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Two-Stage Fractionation of Corn Stover Using Aqueous Ammonia and Hot Water

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Abstract Hot water and aqueous ammonia fractionation of corn stover were used to separate hemicellulose and lignin and improve enzymatic digestibility of cellulose. A two-stage approach was used: The first stage was designed to recover soluble lignin using aqueous ammonia at low temperature, while the second stage was designed to recover xylan using hot water at high temperature. Specifically, the first stage employed a batch reaction using 15 wt.% ammonia at 60 °C, in a 1:10 solid:liquid ratio for 8 h, while the second stage employed a percolation reaction using hot water, 190–210 °C, at a 20 ml/min flow rate for 10 min. After fractionation, the remaining solids were nearly pure cellulose. The two-stage fractionation process achieved 68% lignin purity with 47% lignin recovery in the first stage, and 78% xylan purity, with 65% xylan recovery in the second stage. Two-stage treatment enhanced the enzymatic hydrolysis of remaining cellulose to 96% with 15 FPU/g of glucan using commercial cellulase enzymes. Enzyme hydrolyses were nearly completed within 12–24 h with the remaining solids fraction.

Keywords SAA (soaking in aqueous ammonia) · Biorefinery · Lignocellulosic biomass · Lignin · Xylan

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Introduction

Corn stover, lignocellulosic biomass, is considered one of the most promising feedstocks for the production of biofuels and chemicals since it is the biomass by-product of the single largest quantity in the USA [1]. Corn stover is a heterogeneous material consisting mainly of cellulose, hemicelluloses, and lignin, which can be converted to compounds that either have direct applications or can serve as precursors for important industrial chemicals currently produced from oil refinery. Fractionation of lignocellulosic biomass into cellulose, hemicelluloses, and lignin components has been proposed as the first step of biomass refining to high value-added products [2].

Glucose, which is the main component of cellulose (C6 stream), can be used as a feedstock for bio-based industry such as cellulosic ethanol, food, and pharmaceutical companies. Glucose is currently being produced from sugar cane and corn for ~6 cents/kg (April 2010) [3], but these feedstocks cannot meet the high demand for sugars in the future. C6 (mainly glucose) sugar produced from relatively cheap substrates like lignocellulosic biomass has potential to be a valuable feedstock in the future biotechnology and food industry. Xylose, which is the main component of hemicellulose (mainly C5 sugars) stream, can also be converted to other products besides ethanol. These products include fuels such as hydrogen [4] and chemicals such as succinic acid [5], xylitol [6], and xylo-oligosaccharides [7]. Xylo-oligosaccharides (XO) are of particular interest since they have high values and can potentially be used in a wide range of applications, including food additives, nutraceuticals, and pharmaceuticals [7–12]. Lignin, a complex compound that is comprised of aromatic rings that are linked by C–O–C and C–C, can be broken into smaller segments using existing facilities in oil refinery plants while maintaining the aromatic ring structures.

In order to realize the effective utilization of lignocellulosic material and to develop an economically viable biorefinery process, separation of each component with high purity is essential. Production of high value products from each component will have strong impacts on the future economics of the biofuel and bio-based industry. This study is important since it demonstrates one possible upstream biorefinery concept utilizing biorenewable materials effectively.

Two-stage fractionation process using aqueous ammonia and hot water was developed in this study. This fractionation process consists of low molecular weight of lignin separation using aqueous ammonia at low temperature and hemicelluloses separation with hot water (Fig. 1). Kim and Lee have reported aqueous ammonia pretreatment at low temperature (60–80 °C) retained most of carbohydrates (cellulose+hemicellulose) with solids, while it removed lignin significantly [13–15]. It was also reported that autohydrolysis using hot water treatment (190–210 °C) was effective for hemicellulose hydrolysis [14, 16–18].

Here, the effects of various reaction conditions such as reaction time, flow rate, and reaction temperature in each stage are discussed to determine the proper range of the process conditions. Composition analysis and enzymatic digestibility tests after fractionation process were conducted to evaluate the efficacy of fractionation conditions.

Materials and Methods

Materials

Air-dried ground corn stover was supplied by the National Renewable Energy Laboratory (NREL, Golden, CO, USA). The corn stover was screened to a nominal size of 9–35 mesh. The

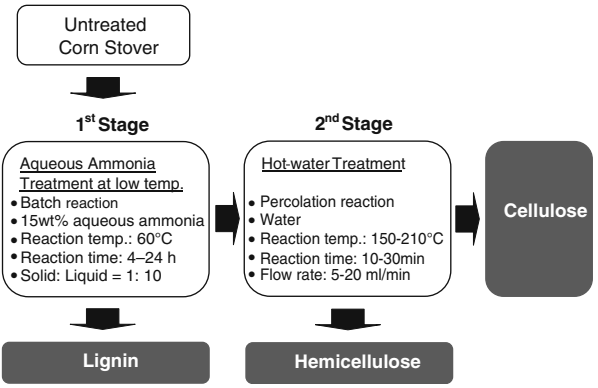

Fig. 1 Flow diagram of multi-stage fractionation process

composition of corn stover was determined by our lab following the chemical analysis and testing standard method developed by NREL [19]. The initial composition of the corn stover was 34.2 wt.% glucan, 22.3 wt.% xylan, 1.6 wt.% galactan, 3.1 wt.% arabinan, 12.2 wt.% lignin (acid insoluble+acid soluble), 3.9 wt.% acetate, 2.2 wt.% sucrose, 1.6 wt.% protein, 4.0 wt.% uronic acid, 1.2 wt.% ash, and 10.7 wt.% other extractives.

Cellulase enzyme, GC 220 (Genencor International Inc., Lot No. 301-04232-162), was provided by Genencor International Inc. The average activity of the enzyme was 45 filter paper unit (FPU)/ml, and the protein was 184 mg/ml. The β-glucosidase enzyme, Novozyme 188 (Novo Inc., Lot No. 11K1088), was purchased from Sigma-Aldrich (St. Louis, MO, USA). Activity of β-glucosidase (Novozyme 188 from Novo Inc., Lot No. 11K1088) was 750 cellobiase unit (CBU)/ml.

Experimental Setup and Operation

Reaction conditions explored and process scheme are summarized in Fig. 1.

First Stage (Aqueous Ammonia Treatment at Low Temperature)

Corn stover was treated with 15 wt.% of aqueous ammonia in glass media bottles (Fisher Cat No. 06-141-1C) at 60 °C for 4–24 h. Solid:liquid ratio was kept at 1:10. After completion of treatment, the solids and liquids were separated by fluted filter paper (Fisher Cat No. 09-790-14F), and solids were washed with de-ionized (DI) water using vacuum filter until the wash water had a neutral pH. Solid cakes were dried in the air in our lab until the moisture content of samples reached approximately 10% (drying conditions: ambient temperature and 48–72 h of drying time) and stored in the refrigerator for the second-stage hot water treatment.

Second Stage (Hot Water Treatment)

The reactor system for the second-stage treatment consists of flow-through column reactor with preheating coil, high performance liquid chromatography (HPLC) pump (Series II pump, Chrom Tech, Inc., Apple Valley, MN, USA), temperature-programmable gas chromatography (GC) oven (Hewlett Packard 5890, HP Inc., Mississauga, ON, Canada), solution reservoir, and sample cylinder tanks Nos. 1 and 2. A schematic diagram of the laboratory reactor setup is shown in Fig. 2. The reactor (70 cm^3 of internal volume) was

constructed out of 6.5 in. of SS-316 tubing with an internal diameter of 0.9 in. Two 1,000-ml SS 304 cylinders were used as receiver tanks for collecting the liquid products.

Corn stover was treated using aqueous ammonia in the first stage and dried in the air as described above. Ten dry-gram of ammonia-treated sample was packed into the flow-through type reactor. The packed bed reactor with preheating coil was placed in a temperature-programmable GC oven for temperature control. The packed bed reactor with preheating coil was preheated to a desired temperature, at which point, the water was pumped into the reactor through a preheating coil by a HPLC pump. After the GC oven was brought up to temperature, the HPLC pump supplied water to fill the preheating coil (not the reactor). The pump was then turned off, while the reactor set and the water in the coil preheated until it reached the target temperature (~15 min). The pump was then turned on again, while 300 psi of N_2 backpressure was applied to the reactor system to maintain the system pressure above the saturated pressure of water. Preheating time was not included in the reaction time. The second-stage fractionations were tested under the following reaction conditions: 170–210 °C of reaction temperature and 5.0–15.0 ml/min of flow rate.

Enzymatic Digestibility Test

The enzymatic digestibilities of solid samples obtained from two-stage fractionation were determined in duplicate according to the NREL Chemical Analysis and Testing Standard Procedure [20]. Treatment conditions used for sample preparations were as follows: two-stage treatment, 24 h, 60 °C; 15 wt.% aqueous ammonia; 1:10 of solid:liquid in the first-stage treatment; and two different temperatures covering 170 °C and 210 °C with the flow rates of 10 and 20 ml/min of hot water in the second stage. The conditions of the enzymatic digestibility test were pH 4.8 (0.05 M sodium citrate buffer) on a shaker bath agitated at 150 rpm at 50 °C. Enzyme loadings were 15 FPU of GC-220/g of glucan supplemented with 30 CBU of β-glucosidase (Novozyme 188)/g-glucan. The initial glucan concentration was 1% (*w*/*v*) based on 100 ml of total liquid and solid [20]. All the samples used in the digestibility tests were wet solid samples as collected from various pretreatments. The 250-ml

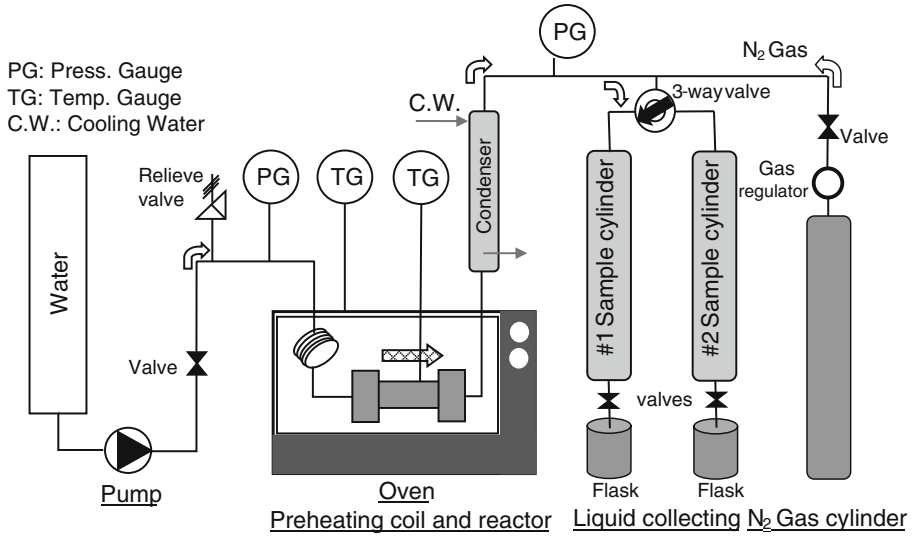

Fig. 2 Laboratory reactor setup

screw-capped Erlenmeyer flasks containing the enzyme hydrolysis preparations were placed in an incubator shaker (New Brunswick Scientific, Edison, NJ, USA). Samples were taken periodically (6, 12, 24, 48, 72, and 96 h) and analyzed for sugar contents using HPLC.

Solid and Liquid Sample Analyses

Solid samples were analyzed for carbohydrates (sugars) and lignin following the two-stage acid-hydrolysis procedures given in NREL Chemical Analysis and Testing (CAT) Standard Procedures [19]. Each sample was analyzed in duplicate. The conditions of the first hydrolysis were 72 wt.% sulfuric acid, 1:10 of solid-to-liquid ratio, and 30 °C for 1 h. The conditions for the secondary hydrolysis were 4 wt.% sulfuric acid and 121 °C for 1 h. Sugars in the hydrolyzates were determined by HPLC using a Bio-Rad Aminex HPX-87P column coupled with a refractive index detector (Varian 356-LC, Varian, Inc., Palo Alto, CA, USA). For the acid-insoluble lignin analysis, the autoclaved hydrolysis solution was vacuum filtered, and the filtered hydrolyzed solid sample was dried and weighed. The dried samples were then combusted in a furnace at 575±25 °C for 3 h to determine the ash content. The difference of the two weights was taken as the acid-insoluble lignin. The absorbance of the hydrolysis liquor in the aliquot obtained from the vacuum filter sample at 320 nm on a UV–Visible spectrophotometer measured the acid-soluble lignin.

Carbohydrates in the liquid samples were determined by secondary acid hydrolysis (conditions: 4 wt.% H_2SO_4, 121 °C, and 1 h) following the NREL Standard Analytical Procedure [21].

Recovery Yield, Purity, and Fractionation Factor (k)

In order to quantitatively evaluate the fractionation effects of various reaction conditions on each component (glucan, xylan, and lignin), recovery ratio (or yield) and purity of each component were defined as follows:

$$\text{Xylan } (R_X) \text{ or lignin recovery}(R_L)[\%] = \frac{\text{Xylan or lignin [wt.\%] in liquid}}{\text{Xylan or lignin [wt.\%] in untreated corn stover}} \times 100$$

$$\text{Xylan (xylooligomer)}(P_X) \text{ or lignin purity (in liquid)}(P_L)[\%] = \frac{\text{Xylan or lignin [wt.\%] in liquid}}{\text{(Glucan + xylan + lignin) [wt.\%] in liquid (hydrolysate)}} \times 100$$

In the liquid hydrolysates, other sugar components such as galactan and arabinan were also released with water or ammonia, but the amounts of these sugars were relatively small compared to glucan, xylan, and lignin components. Therefore, these other sugars were not considered in the calculation of xylan and lignin purities to simplify this study.

In order to combine the above terms, we also introduced a new term, fractionation factor k, which combined purity and recovery in one variable and was defined as follows:

$$k_X = \left[\frac{(R_X \times \eta_1) + (P_X \times \eta_2)}{\eta_1 + \eta_2}\right] / 100$$

$$k_L = \left[\frac{(R_L \times \eta_1) + (P_L \times \eta_2)}{\eta_1 + \eta_2}\right] / 100$$

where η_1 and η_2 are weighting factors of purity and recovery, respectively.

Comparison of recovery yields and purities obtained from various fractionation conditions were simplified with the used of fractionation factors k_X and k_L.

In general, trade-off between purity and recovery yield was observed in our study. Although both purities and recovery yields are considered to be important, further study is needed to determine the range of each weighting factor (e.g., techno-economic analysis). It was unclear how each factor affects the overall fractionation economics. In this study, these weighting factors were assumed to be equally important ($\eta_1=\eta_2=1$). The compositions of solid and liquid samples were analyzed to calculate the purity, recovery, and k factor.

Results and Discussion

Effects of the First-Stage Reaction Time

The compositional changes in solids after first-stage fractionation are summarized in Table 1. In the series of experiments, four different reaction times (4, 8, 12, and 24 h) were attempted at 60 °C to find the lignin recovery keeping solid-to-liquid ratio at 1:10 and ammonia concentration of 15 wt.%. Solubilization of xylan was only 6–15% within 4–24 h of reaction time. Nearly 100% of glucan was retained at all reaction times. Approximately 25% of the lignin was recovered within 4 h of treatment, and the lignin recovery (R_L; 47–62%) increased as reaction time (8–24 h) increased, but effect of reaction time had no significant effect on recovered lignin purity (P_L) (67–70%). When $\eta_1=\eta_2=1$ was assumed, k_L increased as reaction time increased, and 24-h treatment resulted in the highest k_L (0.65). These first-stage fractionation results support the previous reports (Kim and Lee [15]) that the first-stage treatment using aqueous ammonia removes lignins, while hemicellulose, mainly xylan, hydrolysis was not substantial. Data in Table 1 also indicated that 24 h of reaction time in the first stage can be selected as the best reaction time in terms of lignin recovery (R_L).

Although 24 h of reaction time in the first stage was the optimum reaction condition for the lignin fractionation, overall performance of the fractionation process must be determined by considering other factors such as xylan recovery (R_X) and purity (P_X) in

Table 1 Effects of reaction times on the compositions in the first stage

Reaction time [h]	Solid				Yield in liquid		
	SR [%]	Glucan [%]	Xylan [%]	Lignin[a] [%]	Lignin purity [%]	Lignin recovery [%]	k_L
Untreated	100.0	34.2	22.3	12.2	–	–	–
4	71.7	34.1	20.9	9.1	67.4	25.4	0.46
8	69.6	34.0	19.8	6.5	67.9	46.7	0.57
12	68.0	34.4	19.5	6.1	70.1	50.0	0.60
24	65.2	34.0	19.0	4.7	68.2	61.5	0.65

The data in the table show the mean value ($n=2$, SD<0.3 for glucan, xylan, and lignin in solid, SD<1.8 for lignin purity, SD<2.3 for lignin recovery, SD<0.1 for k_L). Data in the table are based on the oven-dried untreated corn stover. Fractionation conditions: 15 wt.% of ammonia concentration, 60 °C of reaction temperature, and solid:liquid ratio=1:10 (based on weight)

SD standard deviation, *SR* solid remaining after reaction

[a] Acid-insoluble lignin+acid-soluble lignin

the second stage and cellulose recovery yield, and digestibility after fractionation. To test effects of first-stage reaction times on xylan fractionation in the second stage, the hot water treatment was then conducted, keeping reaction conditions at 210 °C, 20.0 ml/min, and 10 min. R_X and P_X data are summarized in Fig. 3. P_X increased (63→79%) as the reaction time of the first stage, while the R_X decreased (67→51%), and 8-h treatment in the first stage resulted in the highest k_X value (0.72) in the second stage. This result indicated that 8 h of reaction time in the first stage was the best reaction time in terms of the xylan recovery (R_X) in the second stage.

Effects of Flow Rate in the Second Stage on Xylan Fractionation

The two-stage fractionation experiments of corn stover were conducted; the reaction conditions of the first stage treatment were 4 and 8 h, 60 °C, 15 wt.% aqueous ammonia, and 1:10 of solid:liquid. To evaluate the effects of various flow rates in the second stage, three to four different flow rates—(1) first series of run: 7.5, 10, 15, and 20 ml/min for the 4-h-treated corn stover in the first stage, and (2) second series of run: 10, 15, and 20 ml/min for the 8-h-treated corn stover in the first stage—were applied at 210 °C, maintaining reaction time at 10 min.

The P_X, R_X, and k_X values in the first series of run are presented in Fig. 4a. Low R_X (51%) at 7.5 ml/min was observed with significant amounts of released xylose decomposition. As the flow rate increase from 7.5 to 15 ml/min, R_X increased and reached maximum (72%) with 15 ml/min of flow rate, while P_X (70–71%) did not change much with different flow rate. However, purity, recovery, and k_X of xylan decreased when the flow rate was 20 ml/min. Therefore, 15 ml/min was preferred so as to obtain high R_X and k_X in this study. We assumed that low flow rate (<15 ml/min) resulted in longer residence time for xylan decompositions (43%) in the flow-through reactor. It was also speculated that the excessively higher flow rate reduced the residence time of hot water in the percolation reactor, and thus, xylan was not sufficiently hydrolyzed (67%) from corn stover than 15 ml/min case (72%). It was also able to explain the following results: At 7.5 ml/min, 80% of xylan was hydrolyzed from solid, while 74% was hydrolyzed by hot water at 20 ml/min.

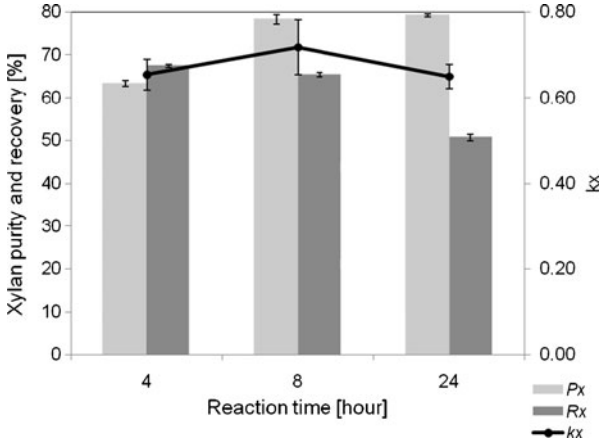

Fig. 3 Effects of first-stage reaction time on xylan fractionation in the second stage. Treatment conditions: 15 wt.% of ammonia, 60 °C, solid:liquid=1:10, 4–24 h; hot water, 20.0 ml/min, 210 °C, 10 min; the *data points and bars* in the graph show the mean value (n=2); data are based on the oven-dried untreated corn stover

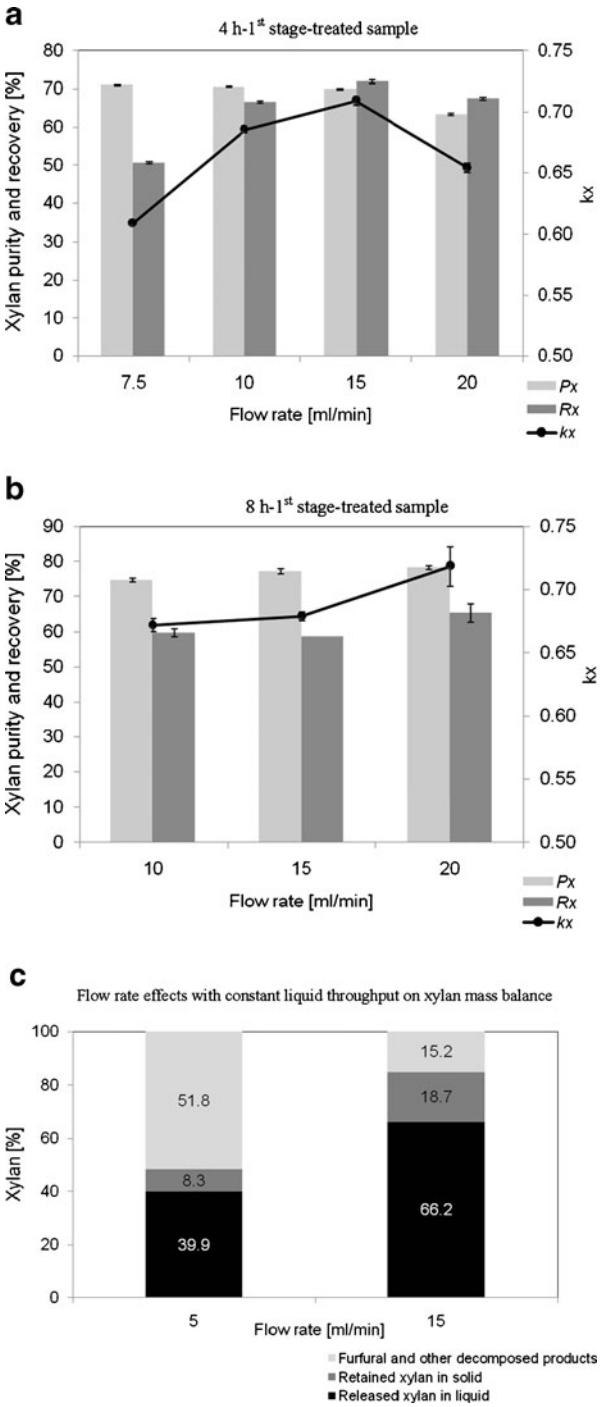

Fig. 4 Effects of flow rate of hot water on xylan fractionation in the second stage. The *data points and bars* in the graph show the mean value (n=2). **a**, **b** Reaction conditions: 15 wt.% of ammonia, 60 °C, solid:liquid=1:10, 4–8 h; hot water, 210 °C, 10 min. **c** Reaction conditions: 15 wt.% of ammonia, 60 °C, solid:liquid ratio=1:10, 8 h; hot water, 210 °C, 10–30 min; total amount of water throughput, 150 ml

The P_X, R_X, and k_X values in the second series of run are presented in Fig. 4b. In this experiment, P_X and R_X increased (78% and 65%, respectively) as the flow rate increased over all attempted flow rate range from 10 to 20 ml/min, which was different from those observed in the earlier experiments. Increased R_X with 20 ml/min may correlate with the reaction severity in the first-stage treatment; 8-h treatment may result in more open structure than 4-h treatment, and then, the residence time with 20 ml/min was still sufficient enough to hydrolyze the xylan in the corn stover structure.

In the earlier series of run using 4-h first-stage treated sample, the degradation of xylan at low flow rate was observed. To compare the decompositions of xylan at different flow rate more clearly, another set of experiments was conducted keeping the amount of hot water throughput constant at 150 ml (Fig. 4c). Two different flow rates were tested, and conditions were 5 ml/min for 30 min and 15 ml/min for 10 min. Figure 4c shows the effects of flow rate of hot water on the amounts of decomposed xylan during the hot water fractionation stage using the same amount of liquid throughput. At these conditions, the residence time with 5 ml/min using flow-through column reactor was longer than the time with 15 ml/min. Therefore, released sugar with 5 ml/min more easily underwent decomposition than with 15 ml/min at the same reaction temperature. At 5 ml/min, 52% of xylan (based on total xylan in untreated corn stover) was degraded to furfural, formic acid, and other decomposed products, while only 15% of xylan was decomposed in 15 ml/min case. Therefore, R_X with 15 ml/min was 59%, which was higher than 35% with 5 ml/min to 30 min.

Effects of Reaction Temperature in the Second Stage on Xylan Fractionation

The effects of reaction temperature of hot water treatment on P_X and R_X were tested using two different sets of reaction conditions: (1) first stage, 4 h, 15 wt.% aqueous ammonia 60 °C, 1:10 of solid:liquid; second stage, three different temperatures covering 170–210 °C with the flow rates of 15.0 ml/min; (2) first stage, 12 h, 15 wt.% aqueous ammonia 60 °C, 1:10 of solid:liquid; second stage, four different temperatures covering 150–210 °C with the flow rates of 5.0 ml/min. The P_X, R_X, and k_X are presented in Fig. 5.

In the first set of runs (Fig. 5a) with 15 ml/min of hot water flow rate, the maximum P_X (74%) and R_X (72%) were obtained under conditions of hot water fractionation at 190 °C and 210 °C, respectively. On the other hand, in the second set of runs (Fig. 5b) with 5 ml/min of hot water flow rate, R_X increased as temperature increased; on the contrary, decrease of P_X was observed as temperature increased; this explains that remaining lignin in corn stover also underwent the solubilization reaction. Fractionation factor, k_X, increased with reaction temperature up to 190 °C (k_X=0.62) then decreased at 210 °C. Overall, 5.0 ml/min of hot water fractionation recovered xylan at low yields, which were only in the range of 12.5–43.9%.

Enzymatic Digestibility of Fractionated Corn Stover

With the remaining solid samples after two-stage treatment, enzymatic digestibility tests were conducted using GC-220 cellulase supplemented with Novozyme 188 β-glucosidase enzymes. Enzymatic digestibility test results are presented in Fig. 6. Enzyme digestibilities of two-stage treated corn stover were 91–97% with 15 FPU/g of glucan. Digestibility (at 72 h) of 210 °C-treated corn stover was substantially higher than 170 °C-treated samples, and glucan hydrolysis of 210 °C-treated samples were nearly completed only within 24 h. It should be noted that shorter enzyme hydrolysis time is considered to be important from the economic point of view because enzyme hydrolysis is the limiting step due to the slower

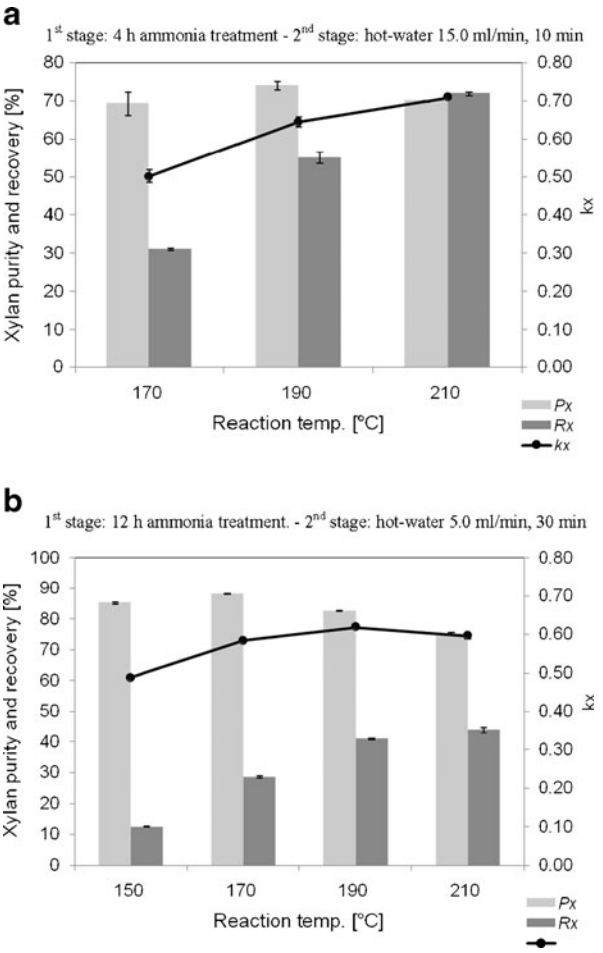

Fig. 5 Effects of reaction temperature of hot water on xylan fractionation in the second stage. Treatment conditions: 15 wt.% of ammonia, 60 °C, solid:liquid= 1:10, 4–12 h; hot water, 5.0–15.0 ml/min, 10–30 min: the *data points and bars* in the graph show the mean value (n=2)

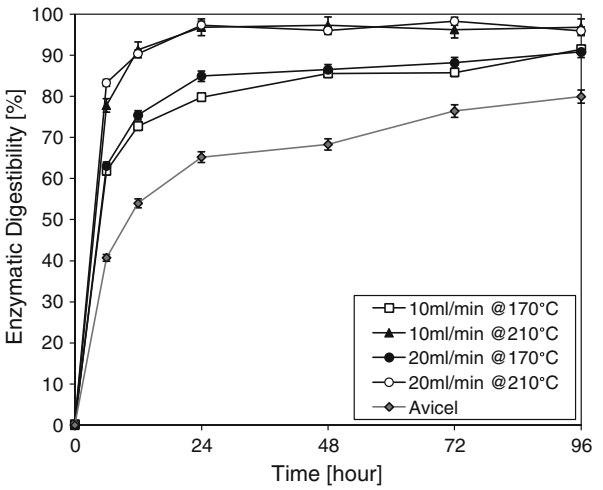

Fig. 6 Enzymatic digestibility of two-stage treated corn stover. Treatment conditions: 15 wt.% of ammonia, 60 °C, solid:liquid= 1:10, 24 h; hot water, 20.0 ml/min, 10 min, 170–210 °C; enzymatic hydrolysis conditions, 15 FPU of GC 220/g-glucan and 30 CBU of Novozyme 188/g-glucan, pH 4.8, 50 °C, 150 rpm; the *data points* in the graph show the mean value (n=2)

reaction rate than that of microbial reaction in bioconversion of cellulosic using simultaneous saccharification and fermentation (SSF).

The impacts of treatment conditions on the composition of solids and liquids, and on the enzymatic digestibility of solids, are summarized in Table 2. Increasing temperature of the second stage from 170 °C to 210 °C reduced the xylan and lignin contents of the treated solids, regardless of flow rate, with the most significant composition change in the xylan: At 10 ml/min, xylan and lignin removals were increased 42% to 80% and 72% to 80%, respectively, while at 20 ml/min, xylan and lignin removals were increased 51% to 85% and 73% to 93%, respectively. These results probably reflect that the xylan was more labile in this temperature range. In contrast, glucan content remained relatively constant.

The glucan and xylan digestibilities of the untreated corn stover were 19% and 13% at 15 FPU/g of glucan, respectively. At both flow rates, the glucan and xylan digestibilities of the 170 °C-treated samples were all above 73%, while those of the 210 °C-treated samples were all above 97%. Second-stage temperature appears far more important than flow rate in determining the enzymatic digestibility of treated solids. It appears that the reduced xylan and lignin contents in the treated solid samples contributed to the improved enzyme digestibility of the remaining solids, but there is no clear evidence regarding which of these components contribute most significantly to the enhanced digestibility.

Conclusion

Three main components of corn stover, glucan, xylan, and lignin, were effectively fractionated by fractionation processes using aqueous ammonia and hot water. The best two-stage treatment conditions were observed as follows: first stage, 15 wt.% aqueous ammonia for 8 h at 60 °C and solid:liquid ratio of 1:10; second stage, 20 ml/min hot water at 190–210 °C for 10 min. Under these conditions, (1) 78% of P_X, 65% of R_X, and 0.72 of

Table 2 Effects of flow rate and temperature on the solid–liquid compositions and enzymatic digestibility after the two-stage fractionation

Flow rate [ml/min]	Temperature [°C]	SR [%]	Solid			Liquid		Enzymatic digestibility[a]	
			Glucan [%]	Xylan [%]	Lignin[b] [%]	Glucan [%]	Xylan [%]	Glucan [%]	Xylan [%]
Untreated		100	34.2	22.3	12.2	–	–	19.0	12.5
10	170	54.4	34.2	13.0	3.4	0.9	6.1	79.8	73.1
	210	50.9	33.0	4.4	2.5	1.9	10.7	96.8	97.4
20	170	40.3	33.6	10.9	3.3	0.8	7.8	84.9	78.4
	210	38.7	32.2	3.4	0.8	1.8	11.3	97.4	100.0

The data in the table show the mean value (n=2, SD<0.5 for glucan, xylan, and lignin in solid). Data in the table are based on the oven-dried untreated corn stover. Fractionation conditions: first stage, 15 wt.% of ammonia concentration, 60 °C of reaction temperature, 24 h or reaction time, and solid:liquid ratio=1:10 (based on weight); second stage, hot water, 10 min, and 170–210 °C

SD standard deviation, *SR* solid remaining after reaction

[a] Digestibility at 72 h, enzymatic hydrolysis conditions: 15 FPU of GC 220/g-glucan and 30 CBU of Novozyme 188/g-glucan, pH 4.8, 50 °C, 150 rpm

[b] Acid-insoluble lignin+acid soluble lignin

k_X and (2) 68% of P_L, 47% of R_L, and 0.57 of k_L were obtained. The remaining solid contains mostly cellulose (>86%) after fractionation process, and it enhanced enzymatic digestibility of treated solid sample (97%).

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