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Comparison of Pretreatment Methods on the Enzymatic Saccharification of Aspen Wood

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ABSTRACT

Five different chemical pretreatments, using dilute sulfuric acid, sodium hydroxide, hydrogen peroxide and sodium hydroxide, peroxymonosulfate, and acetic acid, were applied to aspen thermomechanical fibers. The pretreated fibers were submitted to enzymatic hydrolysis and the liberated glucose was monitored. High glucose concentrations were observed for the peroxymonosulfate and the acetic acid pretreated samples. Glucose concentrations greater than 25 g/L were obtained in these cases. This corresponds to conversions on the order of 90% of the pretreated substrate glucose content.

Index Entries: Chemical pretreatment, enzymatic hydrolysis, biomass, aspen, *Populus tremuloides*, hardwood.

INTRODUCTION

Research on the production of ethanol from renewable lignocellulosics has been performed for more than two decades. This interest in lignocellulose as a raw material can be justified by its low cost and availability (*1*). The major constituents in lignocellulosics are cellulose, lignin, and hemicellulose. Cellulose is a polysaccharide that can be reduced to glucose by chemical or enzymatic hydrolysis. Lignin is a heterogeneous aromatic

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polymer. Hemicellulose is a polysaccharide that, upon hydrolysis, produces xylose and other monosaccharides.

There are a variety of processes for the conversion of cellulose into ethanol (2,3), all of them involving the breakdown of the polysaccharide into glucose. The most promising processes are those employing cellulase enzymes. Enzymes are biodegradable and are not harmful to the environment.

Cellulose and hemicellulose in woody materials and agricultural residues are strongly resistant to enzymatic depolymerization. This is caused by the presence around cellulose of a lignin-hemicellulose shield, which restricts the enzymatic attack. To enhance the enzymatic digestibility of wood substrates, a pretreatment is usually performed. Various types of pretreatments, including biological, mechanical, and physicochemical ones (4–6), have been used to achieve higher glucose yields. For example, a cellulose conversion of 80% was attained in 72 h after pretreatment of aspen with dilute H_2SO_4 at 160°C (7).

The primary objective in this study was to investigate the effect of different pretreatments on the glucose yield during the enzymatic hydrolysis of aspen (*Populus tremuloides*) thermomechanical pulp. Five different types of pretreatment were performed and the corresponding substrates were hydrolyzed enzymatically for 72 h.

MATERIALS AND METHODS

Substrate Pretreatment

Aspen chips were reduced to fibers with a thermochemical pulping process (TMP). Chips were steam-heated for 3 min at 145°C and refined with an energy of 574 KWh/ODT. The yield and the Canadian standard freeness (CSF) of the pulp were 95% and 725 mL, respectively.

In the dilute acid pretreatment (DA), the aspen pulp was mixed with 0.05*M* sulfuric acid and heated for 60 min at 121°C. The liquid-to-solid ratios for this and the other pretreatments are transcribed in Table 1. The sodium hydroxide pretreatment (NA) was performed by adding 0.38*M* sodium hydroxide to the aspen pulp and keeping the slurry at 55°C for 60 min, with thorough agitation every 10 min. The alkaline peroxide pretreatment (AP) was performed at 50°C by adding to the substrate 5% hydrogen peroxide in 0.38*M* sodium hydroxide. The mixture was stirred intermittently during 60 min. The peroxymonosulfate pretreatment (PMS) was conducted by mixing the substrate with a solution of hydrogen peroxide and sulfuric acid in water (8). After 2 h at 50°C with periodic agitation, the fibers were washed with 0.25*M* sodium hydroxide and heated at 55°C for 60 min. For the acetic acid method (AC), the aspen fibers were mixed with a solution containing 17.4*M* acetic acid and 30% hydrogen peroxide and kept at 110°C for 2 h. In all cases, the pretreated aspen pulp was washed with water until the filtrate had a pH of 4.5.

Table 1
Aspen TMP: Pretreatment Conditions and Substrate Recovery Factor

Pretreatment[a]	Temperature, °C	Solids-to-liquid ratio, g/100 mL	Substrate recovery, %
DA	120	10.0	91
NA	50	5.5	83
AP	55	7.5	77
PMS	50	10.0	49
AC	110	8.3	40

[a]DA, dilute acid; NA, sodium hydroxide; AP, alkaline peroxide; PMS, peroxymonosulfate; AC, acetic acid.

Substrate Analysis

The Klason lignin content, determined following the TAPPI standard, was 22 ± 2%, the extractives 2 ± 1%, and the ash content 1.5 ± 0.2%. After pretreatment, the substrate was hydrolyzed and total saccharides were determined using a high-performance liquid chromatography (HPLC) technique (*9*).

Enzymatic Hydrolysis

The saccharification of the pretreated samples was performed at 45°C in a 2.5 L BioFlo llc vessel, from New Brunswick (Hatfield, UK). This temperature controlled reactor had a working volume of 1.5 L and a pH electrode. Cellulase (Celluclast 1.5β) and β-glucosidase (Novozym 188β) were used at concentrations of 7 FPU/g substrate and 54 IU/g substrate, respectively. The solid concentration was 3.5% (w/v) and agitation was set to 200 rpm. A citrate buffer was used to keep the pH at 4.8; streptomycin and cycloheximide (Sigma, St. Louis, MO) were used as preservatives at concentrations of 10 mg/L.

Saccharide Analysis

Samples were collected from the reactor, centrifuged, and the supernatant liquid filtered through a 0.45 μm nylon filter from Cole-Parmer (Niles, IL) before preimmediate analysis. An Aminex HPX-87P column (30 cm × 7.8 mm), and two sets of precolumns (CarboP and Carbohydrate Analysis ion-exclusion Micro-Guard), all from BioRad (Richmond, CA), were employed to monitor glucose concentration using a HPLC technique. Deionized (DI) water was used as the mobile phase. DI water was filtered and degassed before being delivered through a model 510 pump from Waters (Milford, MA) at a flow rate of 0.6 mL/min. The column temperature was kept at 85.0 ± 0.2°C with a heating system from Waters. Samples of 20 μL were injected automatically by an autosampler from Waters, model Wisp 710B. A differential refractometer, model 410 from

Table 2
Chemical Composition of Aspen TMP Fibers After Pretreatment

Pretreatment[a]	Klason lignin, %	Ligin removal, % of original	Selectivity, %	Glucose content, %	Xylose content, %
DA	24	9	2	55	10
NA	26	6	3	55	10
AP	28	2	2	45	<2
PMS	3	93	67	75	10
AC	2	96	55	90	5

[a]DA, dilute acid; NA, sodium hydroxide; AP, alkaline peroxide; PMS, peroxymonosulfate; AC, acetic acid.

Waters, was used as a detector, with a Waters Data Module 730, to record the signals. Calibration curves for glucose, xylose, and cellobiose were constructed periodically.

RESULTS AND DISCUSSION

Pretreatment

The substrate recoveries for all pretreatments are presented in Table 1. These values correspond to the percentage of the original sample recovered after pretreatment. The substrate recovery was determined by weighing the dry sample before and after a given pretreatment. Equation 1 was used to compute the percent substrate recovery, R.

$$R = (w_2 - w_1) / w_1 \times 100 \quad (1)$$

where w_1 is the weight of the sample before pretreatment and w_2 the weight after pretreatment.

Table 1 shows that the highest substrate recovery resulted after pretreatment DA. The lowest values were obtained with pretreatments PMS and AC. The results show that more than 50% of the corresponding original substrate was depleted after pretreatments PMS and AC. To verify the effectiveness on lignin removal of each method, pretreatment selectivities were introduced. The selectivity was obtained by dividing the weight of removed lignin by the weight of other material lost by decomposition during the pretreatment. The percent selectivity is given by Eq. 2.

$$S = (w_1 \cdot L_1 - w_2 \cdot L_2) / [w_1 (1 - L_1) - w_2 (1 - L_2)] \times 100 \quad (2)$$

In Eq. 2, L_1 and L_2 are the lignin content of the sample before and after pretreatment, respectively.

Table 2 contains data on Klason lignin content, percentage of lignin removal, selectivity, and glucose and xylose content of the substrate after

pretreatment. The Klason lignin content of PMS- and AC-pretreated samples were lower than that obtained with pretreatments DA, NA, and AP. These results clearly indicate that there was considerable lignin removal and a higher selectivity for pretreatments PMS and AC, compared to pretreatments DA, NA, and AP. For pretreatments PMS and AC, more than 90% of the lignin initially present in the substrate was removed, but pretreatments DA, NA, and AP did not remove lignin significantly. The samples subjected to pretreatments DA, NA, and AP had less than 10% of their original lignin removed. This is underscored by their low selectivity values. High selectivity corresponds to an extensive lignin removal during the pretreatment. High substrate recovery and high lignin selectivity values, when obtained simultaneously, indicate that lignin was efficiently removed, with a relatively small loss of other material.

The low lignin selectivities and the high substrate recovery values for pretreatments DA, NA, and AP indicate the preferential, limited removal of some wood components other than lignin. This explains why the lignin contents after pretreatments DA, NA, and AP are higher than the original value of 22% for the untreated samples. The reactions involved in the peeling mechanism (*10*), observed in alkaline media, explain the saccharide loss during pretreatments NA and AP. Further hemicellulose removal was favored by the conditions employed under pretreatment AP. This is shown in Table 2 by the low xylose content of the corresponding substrate. In pretreatment DA, the acid released part of the hemicellulose without drastically affecting the lignin portion of the substrate (*11*). The glucose content is higher in the AC-pretreated sample than in the PMS-pretreated one. Inversely, the xylose content is lower in the AC-pretreated sample than in the PMS-pretreated one.

The substrate recoveries for pretreatments PMS and AC were relatively low. In the former, this was caused by the peroxymonosulfate ion HSO_5^-. The peroxymonosulfate ion liberates hydroxyl radicals, HO* (*12*), which are strong oxidants for both lignin and polysaccharides. Peroxyacetic acid, CH_3COOOH, is likely to be formed under the conditions prevailing in pretreatment AC. Decomposition of CH_3COOOH produces the acetate radical CH_3COO^*. This radical leads to the same type of degradation promoted by the HO* radicals in pretreatment PMS.

Saccarification

Table 3 lists the enzymatic saccharification results of the pretreated aspen fibers after 72 h. The highest glucose concentration values were obtained with the PMS- and AC-pretreated samples, for which the lignin content was lower than those of the other pretreated substrates. For the substrates corresponding to pretreatments PMS and AC, there was more hydrolyzable material in the reactor. This is because the initial solids content was the same in all experiments and the selectivity values corresponding to pretreatments PMS and AC were the highest.

Table 3
Glucose Conversion on Three-Day Saccharification at 45°C of Pretreated Aspen TMP Fibers

Substrate pretreatment	Glucose concentration, g L^{-1}	Glucose conversion,		
		%[a]	%[b]	%[c]
DA	1.5	8	3.9	4.3
NA	1.9	10	4.6	5.4
AP	2.5	16	5.5	7.1
PMS	25.5	96	35.7	72.9
AC	30.2	95	33.9	86.3

[a]Percentage of the maximum attainable concentration.
[b]Percentage based on the corresponding substrate weight before treatment.
[c]Percentage based on the pretreated substrate weight.

In this study, a relatively low cellulase loading was used in the saccharification of the AC-pretreated substrate, corresponding to less than 10 FPU/g of potential glucose. The fact that a remarkably high glucose concentration was obtained with this substrate reveals that, in the saccharification experiments of the DA-, NA-, and AP-pretreated substrates, enzyme loading was not a limiting factor to hydrolysis. In the experiments reported here, no atttempt was made to optimize the hydrolysis rate by varying the enzyme loading.

Compared to the other methods, pretreating aspen TMP with hydrogen peroxide in acidic medium resulted in a dramatic increase in the susceptibility of the corresponding substrates to hydrolysis by cellulolytic enzymes. The results in Table 3 reveal that with pretreatments PMS and AC, approx 95% of the glucose content of the corresponding substrate was hydrolyzed. The AP-pretreated sample, for which the hemicellulose removal was the lowest among all substrates, did not produce a satisfactory glucose conversion.

The yield based on the glucose content of the corresponding substrate is, however, a biased measure of the extent of conversion. This is because the material loss of the raw substrate is not taken into account. A more realistic indication of the efficiency of the whole process is given by the glucose amounts released per unit weight of the original substrate; these are displayed in Table 3. These results indicate that, even though the glucose concentration obtained with the AC-pretreated sample was approx 18% higher than that corresponding to the pretreatment PMS, there is no significant difference between these two pretreatments on the glucose released per initial substrate weight. Pretreatment PMS is performed at a lower temperature and employs a higher solids-to-liquid ratio. For these reasons, pretreatment PMS is preferred. The time evolution of the glucose concentration in the saccharification of the PMS-pretreated substrate and for a selected lignin-rich sample, corresponding to pretreatment NA,

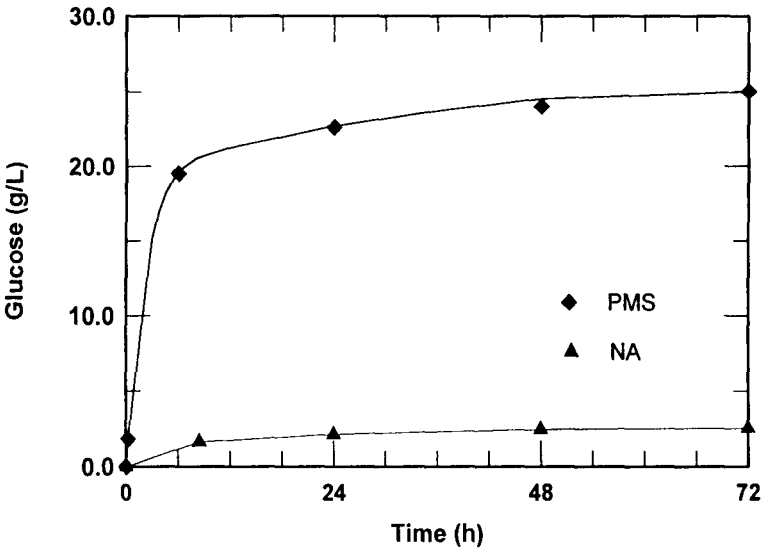

Fig. 1. Glucose concentration vs time during the enzymatic saccharification of the PMS- (◆) and NA-pretreated (▲) aspen TMP.

Table 4
Average Rates of Glucose Formation on Three-Day Saccharification at 45°C of Pretreated Aspen TMP

	Average rate, 10^{-2} g L^{-L} h^{-1}			
Substrate pretreatment	0–24 h	24–48 h	48–72 h	0–72 h
DA	6	~0	~0	2
NA	5	2	1	3
AP	9	1	~0	3
PMS	94	6	4	35
94	6	4	35	
AC	80	26	20	42

is represented in Fig. 1, which clearly shows that the overall glucose production is enhanced by pretreatment PMS.

The average rates of glucose formation are listed in Table 4. A general decrease in those rates is observed after a period of 24 h. For the lignin-rich samples, corresponding to pretreatments DA, NA, and AP, there was no significant increase in the glucose concentration after the first 24 h. The highest rates of glucose formation were obtained in the saccharification of the PMS- and AC-pretreated samples. The average rate corresponding to the PMS-pretreated sample is higher than that obtained with the AC-pretreated substrate in the first 24 h of saccharification. The 72 h average rate obtained with the AC-pretreated sample is higher than that for the PMS-pretreated substrate. The reasons leading to the superior

average rates observed in the saccharification of the AC-pretreated substrate are under investigation. It is possible that a more extensive depolymerization of the substrate took place in pretreatment AC, to favor further hydrolysis.

For the DA-, NA- and AP-pretreated substrates, Table 3 shows that the glucose concentrations after three days correspond to relatively low yields. Enzyme inhibition by glucose accumulation in the reactor could explain the low average rates of glucose formation in these cases. Nevertheless, higher glucose concentrations were attained with the samples pretreated with peroxide in acidic medium. This observation eliminates the hypothesis of enzyme inhibition caused by glucose accumulation. The drop in the enzyme activity due to cellobiose buildup in the reactor is more important than that associated with glucose accumulation (*13,14*). Cellobiose was not detected at any experimental time for all experiments and enzyme inhibition caused by accumulation of this disaccharide cannot explain the low saccharification yields obtained with the DA-, NA-, and AP-pretreated substrates. The failure to attain a higher extent of saccharification could be attributed, for examle, to irreversible or nonspecific adsorption of the enzymes on the substrate (*15*). Cellulase diffusion into the cellulose fibrils (*16*) is another factor which could influence the saccharification process.

Hemicellulose removal alone did not lead to acceptable glucose yields with the saccharification conditions employed in this study, as suggested by the results corresponding to pretreatment AP. The most noticeable outcome of pretreatments AC and PMS, which led to high glucose conversions, is the significant extent of lignin removal. It is assumed that an increase in the surface area available to the enzymes follows lignin removal (*17*). It is plausible that an increase in the swelling capacity of the substrate or a decrease of the average polymerization degree (*18*) facilitated the enzyme action. All these questions relevant to processes for biomass saccharification require further study.

CONCLUSION

In these saccharification experiments, the most effective glucose conversion of the original substrate was obtained by using the peroxymonosulfuric acid pretreatment. The peroxymonosulfuric acid pretreatment appears to be an effective method for improving the enzymatic saccharification of aspen wood. This improvement is related to lignin removal. It is believed that removal of the lignin shield leads to an increase in the area available for enzymatic attack. Optimization of the peroxymonosulfate pretreatment with respect to its duration, pH, and peroxide concentration should lead to improved selectivities. Literature regarding the mechanism of delignification is limited, and more information, especially related to the chemical reactions involved, would be extremely valuable in devising

more favorable conditions. Also, further studies are needed on hemicellulose and cellulose removal during pretreatments AC and PMS. Delignification with peroxymonosulfate is performed at relatively low temperatures and at atmospheric pressure. Its low energy requirement makes this type of pretreatment more attractive. Several components of peroxymonosulfate delignification still have to be analyzed, including cost and recyclability of the chemicals used.

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