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Fractionation of corn stover by hot-water and aqueous ammonia treatment

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Abstract

The efficiency of biomass utilization can be significantly improved by fractionation of biomass. A two-stage percolation process was investigated for pretreatment and fractionation of corn stover. The two-stage process is composed of hot water treatment followed by treatment with aqueous ammonia, both applied in a flow-through (percolation) reactor. The first stage processing is intended for hemicellulose removal whereas the second stage is intended for delignification. The pretreated material was nearly pure cellulose and both reagents are cheap and environmentally friendly. The conditions that achieve satisfactory level of biomass fractionation and acceptable enzymatic hydrolysis were identified in terms of reaction temperature, flow rate (retention time) and reaction time for each stage. With proper operation of two-stage treatment, fractionation of biomass was achieved to the extent that the xylan fraction is hydrolyzed with 92–95% conversion, and recovered with 83–86% yields; and the lignin removal is 75–81%. The remaining solid after two-stage treatment contained 78–85% cellulose. The two-stage treatments enhanced the enzymatic digestibility to 90–96% with 60 FPU/g of glucan, and 87–89% with 15 FPU/g of glucan. In two-stage treatment, the composition and digestibility data indicate that the lignin content in the biomass is one of the major factors controlling the enzymatic digestibility.

Keywords: Corn stover; Pretreatment; Fractionation; Enzymatic hydrolysis; Lignin

1. Introduction

Corn stover is currently regarded as the most promising biomass resource in the US with 60–80 million tons/yr available for conversion into fuels and chemicals (Kadam and McMillan, 2003). Production of corn stover roughly equals the mass of corn kernels (USDOE, 2001). Pretreatment is one of the key elements in the bioconversion of this biomass. It is required for efficient enzymatic hydrolysis of biomass because of the physical and chemical barriers that inhibit the accessibility of enzyme to the cellulose substrate (Saddler, 1993). Among the known chemical barriers are lignin, hemicellulose (Schwald et al., 1988), and acetyl group (Chang and Holtzapple, 2000; Grohmann et al., 1989; Kong et al., 1992). The physical factors of biomass, such as crystallinity (Caufield and Moore, 1974; Cowling and Kirk, 1976; Fan et al., 1980; Polcin and Bezuch, 1977; Sasaki et al., 1979; Schwald et al., 1988), surface area (Burns et al., 1989; Lee et al., 1995), and degree of polymerization (Puri, 1984), have also been known to influence the enzymatic hydrolysis. Among these factors, lignin has been considered as a major impeding factor (Chang

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and Holtzapple, 2000; Cowling and Kirk, 1976; Dulap et al., 1976; Lee et al., 1995; Mooney et al., 1998; Schwald et al., 1988). Main chemical bonds found in lignin are C–O–C and C–C linkages. Moreover, the existence of covalent bonds between lignin and carbohydrates, has also been verified by various researchers (Karlsson, 1997; Nikitin et al., 1971; Polcin and Bezuch, 1977), thus forming what is known as "lignin–carbohydrate complex (LCC)". They further postulated that there are three different types of linkages (ether, ester, and glycosidic linkage) between lignin and carbohydrate, the ester linkage being prevalent and stable. A number of researchers suggested that early removal of lignin would eliminate the interaction between lignin and cellulase enzyme making the enzymatic hydrolysis more efficient (Converse, 1993; Dulap et al., 1976; Millet, 1974; Van Soest, 1969).

Fractionation of biomass into the three main biomass constituents is a concept being developed as a means to improve the overall biomass utilization. Hemicellulose when separated from the biomass may find broader use for chemicals, fuel, and food application. The lignin separated in the process can be used as a fuel, the energy content being 26.3 MJ/OD kg of lignin (Saddler, 1993). Unlike the lignin generated from pulping process, lignin fractionated from biomass by our approach is relatively clean, free of sulfur or sodium. It is a feedstock suitable for other applications: binder, dispersant, emulsifier, or sequestrant (Adler, 1977; Lin and Lebo, 1995; Northey, 1992; Sarkanen and Ludwig, 1971).

In our laboratory, the ammonia recycle percolation (ARP) method has been investigated as a pretreatment method. It utilizes aqueous ammonia as the pretreatment reagent. This method has been proven to give high degree of delignification while keeping most of the cellulosic component in biomass intact. One of the problems associated with this process is that substantial amount of xylan is also removed along with lignin (Iyer et al., 1996; Kim and Lee, 1996; Kim et al., 2000; Yoon et al., 1995). Partial removal of xylan makes the total fractionation process complicated. To alleviate this problem, we have devised a two-stage process in which hot water treatment and the ARP were operated in succession. This process scheme was designed to separate hemicellulose sugars in the first stage and lignin in the second stage. The remaining solid thus contained mostly cellulose. Upon completion of this process, a total fractionation of biomass was achieved.

In this study, the proposed two-stage percolation process was investigated to assess its effectiveness as a method of pretreatment as well as fractionation scheme. Broad range of reaction and operating conditions were explored seeking optimum range of the process parameters that allowed satisfactory pretreatment and fractionation of corn stover.

2. Methods

2.1. Material

Air-dried ground corn stover was supplied by National Renewable Energy Laboratory (Golden, CO). The corn stover was screened to the nominal size of 9–35 mesh. The initial composition of corn stover as determined by NREL was: 37.5 wt.% glucan, 20.8 wt.% xylan, 2.7 wt.% arabinan, 0.8 wt.% mannan, 1.6 wt.% galactan, 17.6 wt.% Klason lignin, 6.7 wt.% ash, 2.2 wt.% acetyl group, 2.9 wt.% protein, 3.6 wt.% uronic acid and 3.6 wt.% unaccounted for. Alpha-Cellulose (Sigma-Aldrich Cat. No. C-8200, Lot No. 11K0246) was purchased from Sigma-Aldrich Corporate (St. Louis, MO). Cellulase enzyme, Spezyme CP (Lot No. 301-00348-257, Genencor International Corporate, Palo Alto, CA) was obtained from NREL. An average activity of the enzyme, as determined by NREL was: 31.2 filter paper unit (FPU)/ml and 20.0 cellobiase unit (CBU)/ml. Activity of β-glucosidase (Sigma-Aldrich Cat. No. G-0395) was 5.0 CBU/mg.

2.2. Experimental setup and operation

A schematic diagram of the reactor setup is shown in Fig. 1. The reaction and operating conditions are summarized in Fig. 2. The system consists of a stock solution reservoir, pump, temperature-programmable GC oven (Varian 3700, Varian Inc. Corporate, Palo Alto, CA), spring-loaded reactor, and liquid holding tanks #1 and #2, which also served as a backpressure vessel. Aqueous ammonia was pumped by metering piston pump to the reactor. The reactor (101.9 cm^3 of internal volume) was constructed out of 10 inches of SS-316 tubing with an ID of 9/10 inches. A 2.25 l and a 1.0 l SS304 cylinder were used as receiver tanks for the first and second stage treatments. In the ARP experiment, 10 g of dry biomass sample was packed into the reactor and soaked with ammonia solution overnight. The oven was preheated for 16–17 min and 2.5 MPa of N_2 backpressure was applied to the reactor system before reactor startup. After completion of the first stage, temperature shifting was done over 10-min period. A typical temperature profile for the two-stage processing is shown in Fig. 3. In the second series of runs, the biomass feedstocks were subjected to sequential two-stage treatment without intermittent sample taking. At the beginning of the second stage (ARP), the output effluent was switched into the second receiving tank by a 3-way valve. At the completion of the run, the reactor was pumped with water to remove the residual sugar and ammonia trapped in the treated biomass. The wet solids discharged from the reactor were separated into two portions. One was dried by moisture analyzer for measurement of weight loss, and subjected to composition

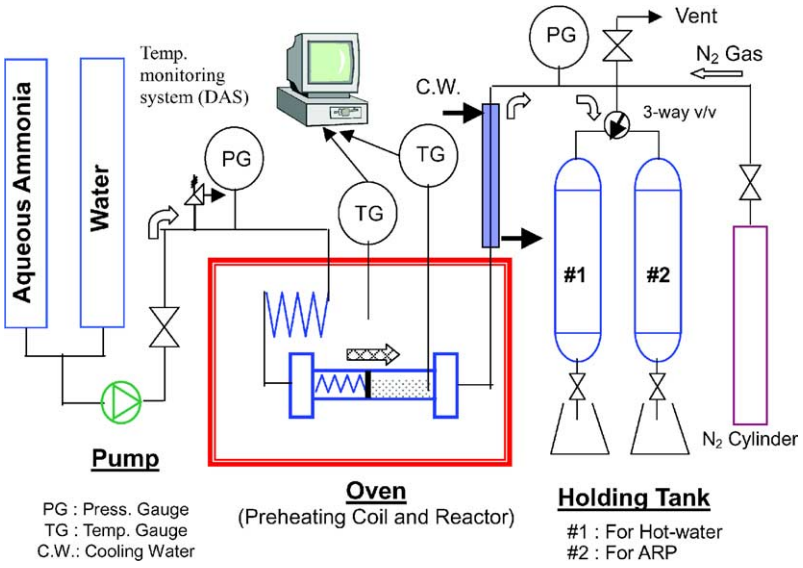

Fig. 1. Experimental set-up of two-stage percolation.

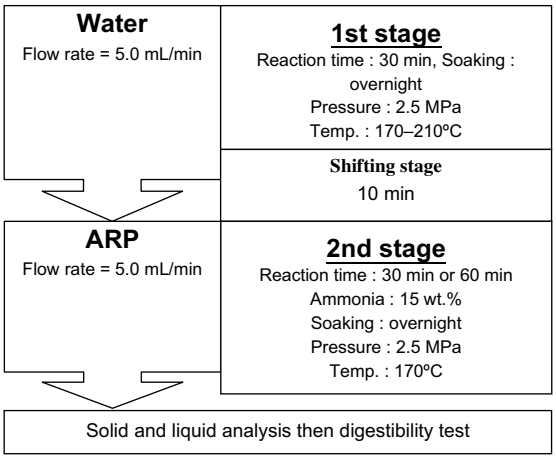

Fig. 2. Experimental conditions. *Note:* Cellulase enzyme (Spezyme CP, Lot 301-00348-257, activity: 31.2 FPU), 60 or 15 FPU/g of glucan, β-glucosidase supplement (Sigma-Aldrich, Cat. No. G-0395, 15 CBU/g glucan), pH 4.8, 50 °C, 150 rpm.

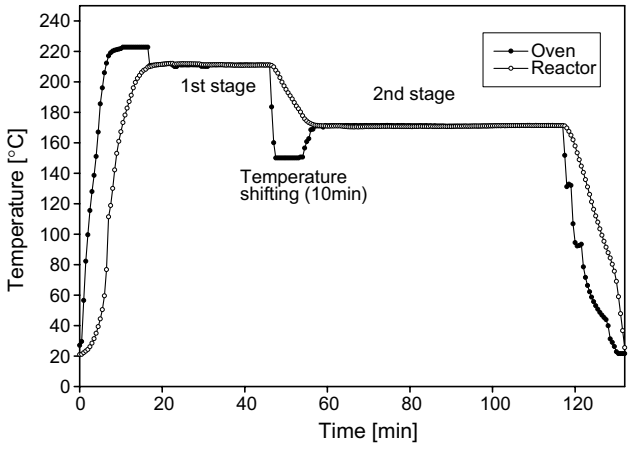

Fig. 3. Temperature profile of a typical two-stage treatment.

analysis. The other was used for the enzymatic digestibility test.

2.3. Digestibility test

The enzymatic digestibility of corn stover was determined in duplicates according to the NREL Chemical Analysis and Testing Standard Procedure No. 009 (NREL, 1996). The conditions of the enzymatic digestibility tests are 50 °C and pH 4.8 (0.05 M sodium citrate buffer) on a shaker bath agitated at 150 rpm. Enzyme loadings of 15 and 60 FPU of Spezyme CP/g of glucan supplemented with 15 CBU of β-glucosidase (Sigma-Aldrich Cat. No. G-0395) were used. The initial glucan concentration was 1% (w/v) based in 100 ml of total liquid. The 250 ml screw-capped Erlenmeyer flasks containing the enzyme hydrolysis preparations were placed in an Innova-4080 incubator shaker (New Brunswick Scientific, Edison, NJ). Samples were taken periodically at appropriate sampling times (6, 12, 24, 48, and 72 h) and analyzed for glucose and cellobiose content using HPLC (Bio-Rad Laboratories, Hercules, CA). Total released glucose after 72 h of hydrolysis was used to calculate the enzymatic digestibility. Alpha-cellulose and untreated corn stover were put through the same procedure as a reference and the control.

2.4. Analytical methods and SEM (scanning electron microscope)

Solid samples were analyzed for sugar and Klason lignin following the procedures of NREL Chemical Analysis and Testing Standard Procedures No. 001–

004 (NREL, 1996). Each sample was analyzed in duplicate. Sugars were determined by HPLC using a Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA), and YSI 2300 Glucose/Lactate analyzer (YSI incorporated, Yellow Springs, OH) (for some of the digestibility hydrolysate samples). A refractive index detector was used for HPLC. Sugars in the liquid samples were determined by secondary acid hydrolysis to account for the oligomer contents. Conditions of the secondary hydrolysis were 4 wt.% sulfuric acid and 121 °C for 1 h.

A scanning electron microscope (Zeiss-DSM940, Carl Zeiss Corporate, Oberkochen, Germany) was used to image the biomass samples.

2.5. Statistical analysis

A mean value and a standard deviation were calculated using JMP software version 5.0 (SAS Institute Inc., Cary, NC). SigmaPlot Version 8.0 (SPSS Inc., Chicago, IL) was used to plotting the results.

3. Results and discussion

3.1. Hot-water treatment

Under the hot-water treatment conditions, the hydronium ion initially causes xylan depolymerization and cleavage of the acetyl group. The autohydrolysis reaction then follows, in which, the acetyl group catalyzes the hydrolysis of the hemicellulose (Casebier et al., 1969; Fernandez-Bolanos et al., 1999; Lora and Wayman, 1978). For hot-water treatment, the effects of flow rate were first investigated to seek the reasonable range of operating conditions. In the first series of experiments, three different flow rates (2.5, 5.0, and 7.5 ml/min) were applied at 180 °C, keeping reaction time constant at 30 min (Table 1). In the second series, four different flow rates (1, 2.5, 5.0, and 7.5 ml/min) were applied 75 ml of total liquid throughput. For example, 75 min reaction time was given for 1 ml/min run and 10 min reaction time was given for 7.5 ml/min run (Table 2).

The first series was conducted to test effects of flow rate, and the second series for determination of optimal flow rate that maximizes the xylan yield. In hot-water treatment, the xylan was recovered mostly in soluble xylose oligomers. Xylan yield by hot water treatment generally increased as the flow rate increased with the highest xylan yield occurring at 7.5 ml/min (Table 1). However, it resulted in lower sugar concentration and high liquid throughput, which raises the processing cost. From the results of the second series of runs (Table 2), it became clear that reaction time of 30 min was required to attain substantial conversion of xylan. From above results, 2.5 and 5.0 ml/min with 30 min reaction time were selected as conditions to be pursued further.

The hot-water-only pretreatment was then tested at five or six different temperatures covering 170–220 °C and applying flow rates of 2.5 and 5.0 ml/min. The composition data after these treatments are summarized in Table 3. The xylan and lignin remaining in solid after treatments generally decreased as temperature is increased. Solubilization of xylan was 53–71% with 2.5 ml/min, and 58–86% with 5.0 ml/min of hot water. The data also indicated that reaction temperature above 190 °C was required for efficient solubilization of xylan,

The glucan content was well preserved. The accountability of glucan (glucan content in the solid plus that in liquid) was 97% for both flow rates. However, the accountability of xylan above 180 °C at 2.5 ml/min was less than 80%, indicating substantial amount of xylan is decomposed under those conditions. The xylan yield in liquid at 2.5 ml/min was lower than that at 5 ml/min for all temperature. Hot water treatment at 190–200 °C and 5 ml/min attained xylan recovery of 85%. The flow rate of 5 ml/min was therefore selected for the subsequent experiments.

Lignin removal in the hot-water-only treatment was in the range of 22–50%. We observed an unusual

Table 1
Effect of flow rate on the compositions in the hot-water-only treatment at 180 °C[a]

Flow rate [ml/m]	Solid				Liquid		Total		Yield in liquid	
	SR[b] (%)	Lignin[c] (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)
Untreated	100.0	17.6	37.5	20.8	–	–	37.5	20.8	–	–
2.5	63.1	12.1	36.5	4.5	0.8	13.9	37.3	18.5	2.0	67.0
5.0	58.1	11.8	35.9	4.3	1.3	15.6	36.8	19.9	3.4	74.9
7.5	58.6	11.9	36.6	4.0	1.2	17.4	37.8	21.5	3.1	83.8

Note: The data in the table show the mean value ($n = 2$; SE < 0.3% for K-lignin, SE < 0.8% for SR, SE < 0.3% for glucan and xylan in solid and liquid, SE: standard error).

[a] Data in the table based on the oven dry untreated biomass. Pretreatment conditions: 30 min, 2.5 MPa. All reactions are carried out in a bed-shrinking flow-through (BSFT) reactor.

[b] SR stands for solid remaining after reaction.

[c] Klason lignin.

Table 2
Effect of flow rate and reaction time on the compositions in the hot-water-only treatment at 180 °C[a]

Flow rate [ml/m]	Reaction time [ml/m]	Solid				Liquid		Total		Yield in liquid	
		SR[b] (%)	Lignin[c] (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)
Untreated	–	100.0	17.6	37.5	20.8	–	–	37.5	20.8	–	–
1.0	75	61.8	14.7	37.4	3.5	0.4	14.3	37.8	17.8	1.1	68.9
2.5	30	63.1	12.1	36.5	4.5	0.8	13.9	37.3	18.5	2.0	67.0
5.0	15	67.2	14.7	37.3	7.5	0.6	12.4	37.9	20.0	1.6	59.8
7.5	10	72.0	15.5	37.9	10.4	0.4	9.1	38.3	19.5	1.0	43.9

Note: The data in the table show the mean value ($n = 2$; SE < 0.3% for K-lignin, SE < 0.8% for SR, SE < 0.3% for glucan and xylan in solid and liquid, SE: standard error).

[a] Data in the table based on the oven dry untreated biomass. Pretreatment conditions: 2.5 MPa. All reactions are carried out in a bed-shrinking flow-through (BSFT) reactor.

[b] SR stands for solid remaining after reaction.

[c] Klason lignin.

Table 3
Effect of temperature on composition in hot-water-only pretreatment[a]

Temperature (°C)	Solid				Liquid		Total		Yield in liquid		Digestibility[b]	
	SR[c] (%)	K-lignin[d] (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	60 FPU (%)	15 FPU (%)
Untreated	100	17.6	37.5	20.8	–	–	37.5	20.8	–	–	21.2	16.1
2.5 ml/min												
170	69.1	13.1	36.9	8.0	0.9	11.9	37.8	19.9	2.3	57.4	–	–
180	63.1	12.1	36.5	4.5	0.8	13.9	37.3	18.5	2.0	67.0	–	–
190	57.9	10.9	36.3	1.9	1.0	14.9	37.3	16.8	2.6	71.4	–	–
200	56.6	10.3	35.8	1.5	1.1	13.8	36.9	15.3	2.8	66.4	–	–
210	56.9	13.6	35.4	1.0	1.2	11.1	36.5	12.1	3.0	53.5	–	–
5.0 ml/min												
170	65.4	13.5	36.8	8.0	1.0	12.1	37.5	20.1	2.7	58.4	59.1	47.5
180	58.1	11.8	35.9	4.3	1.3	15.6	36.8	19.9	3.4	74.9	73.9	62.7
190	55.0	11.3	36.3	2.6	1.5	17.9	37.4	20.5	4.1	86.0	86.8	71.6
200	53.0	10.3	36.2	1.4	1.9	17.7	37.8	19.1	5.1	85.3	90.9	76.6
210	51.0	8.8	35.7	1.1	2.0	17.6	37.3	18.7	5.3	84.7	93.6	88.9
220	50.5	10.1	34.0	0.1	2.8	13.2	36.5	13.3	7.5	63.3	95.0	93.3

Note: The data in the table show the mean value ($n = 2$; SE < 0.2% for K-lignin, SE < 0.9% for SR, SE < 0.3% for glucan and xylan in solid and liquid, SE < 2.0% for digestibilities, SE: standard error).

[a] Data in the table based on the oven dry untreated biomass; hot-water-only treatment conditions: 2.5 or 5.0 ml/min, 30 min, 2.5 MPa. All reactions are carried out in a bed-shrinking flow-through (BSFT) reactor.

[b] Digestibility at 72 h. Enzymatic hydrolysis conditions: 60 or 15 FPU/g of glucan, pH 4.8, 50 °C, 150 rpm.

[c] SR stands for solid remaining after reaction.

[d] Klason lignin.

behavior that Klason lignin content of the treated solid sample decreased with temperature up to a certain point then increased again above that temperature (between 200 and 210 °C with 2.5 ml/min, and 210 and 220 °C with 5.0 ml/min). This behavior appears to be related with other lignin-related reactions. As the hot water traveled through the flow-through reactor, the lignin released into liquid may undergo side reactions at high temperatures. Similar phenomenon was also observed in the ensuing experiments, which is discussed separately in the next section.

The 72-h digestibilities of samples treated at various temperatures are shown in Fig. 4. Here again, an interesting result was observed in that there was a sharp rise of digestibility with 15 FPU/g of glucan between 200 and 210 °C. This seemed to be related with the lignin content in the solid samples because the difference of lignin removal between these two temperatures was higher than the difference of the other temperature intervals. Hot-water treatment at 220 °C gave the highest digestibility although the lignin value increased from that of 210 °C. This is yet another interesting point for which additional discussion is given in the next section. If hot-water-only treatment is to be used as a pretreatment method, the 210–220 °C is the region that deserves further investigation because of the high digestibilities.

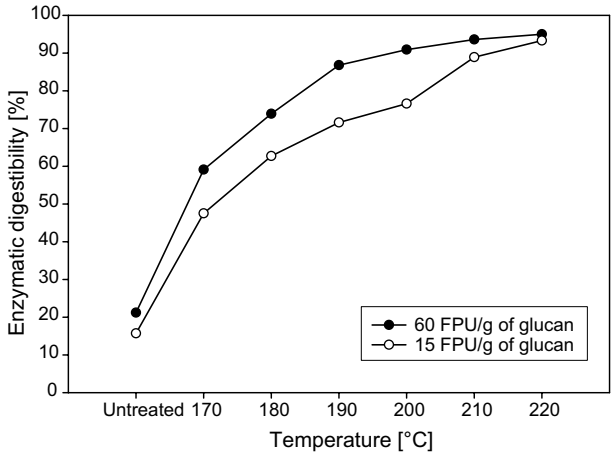

Fig. 4. Effect of temperature on enzymatic hydrolysis in hot-water only treatment. *Note:* (1) Pretreatment conditions: 5.0 ml/min, 30 min, 2.5 MPa. All reactions are carried out in a bed-shrinking flow-through (BSFT) reactor. (2) Digestibility is the percentage at 72 h. (3) Enzymatic hydrolysis conditions: 60 or 15 FPU/g of glucan, pH 4.8, 50 °C, 150 rpm. (4) The data points in the graph show the mean value (SE < 2.0%, $n = 2$).

3.2. Hot-water-ARP treatment

The two-stage percolation process was tested for fractionation and pretreatment of corn stover. In this process, hot water treatment was followed by ARP treatment. Experimental procedure and conditions are summarized in Fig. 2. Five different temperatures covering 170–210 °C were applied in hot-water treatment stage.

In the second series of runs, the biomass feedstocks were subjected to sequential two-stage treatment without intermittent sample taking. Conditions of the ARP treatment were: 170 °C, 15 wt.% NH_3, 30 min or 60 min, selected on the basis of our previous study (Kim et al., 2003). Again, the intent here was to recover hemicellulose in the first stage by autocatalytic hydrolysis and remove lignin in the second stage, and do them in a single reactor successively. Results are summarized in Table 4.

The composition of 30-min and 60-min ARP-treated samples indicates that ARP removed basically lignin, but caused slight decomposition of xylan. Glucan was well protected during the ARP treatment. Lignin removal after 60-min ARP was 70–80%, which was much higher than that of 30-min ARP (53–66%). The digestibilities after two-stage treatment with 60-min ARP were 94–96% with 60 FPU/g of glucan, and 79–87% with 15 FPU/g of glucan. The digestibilities of the 60-min ARP samples were substantially higher than 30-min ARP samples. The glucan hydrolysis rates at two different enzyme loadings are shown in Fig. 5.

Generally the delignification and digestibility increased with the temperature of the hot-water treatment. This trend was reversed in 180–210 °C range (Table 4). As the temperature was increased above 180 °C, the Klason lignin content increased, and the 15-FPU fluctuated corresponding to the lignin content. It is unlikely that the delignification reaction suddenly decreased at certain reaction temperature. The increased Klason lignin content may be linked to other lignin-related

Table 4
Effect of temperature on composition in hot-water–ARP pretreatment[a]

Temperature (°C)	Solid				Liquid		Total		Yield in liquid		Digestibility[b]	
	SR[c] (%)	K-lignin[d] (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	Glucan (%)	Xylan (%)	60 FPU (%)	15 FPU (%)
Untreated	100	17.6	37.5	20.8	–	–	37.5	20.8	–	–	21.2	16.1
Hot-water-30 min of ARP												
170	53.3	8.3	35.9	5.3	1.5	13.9	37.4	19.2	3.9	66.9	82.1	60.7
180	49.5	6.0	35.4	3.8	1.3	15.4	36.7	19.2	3.5	74.0	90.1	76.1
190	47.7	6.0	35.4	2.3	1.7	18.0	37.3	20.3	4.6	86.5	88.7	73.0
200	45.8	6.1	34.1	1.5	1.9	17.4	36.0	18.9	5.1	83.5	94.0	73.0
210	44.2	6.8	33.8	0.7	2.4	15.8	35.2	16.5	6.4	76.0	97.5	77.5
Hot-water-60 min of ARP												
170	47.7	3.7	35.7	3.7	1.5	14.5	37.2	18.2	3.8	69.6	96.3	87.0
180	47.0	3.8	34.8	2.7	1.8	15.5	36.6	18.2	4.7	74.4	95.9	87.1
190	44.9	4.4	34.9	1.6	1.6	17.4	36.5	19.0	4.3	83.4	93.6	84.8
200	44.7	5.7	33.2	1.2	2.1	17.5	35.2	18.7	5.5	84.0	94.5	79.6
210	43.2	5.4	33.2	0.7	2.3	15.4	35.4	16.2	5.9	74.2	94.7	82.7

Note. The data in the table show the mean value ($n = 2$; SE < 0.1% for K-lignin, SE < 0.8% for SR, SE < 0.2% for glucan and xylan in solid and liquid, SE < 2.0% for digestibilities, SE: standard error).

[a] Data in the table based on the oven dry untreated biomass. Pretreatment conditions: (a) hot water: 5.0 ml/min, 30 min, 2.5 MPa. (b) ARP: 15 wt.% of ammonia, 5.0 ml/min, 30 or 60 min, 2.5 MPa. All reactions are carried out in a bed-shrinking flow-through (BSFT) reactor.

[b] Digestibility at 72 h. Enzymatic hydrolysis conditions: 60 or 15 FPU/g of glucan, pH 4.8, 50 °C, 150 rpm.

[c] SR stands for solid remaining after reaction

[d] Klason lignin.

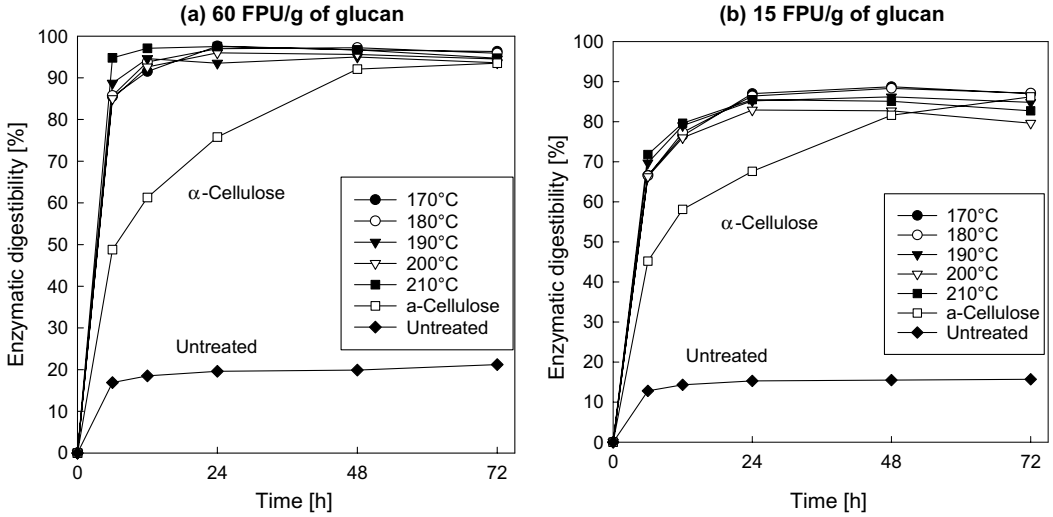

Fig. 5. Enzymatic digestibility of hot-water-ARP treated samples at different enzyme loading. *Note:* (1) All sugar and lignin content based on the oven-dry untreated biomass. Pretreatment conditions: hot-water; 5.0 ml/min, 30 min, 2.5 MPa; ARP; 170 °C, 15 wt.% NH_3, 5.0 ml/min, 60 min, 2.5 MPa. (2) Enzymatic hydrolysis conditions: 72 h, (a) 60 or (b) 15 FPU/g of glucan, pH 4.8, 50 °C, 150 rpm. (3) The data points in the graph show the mean value (SE < 2.0%, $n = 2$).

reactions. Literature information indicated that lignin undergoes condensation and repolymerization, turning into insoluble substances (Genco et al., 1997; Lora and Wayman, 1978; Xu and Lai, 1999). Lignin may also bond to cellulose at high temperatures (Karlsson, 1997). These secondary reactions form complexes that are not hydrolyzed by concentrated sulfuric acid during the carbohydrate analysis procedure. Since it was counted as Klason lignin, it would appear as if delignification rate was reduced at high temperatures. The relation between lignin content in solid and the relevant digestibility with 15 FPU/g of glucan for hot-water-ARP treatment samples is summarized in Fig. 6. The digestibility in this region fluctuated corresponding to the lignin content in a manner that the digestibility was inversely related with lignin content. How the low level lignin affected the digestibility was unknown. We hypothesized that the soluble lignin, as it interacted with cellulose, modified the cellulose surface structure enough to interfere with the cellulase action. The solubilized lignin may also interact with the soluble glucose at high temperatures as suggested by Xiang (2002), which would also reduce the digestibility numbers. However, a direct relation between the enzymatic digestibility with 60 FPU/g of glucan and the lignin content was not evident in the data of Table 4. We speculate that the enzyme activity at 60 FPU/g of glucan was high enough to overcome the negative effect the effect of lignin giving consistently high digestibility values.

The optimum operating condition of the two-stage process, on the basis of fractionation and digestibility, were: 190 °C, 5.0 ml/min, 30 min for hot-water treatment and 170 °C, 5.0 ml/min, 60 min for ARP pretreatment. Upon two-stage processing at the optimum

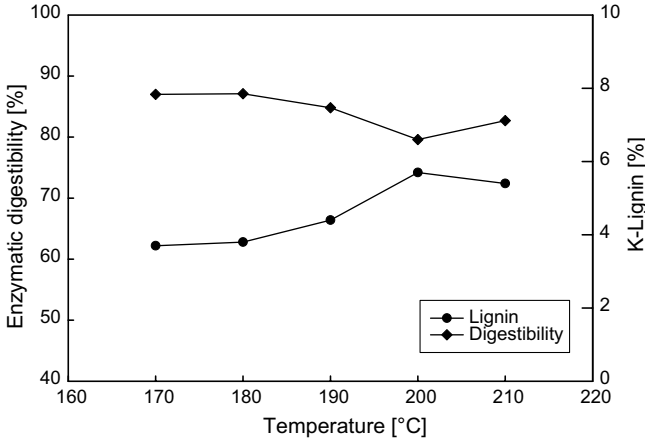

Fig. 6. Relationship between lignin content and enzymatic digestibility (with 15 FPU/g of glucan) on two-stage treated solid sample. *Note:* (1) Pretreatment conditions (hot-water-ARP): hot-water; 5.0 ml/min, 30 min, 2.5 MPa; ARP; 15 wt.% of ammonia, 5.0 ml/min, 60 min, 2.5 MPa. All reactions are carried out in a bed-shrinking flow-through (BSFT) reactor. (2) Enzymatic hydrolysis conditions: 72 h, 15 FPU/g of glucan, pH 4.8, 50 °C, 150 rpm. (3) The data points in the graph show the mean value (SE < 2.0%, $n = 2$ for digestibility; SE < 0.2%, $n = 2$ for lignin).

operating conditions, 92% of xylan was hydrolyzed, of which 83% was recovered, and 75% of delignification was achieved. The treated biomass contained 78% glucan, 3.6% xylan and 9.8% Klason lignin. The digestibility of hot-water-ARP treated sample was 94% with 60 FPU/g-glucan and 85% with 15 FPU/g of glucan.

3.3. SEM

SEM pictures of treated and untreated samples were taken. Two-stage treatment altered the biomass structure

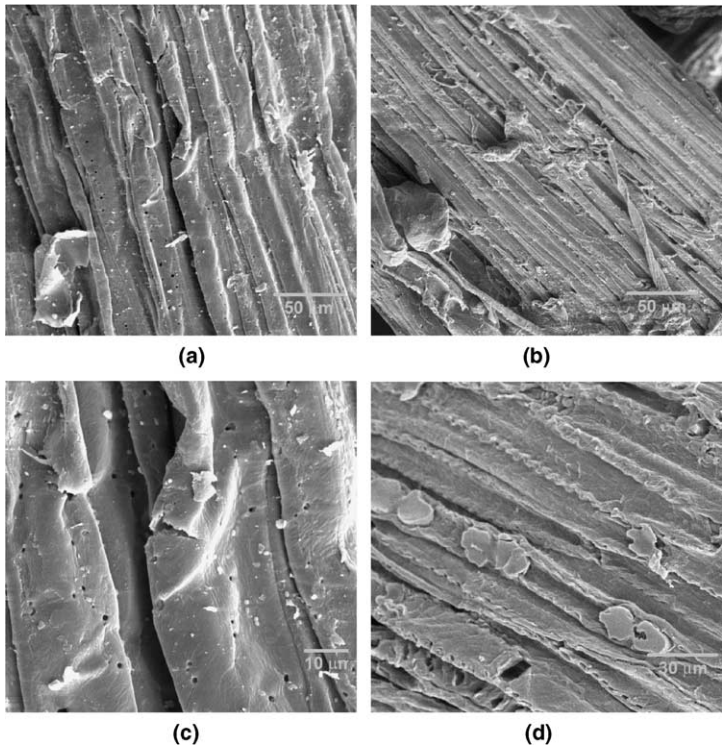

Fig. 7. Scanning electron micrographs (SEM) of treated and untreated corn stover. *Note:* (1) Pretreatment conditions (hot-water-ARP): hot-water; 5.0 ml/min, 30 min, 2.5 MPa; ARP; 15 wt.% of ammonia, 5.0 ml/min, 60 min, 2.5 MPa. All reactions are carried out in a bed-shrinking flow-through (BSFT) reactor. (a) Untreated (×300), (b) hot-water-ARP (×300), (c) untreated (×1000), (d) hot-water-ARP (×500).

substantially (Fig. 7). The untreated sample exhibited rigid and highly ordered fibrils (Fig. 7a and d). The fibers of treated samples were separated from the initial connected structure and fully exposed (Fig. 7b, c, e, and f). By manual touch of the material, the wet treated biomass felt much softer than the untreated biomass.

4. Conclusions

Two-stage percolation processes effectively fractionates corn stover into three main constituents of cellulose, xylan, and lignin. The optimum reaction conditions for fractionation are: 190 °C, 5.0 ml/min, 30 min for hot-water treatment section and 170 °C, 15 wt.% NH_3, 5.0 ml/min, 60 min for the ARP treatment section. Under these conditions, 83% recovery of soluble xylan recovery and 75% of delignification were achieved. The treated residue contained 78% glucan, 3.6% xylan, and 9.8% lignin. This high glucan material may be suitable for use as a filler fiber in papermaking and as other value-added products. The enzymatic digestibilities of the two-stage treated residue were 94% with 60 FPU/g-glucan and 85% with 15 FPU/g of glucan. This compares to the digestibilities 87% and 72% of the samples from hot-water-only treatment. Although there is a noticeable improvement of digestibility for the two-stage processing over one-stage (hot-water-only), the proposed two-stage processing is more meaningful as a fractionation than as a method of pretreatment since there are other one-stage pretreatment methods equally effective. Composition and digestibility data of the treated corn stover indicate that the lignin is one of the prime factors controlling the enzymatic digestibility. Hot-water treatment above 210 °C may induce lignin recondensation and/or lignin–carbohydrate bonding.

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