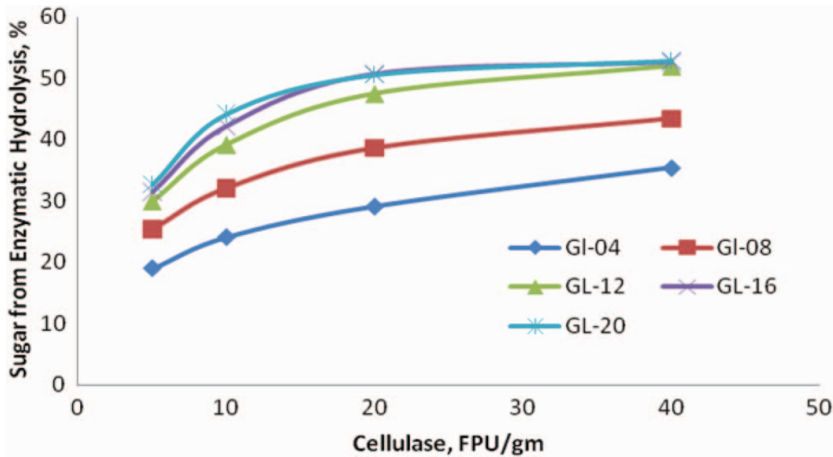


Figure 8. Sugar from enzymatic hydrolysis as a function of enzyme charge for green liquor pulps pretreated with varying TTA. Sugar% based on wood. Figure available online in color.

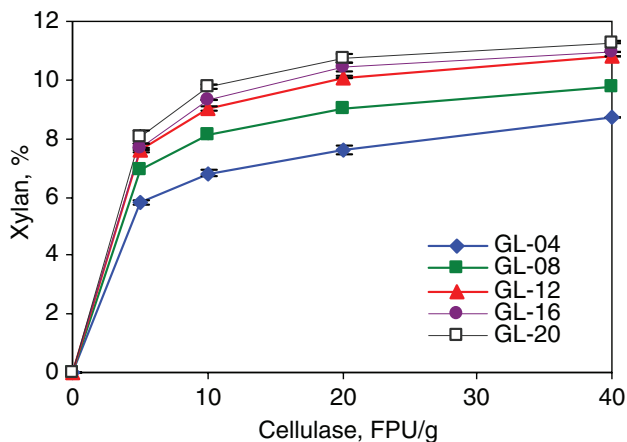


Figure 9. Xylan recovered as a function of the enzyme charge for GL pulp with varying TTA charge. Xylan% based on wood. Figure available online in color.

charge, the weight loss and sugar recovery in enzymatic hydrolysis was about 70% based on pulp and about 50% based on wood.

Figures 9 and 10 show the enzymatic hydrolysis sugar yield for both the xylan and the glucan on the basis of the original wood. The mannan recovery in enzymatic hydrolysis was low since most of the mannan is removed during the green liquor pretreatment. Therefore the glucan and xylan represents the total sugar recovered. It is easier to recover the xylan during enzymatic

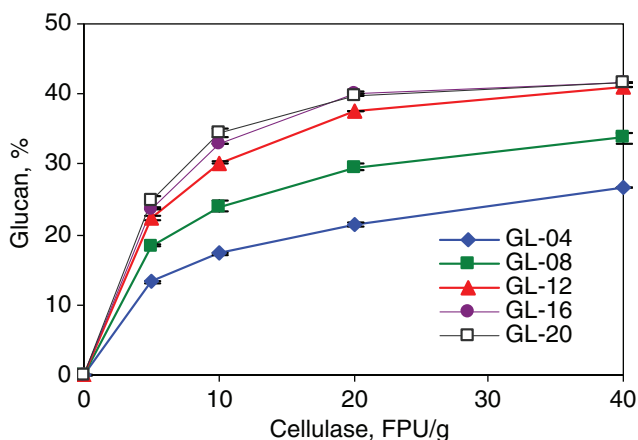


Figure 10. Glucan recovered as a function of the enzyme charge for GL pulp with varying TTA charge. Glucan% based on wood. Figure available online in color.

Table 5. Total sugar recovery of green liquor pretreated hardwood pulp,% on total sugar in original wood

FPU/g pulp	GL-04	GL-08	GL-12	GL-16	GL-20
5	28.7	38.4	45.3	47.5	49.4
10	36.5	48.5	59.3	63.7	66.8
20	43.9	58.4	71.8	76.5	76.5
40	53.5	65.6	78.5	79.5	79.9

hydrolysis and increasing the TTA charge does not have a large effect on overall xylan recovery. However, as the TTA charge is the green liquor stage is increased the glucan recovery increases significantly. At an enzyme charge of 20 FPU/gm, increasing the TTA charge from 8–12% increases the xylan recovered from 9–10% as compared to 30–38% for the glucan. The highest enzymatic saccharification yields of glucan, xylan, and total sugar are 41.6%, 11.3%, and 52.9% based on wood with a TTA charge of 20% in the green liquor pretreatment stage.

Total sugar recovery of green liquor pretreated hardwood pulp at different enzyme loadings is shown in Table 5. Sugar recovery is defined as the percentage of the carbohydrates in the original wood that was recovered during enzymatic hydrolysis. The total sugar recovery with GL-20 at 40 FPU was 79.9%, since the amount of sugar in enzymatic hydrolysis filtrate was 52.9% and the total amount of carbohydrates in wood was 66.4% (Table 2). The sugar

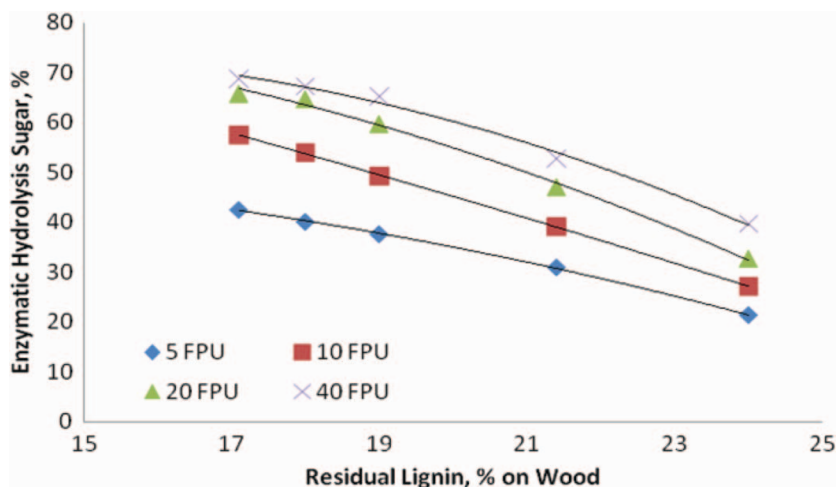


Figure 11. Effect of lignin removal on sugar from enzymatic hydrolysis for GL pretreated pulp with varying TTA. Sugar% based on pulp. Figure available online in color.

recovery measurement incorporates the effect of the yield during green liquor pretreatment and also the efficiency of the enzymatic hydrolysis. Overall sugar recovery is very useful, because it helps determine which combination of green liquor pretreatment and enzymatic hydrolysis gives the best fermentable sugar yield. It can be seen that hardwood pretreated by green liquor at 12–20% TTA charge had a total sugar recovery of about 80% at a cellulase dosage of 40 FPU/g substrate. At 20 FPU, the green liquor pretreatment at 16% TTA resulted in the best sugar recovery. These levels of sugar recovery are competitive with other pretreatments for hardwood.

Residual lignin content in GL pulp plays an important role on the efficiency of enzymatic hydrolysis as shown in Figure 11. As the residual lignin content in green liquor pretreated pulp decreases, sugars from enzymatic hydrolysis increases. However as the lignin content is decreased beyond 19% based on wood, the improvements are smaller especially at the higher enzyme dosages. The 19% lignin on wood represents 33% removal of lignin from the wood. Other researchers have also found that delignification treatment of lignocellulosic biomass increases the monomeric sugar yield by enzymatic hydrolysis.^[25,26] It has been speculated that cellulolytic enzymes are adsorbed non-specifically on the lignin fraction of lignocellulose.^[27–30] Delignification treatment also improves the hydrolysis of cellulose and hemicellulose by increasing the number of substrate sites available to enzymes on these polysaccharides.^[31] Lignin modification can also improve fermentable sugar yields for biofuel production.^[32–34]

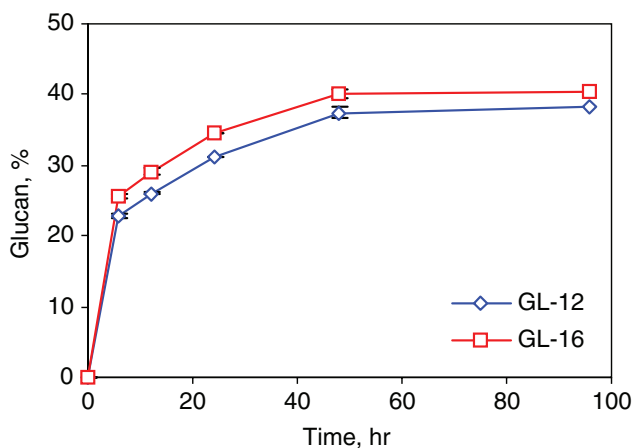


Figure 12. Effect of enzymatic hydrolysis time on glucan yield. Glucan% based on original wood. Figure available online in color.

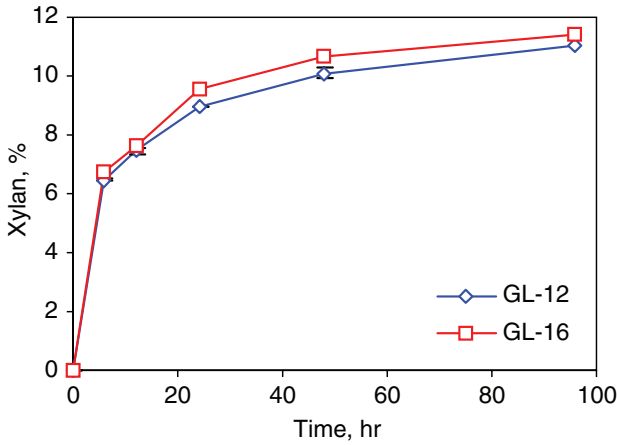


Figure 13. Effect of enzymatic hydrolysis time on xylan yield. Xylan% based on original wood. Figure available online in color.

Effect of Enzymatic Hydrolysis Time on Sugar Yield

Figures 12 to 14 show the effect of enzymatic hydrolysis time on sugar yield. Both glucan and xylan yield increases with enzymatic hydrolysis time. More than a half monomeric sugar is released in the first 6 h. Sugar yield at 96 h is only 1.1% higher than that at 48 h, which means most wood polysaccharides are degraded in 48 h.

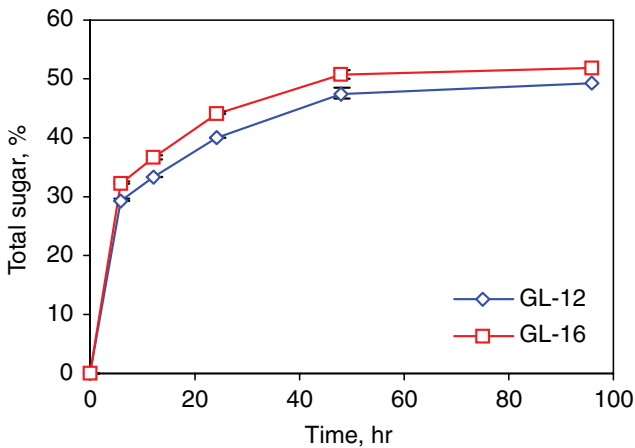


Figure 14. Effect of enzymatic hydrolysis time on total sugar yield. Sugar% based on original wood. Figure available online in color.

CONCLUSIONS

It has been shown that green liquor, which is a mixture of sodium carbonate and sodium sulfide, can be used as an effective pretreatment for hardwood for the production of ethanol using enzymatic hydrolysis. Green liquor is an integral part of the kraft liquor cycle and this mixture is readily available in a repurposed kraft pulp mill. The use of green liquor for pretreatment is simpler than either sodium hydroxide or white liquor (sodium hydroxide and sodium sulfide) since the production of green liquor does not require the causticization process. This will decrease the overall cost for the pretreatment process. In addition the use of green liquor pretreatment uses proven technologies for the recovery of all the chemicals at a very high efficiency and it can be easily implemented in a repurposed kraft pulp mill. A patent application has been submitted with the U.S. Patent Office for this process.

During green liquor pretreatment the cellulose is very stable. After an initial rapid degradation, both xylan and mannan became stable even at high TTA charges. In green liquor pretreatment nearly 100% cellulose and 75% xylan in mixed southern hardwood could be preserved. The low pH prevents the random hydrolysis of polysaccharide and secondary peeling reactions from occurring during the pretreatment, resulting in higher retention of the polysaccharides in pulp. The residual lignin content drops rapidly as the yield decreased from 85–77%. At pulp yield of 77–78%, the lignin content decreased from the original 26.7% to 17–18%, a delignification of about one third of the original lignin in wood. This level of delignification is sufficient to afford efficient enzymatic hydrolysis of the remaining carbohydrates in pulp.

With green liquor pretreatment at 16% TTA, the overall sugar recovery for hardwood was shown to be around 77% at a cellulase charge of 20 FPU/gm of substrate. A sugar recovery of 80% was achieved at higher enzyme charges. These levels of sugar recovery are competitive with other pretreatments for hardwood. The recovery of glucan was about 87% while the recovery of xylan was about 67%. The recovery of mannan was low since most of the mannans are lost during the pretreatment.

Lignin content in pretreated pulp plays an important role on total sugar recovery. Improvement in sugar recovery was measured when the lignin content was decreased to about 19% based on original wood. A lignin content of 19% on wood represents about 33% lignin removal. When lignin content based on original wood is lower than 19%, no significant improvement in sugar recovery was measured, especially at high enzyme loadings.

REFERENCES

1. Ragauskas, A.J.; Williams, C.K.; Davison, B.H.; et al. The path forward for biofuels and biomaterials. *Science*, **2006**, *311*, 484–489.

2. Kadam, K.L.; McMillan, J.D. Availability of corn stover as a sustainable feedstock for bioethanol production. *Bioresource Technol.*, **2003**, *88*, 17–25.
3. Taherzadeh, M.J.; Karimi, K. Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review. *BioResources*, **2007**, *2* (4), 707–738.
4. Öhgren, K.; Bura, R.; Saddler, J.; et al. Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. *Bioresource Technol.*, **2007**, *98*, 2503–2510.
5. Adsul, M.G.; Ghule, J.E.; Shaikh, H.; et al. Enzymatic hydrolysis of delignified bagasse polysaccharides. *Carbohydrate Polym.*, **2005**, *62*, 6–10.
6. Tabka, M.G.; Herpoël-Gimbert, I.; Monod, F.; et al. Enzymatic saccharification of wheat straw for bioethanol production by a combined cellulase xylanase and feruloyl esterase treatment. *Enzyme and Microbial Technol.*, **2006**, *39*, 897–902.
7. Zhao, Y.; Wang, Y.; Zhu, J.Y.; et al. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. *Biotechnol. Bioeng.*, **2008**, *99* (6), 1320–1328.
8. Yu, Z.; Zhang, H.X. Ethanol fermentation of acid-hydrolyzed cellulosic pyrolysate with *Saccharomyces cerevisiae*. *Bioresource Technol.*, **2004**, *93*, 199–204.
9. Sun, Y.; Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technol.*, **2002**, *83*, 1–11.
10. Pan, X.J.; Arato, C.; Gilkes, N.; Gregg, D.; Mabee, W.; Pye, K.; et al. Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuelgrade ethanol and co-products. *Biotechnol. Bioeng.*, **2005**, *90*, 473–481.
11. Galbe, M.; Zacchi, G. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv. Biochem. Eng./Biotechnol.*, **2007**, *108*, 41–65.
12. Litzen, D.; Dixon, D.; Gilcrease, P.; Winter, R. Pretreatment of biomass for ethanol production. U.S. Patent, 2006 0141,584 (2006).
13. Takacs, E.; Wojnarovits, L.; Foldvary, C.; Hargittai, P.; Borsa, J.; Sajo, I. Effect of combined gamma-irradiation and alkali treatment on cotton-cellulose. *Radiat Phys. Chem.*, **2000**, *57*, 399–403.
14. Mabee, W.E.; Gregg, D.J.; Arato, C.; et al. Updates on softwood-to-ethanol process development. *Appl. Biochem. Biotech.*, **2006**, *129* (1–3), 55–70.
15. Pan, X.; Xie, D.; Gilkes, N.; et al. Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. *Appl. Biochem. Biotech.*, **2005**, *121–124*, 1069–1079.
16. Pan, X.; Xie, D.; Yu, R.W.; et al. Pretreatment of lodgepole pine killed by mountain pine beetle using the ethanol organosolv process: Fractionation and process optimization. *Ind. Eng. Chem. Res.*, **2007**, *46* (8), 2609–2617.
17. Wyman, C.E.; Dale, B.E.; Elander, R.T.; et al. Coordinated development of leading biomass pretreatment technologies. *Bioresource Technol.*, **2005**, *96*, 1959–1966.
18. Lee, J. Biological conversion of lignocellulosic biomass to ethanol. *J. Biotechnol.*, **1997**, *56*, 1–24.
19. Emmel, A.; Mathias, A.L.; Wypych, F.; Ramos, L.P. Fractionation of *Eucalyptus grandis* chips by dilute acid-catalysed steam explosion. *Bioresource Technol.*, **2003**, *86* (2), 105–115.
20. Wood, T.M.; Bhat, M. Methods for measuring cellulase activities. In *Methods in Enzymology. Biomass (Part A) Methods in Enzymology. Biomass (Part A)*, vol. 160; Colowick, S.P., Kaplan, N.O., Eds.; Academic Press; New York, 1988; 87–112.

21. Dence, C.W. The determination of lignin. In *Methods in Lignin Chemistry*; Lin, S.Y., Dence, C.W., Eds.; Springer; Heidelberg, Germany, 1992; 33–61.
22. Sjöström, E. *Wood Chemistry: Fundamentals and Applications*; Academic Press, New York, 1993; 137.
23. *Ibid.*, p. 65.
24. *Ibid.*, p. 46.
25. Mooney, C.A.; Mansfield, S.D.; Touhy, M.G.; Saddler, J.N. The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. *Bioresource Technol.*, **1998**, *64*, 113–119.
26. Draude, K.M.; Kurniawan, C.B.; and Duff, S.J.B. Effect of oxygen delignification on the rate and extent of enzymatic hydrolysis of lignocellulosic material. *Bioresource Technol.*, **2001**, *79*, 113–120.
27. Sutcliffe, R.; Saddler, J.N. The role of lignin in the adsorption of cellulases during enzymatic treatment of lignocellulose material. *Biotechnol. Bioeng. Symp.*, **1986**, *17*, 749–762.
28. Chernoglazov, V.M.; Ermolova, O.V.; Klyosov, A.A. Adsorption of high-purity endo-1,4- β -glucanases from *Trichoderma reesei* on components of lignocellulosic materials: cellulose, lignin, and xylan. *Enzyme Microb. Technol.*, **1988**, *10*, 503–507.
29. Converse, A.O.; Ooshima, H.; Burns, D.S. Kinetics of enzymatic hydrolysis of lignocellulosic materials based on surface area of cellulose accessible to enzyme and enzyme adsorption on lignin and cellulose. *Appl. Biochem. Biotechnol.*, **1990**, *24–25*, 67–73.
30. Osshida, H.; Burns, D.; Converse, A.O. Adsorption of cellulase from *Trichoderma reesei* on cellulose and lignaceous residue in wood pretreated by dilute sulfuric acid with explosive decompression. *Biotechnol. Bioeng.*, **1990**, *36*, 446–452.
31. Yoshida, M.; Liu, Y.; Uchida, S. Effects of cellulose crystallinity, hemicellulose, and lignin on the enzymatic hydrolysis of *Miscanthus sinensis* to monosaccharides. *Biosci. Biotechnol. Biochem.*, **2008**, *72* (3), 805–810.
32. Davison, B.H.; Drescher, S.R.; Tuskan, G.A.; Davis, M.F.; Nghiem, N.P. Variation of S/G ratio and lignin content in a *Populus* family influences the release of xylose by dilute acid hydrolysis. *Appl. Biochem. Biotechnol.*, **2006**, *130*, 427–435.
33. Dien, B.; Jung, H.G.; Vogel, K.P.; Casler, M.D.; Lamb, J.F.S.; Weimer, P.J.; et al. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass Bioenergy*, **2006**, *30*, 880–891.
34. Chen, F.; Dixon, R.A. Lignin modification improves fermentable sugar yields for biofuel production. *Nature Biotechnol.*, **2007**, *25*, 759–761.