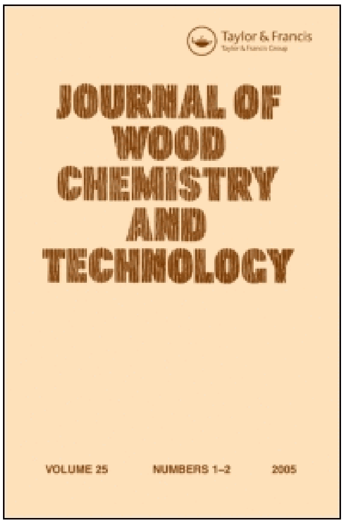

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Novel Green Liquor Pretreatment of Loblolly Pine Chips to Facilitate Enzymatic Hydrolysis into Fermentable Sugars for Ethanol Production

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Abstract: Softwood species generally have been found very recalcitrant to enzymatic hydrolysis of the carbohydrate fractions to monomeric sugars. To solve this problem, loblolly pine chips were pretreated with green liquor at 12–20% Total Titratable Alkali (TTA) (as Na_2O on wood) at 170°C for 800 H-factor. The yield of resulting pulp was 76.5–78.6% and the lignin content decreased from 29.2 to 20.2–22.4% and the total polysaccharide decreased from 62.6 to 53.8–55.0%, all based on the weight of original wood. When the pulp was subjected to enzymatic hydrolysis using 40 Filter Paper Unit (FPU)/g pulp, only 41% of the polysaccharides in wood were converted to monomeric sugars. This conversion figure is much lower than that of mixed southern hardwoods (80%) treated under similar conditions. If the green liquor treated pulp was further subjected to either oxygen delignification or mechanical refining prior to the enzymatic hydrolysis, the conversion rate increased to around 55% and 60%, respectively. Furthermore, combination of oxygen delignification and refining further increased the total sugar conversion to 78% of the total sugar in wood, approximately equal to that of the mixed southern hardwoods.

Keywords: Biofuel, green liquor, pretreatment, loblolly pine

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INTRODUCTION

Lignocellulosic biomass is the most abundant renewable resource on earth. In the past decade, there has been a growing interest in using this biomass as feed stock for the production of bioethanol.[1–7] Lignocellulosic biomass cannot be converted directly to ethanol, requiring several unit operations. First the polysaccharides (cellulose and hemicelluloses) are hydrolyzed to fermentable sugars, followed by fermentation of the resulting sugars to ethanol and distillation of the dilute ethanol broth to the final product. Utilization of cellulolytic enzyme systems for hydrolysis of the polysaccharides is currently an active research and development topic for bioethanol production technology.[8] However, lignocellulosic biomass, especially softwood, has natural resistance to biological degradation because of its morphological structure and chemical composition. Thus, some kind of pretreatment is required to obtain high conversion of polysaccharides to fermentable sugars.[9–12] Many pretreatment methods have been researched, including acid hydrolysis,[13–15] wet oxidation,[13,16–17] steam explosion,[13,18–20] SO_2 pretreatment,[10] oxygen delignification,[11] peracetic acid pretreatment,[12] ammonia explosion,[10, 24–25] aqueous ammonia soaking,[26–27] sulfite pretreatment[28] and lime pretreatment.[10] These pretreatment methods affect enzymatic hydrolysis of biomass differently, and also introduce different degrees of complexity in process technology, capital investment, and chemical and other operating costs.

A novel pretreatment method based on green liquor ($Na_2CO_3 + Na_2S$) was developed in our laboratory at North Carolina State University[29–31] (patent applied). The green liquor pretreatment process was developed with the concept of repurposing an old kraft pulp mill for ethanol production, taking advantage of the recent pulp mill closures as a result of declining demand in pulp and paper.[29] A conceptual diagram of repurposing a kraft mill for ethanol production is shown in Figure 1. Wood chips are cooked in the digester with green liquor and then fiberized by the inline refiner. The black liquor and the pulp are separated in the subsequent washing operation. The washed pulp is sent to enzyme hydrolysis, taking advantage of many pulp storage and bleaching towers available in the mill. The enzymatic hydrolysate, after the residue (mainly lignin) is separated by the lignin filter, is fermented and distilled to produce ethanol. The black liquor from washer is evaporated to a solid content of over 70% and, together with lignin from the lignin filter, is burnt in the recovery boiler to recover inorganic chemicals and energy (as high pressure steam). The steam is sent to a turbine generator to produce power. Some low pressure steam is extracted from the turbine for use as process steam.

One special feature of the present process that differs from other pretreatment methods is that all fermentable sugars are recovered in a single step, the enzymatic hydrolysis stage, without production of fermentation inhibtors. This feature avoids the need to collect fermentable sugars from both pretreatment and enzymatic hydrolysis stages, resulting in diluted sugar concentration for

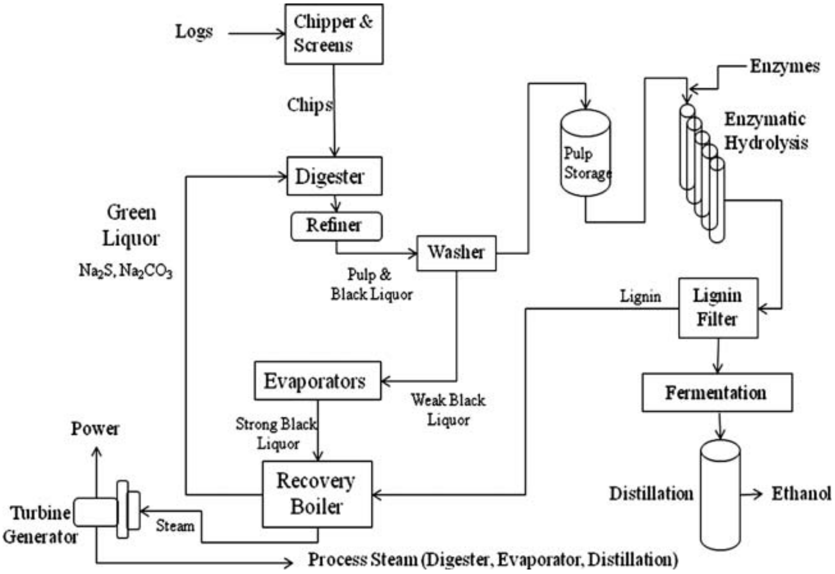

Figure 1. Conceptual diagram of repurposing a kraft mill for ethanol production.

fermentation to ethanol. This feature requires that the pretreatment retains as much polysaccharides as possible in the pulp while at the same time facilitate the subsequent enzymatic hydrolysis for high sugar conversion. The second special feature is that, unlike other pretreatment methods, the inorganic chemicals used are recovered and the dissolved organics including lignin are burned to produce energy, thereby the operating costs are minimized. The process and the equipment used are all proven technologies used in many pulp mills and thus involve low risk for investment.

While the green liquor pretreatment alone works well with hardwoods,[29–30] it gave relatively low fermentable sugars under the same conditions for softwood.[31] Loblolly pine is a major wood resource in the southeastern United States. Any repurposing of the old kraft mills in this region would have to include loblolly pine for economical reason. In this article, we report a novel combination of the green liquor pretreatment with two additional unit operations, oxygen delignification and mechanical refining for softwood pulp that gives high conversion rate of fermentable sugars in the subsequent enzymatic hydrolysis, and requires only proven process steps, with chemicals completely compatible with a conventional kraft recovery system. Also, 25% sulfidity was used exclusively in this article since this is the sulfidity used commonly in most kraft mills in this country. The effect of higher sulfidity is being studied and will be reported in a separate article.

MATERIALS AND METHODS

Raw Materials

Loblolly pine from a mill in the southeastern United States was used in this study. The chips were screened and the accepts from 3/8″ and 5/8″ holes were used for pretreatment.

Pretreatment

Pretreatment was carried out in a 7-liter M&K Digester with 700 OD grams of chips. The chips were cooked with green liquor at three different alkali charges (12, 16, and 20% Total Titratable Alkali (TTA) as Na_2O on wood, 25% sulfidity) and the contents were pulped to the target H Factor of 800 at a maximum temperature of 170°C with liquor to wood ratio of 4. After pulping the samples (still in chip form) were washed overnight to remove dissolved organic and inorganic components so that yield can be determined accurately. The yield was measured by centrifuging the chip mass and measuring the consistency and total weight. The chips were then disintegrated using a refiner at .005 inch gap and then screened using a 0.008 inch screen plate. The rejects were refined with a disk gap of 0.001 inch and then added back to the accepts. The pulp was then centrifuged and fluffed for further processing.

Oxygen Delignification

Oxygen delignification was carried out in a 2.8 L reactor in an oven heated by blowing hot air. GL pulp (100 g OD) was treated with 5% NaOH on pulp at 10% consistency under100 psig oxygen pressure and at 110°C for 60 min (excluding time to temperature of 45 min). After delignification, the pulp was washed with cold water, centrifuged, and fluffed. Pulp yield was measured by the consistency and total weight.

Refining

Pulp (30 g OD) was refined in a PFI mill at 10% consistency for 9000 revolutions. After refining, pulp was collected and hydrolyzed with enzyme without washing.

Enzymatic Hydrolysis

Enzymes used in this work were kindly provided by Novozymes. An enzyme mixture was prepared by mixing cellulase (NS50013), xylanase (NS50014), and

β-glucosidase (NS50010) in the ratio of 1 FPU:1.2 FXU:1 CBU, respectively, as suggested by Novozymes. This enzyme mixture was used throughout this work. Enzyme dosage is expressed in Filter Paper Unit (FPU), but it includes the other two enzymes in the ratio shown earlier. FPU,[32] FXU (Fabre Xylanase Unit),[33] and CBU (Cellubiose Unit)[32] are measures of enzyme activities for cellulose, xylanase, and β-glucosidase, respectively. Definition of these units can be found in the references cited.

Enzymatic hydrolysis was carried out with 2 g of pulp at 5% consistency with 40 FPU/g pulp at pH 4.8 (acetate buffer) and 50°C for 48 h. After enzymatic hydrolysis, the mixture was centrifuged. The supernatant was collected, boiled for 5 min and centrifuged again. The aliquot of the supernatant was used for determination of sugar content. The residue from the enzymatic hydrolysis was washed and centrifuged (3 times), freeze-dried in water suspension, and weighted for weight loss.

Efficiency of enzymatic hydrolysis was estimated by weight loss (WL) and total sugar in enzymatic hydrolysate (TS).

Analytical Methods

Sugar analysis was carried out with ion chromatography (ICS-3000, Dionex) using CarboPac^{TM}PA1 (2 × 250 mm) as analytical and CarboPac^{TM}PA1 (4 × 50 mm) as guard column. Sodium hydroxide solution (0.1 mole/L) was used as eluent. Aliquots of Klason lignin filtrate and enzyme hydrolysate were used to determine sugar content for GL pulps and enzymatic hydrolysate, respectively.

Lignin content is determined according to the TAPPI Standard Method T222.

RESULTS AND DISCUSSION

Chemical Composition of Wood and Green Liquor Treated Pulps

A special feature of the green liquor pretreatment technology is that, unlike other pretreatment methods, all fermentable sugars are recovered in a single step, the enzymatic hydrolysis stage. This feature avoids the need to collect fermentable sugars from both pretreatment and enzymatic hydrolysis stages, resulting in diluted sugar concentration for fermentation to ethanol. This feature requires that the pretreatment retains as much polysaccharides as possible in the pulp while at the same time facilitating the subsequent enzymatic hydrolysis for high sugar conversion.

Chemical composition of wood, green liquor pretreated pulps at 3 different alkali charges (GL 12, GL16 and GL20) as well as oxygen delignified GL pulps (GL-O) are given in Table 1. All data are based on oven dried weight of unextracted wood chips. The total weight percent of polysaccharides and lignin in

Table 1. Yield and chemical composition of GL pulps and oxygen delignified GL pulps (all data are percent weight based on original wood weight)

	Polysaccharides (% on wood)							
	Glucan	Mannan	Other Hexosan	Pentosan	Sum	Lignin%	Total%	Cooking Yield%
Wood	44.3 ± 0.7	10.0 ± 0.1	1.8 ± 0.1	6.5 ± 0.1	62.6 ± 0.4	29.2 ± 0.1	91.8 ± 0.3	
GL20	44.0 ± 0.9	3.4 ± 0.2	1.0 ± 0.1	5.4 ± 0.2	53.8 ± 0.4	20.2 ± 0.3	74.0 ± 0.6	76.5
GL16	44.4 ± 0.8	3.6 ± 0.2	1.0 ± 0.2	5.5 ± 0.2	53.8 ± 0.2	21.6 ± 0.4	75.4 ± 0.6	77.0
GL12	44.0 ± 0.8	4.1 ± 0.3	1.1 ± 0.2	5.8 ± 0.2	55.0 ± 0.4	22.4 ± 0.8	77.4 ± 0.8	78.6
GL20-O	41.4 ± 1.0	3.3 ± 0.1	0.9 ± 0.1	5.3 ± 0.1	50.4 ± 0.3	18.5 ± 0.1	68.9 ± 0.5	72.3
GL16-O	41.2 ± 1.2	3.3 ± 0.1	0.9 ± 0.1	5.3 ± 0.2	50.7 ± 0.2	19.5 ± 0.1	70.2 ± 0.8	73.8
GL12-O	43.4 ± 1.2	3.9 ± 0.3	1.1 ± 0.1	5.5 ± 0.2	53.9 ± 0.2	20.1 ± 0.2	74.0 ± 0.9	77.0

original wood chips amounts to 92%. This is not unusual since extractives (3%), acetyl (1%), ash (<0.5%) and uronic anhydrides (1–3%) were not included.[34] While acetyl and some extractives will be removed during the green liquor pretreatment, some extractives, uronic anhydride, and ash remained with the pulp and may account for the discrepancies between total carbohydrates and lignin and the cooking yield in Table 1.

As can be seen, during the green liquor pretreatment, the lignin content decreased from 29.2% to 20.2–22.4%, a decrease of 7–9% based on wood, depending on TTA charged. Approximately about equal amounts of carbohydrates were lost with lignin during the pretreatment, mainly mannan as expected due to well-known peeling reactions. Pentosan, mainly xylan, on the other hand, was largely retained. It is noteworthy that little glucan was lost during the GL pretreatment. This is not unexpected as it has been demonstrated that random hydrolysis and secondary peeling reactions do not occur in the green liquor pretreatment as a result of the much lower pH than the conventional kraft cooking.[30] Surprisingly, subsequent oxygen delignification only removed an additional 2% lignin (on wood) and is accompanied by 1–4% lost in polysaccharides.

Enzymatic Hydrolysis of Green Liquor Treated Pulps: Comparison between Loblolly Pine and Mixed Southern Hardwood

Efficiency of enzymatic hydrolysis can be evaluated by either the weight loss of the enzymatic hydrolysis or the total sugars in the enzymatic hydrolysate. As shown in Table 2, green liquor treated pulps of loblolly pine gave only about one half of the amount of total sugars as compared with the corresponding pulps of mixed southern hardwood pulped under the same alkali charge. These results are consistent with our earlier results on enzymatic hydrolysis of kraft pulp.[31,35] Even though the lignin content had an impact on enzymatic hydrolysis, good hydrolysis efficiency (90+%, based on the total sugars in pulp) was possible with hardwoods up to a kappa number of 90 with an enzyme charge of 20 FPU/g pulp. The degree of hydrolysis for softwoods was significantly lower than that

Table 2. Enzymatic hydrolysis of green liquor treated pulps: comparison between loblolly pine and mixed southern hardwood (All numbers are weight% based on original weight of wood, all based on enzyme dosage of 40 FPU)

	Loblolly pine		Mixed southern hardwood	
	Weight loss	Total sugars	Weight loss	Total sugars
GL-12	24.2	26.7	48.6	52.0
GL-16	25.7	27.4	52.3	52.6
GL-20	29.7	28.8	53.6	52.9

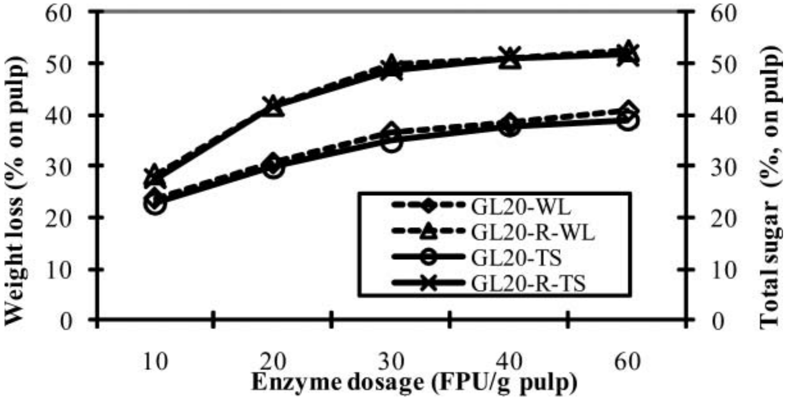

Figure 2. Effect of enzyme dosage on the enzymatic hydrolysis of green liquor treated loblolly pine pulps: WL = weight loss, R-WL = weight loss, refined sample, TS = total sugars, R-TS = total sugars, refined sample.

with hardwoods. At a softwood kappa number of 50, the degree of hydrolysis was about 65% at an enzyme charge of 20 FPU/g pulp. As the softwood kappa number is increased to 160, the degree of hydrolysis decreases to 15% at 20 FPU/g pulp.

In order for the green liquor pretreatment process to be economically viable for ethanol production, a 75–80% conversion of the total polysaccharides in wood to fermentable sugars is estimated to be required. The total polysaccharides content of mixed southern hardwood is 66%. A conversion of 53% on the weight of wood means a conversion of 80% (53/66*100) total polysaccharides in wood to fermentable sugars. Loblolly pine has a total polysaccharides content of 62.6%. As can be seen in Table 2, conversion of total polysaccharides in loblolly pine is less than 50% (29/62.6*100). Thus, it is obvious that other unit operations are required to render higher conversion rate of loblolly pine. Furthermore, it appeared that loblolly pine may require higher enzyme dosage than the mixed southern hardwood. As shown in Figure 2, the GL-20 pulp required 30–40 FPU/g pulp to achieve maximum conversion. This is twice as high as the dosage required for mixed southern hardwood.[30] Since the objective of the present research is to find process suitable for processing loblolly pine, a dosage of 40 FPU/g pulp is used throughout this study leaving the minimization of enzyme dosage for future study.

Improving Enzymatic Hydrolysis by Oxygen Delignification and Pulp Refining

In the same enzyme dosage and time, the effects of GL pretreatment, oxygen delignification, and refining on weight loss of enzymatic hydrolysis and total

Table 3. Enzymatic hydrolysis of GL pretreated pulp

	Weight loss (%)		Total sugar yield in hydrolysate (%)		
	On pulp	On Wood	On pulp	On wood	Sugar conversion* (%)
GL20	38.4 ± 0.8	29.4 ± 0.6	37.7 ± 0.4	28.8 ± 0.3	47
GL16	33.4 ± 0.2	25.7 ± 0.4	35.6 ± 0.4	27.4 ± 0.3	41
GL12	30.8 ± 0.5	24.2 ± 0.4	34.0 ± 0.6	26.7 ± 0.5	39
GL20-R	51.0 ± 0.2	39.0 ± 0.3	51.3 ± 0.3	38.9 ± 0.2	62
GL16-R	47.2 ± 0.1	36.3 ± 0.2	47.4 ± 0.2	37.0 ± 0.2	58
GL12-R	43.8 ± 0.2	34.4 ± 0.2	42.6 ± 0.2	33.1 ± 0.2	55
GL20-O	49.3 ± 0.4	35.6 ± 0.3	47.0 ± 0.2	34.0 ± 0.1	57
GL16-O	44.5 ± 0.4	32.8 ± 0.3	43.3 ± 0.3	32.0 ± 0.2	52
GL12-O	35.1 ± 0.3	27.0 ± 0.2	33.2 ± 0.7	28.6 ± 0.5	43
GL20-O-R	65.9 ± 0.1	47.7 ± 0.1	64.2 ± 0.3	46.4 ± 0.2	76
GL16-O-R	66.4 ± 0.2	49.0 ± 0.2	63.6 ± 0.2	46.9 ± 0.2	78
GL12-O-R	59.4 ± 0.4	45.7 ± 0.3	60.0 ± 0.4	46.2 ± 0.3	73

*Percentage Sugar conversion = Weight loss (on wood)/Total sugar in wood × 100.

fermentable sugar yield in the hydrolysate are shown in Table 3. Both weight loss and total sugar yield are indicators of the enzymatic hydrolysis efficiency. As can be seen in Figure 2 and Table 3, the numbers are very close in every treatment, indicating that weight loss is as good an indicator of enzymatic hydrolysis as the total sugar yield in hydrolysate. These results also indicate that little lignin becomes soluble and little polysaccharides remain as oligomer at the end of the enzymatic hydrolysis. Indeed, little oligomer or dimer was found in the enzymatic hydrolysate. Green liquor pretreatments resulted in conversion of 41% of the polysaccharides in wood into fermentable sugars. Total alkali charge (TTA) in GL pretreatment appeared to have only minor effect. Both refining (R) and oxygen delignification (O), each carried out alone, significantly improved enzymatic hydrolysis efficiency. In both cases, the effect of TTA in GL pretreatment became significant, higher TTA giving higher sugar conversion. When both refining and oxygen delignification were employed with GL pretreatment, over 78% of the total polysaccharides in wood were converted to fermentable sugars.

Oxygen delignification serves as an extension of delignification in green liquor pulps. As shown in Figure 3, the sugar recovery is a function of lignin content in pulp for both green liquor treated pulps and oxygen-delignified pulps. Refining, on the other hand, presumably improves the accessibility of enzymes to polysaccharides in pulp through delamination of fiber. The effect of refining on enzymatic hydrolysis is obvious from both Figures 2 and 3.

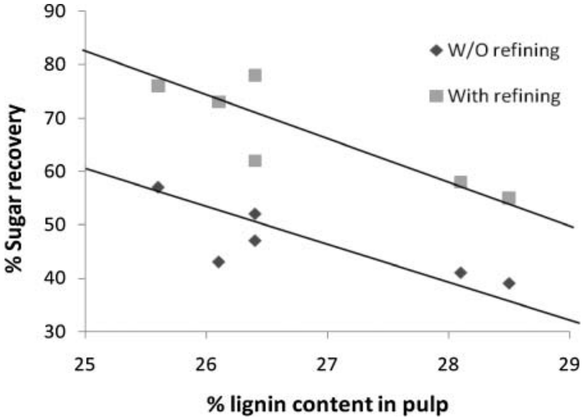

Figure 3. Effect of pulp lignin content and refining on sugar recovery.

Both oxygen delignification and pulp refining are well-known technologies used commonly in a kraft pulp and paper mills, especially when processing softwood bleached kraft pulp. Hence the employment of these two unit operation will not cause any undue risk to the investor, albeit some potential increase in some locations in capital and operational costs as a result of their applications. While oxygen delignification is not always available in every kraft pulp mill, pulp refiners are common equipment used by every mill.

Conversion of Various Polysaccharides to Mono Sugars during Enzyme Hydrolysis

Various polysaccharides in the green liquor pretreated pulps behaved differently during the subsequent enzymatic hydrolysis. As shown in Figure 4, more than 80% of pentosan in the GL pulps was hydrolyzed to mono sugars whereas only less than 50% of hexosans including glucan were transferred to mono sugars. The enzymatic hydrolysis of hexosans was further improved by either oxygen delignification or mechanical refining, each individually improving the recovery of fermentable sugars to about 60%. However, oxygen delignification appeared to do little for the recovery of pentosans, whereas pulp refining further improved the pentosan recovery to more than 95%. Combination of oxygen delignification and mechanical refining further improve enzymatic hydrolysis to the extent that almost all the pentosan and around 90% of hexosan were transferred to mono sugars. The synergetic effect of the combined oxygen delignification and refining on total sugar conversion is especially noteworthy.

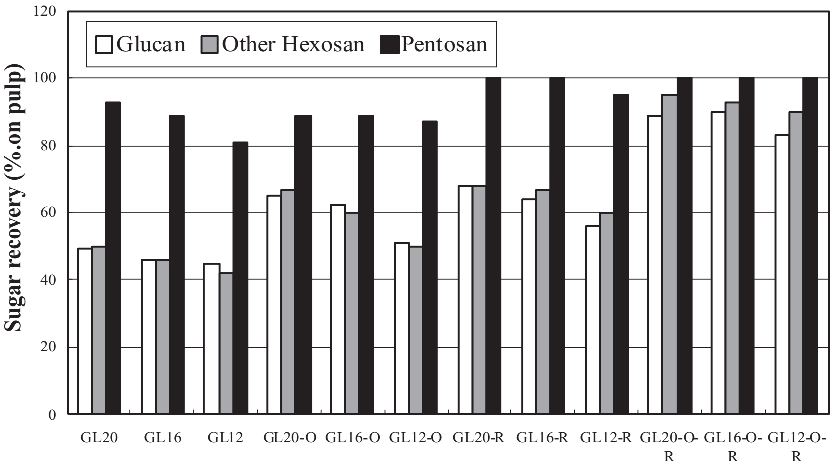

Figure 4. Pattern of enzyme hydrolysis of various polysaccharides in pulps.

The pattern of enzymatic hydrolysis of green liquor pretreated pulps are quite different between loblolly pine and mixed southern hardwood. The difference in ease of enzyme hydrolysis was much smaller between hexosan and pentosan in hardwood. Without the use of either oxygen delignification or pulp refining, about 85% of the glucan and 90% of the xylan in the green liquor treated hardwood pulps were hydrolyzed to mono sugars.[30]

The efficacy of a process combining green pretreatment, oxygen delignification and refining has been demonstrated for the repurposing old kraft pulp mill to produce ethanol from loblolly pine. A preliminary economic analysis revealed that ethanol can be produced at $2.27 per gallon.[31] Based on the assumption of $2.50 per gallon sale price and an enzyme cost of $0.60 per gallon, internal rates of return of 13% and 15% can be realized depending on whether or not the repurposing mill had oxygen delignification facility in hand.[31] The preliminary economic analysis was carried out without the optimization of all three unit operations involved. We are currently optimizing the green liquor process to increase retention polysaccharides, combining green liquor process with optimized oxygen delignification and refining to increase enzymatic hydrolysis efficiency while minimizing enzyme dosage.

CONCLUSION

Unlike hardwood, green liquor pretreatment of loblolly pine gives poor enzymatic hydrolysis efficiency, only less than 50% of polysaccharides being converted to fermentable sugars. Enzymatic hydrolysis efficiency of loblolly pine can be greatly improved if the green liquor pretreatment is followed by oxygen delignification and refining, all being proven technologies. Over 78% of the total polysaccharides in wood can be converted to fermentable sugars. The pattern of enzyme hydrolysis is different between hexosan and pentosan. While pentosan is readily hydrolyzed, only less than 50% of the hexosan is converted to fermentable sugar. Both oxygen delignification and pulp refining greatly improve the enzymatic hydrolysis efficiency.

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