

## CONTINUOUS STEAM EXPLOSION OF WHEAT STRAW BY HIGH PRESSURE MECHANICAL REFINING SYSTEM TO PRODUCE SUGARS FOR BIOCONVERSION

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This study demonstrated the use of a pressurized mechanical refining system for the continuous steam explosion pretreatment of wheat straw. Wheat straw was first impregnated with either dilute acid (0.5% sulfuric acid) or water and then steam exploded in an Andritz pressurized refiner. The effect of a range of pretreatment conditions, including refining retention time and steam pressure/temperature on the resulting substrate composition and hydrolysability as well as overall sugars yield was investigated. For autohydrolysis, the optimum conditions, 198 °C/6 min gave an enzymatic hydrolysis yield of 93.3% and an overall glucose yield of 85.8%, while 198 °C/4 min gave an enzymatic hydrolysis yield of 88.7% and overall glucose yield of 88.4%. Longer retention time increased the enzymatic hydrolysability but reduced the overall glucose yield owing to the degradation reaction during pretreatment. For acid pretreatment, the most favourable condition for enzymatic hydrolysis and overall glucose yield coincided at 178 °C /6 min.

*Keywords:* Wheat straw; Steam explosion; Autohydrolysis; Acid pretreatment; Enzymatic hydrolysis

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### INTRODUCTION

Producing bioethanol from lignocellulosic raw materials has gained increasing global attention over the last years. During the past 50 years, there has been a dramatic increase in the emission of greenhouse gases. The content of CO<sub>2</sub> in the air has risen from 320 ppm to 390 ppm during this period, and it is still increasing from year to year according to the data released from the Mauna Loa Observatory. Compared to the fossil fuels, biofuel from biomass contributes little or no net CO<sub>2</sub> to the earth's atmosphere. A typical biochemical conversion process from lignocellulosic biomass to bioethanol involves at least four main steps: 1) pretreatment of biomass to disintegrate biomass feedstock and fractionate its components, 2) enzymatic hydrolysis to break down polysaccharides, chiefly cellulose, 3) fermentation of sugar monomers to bioethanol, and 4) ethanol recovery and purification. The intricate nature of the lignocellulosic biomass cell wall structure and chemistry significantly hinder the cellulose hydrolysis and bioethanol conversion. Hence, it is always necessary to carry out pretreatment to break down the hemicellulose and lignin surrounding the cellulose prior to the enzymatic hydrolysis. From the point of economic cost, pretreatment is one of the most costly steps

in the bioethanol production (Sanderson 2006). In order to achieve cost-competitive bioethanol production, it will be necessary to improve the critical pretreatment efficiency.

Pretreatment steps play a critical role in determining the efficiency and economics of the biomass to ethanol conversion process. Wheat straw is the second most abundant non-woody lignocellulose biomass feedstock in the world after rice straw (Kim and Dale 2004). A number of pretreatment methods have been developed and evaluated for bio-conversion of wheat straw (Talebnia et al. 2010). Among these, steam explosion has been demonstrated as an efficient and cost-effective pretreatment method for wheat straw. Steam explosion can be carried out at short resident time and relative lower reaction temperature with or without the addition of acid catalysts such as H<sub>2</sub>SO<sub>4</sub> or SO<sub>2</sub> (Linde et al. 2008; Öhgren et al. 2007; Chandra et al. 2007; Schell et al. 1991). During steam explosion, a significant amount of hemicellulose sugars are released and fiber cell wall structures are disrupted, which significantly increases the proportion of accessible surface area of the substrate.

Optimizing steam explosion for efficient hydrolysis of hemicellulose, and improving the following cellulose hydrolysability by enzymes have been the subject of intensive investigations. However, most previous investigations on steam explosion pretreatments of wheat straw were carried out in batch reactors (Ballesteros et al. 2006; Linde et al. 2008). One challenge facing the industrial application of steam explosion pretreatment is the development and deployment of a continuous steam explosion device. In a recent study, the use of an Andritz Pressurized Refining System (Fig. 1) for continuous explosion of spruce was demonstrated (Fang et al. 2010). This system was initially designed to refine the raw biomass (both woody and non-woody) materials by applying heat, pressure, and mechanical friction force to produce fibers. The raw materials (e.g. chips, sawdust, or agricultural residues), are first pre-steamed in a pre-steaming bin (Fig. 1), then discharged and fed into a plug feeder located below the bin by the vibrating discharger. A subsequent plug screw feeder is used to squeeze out moisture and condensate contained in the materials after pre-steaming prior to feeding into the digester (or refiner). The retention time of materials in the refiner is controlled by adjusting rotation speed of the cooking screw inside the refiner. The refining process takes place in the refiner between the rotating disc and the stationary disc, on which refining plates are mounted. Specific refining energy can be adjusted by changing the gap between two disc plates and rotation speed. After refining, materials are transferred through the blow line into the cyclone, where the steam explosion effect is created by the large steam pressure differential between the two segments. The objective of the present study is to investigate the effectiveness and optimise the pretreatment parameters of using a pilot scale Andritz pressurized refiner system for pretreatment of wheat straw.

## EXPERIMENTAL

### Raw Material

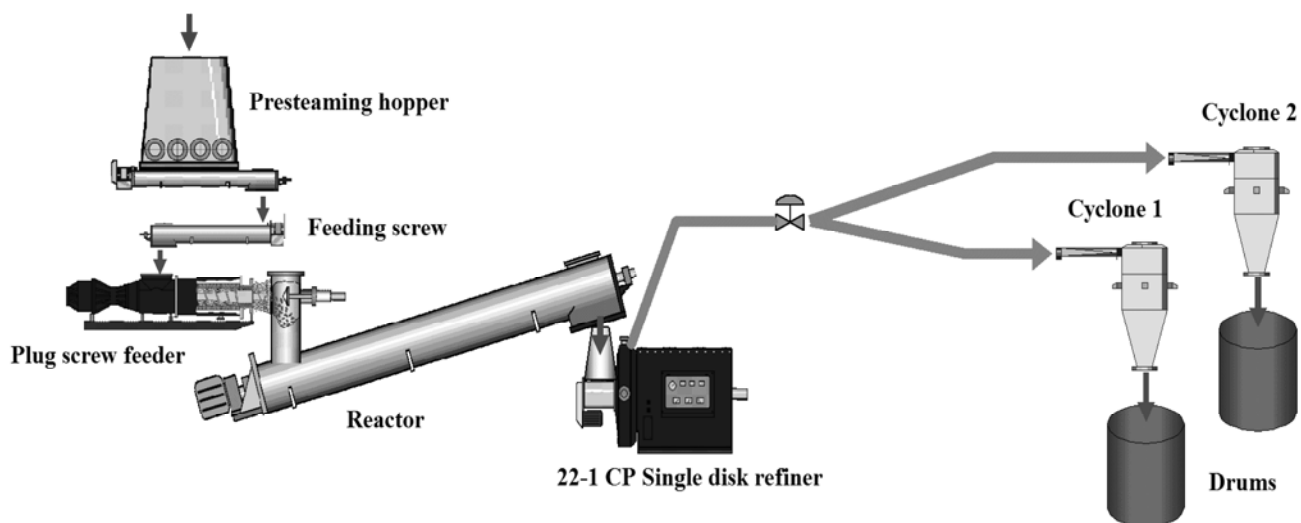
Wheat straw was milled into small pieces and screened. The pieces obtained, of size 2 to 20 mm, were stored at ambient temperature. The chemical composition of the untreated wheat straw is shown in Table 1.

**Table 1.** Chemical Composition of Untreated Wheat Straw

	g/100g DM
Glucose	38.8(1.9)
Xylose	13.4 (1.1)
Arabinose	4.7(0.5)
Acid insoluble lignin	26.6 (0.3)
Soluble lignin	1.8(0.2)
Ash	9.7 (0.2)
Extractives	3.4 (0.2)
Others	11.3

### Pretreatment

The pretreatment was conducted in an Andritz 22-inch pressurized refining system (Fig. 1) at FPInnovations' pilot plant located in Quebec City (Canada). Wheat straw was immersed in 0.5wt% sulfuric acid solution or pure water overnight and drained. The solid to liquid ratio was 1:15. The steam temperature in the pre-steaming hopper was maintained at 80°C. Three different steam pressures (9.5, 12, and 15 bar, corresponding to 178, 188, and 198°C) and three different retention times (2, 4, and 6 minutes) were selected for the pretreatment. Autohydrolysis (without acid presoaking) was carried out only at high steam pressure of 15 bar. The rotation speed of the refining disk was set at 2500 rpm, and the plate gap was adjusted to 0.1 mm. All the pretreatment parameters and corresponding pretreated sample codes are listed in Table 2. The steam-exploded samples were stored frozen (-20°C) prior to subsequent analysis.



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

**Table 2.** Pretreatment Conditions

Pressure(bar)	Temperature (°C)	Time (min)	Acid concentration (wt%)
9.5	178	2	0.5
9.5	178	4	0.5
9.5	178	6	0.5
12	188	2	0.5
12	188	4	0.5
15	198	2	0.5
15	198	4	0.5
15	198	2	0
15	198	4	0
15	198	6	0

### Enzymatic Hydrolysis

The samples collected from the cyclone were washed with 20 times their weight of distilled water and filtered. Solid residues were collected for subsequent enzymatic hydrolysis. The enzyme hydrolysis was carried out at 5% (w/v) substrate consistency in sodium citrate buffer (pH 4.8). A commercial cellulase mixture, Celluclast 1.5L supplemented with  $\beta$ -glucosidase, Novozyme 188 (weight ratio of 2:1) was used for hydrolysis of the steam exploded substrates (enzymatic activity of 60 FPU/mL). 100  $\mu$ L sodium azide (20 mg/mL) was added at the beginning of the hydrolysis to prevent microbial contamination. The hydrolysis was carried out in 150 mL flasks placed in a shaking incubator at 50 °C and 150 rpm. The enzyme loading was 30 FPU per gram of dry substrate.

### Analysis

All chemical analysis procedures were based on the Laboratory Analytical Procedures (LAP) established by NREL. Dry matter content was measured by drying samples in an oven at 105°C overnight (LAP-001). The ash content of raw material was measured by incineration at 575°C for 3 hours (LAP-005). The ethanol extractive extent was measured following standard LAP-010. The two-step acid hydrolysis was carried out on the raw material and acid insoluble solid to measure their chemical composition by HPLC method (LAP-002). The Klason lignin content was determined as the amount of insoluble residual after two-step acid hydrolysis. The acid-soluble lignin was determined by using the UV absorbance at 320 nm (LAP-002).

The pretreated solid was washed with 20 times its weight of 50°C water and filtered through a Whatman filter paper. The filtrates were collected for acid-soluble sugar content determination. The liquid fractions were composed of monomer and oligomers, so the filtrate was hydrolyzed by 3.0% H<sub>2</sub>SO<sub>4</sub> at 121°C for 20 min to convert all oligosaccharides to monosaccharides. The amounts of sugars in liquid fraction after

pretreatment and enzymatic hydrolysis, and in the solid fraction after pretreatment and two-step hydrolysis, were analysed by HPLC (LC1200, Agilent). The sugar components were separated by a Rezex RPM-Monosaccharide Pb++ (8%) column and 0.2 µm filtered distilled water as mobile phase and monitored by a refractive index detector. The flow rate was 0.6 mL/min.

## RESULTS AND DISCUSSION

The dry matter content of wheat straw after acid pre-soaking was 26%. It increased to 31% after the screw feeding. The steam condensation during the cooking reactor decreased the dry matter of the pretreated samples to 20 to 25%. In order to evaluate the efficiency of pretreatment of wheat straw on the conversion to ethanol, the slurry was separated into water-insoluble solid (WIS) and liquid prehydrolysate (LP). The pretreatment without and with acid addition was designated as autohydrolysis and acid pretreatment, respectively.

### Solid Recovery and WIS Composition

During refining steam explosion pretreatment, hemicelluloses were hydrolyzed and released into the filtrate. Degradation of released sugars to low molecular weight compounds, such as furfural and 5-hydroxy-2-methyl furfural (HMF), also took place during the pretreatment. Other reactions also occur, including acetic acid generation from the hydrolysis of the acetyl groups on hemicellulose, extractives removal, partial dissolution of lignin (acid soluble lignin) and ashes, and solubilization of proteins. These reactions will produce volatile substrates, leading to the loss of biomass materials. Table 3 summarizes the glucose and lignin contents of the WIS obtained from different pretreatment conditions. The solubilisation of hemicellulose and extractives during pretreatment resulted in a higher cellulose content in the WIS fraction ranging between 52.3% and 57.9%, depending on the treatment conditions. Increasing steam explosion severity led to lower xylan content in WIS. Similarly to glucan, the lignin content increased along with the increase in treatment severity. Besides cellulose and lignin, a variable amount of other components (referred to as others) were also present in the WIS. Residual xylan and extractives were the main constituents of the “others” fraction. The amount of “others” decreased from approximately 15% at the mildest treatment condition (198°C/2min/no acid) to zero at the most severe treatment condition (198°C, 4 min/acid). This result demonstrated that refining steam explosion can remove all the hemicellulose, extractive and proteins from wheat straw.

The formation of volatile gas was a main factor accounting for the biomass yield loss during steam explosion. As shown by Zimbardi et al. (1999), pretreating wheat straw at 225°C for 6 min led to a mass loss of 59.5%, among which organic matter loss accounted for 40.8%. Even at the lowest pretreatment severity (193°C and 2 min), a 15% of organic matter was still observed. The organic matter loss was evaluated from the ash content in the insoluble solid after pretreatment with the assumption that little loss of ash would occur during pretreatment. However, it was reported that thermomechanical refining was effective to remove the silicon in the wheat straw (Han et al. 2010). More

than half of the silicon and ash were removed by steam explosion at 190°C/3 min and 200°C/2 min (Han et al. 2010). Thomsen et al. (2009) also observed lowering of ash content in the pretreated samples, and the increasing of lignin content at the same time.

Precisely measuring the mass balance before and after steam pretreatment presented a challenge, due to the formation and loss of volatile products. In this work, lignin content was chosen as a calibration factor. The removal of lignin from wheat straw during steam explosion has been determined by a number of groups. Sun et al. (2004) applied higher steam pressure from 198 to 210 °C for steam explosion of wheat straw and found the lignin loss to be consistent in range between 11% and 12%. Zhang et al. (2008) showed that the lignin degradation plateaued between 13 and 14% at a steam pressure above 8 bar. Carvalho et al. (2009) dissolved 15% of the original lignin (maximum solubility) with autohydrolysis severity in the range 3.4 to 4.0. Pedersen et al. (2010) removed 12% of lignin when wheat straw was pretreated at 140°C for 10 min at a pH value of 1.0. From the previous studies, the lignin removal was in the range of 11 to 15% by varying the pretreatment severities. Therefore, in this study, a 13% reduction in lignin was assigned to all samples for estimating the solid recovery after steam explosion. This recovery value is presented in the last column of Table 3. The solid recovery based on lignin content in solid residue will be between 53.4% and 68.3 %, and the glucose recovery in WIS will be ranging from 100.1% to 83.0% for the investigated pretreatment conditions.

**Table 3.** Solid Recovery and Composition of the Water-Insoluble Solid and Water-Soluble Fraction

Pretreatment conditions	Solid composition (% dry WIS)			Soluble fraction (g/100g dry pretreated solid)			Solid recovery (%)	Glucose recovery in solid (%)
	Glucose	Lignin	Others	glucose	xylose	lignin		
198°C/2min/no acid	52.3(0.9)	31.8	15.9	3.1(0.2)	9.1(0.1)	2.1(0.1)	68.3	93.7
198°C/4min/no acid	53.7(1.4)	32.6	13.7	3.3(0.3)	8.6(0.3)	2.0(0.05)	66.8	92.5
198°C/6min/no acid	55.3(0.2)	36.2	8.5	2.9(0.2)	7.5(0.4)	1.8(0.03)	60.8	86.7
178°C/2min/acid	55.0 (0.3)	35.5	9.5	2.9(0.2)	10.6(2.0)	1.5(0.05)	62.5	88.7
178°C/4min/acid	53.5(2.4)	37.5	8.9	4.5(0.3)	10.0(0.5)	1.5(0.01)	59.3	81.8
178°C/6min/acid	54(1.8)	38.1	7.9	4.0(0.4)	10.1(0.5)	1.6(0.04)	58.3	81.2
188°C/2min/acid	55.6(2.7)	40.5	3.9	3.7(0.2)	5.0 (0.2)	0.92(0.1)	55.9	80.0
188°C/4min/acid	53.0(1.1)	39.9	7.1	2.3(0.1)	3.0(0.2)	0.86(0.3)	56.8	77.5
198°C/2min/acid	56.9(2.2)	42.1	1.0	2.4 (0.2)	3.4(0.1)	0.83(0.04)	53.6	78.7
198°C/4min/acid	57.9(3.9)	42.4	0	2.5 (0.1)	3.4(0.3)	0.81(0.03)	53.4	79.7

### Sugar and Lignin Recovery in Liquid Fraction

Xylan is the main hemicellulose component in wheat straw and the predominant sugar in the liquid fraction. As shown in Table 4, the xylose recovered in the liquid solution was very low and it decreased with increasing retention time and steam pressure.

It is no surprise that the degradation of xylose contributed to a significant part of the mass loss. The highest xylose recovery (53.7%) was observed when wheat straw was pretreated at the lowest steam pressure and retention time (178°C/2min/acid). Comparing samples pretreated at 198 °C with and without acid impregnation, it became apparent that the use of acid catalyst likely caused much severe sugar degradation and mass loss during steam explosion of wheat straw. This implies that devising a method to pre-extract xylan prior to steam explosion will likely improve the recovery of xylose in the biomass feedstock.

**Table 4.** Sugar and Lignin Recovery in Liquid Fraction \*

Pretreatment conditions	Glucose	Xylose	Lignin
198°C/2min/no acid	6.2	51.4	6.8
198°C/4min/ no acid	6.4	47.6	6.5
198°C/6min/ no acid	5.0	36.4	5.1
178°C/2min/acid	5.2	53.7	4.4
178°C/4min/acid	8.1	51.0	4.4
178°C/6min/acid	6.5	46.6	4.2
188°C/2min/acid	5.4	20.4	2.1
188°C/4min/acid	3.4	12.8	2.1
198°C/2min/acid	3.3	13.4	2.3
198°C/4min/acid	3.5	13.8	2.1

\* g/100g potential sugar and lignin in raw material

The glucan was more stable during the steam explosion and the amounts of glucose released from pretreatment were less than 8.1%, which corresponded to a glucose yield of 3.5 g glucose per 100 g raw material. In general, the samples pretreated at low steam pressure with addition of acid presented the highest glucose yield in the liquid fraction. Then the autohydrolysis at high steam pressure produced higher glucose yield than the other acid pretreatments.

During pretreatment, lignin undergoes both degradation and repolymerization reactions. A two-step mechanism has been proposed for lignin solubilisation (Lora 1978; Montané 1994; Aoyama 1995). In the first, fast step fragments of lignin with low molecular weight and high reactivity are solubilized by breaking lignin-carbohydrate bonds. More lignin is solubilised with the increase in treatment severity (e.g. steam pressure, retention time) and then the system approaches a plateau. In the second, slower step, lignin polymerisation occurs in the presence of organic acids released by autohydrolysis treatments to yield insoluble condensation products. The condensation of lignin with sugars and/or degradation products, also called pseudo-lignin, increased Klason lignin content in WIS from pretreatment. This two-step mechanism explains the decreasing of lignin recovery in liquid fraction with increasing pretreatment severity. The result also suggested that less lignin was released with addition of acid during pretreatment.

### Enzymatic Hydrolysis

The enzymatic hydrolysis was carried out to determine the effects of different pretreatment conditions on the digestibility of WIS fractions. The enzymatic hydrolysis yields were expressed as percentage of glucose released based on the total amount of potential glucose in the solid residue after 48 hours of incubation ( $Y_p$ , grams of glucose per 100 grams potential glucose in the pretreated material). The overall glucose yield was calculated using the percentage of combined glucose content both from enzymatic hydrolysis and collected in the liquid fraction based on available glucose presented in initial raw materials ( $Y$ , grams of glucose per 100 grams potential glucose of raw material). Both yield values obtained from hydrolysis of WIS are presented in Table 5.

**Table 5.** Enzymatic Hydrolysability and Total Glucose Yield

Pretreatment conditions	Hydrolysability, $Y_p$	Overall glucose yield, $Y$
198°C/2min/no acid	79.8(0.7)	79.6
198°C/4min/ no acid	88.7(4.5)	88.4
198°C/6min/ no acid	93.3(3.5)	85.8
178°C/2min/acid	76.2(3.1)	72.9
178°C/4min/acid	88.7(0.3)	80.8
178°C/6min/acid	97.5(1.8)	85.6
188°C/2min/acid	77.5(3.1)	67.4
188°C/4min/acid	83.8(4.9)	68.4
198°C/2min/acid	75.8(3.4)	62.9
198°C/4min/acid	62.4(2.6)	53.3

#### *Hydrolysability of WIS ( $Y_p$ )*

Higher  $Y_p$  values were obtained with WIS samples pretreated at 198°C/6 minutes/ no acid and 178°C/6 minutes/0.5% acid at 93.3% and 97.5%, respectively. These results suggested that the hydrolysability of WIS improved with the increasing of refining time. The hydrolysis results obtained at 178 and 188 °C with acid addition as well as at 198 °C without acid addition confirm this hypothesis (Fig. 2). The effect of longer refining retention time on improving WIS hydrolysability had also been previously observed from refining steam explosion of spruce (Fang et al. 2010). However, an opposite trend was obtained on the samples pretreated at high temperatures (198 °C), with acid pre-impregnation, which indicated that this condition is probably too severe for treating wheat straw. It was above mentioned that at pretreatment conditions, lower lignin concentration was detected in the liquid fraction and higher lignin content in the WIS. Lignin has a great impact on the rate and extent of cellulose enzymatic hydrolysis. The inhibitory role of lignin to enzymatic accessibility was attributed to the nonspecific adsorption of enzyme to lignin and steric hindrance. The cellulose digestibility was observed to increase with the lignin removal (Chang and Holtzapple 2000). The decrease of WIS hydrolysability pretreated at 198 °C was correlated to its high lignin content in

WIS. The condensation of lignin blocked the pores previously formed, which also decreased the hydrolysability.

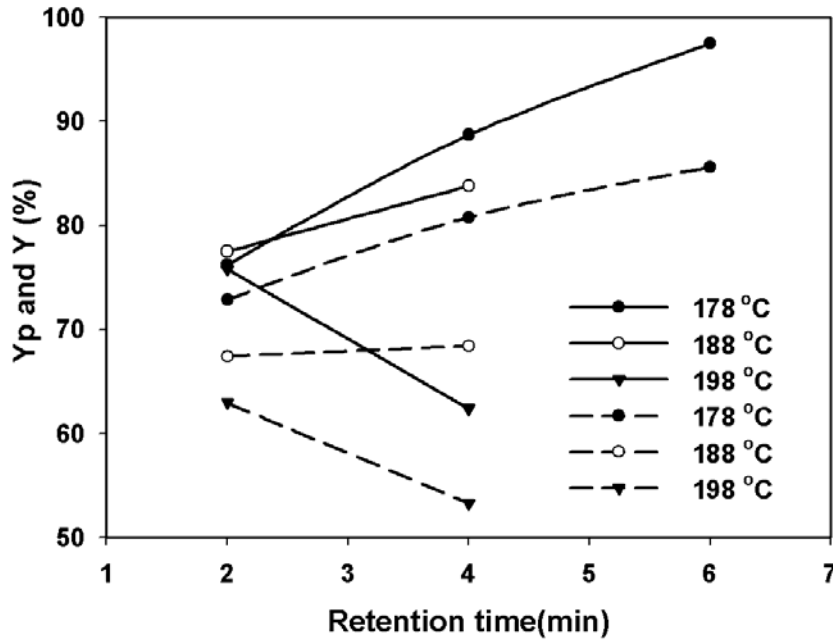


Fig. 2. Variations of  $Y_p$  and  $Y$  as a function of retention time at same steam temperature ( $Y_p$ : solid line and  $Y$ : dash line)

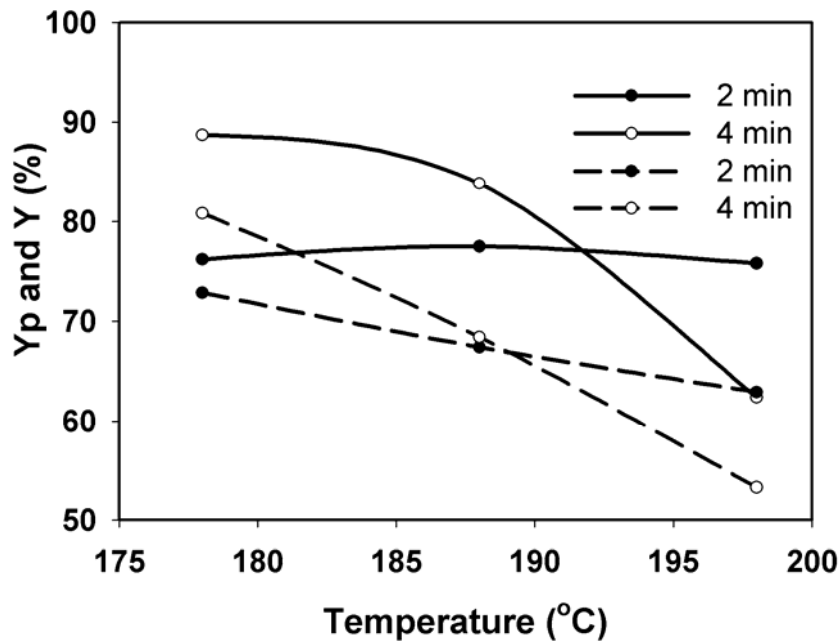


Fig. 3. Variations of  $Y_p$  and  $Y$  as a function of steam temperature at same retention time ( $Y_p$ : solid line and  $Y$ : dash line).

The effect of steam pressure, which is also associated with temperature on WIS hydrolysability, is demonstrated in Fig. 3. It appeared that the increasing of steam temperature had little effect on hydrolysability of solid residue at short refining retention time (2 min) while, at the longer refining retention times (4 and 6 minutes),  $Y_p$  decreased with the increase in temperature. Therefore a low steam temperature (178 °C) is preferred for pretreating wheat straw with sulfuric acid concentration of 0.5wt%.

#### *Overall glucose yield (Y)*

As well as having a good enzymatic hydrolysability, a high recovery yield of overall glucose was found with the steam explosion condition at 198°C/4 minutes/no acid and 178°C/6 minutes/0.5% acid. Again, increasing refining retention time led to a high overall glucose yield, except for treatment conditions with both high pressure and acid impregnation applied (198 °C/2 min/acid and 198 °C/4 min/acid) (Fig. 2). It is clear that under the presence of sulfuric acid, increasing steam pressure (temperature) inevitably decreased the overall glucose yield (Fig. 3). The high temperature caused sugar degradation during steam explosion, and thus reduced overall sugar yield. The samples obtained from acid catalyzed steam explosion at 178 °C (2,4 and 6 min) seemed to have comparable  $Y_p$  and  $Y$  values to those obtained from steam explosion at 198 °C without acid impregnation. This finding provides valuable insight to further optimizing the wheat straw steam explosion process to produce suitable substrates for sugar productions.

Compared to one previous study (Fang et al. 2010), it is certain that wheat straw requires relatively lower steam explosion treatment severity than woody material to achieve an optimized hydrolysability of WIS. With sulfuric acid impregnation, the pretreatment at 178°C for 6 minutes gave the best hydrolysability, at 97.5%, among all the WIS samples tested. Optimizing wheat straw steam explosion conditions to attain a good enzymatic hydrolysability has been investigated by a number of groups using batch systems. Beltrame et al. (1992) reported that the maximum enzymatic hydrolysis yield (93.5%) can be obtained by pretreatment at 230°C for 1 min while the maximum overall glucose yield (83.7%) can be obtained following pretreatment at 210°C for 1 to 2 min. Ballesteros et al. (2006) presented the best pretreatment condition for the highest enzymatic hydrolysability (180°C/10 min, or 200 °C/5 min, 0.9% sulfuric acid) and the former provided the highest ethanol yield with respect to the raw material. In the study by Linde et al. (2008), the wheat straw was impregnated in lower sulfuric acid concentration (0.2%). The highest overall glucose and xylose was obtained after pretreatment at 190°C/10 min. The high steam pressure and longer retention time decreased the enzymatic hydrolysis yield and overall yield. Petersen et al. (2009) found that the highest conversion of cellulose into ethanol was obtained from pretreating wheat straw at 205°C for 6 min. Díaz et al. (2010) have shown that a nearly complete enzymatic hydrolysis can be achieved after steam explosion of wheat straw at a temperature between 210 and 220°C for 30 to 50 min. The overall glucose recovery was approximately 70%.

Based on the results from this study, it is apparent that the continuous refining steam explosion process can dramatically decrease the steam pressure and retention time to achieve a better hydrolysability of the pretreated substrates and high overall glucose recovery yield. The mechanical refining following high pressure steam treatment further decreased the fiber size and increased the fiber surface area by the defibration effect

(Cullis et al. 2004), which resulted higher enzymatic conversion than the samples which had not been refined. For agricultural biomass feedstocks, either acid catalyzed or autohydrolysis strategies can be applied for optimizing sugar production.

## CONCLUSIONS

1. This work has demonstrated that a pressurized mechanical refining system can be readily applied for continuous steam explosion of wheat straw. The best pretreatment condition identified from this experimental study is 178 °C/6 min/0.5% acid impregnation, and 198°C/4min/water impregnation).
2. The refining steam explosion process brings a number of advantages over batch steam explosion process. It can achieve a high substrates hydrolysability and overall sugar recovery at relatively low steam pressure and short residence time. The application of refining steam explosion provides a promising potential for the commercialization of steam explosion pretreatment technology.

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