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Dilute Acid Pretreatment of Black Spruce Using Continuous Steam Explosion System

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Abstract The pretreatment of lignocellulosic materials prior to the enzymatic hydrolysis is essential to the sugar yield and bioethanol production. Dilute acid hydrolysis of black spruce softwood chip was performed in a continuous high temperature reactor followed with steam explosion and mechanical refining. The acid-soaked wood chips were pretreated under different feeding rates (60 and 92 kg/h), cooking screw rotation speeds (7.2 and 14.4 rpm), and steam pressures (12 and 15 bar). The enzymatic hydrolysis was carried out on the acid-insoluble fraction of pretreated material. At lower feeding rate, the pretreatment at low steam pressure and short retention time favored the recovery of hemicellulose. The pretreatment at high steam pressure and longer retention time recovered less hemicellulose but improved the enzymatic accessibility. As a result, the overall sugar yields became similar no matter what levels of the retention time or steam pressure. Comparing with lower feeding rate, higher feeding rate resulted in consistently higher glucose yield in both liquid fraction after pretreatment and that released after enzymatic hydrolysis.

Keywords Dilute acid pretreatment · Enzymatic hydrolysis · Softwood · Continuous steam explosion · Refining

Introduction

Bioethanol could be used as a partial gasoline replacement, which can be produced from a variety of feedstocks, including sugars, starches, and lignocellulosic materials and algae. The first-generation technologies are based on the conversion of sugars and starches (e.g., corn and wheat grain), which is limited by the availability and sustainability of "food" feedstocks. The second generation of technologies will produce biofuels from non-edible and waste biomass streams. Canada has an abundant supply of softwood residues generated from activities in its forest sector. Canadian government expects to include at least 5% of

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bioethanol in the gasoline in 2010 [1]. In order to achieve this target, further work is needed to improve the conversion technologies and to reduce the cost of ethanol production.

Process of converting lignocellulosic biomass to ethanol involves pretreatment, enzymatic hydrolysis, and fermentation. However, this biological conversion is severely limited due to the intense cross-linking of cellulose with hemicellulose and lignin. Pretreatment is recognized as a key step in enzyme-based conversion processes, and the efficient one must meet the following requirements: (1) good accessibility of the cellulose component to hydrolytic enzymes; (2) little or no degradation of solubilised hemicellulose and cellulose; (3) insignificant formation of byproducts inhibitory to the subsequent hydrolysis and fermentation process; and (4) cost effectiveness.

The structural and chemical composition has been modified by a variety of pretreatment methods utilizing mechanical, thermomechanical, and thermochemical processing. Among all the pretreatment methods, steam pretreatment with either H_2SO_4 or SO_2 impregnation was widely studied because it is effective and inexpensive [2–4]. Dilute acid pretreatment is an effective way of hydrolyzing hemicellulose, softening the lignin structure, reducing cellulose crystallinity, and increasing surface area and pore volume of the substrate, hence increasing the accessibility of the cellulose substrate to enzymatic attack. The important variables in steam explosion pretreatment are time (minutes) and temperature (°C). The effect of time and temperature on the pretreatment can be evaluated with the severity parameter defined by Overend and Chornet [5]:

$$\mathrm{Log}(R_0) = \mathrm{Log}\left[t \times \exp\left(\frac{T - T_{\mathrm{ref}}}{14.75}\right)\right] \quad (1)$$

where T_{ref} is 100 °C.

When the pretreatment is performed under acid conditions, the pH can be taken into consideration by the combined severity defined as:

$$\text{Combined severity(CS)} = \mathrm{Log}(R_0) - \mathrm{pH} \quad (2)$$

Most of the steam explosion pretreatments were carried out with small-size batch reactor. As a matter of fact, the continuous processing is of major interest for the industrial application. Zimbardi et al. [6] compared the steam explosion efficiency of wheat straw in batch and continuous reactors. The xylose recovery in aqueous phase from continuous refiner was always lower than that from batch reactor. However, the higher cellulose content in the solid phase was obtained from continuous reactor as a consequence of lower decomposition. The enzymatic hydrolysis yield of samples from continuous process was higher than those from batch process at low pretreatment severity. The opposite result was found at high treatment severity. It was explained by the hypothesis that the disruption and hydrolysis of cellulose fibers is more effective in the continuous reactor, but mechanical compression causes reduction of fiber accessibility to the enzyme at high pretreatment severity. Moreover, the lignin tends to fill up the pores generated during process.

Cullis et al. [7] investigated the post-steam explosion refining on the bioconversion efficiency of Douglas fir by using a mechanical pulping refiner. The refining of substrate decreased the fiber size and increased the pore volume due to the removal of hemicellulose and lignin. The enzymatic conversion was accordingly higher than the samples which were not refined.

In this work, the Andritz pressurized refiner is used as the pretreatment equipment, which is a pilot scale continuous pressurized reactor combining the steam explosion and mechanical refining. The study examined the pretreatment of acid-impregnated black spruce chips at high solid concentration. The effects of pretreatment were assessed by the sugar formation in the aqueous fraction after pretreatment and enzymatic hydrolysis yield of the solid fraction. The goal of the present work is to investigate the effectiveness and optimize the pretreatment parameters in this pilot scale equipment.

Methods

Raw Material

Black spruce logs were chipped and screened, and the fraction retained on the 20 mm screen were milled again. The obtained chips were then screened to remove the fines smaller than 1 mm. The wood chips with a size of 1–20 mm were used as the raw material for this study. The composition of the raw material was 39.4% of glucan, 5.1% of xylan, 12.2% of mannan, 2.0% of galactan, 1.0% of arabinan, 28.2% of acid-insoluble lignin, 0.8% of acid-soluble lignin, and 11.3% of other compounds based on oven-dried material.

Pretreatment System

The pretreatment was conducted in a continuous pressured reactor with steam explosion device modified from an Andritz 22-in. refiner system at the laboratory of FPInnovations (Quebec, Canada). The throughput can vary from 50–200 kg of dry material per hour, depending on the bulk density of the raw material and processing conditions. The steam pressure in the system can be up to 16 bar. The schematic diagram of the pretreatment system is shown in Fig. 1.

The acid-soaked wood chips were firstly conveyed to the pre-steaming hopper via a belt conveyor, where the temperature is 80 °C. The chips were then pressed and loaded into the reactor by the plug screw. The feeding rate is controlled by the rotation speed of the feeding screw. The high pressure steam pretreatment was conducted in the continuous reactor (the cooking screw) where the reaction time was controlled by the rotation speed of the cooking screw. The hydrolyzed materials passed through the refiner and were exploded through the

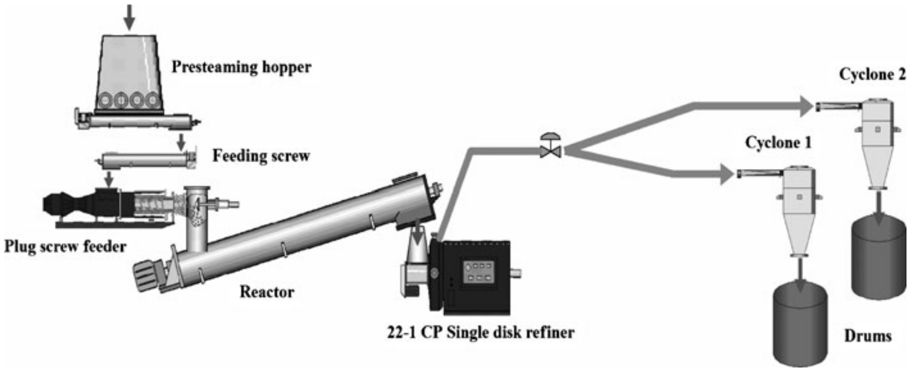


Fig. 1 Schematic diagram of the biomass pretreatment and continuous steam explosion device at the pilot plant used in the experiment

blow line into the cyclone with large steam pressure differential. The steam exploded materials were collected at the bottom of the cyclone.

Soaking of Wood Chips with Diluted Acid

All the black spruce chips were immersed in 0.75 wt.% sulfuric acid solution overnight and drained. The final acid concentration in the chips was about 0.6 wt.% (based on the water content in the chips). The acid concentration was measured by titration with standard sodium hydroxide solution after chips extracted with water overnight in a 50 °C shaking incubator [8]. The dry matter content of the soaked and drained chips was 42%.

Pretreatment Conditions

The pretreatment of acid soaked black spruce chips was carried out by varying the steam pressure (12 and 15 bar) in the reactor (the cooking screw), the rotation speed of cooking screw (14.4 and 7.2 rpm), and feeding rate (60 and 92 kg (DM)/h; Table 1). The high rotation speed of cooking screw decreases the mean residence time of the materials inside the cooking screw. The high feeding rate at the same rotation speed of cooking screw decreases the residence time variance. The dry matter content of the raw material was 86%, and the acid soaked materials were approximately 42%.

The reactor was firstly preheated to the target temperature (12 bar) before feeding the acid soaked chips (1 rpm of feeding screw). The rotation speed of cooking screw was set at 14.38 rpm. When the pretreatment system reached steady-state conditions after 6 min of running, the first batch samples (AS12) were then collected six times in the interval of 30 s. Then, the rotation speed of the cooking screw was changed to 7.2 rpm and waiting time to 8 min before collecting the second batch samples (AL12). Same process procedures were used for the rest of the experiment. The samples for each batch were collected after 8 min of transition between two different pretreatment conditions. The gap distance of the refiner was adjusted to 0.1 mm, and the rotation speed was 2,500 rpm through the entire experiment.

Enzymatic Hydrolysis

Enzymatic hydrolysis of acid-insoluble fraction from pretreatment step was carried out to investigate the effect of pretreatment and to determine the potential sugar yield. The solid

Table 1 Conditions of pretreatment used in the experiment

Feeding screw rotation speed	Feeding rate	Cooking screw rotation speed	Pressure	Sample code
1 rpm	60 kg/h (DM) 144 kg/h (wet)	14.4 rpm	12 bar	AS12
		14.4 rpm	15 bar	AS15
		7.2 rpm	12 bar	AL12
		7.2 rpm	15 bar	AL15
1.3 rpm	92 kg/h (DM) 220 kg/h (wet)	14.38 rpm	15 bar	BS15
		7.2 rpm	15 bar	BL15

A low feeding rate (60 kg/h), *B* high feeding rate (92 kg/h), *S* short residence time, *L* long residence time concentration of washed pretreated solids was 3 wt.%. The enzyme was a commercial cellulase mixture, Celluclast 1.5 L (60 FPU/g, and 17 β-glucosidase IU/g) supplemented with Novozyme 188. Sodium citrate buffer was used to maintain the pH at 4.8. A 100-μl sodium azide (20 mg/ml) was added to inhibit the microbial infections. The enzyme loading was 20 FPU/g dry mass with a Celluclast 1.5 L to Novozym 188 ratio of 2:1. All hydrolysis experiments were carried out at 50 °C for 96 h in a 250-ml flask in a shaking incubator. The reducing sugars were analyzed after 96 h of treatment.

Analysis

Dry matter content was determined by drying samples in an oven at 105 °C overnight. The two-step acid hydrolysis (LAP-002) was carried out on the raw material and acid-insoluble solid to measure their chemical composition by HPLC method. The acid-insoluble lignin content was determined by the filtered solid weight subtracted the ash amount (LAP-002). The acid-soluble lignin was determined by using the absorbance at 205 nm (LAP-004).

The pretreated solid was washed six times with warm water (50 °C) and filtered through a Whatman filter paper. The resultant filtrates were characterized and quantified for acid-soluble sugar content. The liquid fractions were composed of monomers and oligomers; thus, the samples were analyzed directly and after 20 min hydrolysis in the autoclave (121 °C 3.0% H_2SO_4). The oligomer concentration was calculated by the difference. Both the solid after pretreatment and after washing and filtering were dried. The compositional removal after washing was obtained.

The pretreated solid was thoroughly washed to remove the soluble substances, then air-dried at 45 °C, and acid hydrolyzed (LAP-002) to determine the sugar composition. The thoroughly washed solid was also used for the enzymatic hydrolysis.

The raw material, acid-soluble fraction, acid-insoluble fraction after acid hydrolysis from each pretreatment, and all samples from the enzymatic hydrolysis of acid-insoluble fraction were analyzed on a Dionex DX-600 Ion Chromatograph system (Dionex, Sunnyvale, CA). This system consists of an anion exchange column (Dionex CarboPac^{TM} PA1), an AS50 autosampler, and a GP50 gradient pump. Deionized water was used as an eluent at a flow rate of 1 ml/min; 1 M NaOH was used to equilibrate the column after each elution of sugars. To optimize baseline stability and detector sensitivity, 0.2 M NaOH was added post-column. After being filtered through 0.45-μm nylon syringe filters (Chromatographic Specialties Inc.), a 20-μl sample was injected on the column. The sugar monomers were monitored by an ED50 electrochemical detector with parameters set for pulsed amperometric detection. Sugar standards were prepared and analyzed using the same procedure in order to calibrate the instrument before sample analysis.

Results and Discussion

The dry matter content of the pretreated material was between 40% and 43%, which was approximately similar to the materials fed into the refiner. The steam condensed in the cooking screw reactor compensated the water loss during feeding the acid-soaked chips through the press screw feeder where the chips were pressed and part of the water was squeezed out of the chips.

The volatile gases after the cyclone were not collected, so the mass recovery after pretreatment as a percentage of the original dry weight is not applicable in this work. The sugar yield and recovery after pretreatment and enzymatic hydrolysis was based on the pretreated materials collected from cyclone.

Composition of the Liquid Fraction after Pretreatment

The glucose, xylose, and mannose were recovered both as monomer and oligomer. The pretreated materials were washed and filtered. The sugar concentrations in the filtrate are shown in Figs. 2, 3, and 4. With increasing of pretreatment temperature and retention time, more oligomers were decomposed into monomers for both hemicellulose and cellulose, which was especially evident for xylose.

At low feeding rate, the variation of pretreatment conditions greatly influenced on the recovery of xylan, less on the recovery of mannan (Fig. 5). The highest glucose concentration was achieved at 15 bar and short residence time (higher cooking screw rotation speed and steam pressure), which gave the recovery of 8.8% in the liquid fraction. The mannose recovery in the liquid fraction was almost the same at the medium severity (49.7% for AL12 and 48.4% for AS15). The downward trend was observed for the xylose concentration in the liquid fraction. It decreased the xylose recovery from 102.6% (AS12) with the increasing of cooking temperature and residence time to 35.5% (AL15). The harsher pretreatment conditions accelerated the degradation of xylose to furfural and other degradation products [9–11].

The high feeding rate was favorable to recover both the hemicellulose and cellulose. Comparing AS15 and AL15 samples, the sugar yield of latter was lower than the former, due to the degradation reactions with longer retention time in the cooking reactor. However, at higher feeding rate, the sugar yield of BL15 was higher than BS15. The glucose and xylose yields were increased by 6% and 16.7%, respectively, by increasing the feeding rate with long retention time. The pretreatment at high feeding rate improved the glucose yield by lessening the degradation reactions. The increase of feeding rate decreased the mean residence time and the residence time distribution at the same cooking screw rotation speed. The studies on the residence time distribution of biomass in the thermomechanical refiner were hardly reported. In the work of Sievers et al. [12], increasing rotation speed of cooking screw decreased the mean residence time, while it increased the residence time distribution. Lower xylose yield along with excess xylose degradation products were observed at pretreatment conditions with broad residence time distribution. As a matter of fact, the residence time distribution of polymer extruding in a twin or single extruder was widely investigated [13–16]. At the given feeding rate, the mean residence time always decreases

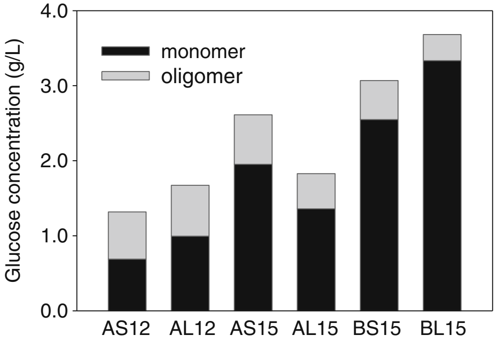
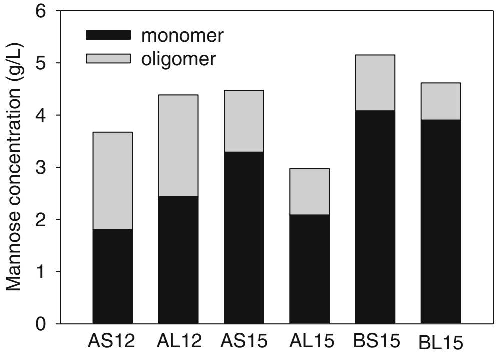


Fig. 2 Glucose concentrations in the acid-soluble fraction after pretreatment at different conditions

Fig. 3 Mannose concentrations in the acid-soluble fraction after pretreatment at different conditions



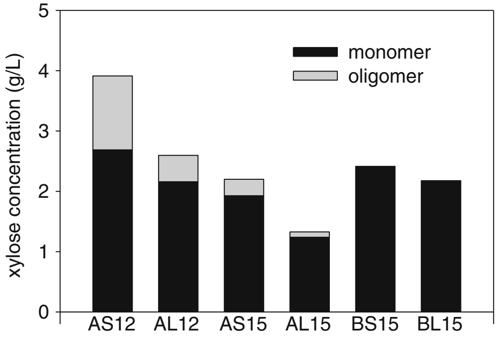
with the increasing of screw rotation rate. However, the residence time distribution increases or decreases with increasing of screw rotation speed, depending on the screw configuration, filling degree, and other factors. In our study, the favorite pretreatment condition of softwood is high steam pressure, low residence time, and high feeding rate.

The pretreated and washed solids were oven-dried overnight, and the composition removal at each condition was obtained. The sum of the hydrolyzed sugars and the composition removal are listed in Table 2. It was found that the sum of hydrolyzed sugars was lower than the composition removal. The large gap was observed for the materials pretreated with higher severity (15 bar). The difference might be the solubilization of lignin and the formation of other byproducts, furfural, and HMF [17]. The volatile organic acid contents of formic acid and acetic acid are also presented in Table 2. More volatile organic acid products were detected at more severe pretreatment conditions with the exception of AL15, which followed the same trend as sugar production. It is explained that the hemicellulose were hydrolyzed and degraded as volatile products but were not collected from cyclone.

Composition of the Solid Fraction after Pretreatment

The acid-insoluble fraction of pretreated material was washed thoroughly and air-dried at 45 °C overnight. Then, the solid was analyzed by two-step acid hydrolysis and followed by HPLC measurement. The xylose concentration was too low to be detected. The glucose and lignin content are shown in Table 3. The glucan content of all pretreated material was much

Fig. 4 Xylose concentrations in the acid-soluble fraction after pretreatment at different conditions



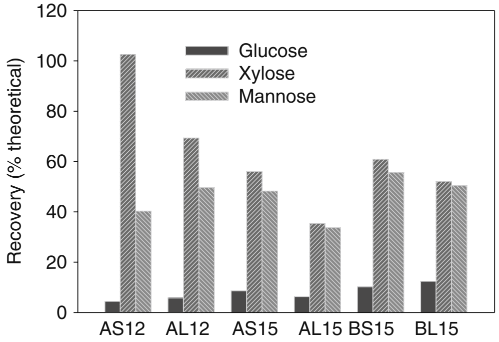


Fig. 5 Recovery yields after pretreatment at different conditions

higher than the raw material (39.4%), which is reasonable because little of glucan was solubilized, whereas much more fraction of hemicellulose was solubilized. This increased the relative amount of glucan.

At low feeding rate, the glucan content in the solid fraction decreased with the increasing of pretreatment severity, which was consistent with the increasing of solubilization of glucose in the soluble fraction. At the same steam pressure, the glucan retained in the solid fraction was higher in the samples pretreated at higher feeding rate. Glucose content was higher in both the solid and liquid fractions when it was processed at high feeding rate. It can be concluded that more glucan was hydrolyzed and degraded at lower feeding rate. So, the net yield of glucose in the soluble fraction was lower at lower feeding rate. The higher feeding rate decreased the mean residence time and variance of residence time distribution, which were favorable to retain the glucose in both insoluble and soluble fraction.

The content of acid-insoluble lignin (38–44%) was much higher than in the raw material (28.2%). This is due to the solubilization of sugar component. The AIL contents were higher for the samples with more sugar component hydrolyzed when they were pretreated at higher pressure and longer retention time.

Enzymatic Hydrolysis

Enzymatic hydrolysis on the pretreated and washed insoluble fraction was performed to assess the pretreatment effect on the overall sugar yield. The conversion was based on the dry weight of collected sludge after pretreatment but not on the raw material. The glucose

Table 2 Compositional analysis of the acid-soluble fraction (g/100 g dry pretreated material)

Sample code	Composition removal	Sum of hydrolyzed sugars	Formic acid	Acetic acid
AS12	15.0	14.58	0.089	0.279
AL12	15.4	14.59	0.158	0.380
AS15	16.6	15.02	0.251	0.652
AL15	13.6	10.41	0.175	0.379
BS15	18.7	16.98	0.169	0.461
BL15	20.3	16.85	0.201	0.557

A low feeding rate (60 kg/h), *B* high feeding rate (92 kg/h), *S* short residence time, *L* long residence time

Table 3 Chemical composition of acid-insoluble fraction after pretreatment (% of dry matter)

Sample code	Glucan	AIL	ASL	Other
AS12	56.6	37.9	0.70	4.8
AL12	54.4	38.5	0.61	6.5
AS15	51.9	41.0	0.57	6.5
AL15	48.3	42.5	0.64	8.6
BS15	54.9	41.5	0.63	3.0
BL15	50.9	43.9	0.77	4.4

A low feeding rate (60 kg/h), *B* high feeding rate (92 kg/h), *S* short residence time, *L* long residence time

and hemicellulose yields after pretreatment and enzymatic hydrolysis for different pretreated materials are presented in Table 4.

Glucose was mostly released during enzymatic hydrolysis. For materials pretreated at low steam pressure or short retention time (AS12, AL12, and AS15), the glucose yield was almost the same. The better yield was obtained for the material that was pretreated at high steam pressure and long retention time (AL15). Increasing feeding rate of raw material increased the glucose yield from enzymatic hydrolysis by 4.1 and 5.6 g/100 g dry solid comparing the pretreated materials at the same steam pressure and cooking screw rotation speed. The higher throughput may intensify the refining force when the materials pass through the refiner. The resulted physical structure of pretreated material was more favorable to be digested by cellulase. On the other hand, the higher glucose yield was on account of the higher glucose concentration in acid-insoluble fraction (Table 3).

The hydrolyzability of insoluble solid based on the glucan concentration in the solid fraction was also listed in Table 4. The higher conversion was obtained at higher steam pressure and longer retention time. Pretreatment of softwood is less efficient due to the more rigid structure and containing more lignin comparing with hardwood and biomass. In the review [18], the highest overall yields are 66% and 67% of the theoretical value using impregnation with SO_2 and H_2SO_4, respectively, for spruce. The hydrolyzability of the glucan in the acid-insoluble fraction was always below 50% [19–21]. By using mechanical

Table 4 Yield of glucose and hemicellulose from enzymatic hydrolysis and pretreatment (g/100 g dry pretreated material)

Sample code	Glucose			Xylose+mannose	Overall sugar
	Enzymatic hydrolysis (hydrolyzability[a])	Pretreatment	Overall glucose	Pretreatment	
AS12	18.1 (28.8%)	1.9	20.0	11.2	31.2
AL12	18.8 (31.1%)	2.5	21.4	10.5	31.9
AS15	17.1 (29.7)	3.8	20.9	9.6	30.5
AL15	22.8 (42.5%)	2.6	25.5	6.2	31.7
BS15	21.2 (34.8%)	4.4	25.6	10.7	36.3
BL15	26.4 (46.7)	5.4	31.8	9.7	41.5

A low feeding rate (60 kg/h), *B* high feeding rate (92 kg/h), *S* short residence time, *L* long residence time

[a] Hydrolyzability is based on the glucan concentration in the acid-insoluble fraction of pretreated samples

refining, the similar conversion was achieved at the pretreatment conditions of lower temperature and acid concentration.

The benefit of pretreatment with higher feeding rate was evidently observed from the overall sugar yield. BL15 produced 10 g more overall sugar than AL15. All the pretreated materials at low feeding rate (12 and 15 bar) produced similar overall sugar. The low severity pretreatment (12 bar) produced less glucose but more hemicellulose, in reverse for the harsh pretreatment (15 bar).

Conclusions

The thermomechanical pretreatment of sulfuric-acid-soaked softwood was carried out in a continuous reactor with steam explosion device modified from the pilot scale MDF refiner. The pretreatment conditions were optimized by varying the steam pressure, retention time, and feeding rate. Under the investigated conditions, the higher steam pressure and longer retention time resulted in high glucose yield after enzymatic hydrolysis. The higher feeding rate was another important consideration for improving the sugar yield. Materials pretreated at higher feeding rate increased the overall sugar yield, resulting in more glucose yield after enzymatic hydrolysis and hemicellulose yield in liquid fraction of pretreated sludge.

Variations in rotation speed of cooking screw and feeding rate change the mean residence time and its distribution in the cooking screw and then the pretreatment efficiency. In the future studies, the influences of pretreatment parameters on the residence time distribution should be further investigated.

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