

# Consecutive Recovery of Non-Structural Sugars and Xylooligomers from Corn Stover using Hot Water and Acidified Calcium Chloride

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Corn stover, which has a high non-structural sugar content, was treated by two-stage fractionation using water and acidified CaCl<sub>2</sub> for the consecutive recovery of non-structural sugars and xylooligomers. In this process, water treatment under mild conditions (60 °C to 140 °C) was used for the recovery of non-structural sugars in the first stage, followed by the recovery of xylooligomers using acidified CaCl<sub>2</sub> in the second stage under severe conditions (160 °C to 180 °C). For the recovery of non-structural sugars, a water treatment at 80 °C was observed to be effective, recovering 95.6% of the non-structural sugars. With a non-structural sugar-free solid, the reaction conditions for the second stage of treatment using acidified CaCl<sub>2</sub> were statistically optimized. The highest recovery yield of xylooligomers (72.5%) was obtained under optimum conditions (172.9 °C, 22.2 min, 4.7% CaCl<sub>2</sub>). With the two-stage fractionation process, the glucan digestibility of treated solid was enhanced from 34.0% for untreated corn stover to 91.0% for the treated solid (with 15 FPU/g-glucan).

*Keywords:* Lignocellulosic biomass; Fractionation; Pretreatment; Percolation; Hemicellulose; Biorefinery

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## INTRODUCTION

Most of the energy consumed by humans is based on finite fossil resources, such as oil, coal, and natural gas. Although fossil resources have been a major raw material for the chemical industry during the last two centuries, they are not considered sustainable resources because they typically take millions of years to form. It has been reported that the use of fossil resources releases carbon dioxide, a major greenhouse gas, which accumulates in the atmosphere, causing climate change (Escobar *et al.* 2009). On the contrary, a bio-based industry built on renewable resources can produce a wide range of fuels and chemicals, which can provide environmental and socio-economic benefits in comparison with petroleum-based industry products (Sener *et al.* 2018). In particular, lignocellulosic biomass, including agricultural by-products, woody biomass, and various cellulosic wastes, have significant potential to replace conventional fuels such as oil and coal for the production of fuels and chemicals (Zabed *et al.* 2016).

Lignocellulosic biomass consists of three primary constituents: cellulose, hemicellulose, and lignin (Kang and Tan 2016). If the aforementioned three components could be separated with high yield and purity before further conversion, effective utilization of lignocellulosic biomass could be achieved (Kim *et al.* 2009; Bhutto *et al.* 2017). For example, pure cellulose can be effectively converted into various fuels/energy, and hemicellulose can be a source for the production of furfural, succinic acid, xylitol, or

xylooligosaccharides (Vazquez *et al.* 2000; Nghiem *et al.* 2005; Shi *et al.* 2011). Lignin can be used as a fuel simply *via* direct burning, and it can be further converted into value-added products such as activated carbon, binders, dispersants, and emulsifiers (Dizhbite *et al.* 1999).

Because various lignocellulosic materials have different chemical and physical characteristics, the effective utilization of biomass can be achieved by the development of an effective fractionation method for each biomass species (Biagini *et al.* 2006). In previous efforts, various fractionation methods using chemical reagents have been attempted, with acids and bases commonly utilized as catalysts (Kim and Lee 2006; Victor *et al.* 2016). Although some of these methods were observed to be effective for the fractionation of valuable components of biomass, several problems have been associated with them; for example, alkaline methods generally require long reaction times because of the slow reaction rate, whereas acid methods use highly corrosive reagents to react and usually result in severe sugar degradation. Autohydrolysis using hot water effectively hydrolyzes hemicellulose, but it depolymerizes a considerable amount of lignin along with hemicellulose (Casebier *et al.* 1969; Kim and Lee 2006). Inorganic salts are generally economical because they are less corrosive and easier to recycle than inorganic acids (Liu *et al.* 2009). In addition, metal cations react with carbohydrates to form a metal cation–cellulose complex, which accelerates the hydrolysis of hemicellulose and inhibits the further degradation of sugar released during hydrolysis (Tajmir-Riahi 1986; Cao *et al.* 1995). In this study, a two-stage fractionation method using water and acidified CaCl<sub>2</sub> was investigated for the consecutive recovery of non-structural sugars and xylooligomers from corn stover. Essentially, CaCl<sub>2</sub> is an effective swelling reagent, which can be expected to improve the enzymatic digestibility of cellulose. Moreover, CaCl<sub>2</sub> is inexpensive; for example, it is three times less expensive than zinc chloride. Nevertheless, there have been few reports on pretreatment methods using CaCl<sub>2</sub>.

In this study, residues of sweet corn, stalks, and leaves were used as feedstock; they contained approximately 30 wt.% of non-structural sugars (sucrose, glucose, and fructose), which can be recovered *via* water treatment even at mild temperatures. In contrast to non-structural sugars, structural sugars (sugar in cellulose and hemicellulose) account for approximately 40 wt.% of total corn stover and include glucan, xylan, galactan, arabinan, and mannan. To recover non-structural sugars and hemicellulose (mostly xylan) in this corn stover, the proposed two-stage flow-through (percolation) process was investigated to assess its effectiveness as a method of pretreatment and fractionation. In this method, non-structural sugars were recovered at moderate temperatures (60 °C to 140 °C) using water in the first stage, and acidified CaCl<sub>2</sub> was used for the recovery of hemicellulose in the form of xylooligomers in the following stage. After the two-stage fractionation, the remaining solid residue contained mostly cellulose and lignin, which are highly digestible by commercial cellulase enzymes.

The primary goal of this study is to develop a fractionation method for the effective recovery of sugars with minimal loss and to improve the enzymatic digestibility of treated biomass. The process conditions for effective fractionation and pretreatment were explored and statistically optimized. The influences of reaction parameters such as temperature, time, catalyst concentration, rate of increase in temperature, and flow rate were investigated. In addition, the effectiveness of the method for enhanced enzymatic digestibility was also evaluated for the production of monomeric sugar.

## EXPERIMENTAL

### Materials

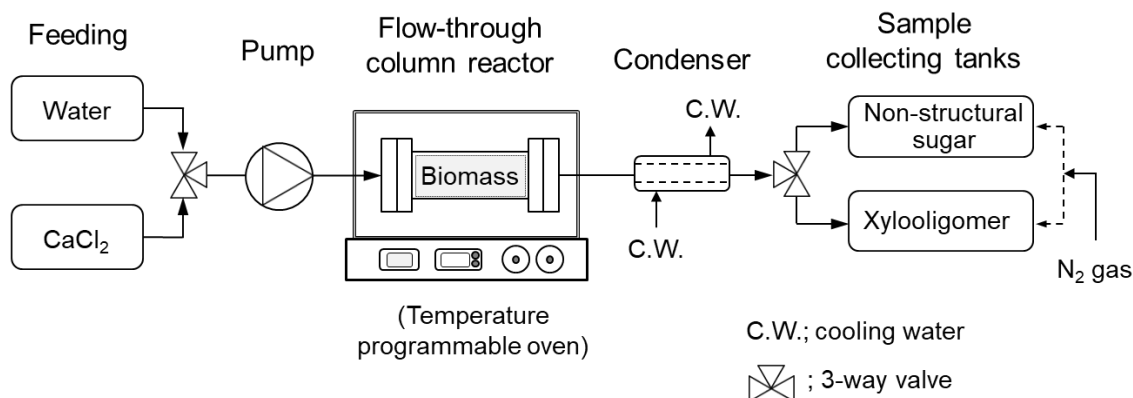
Sweet corn (GCB70 (Golden Cross bantam 70)) was grown and harvested in early June, 2012 in Korea and chopped into lengths of 2.0 cm in the field. The air-dried corn stover was ground and screened in our laboratory. The fraction with particle sizes of 0.50 to 1.41 mm was collected and placed in a convection oven at  $45 \pm 5$  °C for 48 h. The moisture content was maintained at lower than 4.5% during the experiment. The composition of the corn stover was determined by the National Renewable Energy Laboratory (NREL, Golden, CO, USA) Laboratory Standard Procedure (LAP), which was confirmed by SugarEn Inc. (Yongin, Gyeonggi-do, Korea) (Sluiter *et al.* 2008a, 2012). The chemical composition of the untreated corn stover was 29.7% non-structural sugars (9.9% sucrose, 9.7% glucose, and 10.1% fructose), 41.3% structural sugars (22.7% glucan, 13.7% xylan, 2.1% galactan, 2.6% arabinan, and 0.2% mannan), 5.2% acid-insoluble lignin (AIL), 2.1% acid-soluble lignin (ASL), 1.8% organosolv extractives, 16.2% water-soluble extractives, 2.0% ash, and 1.7% others ( $n = 2$ , standard deviations  $< 0.5$ ).

Additionally,  $\alpha$ -cellulose (cat. no. C8002) and  $\text{CaCl}_2$  (cat. no. 746495) were purchased from Sigma–Aldrich Korea (Yongin, Gyeonggi-do, Korea). A commercial cellulase, Celluclast<sup>®</sup> 1.5 L (cat. no. C2730), and a  $\beta$ -glucosidase, Novozym 188 (cat. no. C2730), were purchased from Sigma–Aldrich Korea. The average activities of the cellulase and  $\beta$ -glucosidase were determined to be 119.4 filter paper units (FPU)/mL and 750 cellobiase units (CBU)/mL, respectively. The protein contents of the cellulase were 184 mg/mL.

### Methods

#### *Experimental set-up and operation*

A process flow diagram of the experimental set-up is shown in Fig. 1. The flow-through column reactor was made of SS-316 (internal diameter (ID) 2.3 cm, length (L) 25.4 cm, and internal volume 104.3 cm<sup>3</sup>). The system consisted of a high-performance liquid chromatography (HPLC) pump (Series II pump, Scientific Systems, Inc.), a temperature-programmable oven (M600D, Younglin Co., Anyang, Gyeonggi-do, Korea), solution reservoirs, and two sample-collecting tanks (304 stainless steel cylinder; internal volume 1000 mL).



**Fig. 1.** Laboratory reactor system of two-stage flow-through process

### *Non-structural sugar recovery in the first stage*

Water treatment was performed under moderate conditions (60 °C to 140 °C) to determine the optimal recovery temperature for non-structural sugars. Ten grams (oven-dry weight) of corn stover was packed into the flow-through type reactor. The preheating coil was filled with deionized (DI) water. The reactor system was pressurized with 2.1 MPa of nitrogen backpressure to prevent evaporation of the liquid. The reactor and preheating coil were placed in a temperature-programmable oven and preheated to the desired temperature for 14 min, regardless of the target temperature. The DI water was pumped into the flow-through reactor when the reactor reached the desired reaction temperature; preheating was not counted as reaction time. The reaction time and flow rate were maintained at 20 min and 5.0 mL/min, respectively. At the completion of the recovery of non-structural sugars, the residual solid in the reactor was flushed with water to remove any residual sugars. The wet solids discharged from the reactor were separated into two portions. One portion was dried using a moisture analyzer for the measurement of weight loss and subjected to composition analysis. The other portion was used for the enzymatic digestibility test.

### *Xylooligomer recovery in the second stage*

*Hot water treatment:* Three different flow rates (5.0, 7.5, and 10.0 mL/min) and a range of temperatures (160 to 210 °C) were tested. After completion of the first stage of treatment using hot water at the preselected temperature (80 °C), the temperature was immediately increased at various rates (0.5, 1.0, and 2.0 °C/min). During the increase in temperature at a constant rate, hydrolysate samples were obtained from four different temperature zones (80 to 150 °C, 150 to 170 °C, 170 to 190 °C, 190 to 210 °C, after cooling and flushing) to determine the xylan recovery trend with increasing temperature. After the two-stage treatment, the wet solid parts were subjected to composition analysis and moisture content measurement to determine weight loss.

*CaCl<sub>2</sub> treatment:* After the completion of the first stage of treatment using water at the preselected temperature (80 °C), the temperature was increased up to the target temperature for 10 min. Three different temperatures (160, 170, and 180 °C) were employed while maintaining the other conditions at a constant, *i.e.*, the flow rate of 5.0 mL/min and the reaction time of 20 min. For the acidified CaCl<sub>2</sub> treatment, a solution of 5.0% CaCl<sub>2</sub> with 0.06% hydrochloric acid was used. After the two-stage treatment, the wet solid parts were subjected to composition analysis and moisture content measurement to determine the weight loss.

### *Optimization of acidified CaCl<sub>2</sub> treatment in the second stage*

The acidified CaCl<sub>2</sub> treatment conditions of the second stage were optimized using response surface methodology (RSM) based on central composite design (CCD). The statistical analysis of the data was performed using SAS<sup>®</sup> software (Version 9.4, SAS Institute Inc., Cary, NC). With the non-structural sugar-free sample, acidified CaCl<sub>2</sub> treatment was carried out to fractionate xylan at various temperatures (153.2 to 186.8 °C), times (11.6 to 28.4 min), and CaCl<sub>2</sub> concentrations (0.8 to 9.2%). The duration for which the temperature was increased (from 80 °C in the first stage to the target temperature in the second stage) and the flow rate were fixed at 10 min and 5.0 mL/min, respectively. To achieve high xylan recovery yield, the three variables mentioned above were optimized using RSM. A second-order model was employed to fit the data individually for the

response  $Y$  (xylan recovery yield) with the general model with three variables, *i.e.*,  $X_1$  (reaction temperature),  $X_2$  (reaction time), and  $X_3$  ( $\text{CaCl}_2$  concentration). They were coded in five stages, as presented in Table 1. Experiments on six star points and center points were repeated three times according to a  $2^3$ -fractional factorial design. Seventeen experiments were carried out with three variables, and each variable was varied at five levels ( $\alpha - 1.68$ ) for the xylan recovery yield, which was the response (dependent) variable. The quadratic polynomial model was fitted for the xylan recovery yield, and the purity of xylan is given by the following equation,

$$Y = b_0 + \sum_{i=1}^k b_i x_i + \sum_{i=1}^k b_{ii} x_i^2 + \sum_{i<j} b_{ij} x_i x_j \quad (1)$$

where  $x_i$  and  $x_j$  are the coded values of the independent variable,  $Y$  is the response,  $b_0$  is a constant,  $b_i$  is a linear coefficient,  $b_{ii}$  is a quadratic coefficient, and  $b_{ij}$  is an interactive coefficient.

**Table 1.** Coded Levels and Real Values of Tested Variables in the CCD

Process Variables	Coded Factor Level				
	-1.68	-1	0	1	1.68
Reaction Temperature ( $^{\circ}\text{C}$ ), $X_1$	153.2	160	170	180	186.8
Reaction Time (min), $X_2$	11.6	15	20	25	28.4
$\text{CaCl}_2$ Concentration (%), $X_3$	0.8	2.5	5	7.5	9.2

#### *Enzymatic digestibility test*

The enzymatic digestibility of corn stover was determined according to the NREL LAP (Selig *et al.* 2008). Celluclast<sup>®</sup> 1.5 L (Novozyme A/S Bagsvaerd, Denmark), and  $\beta$ -glucosidase, Novozym 188 (Novozyme A/S Bagsvaerd, Denmark), were used. The enzymatic digestibility tests were performed at  $50^{\circ}\text{C}$ , 150 rpm, and pH 4.8 (0.05 M sodium citrate buffer) in a shaking incubator (Vision, model VS-8480SFN, Scientific Co., Ltd., Bucheon, Gyeonggi-do, Korea). Enzyme loadings were 15 FPU (filter paper unit) of cellulase/g-glucan supplemented with 30 CBU (cellobiase unit) of  $\beta$ -glucosidase/g-glucan. The initial glucan concentration was 1.0% (w/v), based on 100 mL of total liquid in 250-mL Erlenmeyer flasks. To prevent microbial contamination, 40 and 30 mg/L of tetracycline and cyclohexamide, respectively, were added. Samples were removed periodically at appropriate sampling times (6, 12, 24, 48, and 72 h) and analyzed to determine glucose content using HPLC with a HPX-87P column (cat. no. 125-0098, Bio-Rad Laboratories, Hercules, CA). The total released glucose after 72 h of hydrolysis was used to calculate the enzymatic digestibility for glucan as follows,

$$\text{Glucan digestibility (\%)} = \left[ \frac{\text{Total released glucose (g)} \times 0.9}{\text{Initial glucan loading (g)}} \right] \times 100 \quad (2)$$

where 0.9 is the conversion factor of glucose to equivalent glucan.

Untreated corn stover and  $\alpha$ -cellulose were processed *via* the same procedure to obtain a reference and control, respectively.

*Analytical method*

The chemical compositions of the solid and liquid samples were determined following the procedures of the NREL-LAP (Sluiter *et al.* 2008b). The extraction process was carried out to remove the non-structural material and to facilitate the process after the analysis of the biomass composition. This process was carried out in two steps using water and ethanol. After extraction, the solid was used for the analysis of structural carbohydrate composition and liquid was used to analyze the non-structural carbohydrates. For the composition analysis of extractives-free and treated solids, the two-step acid hydrolysis method was applied. Approximately  $300.0 \pm 10.0$  mg of biomass (dry weight) was placed in a water bath at 30 °C for one hour for primary acid hydrolysis. Hydrolyzed liquor was diluted into 4.0% sulfuric acid with DI water and subjected to secondary acid hydrolysis at 121 °C for 1 h. After secondary hydrolysis, the sample was separated into solid and liquid parts. The solid part was used for the analysis of acid-insoluble lignin and ash, and the liquid part was used to determine the content of carbohydrates using HPLC and that of acid-soluble lignin using UV-vis spectroscopy (UV-mini 1240, Shimadzu, Inc., Kyoto, Japan). An HPLC system (LC-10A, Shimadzu Inc., Kyoto, Japan) was used to determine the chemical compositions of the liquid samples. The analytical column and detector used were a Bio-Rad Aminex HPX-87P and refractive index detector (RID-10A, Shimadzu Inc., Kyoto, Japan), respectively. The operating temperature of the HPLC oven was 85 °C. HPLC-grade water was used as the mobile phase with a volumetric flow rate of 0.6 mL/min.

**RESULTS AND DISCUSSION****First Stage: Non-Structural Sugar Recovery via Water Treatment at Moderate Temperature**

To recover a considerable amount of non-structural sugars (~30 wt %), DI water was applied at various temperatures. Six different temperatures (60 to 140 °C) were considered with other conditions remaining constant (20 min and 5.0 mL/min).

**Table 2.** Composition of the Treated Solid and the Recovery Yields of Non-Structural Sugars in Liquid at Different Water Treatment Temperatures <sup>a</sup>

Temp.	S.R. <sup>b</sup>	Solid			Non-structural Sugars in Liquid			
		Glucan	Xylan	Lignin <sup>c</sup>	Sucrose	Glucose	Fructose	Recovery Yield <sup>d</sup>
[°C]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[%]
Untreated	100.0	22.7	13.7	7.3	9.9 <sup>e</sup>	9.7 <sup>e</sup>	10.1 <sup>e</sup>	-
60	50.9	21.2	11.0	7.3	8.6	8.7	9.8	91.2
80	49.4	20.3	11.4	7.3	10.1	8.6	9.9	95.6
100	48.0	19.9	10.6	7.3	10.7	8.6	10.0	96.0
120	48.4	20.5	10.8	7.3	10.5	8.5	10.0	95.6
140	47.2	20.7	10.9	7.3	9.8	8.6	9.8	94.9

<sup>a</sup> The data in the table show the mean value (n = 3, SD < 0.4, SD: standard deviation); <sup>b</sup> S.R.; solid remaining after reaction; <sup>c</sup> acid-insoluble lignin (AIL) + acid-soluble lignin (ASL); <sup>d</sup> recovery yield of non-structural sugars; <sup>e</sup> non-structural sugars in untreated solid

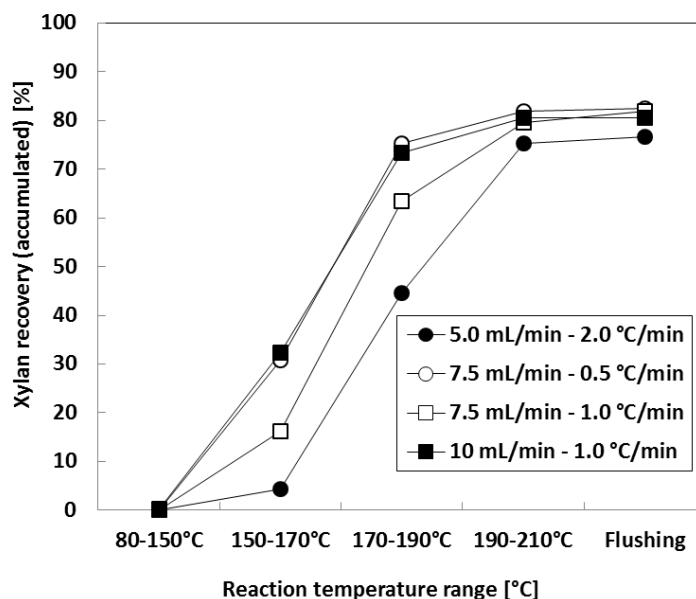
Table 2 summarizes the effect of reaction temperature on the recovery of non-structural sugars and the chemical composition of the treated (residual) solid. At 60 °C, 91.2% of non-structural sugars were recovered, which increased to near complete recovery (95.6% to 96.0%) at 80 to 100 °C. Furthermore, recovery was reduced to 94.9% with treatment at a high temperature (140 °C). This indicated that the treatment at  $\geq 80$  °C could recover most of the non-structural sugars from corn stover. Therefore, the conditions for the water treatment were selected to be 80 °C, 20 min, and 5.0 mL/min for the recovery of non-structural sugars in the first stage.

As the treatment temperature increased (60 to 140 °C), 87.7% to 93.4% of glucan and 77.4% to 83.2% of xylan were retained with the treated solid. Notably, nearly no lignin was solubilized at all at the tested temperatures because the hydrolysate obtained in this test was a relatively pure sugar solution.

## Two-stage Treatment: Hot Water Treatment in the Second Stage

### *Effects of flow rate and rate of increase in temperature on xylan recovery*

Using the non-structural sugar-free sample (removed at 80 °C), the recovery of xylan using the hot water treatment in the second stage was conducted by increasing the temperature gradually (at a constant rate) in a flow-through column reactor. Figure 2 shows the accumulated xylan recovery as the reaction temperature increases in the second stage using the hot water treatment. As the temperature was raised at a constant rate (0.5 to 2.0 °C/min), liquid samples were collected over four temperature ranges; thereafter, each of them was analyzed to determine the recovery rate of xylooligomers. The final xylan recoveries for all the samples were in the range of 76.7% to 82.5%. Xylan recovery *via* hot water treatment generally increased as the flow rate increased, with the most significant xylan solubilization and recovery occurring in the range of 170 to 190 °C.



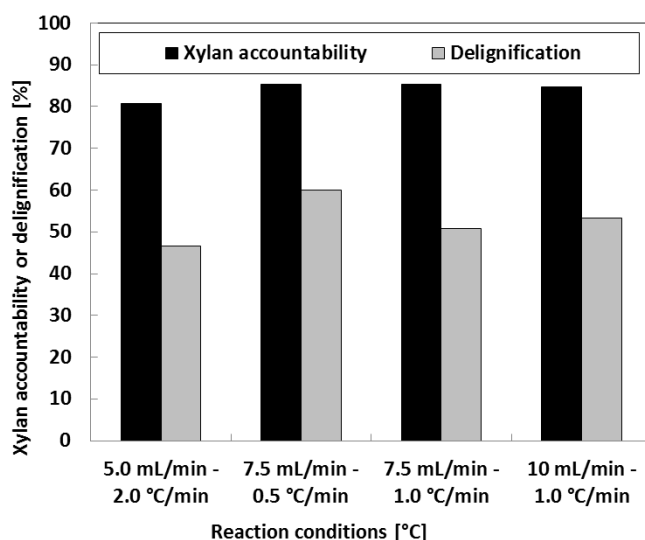
**Fig. 2.** Xylan recovery *via* hot water treatment for various flow rates and rates of increase of temperature

With a low flow rate and high rate of increase in temperature (5.0 mL/min, 2.0 °C/min), the solubilization of xylan in the lower temperature range (150 to 170 °C) was

only 4.4%, which was less significant than those under other conditions. It was assumed that an increase in temperature of 2.0 °C/min was too fast to facilitate the hydrolysis of xylan from biomass.

For two different rates of increase in temperature (0.5 and 1.0 °C/min), with water being pumped at the same flow rate (7.5 mL/min), the treatment at 0.5 °C/min was more effective than the treatment at 1.0 °C/min in the lower temperature range, even though the rate of increase in temperature generally affects the reaction time and total liquid throughput. Similarly, for two different flow rates (7.5 and 10.0 mL/min) at the same rate of increase in temperature (1.0 °C/min), the treatment at 10.0 mL/min was more effective for the recovery of xylan than the treatment at 7.5 mL/min. These results collectively indicate that both factors, the rate of increase in temperature and flow rate, affected the recovery of xylan, and a combination of these two factors can improve the recovery yield.

Figure 3 presents the total accountability of xylan and lignin removal during the hot water treatment of corn stover under the aforementioned conditions. Hot water treatment removed 46.6% to 60.0% of lignin from corn stover and treatment at 5.0 mL/min and 2.0 °C/min resulted in the lowest delignification (46.6%), which also led to the recovery of the least amount of xylan from corn stover.



**Fig. 3.** Xylan accountability and lignin removal during hot water treatment at various flow rates and rates of increase of temperature

The accountability of xylan (xylan content in the solid in addition to that in the liquid) ranged from 80.7% to 85.4%, showing no significant variation under different reaction conditions. However, the accountability of xylan at 5.0 mL/min and 2.0 °C/min was 80.7%, which was lower than that under other reaction conditions, indicating that a considerable amount of xylan was decomposed under these conditions.

#### *Effect of hot water treatment at fixed temperature*

Based on the first series of runs using hot water at various rates of increase in temperature (Table 2), it was observed that treatment temperature between 160 and 210 °C was effective for the dissolution and recovery of xylan. Therefore, the effect of hot water



treatment at fixed temperatures (160, 170, 180, 190, 200, and 210 °C) was evaluated, and the results are summarized in Table 3.

The recovery of xylan using hot water treatment was in the range of 39.4% to 70.8%. The recovery of xylan increased with temperature up to 180 °C, and thereafter decreased at higher temperatures. This was because the xylan released into the liquid may have undergone degradation reactions at high temperatures. The enzymatic digestibility (after 72 h of hydrolysis) of the treated solid gradually increased from 34.0% for the untreated solid to 96.6% for the solid treated at 210 °C as the treatment temperature increased.

**Table 3.** Effects of Hot Water Treatment in the Second Stage on the Composition of the Treated Solids <sup>a</sup>

Reaction Temp.	S.R. <sup>b</sup>	Solid				Liquid		Enzymatic Digestibility <sup>e</sup>
		Glucan	Xylan	Lignin <sup>c</sup>	Glucan Retention <sup>d</sup>	Xylan	Xylan Recovery	
[°C]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[%]	[wt.%]	[%]	[%]
Untreated	100.0	22.7	13.7	7.3	-	-	-	34.0
160	36.1	15.9	6.2	6.8	70.0	5.4	39.4	55.4
170	30.0	15.2	3.7	6.7	67.0	8.1	59.1	64.3
180	26.8	15.0	1.9	6.7	66.1	9.7	70.8	76.2
190	24.0	15.1	0.3	5.0	66.5	9.4	68.6	91.1
200	23.2	14.7	0.1	5.4	64.8	8.8	64.2	95.5
210	22.5	14.7	0.0	4.7	64.8	8.0	58.4	96.6

<sup>a</sup> The data in the table show the mean value (n = 3, SD < 0.5, SD: standard deviation); reaction condition (first stage = water, 80 °C, 20 min, second stage = 20 min, 5.0 mL/min); <sup>b</sup> solid remaining; <sup>c</sup> acid-insoluble lignin (AIL) + acid-soluble lignin (ASL); <sup>d</sup> glucan (in residual solid)/glucan (in untreated biomass) x 100; <sup>e</sup> enzymatic hydrolysis conditions: 15 FPU/g-glucan, pH 4.8, 150 rpm, 50 °C, 72 h

Notably, hot water treatment at a high temperature removed xylan and some glucan. Glucan retention in the solid decreased from 70.0% for the solid treated at 160 °C to 64.8% for the solid treated at 210 °C. The solubilization of lignin was 6.8% to 35.6% over the tested temperature range. In particular, the solubilization of lignin in the lower reaction temperature range (160 to 180 °C) was less significant (6.8% to 8.2%) than that (31.5% to 35.6%) at higher reaction temperatures, in the range 190 to 210 °C. This also indicated that more lignin was removed as the dissolution of xylan increased. This may have occurred because of the lignin–hemicellulose network in lignocellulosic material; *i.e.*, hot water treatment hydrolyzes either hemicellulose or lignin and thus loosens the whole structure of the lignin–hemicellulose network (Brosse *et al.* 2009; Wildschut *et al.* 2012). This makes the recovery of xylan with high purity very difficult.

### Two-stage Treatment: CaCl<sub>2</sub> Treatment in the Second Stage

After the removal of non-structural sugars using water at 80 °C, fractionation of xylan using CaCl<sub>2</sub> treatment in the second stage was conducted in a flow-through column reactor. Three different temperatures were applied while maintaining the other reaction conditions constant as follows: 5% acidified CaCl<sub>2</sub>, 20 min, and 5.0 mL/min. The composition data and enzymatic digestibility (72 h) after the two-stage treatments are summarized in Table 4.

**Table 4.** Effects of CaCl<sub>2</sub> Treatment in the Second Stage on the Composition of the Treated Solids <sup>a</sup>

Reaction temp.	S.R. <sup>b</sup>	Solid				Liquid		Enzymatic Digestibility <sup>e</sup>
		Glucan	Xylan	Lignin <sup>c</sup>	Glucan Retention <sup>d</sup>	Xylan	Xylan Recovery	
[°C]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[%]	[wt.%]	[%]	[%]
Untreated	100.0	22.7	13.7	7.3	-	-	-	34.0
160	28.4	15.3	2.7	6.8	67.4	9.2	67.2	69.5
170	26.2	16.1	1.6	6.7	70.9	9.5	69.3	82.0
180	24.0	16.0	0.7	6.7	70.5	9.9	72.3	90.1

<sup>a</sup> The data in the table show the mean value (n = 3, SD < 0.5, SD: standard deviation); reaction condition (first stage = water, 80 °C, 20 min, second stage = 5% acidified CaCl<sub>2</sub>, 20 min, 5.0 mL/min); <sup>b</sup> solid remaining; <sup>c</sup> acid-insoluble lignin (AIL) + acid-soluble lignin (ASL); <sup>d</sup> glucan (in residual solid)/glucan (in untreated biomass) x 100; <sup>e</sup> enzymatic hydrolysis conditions: 15 FPU/g-glucan, pH 4.8, 150 rpm, 50 °C, 72 h

The remaining glucan contents in the treated solids did not change significantly with reaction temperature (67.4% to 70.9%). After the two-stage fractionation, most of the xylan in the solid was solubilized and 67.2% to 72.3% of the xylan was recovered. The removal of lignin was in the range of 6.8% to 8.2% at all tested temperatures. However, glucan digestibility (72-h enzymatic digestibility) was substantially improved with increasing temperature ( $\geq 180$  °C). For example, it was observed that the digestibility was enhanced from 69.5% for the solid treated at 160 °C to 90.1% for the solid treated at 180 °C. The trend was similar to the results of the two-stage treatment using hot water in the second stage presented in Table 3, but when samples treated at 170 and 180 °C were compared, CaCl<sub>2</sub> were more effective than those of hot water treatments in terms of digestibility because the 72-h digestibilities of samples treated at 170 and 180 °C were 82.0% and 90.1% for CaCl<sub>2</sub>-treated samples and 64.3% and 76.2% for hot water-treated samples (Table 3), respectively. Therefore, the two-stage treatment using CaCl<sub>2</sub> at 180 °C in the second stage not only improved glucan digestibility (> 90%), but, more significantly, also recovered xylan in the liquid more effectively (> 70%).

### Optimization of Two-stage Fractionation Process Using Water Followed by CaCl<sub>2</sub>

The optimal reaction conditions of the first stage treatment using water were chosen earlier (Section 3.1) as follows: 80 °C, 20 min, and 5.0 mL/min. To optimize the second stage using CaCl<sub>2</sub> in the two-stage fractionation, the effects of various correlations among three different parameters (reaction temperature, reaction time, and CaCl<sub>2</sub> concentration) on the recovery of xylan were evaluated. The optimum reaction conditions for the second stage of treatment were predicted using RSM based on the CCD as presented in Table 5.

Table 5 also summarizes the results of various combinations of fractionation conditions. The second-order polynomial equation describing the xylan recovery yield ( $Y_1$ ) as a simultaneous function of reaction temperature ( $X_1$ ), reaction time ( $X_2$ ), and CaCl<sub>2</sub> concentration ( $X_3$ ) is shown in Eq. (3). Its regression coefficient  $R^2$  was 0.98.

$$Y = 67.7 + 14.38X_1 + 8.71X_2 + 4.02X_3 - 20.24X_1^2 - 11.32X_1X_2 - 5.79X_2^2 - 18.75X_1X_3 + 2.08X_2X_3 + 2.04X_3^2 \quad (3)$$

**Table 5.** Estimated Response (Xylan Content) using the CCD for the Fractionation of Corn Stover

No	Process Variables			Response						
	Coded Variables			Xylan Recovery			Residual Solid			Enzymatic Digestibility <sup>b</sup>
	Temp. (X <sub>1</sub> )	Time (X <sub>2</sub> )	Conc. (X <sub>3</sub> )	Yield (Y)	Selec-tivity	Pur-ity	Glucan	Xylan	Lignin <sup>a</sup>	
[°C]	[min]	[%]	[%]	[-]	[-]	[wt.%]	[wt.%]	[wt.%]	[%]	
1	186.8	20.0	5.0	61.9	1.2	52.9	14.3	1.0	4.7	98.2
2	180.0	15.0	2.5	71.9	1.3	57.7	15.8	1.8	5.1	91.4
3	180.0	20.0	7.5	62.7	1.3	56.7	15.4	1.3	5.4	94.6
4	180.0	25.0	5.0	58.8	1.4	57.5	15.9	1.5	5.1	90.3
5	170.0	28.4	5.0	69.8	1.0	52.0	15.0	1.9	3.3	85.1
6	170.0	11.6	5.0	52.2	1.4	60.6	17.2	5.8	7.1	54.5
7	170.0	20.0	9.2	71.3	1.5	60.4	15.9	1.1	5.6	94.7
8	170.0	20.0	0.8	58.2	1.7	63.7	16.5	4.2	7.9	65.5
9	170.0	15.0	7.5	61.2	1.7	63.5	16.4	3.1	7.5	67.5
10	170.0	20.0	5.0	66.3	2.2	63.6	17.5	1.8	7.0	85.1
11	170.0	20.0	5.0	68.3	2.9	65.8	18.3	1.2	7.4	84.9
12	170.0	20.0	5.0	67.1	2.0	64.4	16.9	1.9	7.1	86.4
13	170.0	25.0	2.5	68.9	1.6	63.5	16.6	2.6	6.5	77.5
14	160.0	15.0	5.0	41.9	0.8	54.8	15.2	7.2	7.1	62.6
15	160.0	20.0	2.5	45.4	1.1	55.0	16.0	5.9	7.1	58.4
16	160.0	25.0	7.5	68.1	1.6	61.0	15.8	2.4	7.2	75.3
17	153.2	20.0	5.0	31.2	1.0	50.9	16.4	7.6	7.5	54.0

<sup>a</sup> Acid-soluble lignin + acid-insoluble lignin; <sup>b</sup> 72 h-enzymatic digestibility with enzyme loading of 15 FPU/g-glucan

The lowest xylan yield (31.2%) and purity (50.9%) were observed at 153.2 °C, 20 min, and 5.0% CaCl<sub>2</sub> (run no. 17 in Table 5). However, when the fractionation temperature increased to 170 °C (run no. 10, 11, 12), the xylan recovery yield increased more than two-fold (66.3% to 68.3%). Although the CaCl<sub>2</sub> treatment at a high temperature (186.8 °C, run no. 1) removed xylan significantly from the solid (92.7%), it provided a relatively low xylan recovery yield (61.9%). It was assumed that, during the hydrolysis of hemicellulose by CaCl<sub>2</sub> at a high temperature in the flow-through column reactor, solubilized xylan underwent a further reaction to decompose xylooligomers to furfural or other degraded products (Rasmussen *et al.* 2014).

To quantitatively evaluate the fractionation effects of various reaction conditions on each component, the purity and selectivity of xylan over other components is defined as follows,

$$Selectivity = \frac{m_{Xylan}}{m_{Glucan} + m_{Lignin}} \quad (4)$$

where  $m_{Xylan}$ ,  $m_{Glucan}$ , and  $m_{Lignin}$  are the mass loss (g) of xylan, glucan, and lignin from the solid, respectively.

$$\text{Xylan Purity [\%]} = \frac{\text{Xylan [g] in liquid}}{\text{Glucan} + \text{Xylan} + \text{Lignin [g] in liquid}} \times 100 \quad (5)$$

Over the entire range of treatment conditions, the selectivity and purity were in the ranges of 0.8 to 2.9 and 50.9 to 65.8, respectively. The selectivity and purity of xylan are closely related, as shown in the Methods section. The purity of xylooligomers in the recovered hydrolysate increased as the selectivity of xylan increased.

The analysis of variance (ANOVA) for the above quadratic model is summarized in Table 6. The *F*-value was 38.87, with a low *p*-value (< 0.0001), which represents statistically significant levels of confidence more than 99.99%. In other words, it shows that the overall model is significant. The *p*-values of each variable ( $X_1$ ,  $X_2$ ,  $X_3$ ) are < 0.0001, 0.0002, and 0.0132, respectively, indicating that, among the three variables, the reaction temperature was the most influential variable for xylan recovery yield in the range used in this study. The mutual level of reaction temperature and reaction time ( $X_1X_2$ ) was  $p < 0.05$ , and the mutual level of reaction temperature and  $\text{CaCl}_2$  concentration ( $X_1X_3$ ) was  $p < 0.01$ .

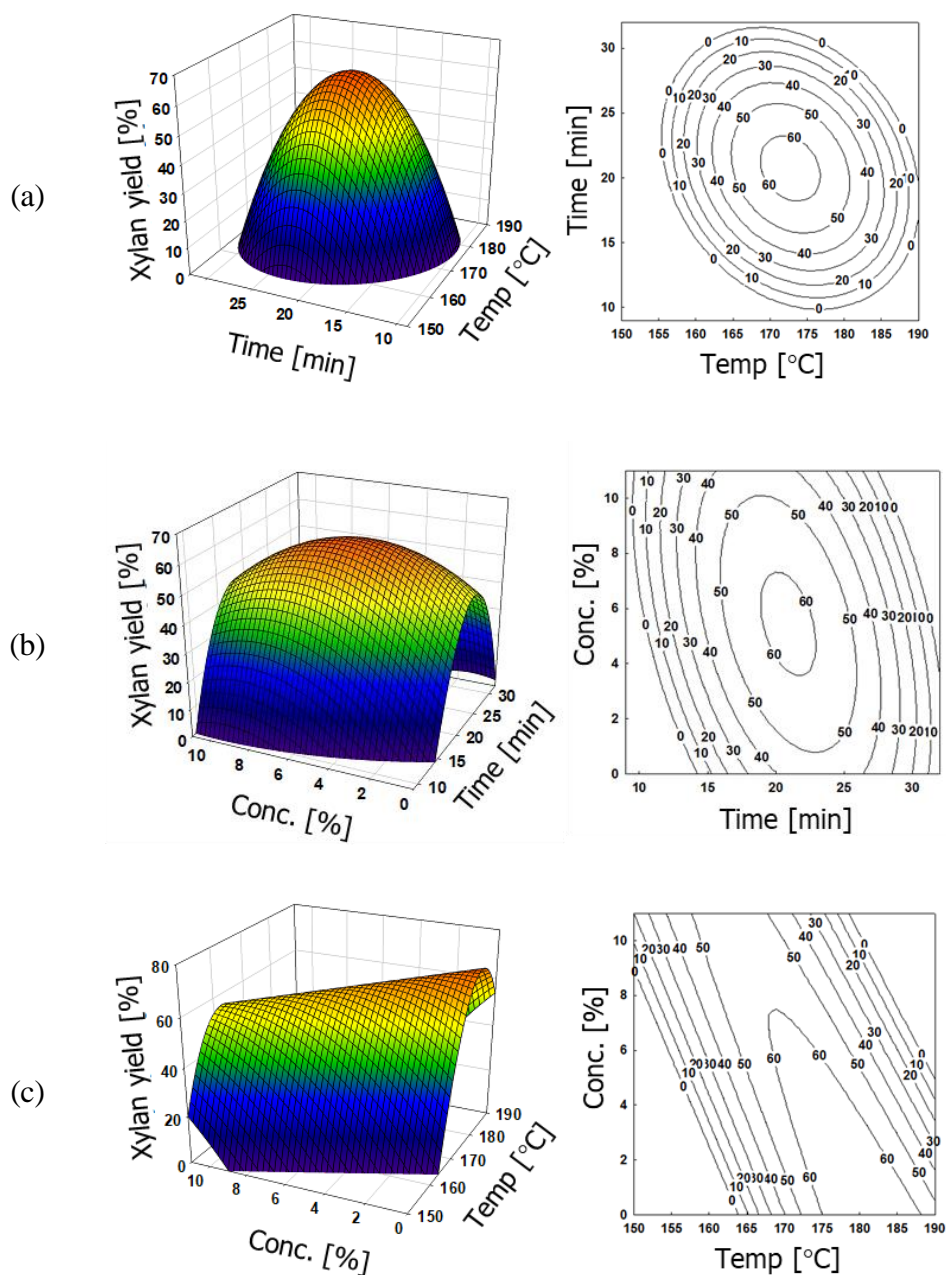
**Table 6.** ANOVA for Response Y (Xylan Recovery Yield [%])

Source	DF <sup>a</sup>	Sum of Squares	Mean Squares	<i>F</i> -Value	<i>Prob</i> <sup>b</sup> > <i>F</i>
Model	9	2136.87	237.43	38.87	< 0.0001
$X_1$	1	851.55	851.55	139.4	< 0.0001
$X_2$	1	312.96	312.96	51.23	0.0002
$X_3$	1	66.45	66.45	10.88	0.0132
$X_1 X_2$	1	38.12	38.12	6.24	0.0411
$X_1 X_3$	1	104.52	104.52	17.11	0.0044
$X_2 X_3$	1	1.29	1.29	0.21	0.6598
$X_1^2$	1	501.93	501.93	82.17	<.0001
$X_2^2$	1	41.11	41.11	6.73	0.0357
$X_3^2$	1	5.11	5.11	0.84	0.3907
Residual	7	42.76	6.11	-	-
Lack of Fit	5	40.73	8.15	8.04	0.1143
Pure Error	2	2.03	1.01	-	-

<sup>a</sup> Degrees of freedom; <sup>b</sup> probability values (*p*-values)

To examine the interactions among the three variables, the predicted values of xylan recovery yield with a change in the three variables are shown by response surface plots and contour lines (Fig. 4). The effects of reaction temperature and time on the xylan recovery yield while maintaining the concentration of  $\text{CaCl}_2$  at a constant are shown in Fig. 4(a). During this process, increases in reaction temperature and time led to a rise in the xylan recovery yield. However, the further increase of these two variables resulted in a decrease of xylan yield. Similarly, Fig. 4(b) (reaction time and  $\text{CaCl}_2$  concentration) also shows a contour plot. Figure 4(c) summarizes the effects of reaction temperature and concentration of  $\text{CaCl}_2$  on the xylan recovery yield. An increase in the concentration of  $\text{CaCl}_2$  led to a rise in the xylan recovery yield, whereas an increase in reaction temperature up to a certain

value resulted in a rise in the xylan recovery yield, which thereafter decreased with a further increase in temperature. This indicates that neither severe nor overly moderate conditions have positive impacts on the xylan yield. In addition, the steep surface curve indicates that the xylan yield is very sensitive to two variables (temperature and time) (Fig. 4(a)).



**Fig. 4.** Response surface combined effects of (a) reaction time and reaction temperature with 5.0% (w/v)  $\text{CaCl}_2$  concentration, (b)  $\text{CaCl}_2$  concentration and reaction time at the reaction temperature of 170 °C, (c)  $\text{CaCl}_2$  concentration and reaction temperature at the reaction time of 20 min

The optimum reaction conditions predicted from the regression equation (Eq. (3)) for the independent variable  $Y$  are reaction temperature 172.9 °C, reaction time 22.2 min,

and  $\text{CaCl}_2$  concentration 4.7%. The predicted values of xylan recovery yield and purity were 71.5% and 64.6%, respectively. After the confirmation run under the optimal conditions, the xylan recovery yield and purity were 72.5% and 65.8%, respectively, which were 1.0% and 1.2% higher than their respective predicted values. After two-stage fractionation under the optimum conditions, the remaining solid contained 17.8 wt.% glucan, 1.1 wt.% xylan, and 6.9 wt.% lignin (based on the oven-dry weight of untreated biomass), and the enzyme digestibility was significantly improved from 34.0% for the untreated biomass to 91.0% for the treated biomass.

### Total Mass Balance

Figure 5 summarizes the overall mass balance of the biomass-to-sugar conversion using two-stage fractionation (water followed by acidified  $\text{CaCl}_2$ ) and enzymatic hydrolysis under the optimum conditions. If 100 g of corn stover is subjected to the two-stage fractionation, 28.6 g of non-structural sugars will be recovered in the first stage, with a yield of 95.6%; these sugars can be used directly for the production of fuels and chemicals. After the first stage, a non-structural sugar-free solid is processed in the second stage using acidified  $\text{CaCl}_2$  (reaction temperature 172.9 °C, reaction time 22.2 min, and  $\text{CaCl}_2$  concentration 4.7 wt.%). From the second stage process, two different products streams are generated: solid and liquid; the liquid stream contains 2.6 g of glucan, 9.9 g of xylan, and 0.4 g of lignin, and the solid product, which contains 16.2 g of glucan, 1.1 g of xylan, and 6.9 g of lignin, is further subjected to enzymatic hydrolysis. After 72 h of enzymatic hydrolysis, 16.4 g of glucose can be obtained, with a yield of 91.0%. If this product is fermented, 8.4 g of ethanol can theoretically be produced.

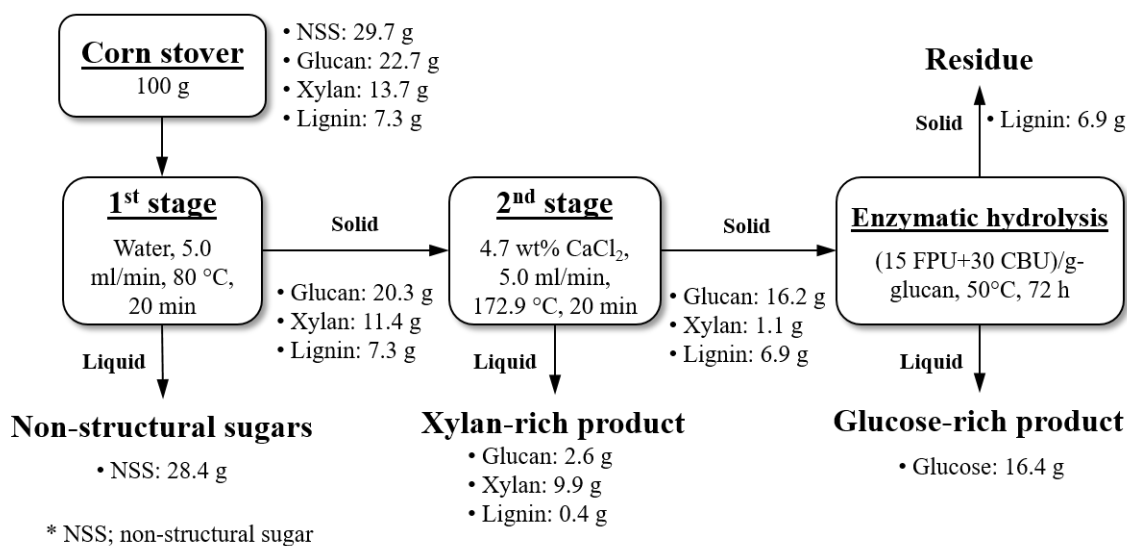


Fig. 5. Mass balance of two-stage processing of corn stover under optimum conditions

### CONCLUSIONS

1. The first stage of treatment using water at 80 °C effectively recovered non-structural sugars with a yield of 95.6%, and the second stage of treatment recovered xylan in the form of xylooligomers with a yield of 72.5% in the presence of acidified  $\text{CaCl}_2$  as the catalyst.

2. In addition, after 72 h of enzymatic hydrolysis, 91.0% of the saccharification yield was obtained, and the unsaccharified residue mostly contained lignin.
3. Two-stage fractionation using water and acidified CaCl<sub>2</sub> effectively recovered both non-structural sugars and xylooligomers; moreover, the treated solid was highly digestible in enzymatic hydrolysis.

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## REFERENCES CITED

- Bhutto, A. W., Qureshi, K., Harijan, K., Abro, R., Abbas, T., Bazmi, A. A., Karim, S., and Yu, G. (2017). "Insight into progress in pre-treatment of lignocellulosic biomass," *Energy* 122(1), 724-745. DOI: 10.1016/j.energy.2017.01.005
- Biagini, E., Barontini, F., and Tognotti, L. (2006). "Devolatilization of biomass fuels and biomass components studied by TG/FTIR technique," *Ind. Eng. Chem. Res.* 45(13), 4486-4493. DOI: 10.1021/ie0514049
- Brosse, N., Sannigrahi, P., and Ragauskas, A. (2009). "Pretreatment of *Miscanthus x giganteus* using the ethanol organosolv process for ethanol production," *Ind. Eng. Chem. Res.* 48(18), 8328-8334. DOI: 10.1021/ie9006672
- Cao, N. J., Xu, Q., and Chen, L. F. (1995). "Xylan hydrolysis in zinc chloride solution," *Appl. Biochem. Biotechnol.* 51(1), 97-104. DOI: 10.1007/BF02933414
- Casebier, R. L., Hamilton, J. K., and Hegert, H. L. (1969). "Chemistry and mechanism of water prehydrolysis on Southern pine wood," *TAPPI* 52(12), 2369-2377.
- Dizhbite, T., Zakis, G., Kizima, A., Lazareva, E., Rossinskaya, G., Jurkjane, V., Telysheva, G., and Viesturs, U. (1999). "Lignin – A useful bioresource for the production of sorption-active materials," *Bioresour. Technol.* 67(3), 221-228. DOI: 10.1016/S0960-8524(98)80004-7
- Escobar, J. C., Lora, E. S., Venturini, O. J., Yanez, E. E., Castillo, E. F., and Almazan, O. (2009). "Biofuels: Environment, technology and food security," *Renew. Sust. Energ. Rev.* 13(6-7), 1275-1287. DOI: 10.1016/j.rser.2008.08.014
- Kang, Q., and Tan, T. (2016). "Exergy and CO<sub>2</sub> analyses as key tools for the evaluation of bio-ethanol production," *Sustainability* 8(1), 76. DOI: 10.3390/su8010076
- Kim, T. H., and Lee, Y. Y. (2006). "Fractionation of corn stover by hot-water and aqueous ammonia treatment," *Bioresour. Technol.* 97(2), 224-232. DOI: 10.1016/j.biortech.2005.02.040
- Kim, T. H., Nghiem, N. P., and Hicks, K. B. (2009). "Pretreatment and fractionation of corn stover by soaking in ethanol and aqueous ammonia," *Appl. Biochem. Biotechnol.* 153(1-3), 171-179. DOI: 10.1007/s12010-009-8524-0
- Liu, L., Sun, J., Cai, C., Wang, S., Pei, H., and Zhang, J. (2009). "Corn stover pretreatment by inorganic salts and its effects on hemicellulose and cellulose degradation," *Bioresour. Technol.* 100(23), 5865-5871. DOI: 10.1016/j.biortech.2009.06.048

- Nghiem, N. P., Donnelly, M., and Sanville-Millard, C. Y. (2005). "A method to produce succinic acid from raw hydrolysates," World Intellectual Property Organization Patent No. 2005116227.
- Rasmussen, H., Sorensen, H. R., and Meyer, A. S. (2014). "Formation of degradation compounds from lignocellulosic biomass in the biorefinery: Sugar reaction mechanisms," *Carbohydr. Res.* 385(19), 45-57. DOI: 10.1016/j.carres.2013.08.029
- Selig, M., Weiss, N., and Ji, Y. (2008). *Enzymatic Saccharification of Lignocellulosic Biomass* (NREL/TP-510-42629), National Renewable Energy Laboratory, Golden, CO.
- Sener, S. E. C., Sharp, J. L., and Anctil, A. (2018). "Factors impacting diverging paths of renewable energy: A review," *Renew. Sust. Energ. Rev.* 81(2), 2335-2342. DOI: 10.1016/j.rser.2017.06.042
- Shi, X., Wu, Y., Yi, H., Rui, G., Li, P., Yang, M., and Wang, G. (2011). "Selective preparation of furfural from xylose over sulfonic acid functionalized mesoporous Sba-15 materials," *Energies* 4(4), 669-684. DOI: 10.3390/en4040669
- Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2008a). *Determination of Extractives in Biomass* (NREL/TP-510-42619), National Renewable Energy Laboratory, Golden, CO.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2008b). *Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples* (NREL/TP-510-42623), National Renewable Energy Laboratory, Golden, CO.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2012). *Determination of Structural Carbohydrates and Lignin in Biomass* (NREL/TP-510-42618), National Renewable Energy Laboratory, Golden, CO.
- Tajmir-Riahi, H. A. (1986). "Sugar-metal ion interaction. Synthesis, spectroscopic and structural analysis of Zn(II), Cd(II), and Hg(II) sugar complexes containing L-arabinose," *J. Inorg. Biochem.* 27(1), 65-74. DOI: 10.1016/0162-0134(86)80109-X
- Vazquez, M. J., Alonso, J. L., Donminguez, H., and Parajo, J. C. (2000). "Xylooligosaccharides: Manufacture and applications," *Trends Food Sci. Technol.* 11(11), 387-393. DOI: 10.1016/S0924-2244(01)00031-0
- Victor, A., Pulidindi, I. N., Kim, T. H., and Gedanken, A. (2016). "Design of a selective solid acid catalyst for the optimization of glucose production from *Oryza sativa* straw," *RSC Adv.* 6(1), 31-38. DOI: 10.1039/C5RA20121G
- Wildschut, J., Smit, A. T., Reith, J. H., and Huijgen, W. J. (2012). "Ethanol-based organosolv fractionation of wheat straw for the production of lignin and enzymatically digestible cellulose," *Bioresour. Technol.* 135, 58-66. DOI: 10.1016/j.biortech.2012.10.050
- Zabed, H., Sahu, J. N., Boyce, A. N., and Faruq, G. (2016). "Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches," *Renew. Sust. Energ. Rev.* 66, 751-774. DOI: 10.1016/j.rser.2016.08.038

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