

# Optimization of Steam Pretreatment of Corn Stover to Enhance Enzymatic Digestibility

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## Abstract

Among the available agricultural byproducts, corn stover, with its yearly production of 10 million t (dry basis), is the most abundant promising raw material for fuel ethanol production in Hungary. In the United States, more than 216 million t of corn stover is produced annually, of which a portion also could possibly be collected for conversion to ethanol. However, a network of lignin and hemicellulose protects cellulose, which is the major source of fermentable sugars in corn stover (approx 40% of the dry matter [DM]). Steam pretreatment removes the major part of the hemicellulose from the solid material and makes the cellulose more susceptible to enzymatic digestion. We studied 12 different combinations of reaction temperature, time, and pH during steam pretreatment. The best conditions (200°C, 5 min, 2% H<sub>2</sub>SO<sub>4</sub>) increased the enzymatic conversion (from cellulose to glucose) of corn stover more than four times, compared to untreated material. However, steam pretreatment at 190°C for 5 min with 2% sulfuric acid resulted in the highest overall yield of sugars, 56.1 g from 100 g of untreated material (DM), corresponding to 73% of the theoretical. The liquor following steam explosion was fermented using *Saccharomyces cerevisiae* to investigate the inhibitory effect of the pretreatment. The achieved ethanol yield was slightly higher than that obtained with a reference sugar solution. This demonstrates that baker's yeast could adapt to the pretreated liquor and ferment the glucose to ethanol efficiently.

**Index Entries:** Corn stover; pretreatment; steam explosion; hydrolysis; bioethanol.

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## Introduction

The inevitable depletion of the world's fossil energy supply and the increasing greenhouse effect have resulted in an increasing worldwide interest in alternative nonfossil-based sources of energy, including bioethanol. Bioethanol is currently made by large-scale yeast fermentation of glucose or sucrose that is mainly extracted or prepared from sugars or starch-containing crops (1). The world-wide bioethanol production in 2001 was approx 22 billion L (2); however, bioethanol will remain uncompetitive compared with fossil fuels unless a significant reduction in the production cost is achieved.

A potential source for cost-effective ethanol production is lignocellulosic feedstock, because it is available in bulk quantities at a relatively low cost (3). During corn harvesting, the amount of byproducts, such as corn stover and corncobs, is two times higher than the amount of crop (4). This, together with the fact that nearly half of the world's bioethanol production is based on corn, makes corn stover one of the most important agricultural byproducts in every corn-growing country. In the US, more than 216 million tons of corn stover (dry basis) is produced annually (5); its heat value could replace about 40% of the energy content of the US gasoline market (6).

Corn stover, like lignocellulosic materials in general, is resistant to enzymatic hydrolysis, because of both the tight network in the lignocellulose complex and the crystalline structure of the native cellulose. These difficulties can be overcome by employing a suitable pretreatment (7).

Steam pretreatment is an extensively investigated method that is especially used for treatment of several types of wood (8,9). However, the literature on the treatment of herbaceous materials is scarce (10,11). Steam pretreatment effectively enhances the conversion of carbohydrates to ethanol using combined enzymatic hydrolysis and fermentation (12). Several previous studies have shown that impregnation prior to steam pretreatment improves the enzymatic saccharification of both lignocellulosic biomass and softwood (12). However, the addition of external catalyst could be omitted in the case of herbaceous plants, such as rice straw (13,14). The three most important pretreating parameters, which affect the total sugar yields, are the amount of the impregnating agent, such as  $\text{SO}_2$  or  $\text{H}_2\text{SO}_4$ ; the temperature; and the residence time (15). During steam pretreatment, sugars and other degradation products can be formed from the xylan and the lignin fraction, which may be inhibitory to microorganisms used in the downstream processing (16–18).

The aim of the present study was to apply steam pretreatment to corn stover and investigate the influence of different pretreatment conditions—temperature, residence time, and concentration of  $\text{H}_2\text{SO}_4$ —on sugar yield and the effect of inhibitors on subsequent ethanol yield.

## Materials and Method

### *Experimental Procedure*

Figure 1 is a schematic of the experimental procedure used. The ground corn stover was impregnated with  $\text{H}_2\text{SO}_4$  (0.5 or 2%) and then pretreated

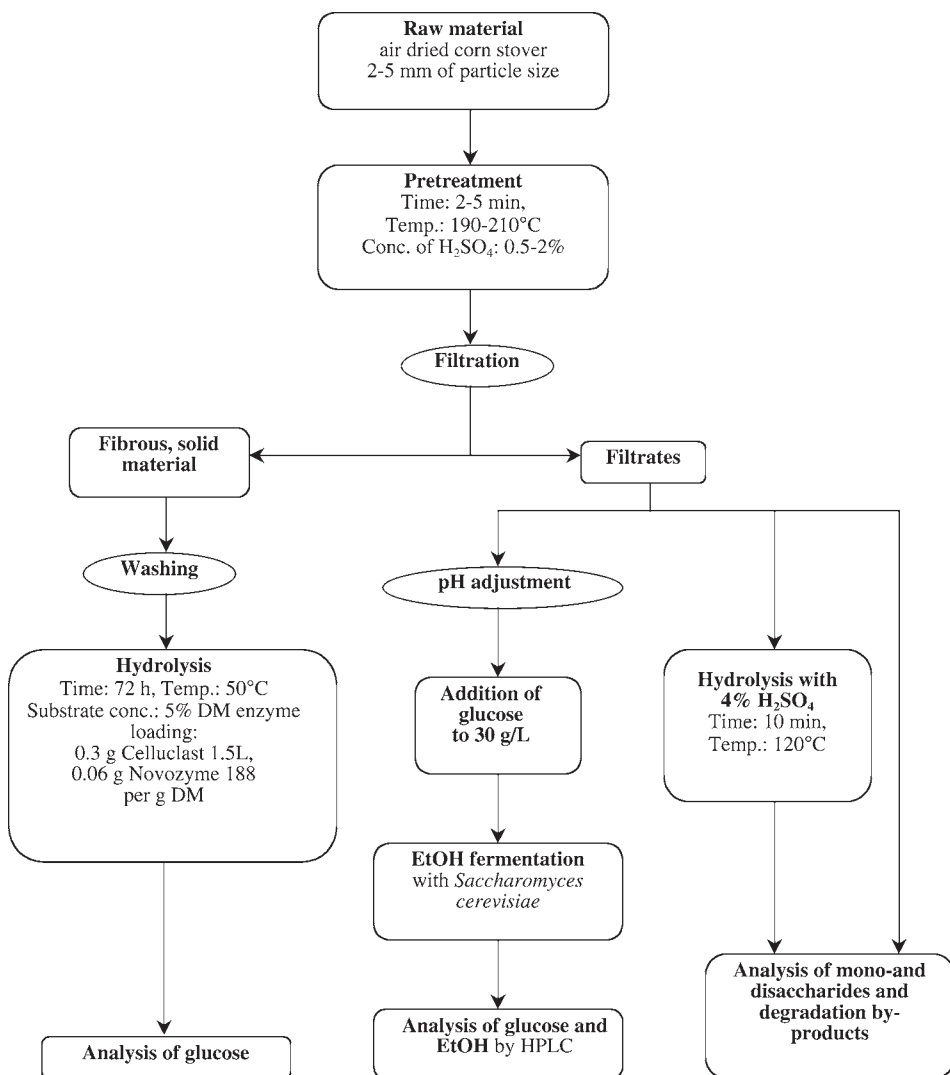


Fig. 1. Schematic procedure for assessment of pretreatment.

with steam at various temperatures (190, 200, and 210°C) and residence times (2 and 5 min). The pretreated material was separated into a solid fibrous fraction and filtrate. The solid residue was washed with distilled water to remove water-soluble components and the insoluble fiber fraction was enzymatically hydrolyzed. A portion of the filtrate was hydrolyzed with H<sub>2</sub>SO<sub>4</sub> to determine the amount of released sugars in oligomer form during the pretreatment. Another portion of the filtrate was complemented with glucose and used in a fermentability test with *Saccharomyces cerevisiae* in the form of compressed baker's yeast. Sugar yield and fermentability were used to estimate the optimal pretreatment conditions.

### *Raw Material*

Corn stover was harvested from south Hungary in the fall of 2001. The selected and washed straw was air-dried to an average 90% dry matter (DM) content. The air-dried material was ground and sieved, and the fraction with a particle size of 2–5 mm was used. The composition of this material and also the washed, solid fibrous fraction remaining after pretreatment was determined using Kaar's (19) method. To measure the total ash content, approx 0.5 g of dried sample was placed in a crucible, ignited at 550°C for 3 h, cooled in a desiccator, and weighed.

### *Steam Pretreatment*

Corn stover (180 g [DM]) was mixed with 1720 mL of diluted H<sub>2</sub>SO<sub>4</sub> or with distilled water and was swollen for one night. Then the material was steam treated in an equipment previously described (20). The pretreatment vessel (2-L volume) was preheated with steam prior to loading the impregnated corn stover. The corn stover was heated by steam to the desired temperature, and when the preset pretreatment time had elapsed, the material was discharged into a flash drum. The material was then separated, by filtration, into a solid fraction and a filtrate. Three variable factors were investigated: the concentration of the H<sub>2</sub>SO<sub>4</sub> (0.5 and 2%), the reaction temperature (190, 200, and 210°C), and the reaction time (2 and 5 min). Each experiment was performed in duplicate and the order of experiments was randomized. After the pretreatments, the samples were separated into a liquid and a solid fraction, and both fractions were analyzed. Both the filtrates and the separated solid fraction were stored frozen (–20°C) for further analysis, including enzymatic hydrolysis and fermentability testing.

### *Determination of Poly- and Monosaccharides (High-Performance Liquid Chromatography)*

The sugars in the liquid after pretreatment are partly obtained in oligomer form. To convert the residual oligosaccharides to mono- and disaccharides, the samples were hydrolyzed with 4% H<sub>2</sub>SO<sub>4</sub> at 121°C for 10 min. To determine the potential sugar degradation during the acid hydrolysis, the experiments were carried out in triplicate, and known amounts of monosaccharides were added to one of the hydrolysate samples.

The amount of mono- and disaccharides released (glucose, xylose, cellobiose, and arabinose) by pretreatment utilizing acid and enzymatic hydrolysis was analyzed by high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan), using an Aminex HPX-87H column with a matching precolumn (Bio-Rad, Hercules, CA) at 65°C. The eluent was 5 mM H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.5 mL/min with detection by refractive index.

For yield calculations, the measured amount of xylose and arabinose was used as a measure of the hemicellulose fraction, while the cellulose fraction was calculated from the amount of glucose and cellobiose.

### Enzymatic Hydrolysis

The pretreated, washed, solid materials were enzymatically hydrolyzed to determine the efficiency of cellulose conversion. The fibrous material was diluted to 5% (DM) using 0.5 M acetate buffer (pH 4.8). Hydrolysis was performed in 250-mL shake flasks using a total working weight of 150 g. Hydrolysis was carried out in duplicate at 50°C for 72 h, and the flasks were agitated at 300 rpm in a shaker incubator (LAB-Therm; Kühner, Birsfeldon, Switzerland). Commercially available enzyme solution, Celluclast 1.5L (cellulase from *Trichoderma reesei*) and Novozym 188 ( $\beta$ -glucosidase; Novozymes A/S, Bagsværd, Denmark), were applied in the enzymatic hydrolysis. The enzyme activity of Celluclast 1.5L was 82 filter paper units (FPU)/g, the  $\beta$ -glucosidase activity of Novozyme 188 was 450 IU/g, and the enzyme loading applied was 0.3 and 0.06 g/g of DM, respectively.

To determine the time required for total hydrolysis, the samples were hydrolyzed for 72 h. Samples were taken after 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 48, and 72 h. The samples were centrifuged at 1400g for 10 min, and the composition of monosaccharides was analyzed by HPLC. The percentage of cellulose enzymatically converted to glucose (ECC) was calculated as a quotient of liberated glucose (g) during the hydrolysis and weight of cellulose (g) before enzymatic hydrolysis. The ECC value based on the glucose concentration measured by HPLC was calculated as follows:

$$\text{ECC} = \frac{c \cdot V}{m \cdot 1.11} \cdot 100\% \quad (1)$$

in which  $c$  is the concentration of D-glucose after enzymatic hydrolysis (g/L),  $V$  is the total volume (L), and  $m$  is the weight of cellulose before enzymatic hydrolysis (g). The factor 1.11 converts the cellulose concentration to the equivalent glucose concentration.

### Measurement of Enzyme Activity

The activity of the cellulytic enzymes was measured as filter paper activity units. A 1  $\times$  6 cm strip of Whatman No. 1 filter paper was added to a total volume of 1.5 mL of enzyme solution containing 0.05 M citrate buffer, pH 4.8. All enzyme activity measurements were carried out in triplicate. The samples were incubated for 1 h at 50°C. Reducing sugars were determined after stopping the hydrolysis by adding 3 mL of dinitrosalicylic acid solution followed by boiling for 5 min. After cooling, 20.0 mL of distilled water was added and the absorbance was read at 540 nm (21).

$\beta$ -Glucosidase activity was measured by incubating the enzyme solution with 10  $\mu$ M *p*-nitrophenyl- $\beta$ -D-glucopyranoside and 0.05 M citrate buffer, pH 4.5, at 50°C for 10 min. The reaction was stopped by adding 0.1 M Na<sub>2</sub>CO<sub>3</sub>, and the liberated *p*-nitrophenol was measured spectrophotometrically at 400 nm. One unit of activity was defined as the release of 1  $\mu$ mol of *p*-nitrophenol/min (22).

Table 1  
Amount of Solids and Yield of Various Components in Solid Fraction  
After Steam Pretreatment, Expressed as g/100 g of Dry, Untreated Corn Stover

Pretreatment conditions			Components in solid fraction <sup>b</sup>						
Temperature (°C)	Acid conc. <sup>a</sup> (%)	Time (min)	Total amount of solid	Lignin	Glucose	Xylose	Arabinose	Ash	
Raw material			100.0						
	190	0.5	2	69.4 ± 2.3	20.2 ± 0.2	41.6 ± 0.3	27.7 ± 0.4	3.6 ± 0.0	4.6 ± 0.0
		5	62.5 ± 2.1	18.5 ± 0.5	33.3 ± 0.5	14.3 ± 0.7	1.0	1.7	
	2	2	48.4 ± 2.8	17.4 ± 0.3	29.6 ± 0.5	11.9 ± 0.6	0.3	1.7	
		5	49.1 ± 1.3	16.6 ± 0.6	24.3 ± 0.4	3.8 ± 0.8	0.1	2.1	
200	0.5	2	67.7 ± 1.7	19.1 ± 0.2	24.2 ± 0.3	1.4 ± 0.5	0.0	2.2	
		5	65.3 ± 0.7	19.5 ± 0.1	32.9 ± 0.2	9.4 ± 0.4	0.0	2.7	
	2	2	50.1 ± 1.1	19.5 ± 0.4	32.6 ± 0.3	8.5 ± 0.5	0.0	3.9	
		5	41.1 ± 0.4	17.9 ± 0.2	24.1 ± 0.4	4.5 ± 0.3	0.1	2.8	
	210	0.5	2	61.4 ± 1.4	19.1 ± 0.3	20.0 ± 0.1	1.1 ± 0.4	0.0	2.2
5			58.2 ± 0.7	19.2 ± 0.4	30.7 ± 0.3	6.4 ± 1.1	0.0	2.4	
2		2	41.2 ± 0.9	18.8 ± 0.2	30.2 ± 0.2	5.7 ± 0.6	0.1	2.6	
		5	36.9 ± 0.7	18.4 ± 0.2	17.4 ± 0.3	2.3 ± 0.2	0.0	2.8	
210		—	2	85.4 ± 1.5	19.4 ± 0.1	13.9 ± 0.1	0.7 ± 0.1	0.0	2.2
	5			19.7 ± 0.5	38.6 ± 0.2	21.6 ± 0.4	2.7	2.2	

<sup>a</sup> Conc., concentration.

<sup>b</sup> Main value ± errors.

## Fermentation

The liquid fractions after pretreatment were fermented using baker's yeast (Jästbolaget AB, Rotebro, Sweden) to determine the toxicity of the samples. Fermentation was carried out in 25-cm<sup>3</sup> glass flasks, sealed with rubber stoppers and equipped with cannulas for removal of produced broth CO<sub>2</sub>. The volume of the fermentation broth was 20 cm<sup>3</sup> (18.5 cm<sup>3</sup> of filtrate, 0.5 cm<sup>3</sup> of nutrients, and 1 cm<sup>3</sup> of inoculum).

The filtrates were adjusted to pH 5.5 with 10 M NaOH solution and supplemented with glucose to a total concentration of 30 g/L, and with nutrients to a final concentration of 2.5 g/L of yeast extract, 0.25 g/L of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 0.025 g/L of MgSO<sub>4</sub>·7H<sub>2</sub>O, and 0.1 g/L of NaH<sub>2</sub>PO<sub>4</sub>. Glucose was added, since the purpose of the fermentation was to investigate the effect of inhibitors on fermentation. The filtrates were inoculated with compressed baker's yeast to a cell concentration of 3 g of DM/L, incubated at 30°C, and stirred with a magnetic stirrer. Reference fermentations, using a pure sugar solution containing 30 g/L of glucose, nutrients, and cells, were run for comparison. Samples were withdrawn from the fermentation broth at the start; before the addition of yeast; and after 1, 2, 4, 6, 8, and 10 h, and a final sample was taken after 20 h. All samples were analyzed for glucose and ethanol by HPLC.

## Results and Discussion

### Pretreatment

After steam pretreatment the yield of solid material ranged from 37 to 85.4 g/100 g and decreased with increased acid concentration at the same temperature (*see* Table 1). The reduction in solid material was owing to the solubilization and/or degradation of hemicellulose and extractives. However, under more severe conditions, a part of the cellulose was also solubilized.

Table 1 summarizes the composition of the solid fibrous fractions after pretreatment, and Table 2 provides the amount of dissolved compounds in the filtrate from 100 g of untreated corn stover. As expected, steam pretreatment preferentially attacked the hemicellulose fraction; the harsher the pretreatment conditions, the higher amount of hemicellulose was solubilized. Under the harshest conditions (210°C, 2% [w/w] H<sub>2</sub>SO<sub>4</sub>, 5 min), almost all hemicellulose was removed from the solid fraction, its amount decreased from 31.3 to 0.7 g. However, the solubilization of hemicellulose was significant, about 85%, also at the lowest temperature (190°C) using 2% H<sub>2</sub>SO<sub>4</sub>. The concentration of H<sub>2</sub>SO<sub>4</sub> had a greater effect on the decrease in the hemicellulose fraction in the solid residues than the temperature (Tables 1 and 2). Without the addition of H<sub>2</sub>SO<sub>4</sub>, even at 210°C for 5 min, the pretreatment solubilized only 20% of the hemicellulose, while 0.5% H<sub>2</sub>SO<sub>4</sub> decreased the hemicellulose content by 82%. It seems that acid hydrolysis is the main mechanism involved, which was also supported by our previous work, in which corn stover was pretreated with 1% H<sub>2</sub>SO<sub>4</sub> at



Table 2  
Sugar Yields in Liquid After Steam Pretreatment, Expressed as g/100 g  
of Dry, Untreated Corn Stover and Concentration of Byproducts (g/L)

Pretreatment conditions			Components in solid fraction						
Temperature (°C)	Acid conc. (%) <sup>a</sup>	Time (min)	Cellobiose	Glucose	Xylose	Arabinose	Concentration (g/L)		
190	0.5	2	0.3	3.3 ± 0.0	9.2 ± 0.2	1.7 ± 0.0	0.7	0.1	0.4
		5	0.6	6.2 ± 0.2	12.5 ± 0.2	1.9 ± 0.0	1.0	0.1	0.7
	2	0.2	9.9 ± 0.2	16.6 ± 0.3	3.2 ± 0.1	2.4	0.2	2.7	
200	0.5	5	0.5	12.8 ± 0.3	18.3 ± 0.4	3.0 ± 0.1	1.8	0.1	1.5
		2	0.5	3.9 ± 0.0	11.8 ± 0.2	1.8 ± 0.0	1.1	0.1	0.9
	5	0.4	2.5 ± 0.0	10.7 ± 0.2	1.6 ± 0.0	1.5	0.1	0.8	
210	2	2	0.5	7.4 ± 0.1	13.8 ± 0.3	2.3 ± 0.0	1.6	0.2	1.5
		5	0.3	12.9 ± 0.2	16.3 ± 0.3	2.7 ± 0.0	2.1	0.3	2.6
	0.5	2	0.3	3.6 ± 0.0	12.7 ± 0.2	1.7 ± 0.0	1.2	0.1	1.0
210	2	5	0.4	4.5 ± 0.1	13.8 ± 0.3	2.1 ± 0.0	1.7	0.2	1.7
		2	0.3	12.8 ± 0.2	13.3 ± 0.2	2.6 ± 0.1	2.2	0.3	2.6
	5	0.3	16.2 ± 0.2	13.7 ± 0.2	3.1 ± 0.1	3.4	0.5	4.6	
210	—	5	0.3	1.0 ± 0.05	5.5 ± 0.1	1.1 ± 0.0	0.7	0.1	0.2



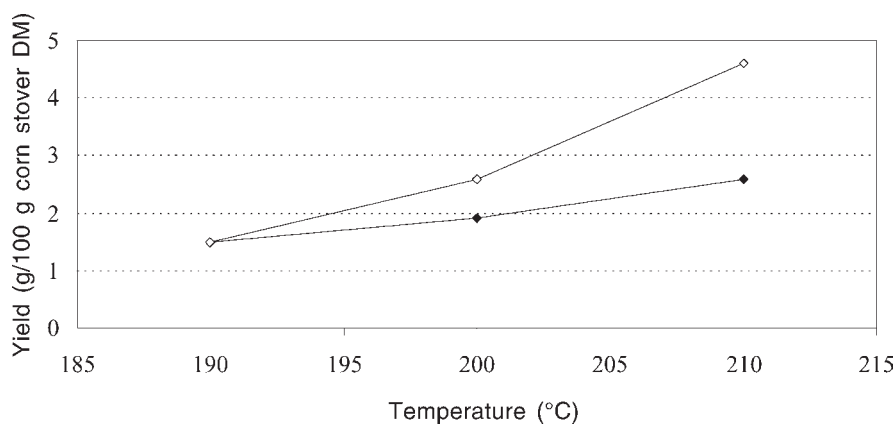


Fig. 2. Amount of formed furfural during pretreatment as a function of reaction temperature and time (2 [◆] and 5 min. [◇] using 2%  $\text{H}_2\text{SO}_4$ ).

120°C and the hemicellulose content decreased by 76% (23). For calculation of the amount of hemicellulose, the amount of mono- and disaccharides (xylose and arabinose) in the liquid was measured by HPLC following the strong acid hydrolysis by Hägglund.

Two percent  $\text{H}_2\text{SO}_4$  was also effective in solubilizing cellulose from the solid material. Even at the lowest temperature (190°C), the cellulose content in the solid fraction decreased by 50%, from 41.6 to 24.3 g. Solubilization and/or degradation of the lignin during steam pretreatment was not significant. The amount of lignin decreased only slightly from 20.2 to 18.4 g, even under the harshest conditions (210°C, 5 min, 2%  $\text{H}_2\text{SO}_4$ ).

The amount of the formed byproducts (acetic acid, furfural, and hydroxymethylfurfural [HMF]) during pretreatment increased with increased temperature and reaction time. Figure 2 shows the formation of furfural as a function of temperature at different pretreatment times. The tendency was similar for all byproducts.

The recovery of both hemicellulose and cellulose sugars is an important point for a suitable pretreatment. The recovery of cellulose and hemicellulose was calculated to estimate their losses during steam pretreatment at different conditions (Table 3). Calculation according to the following equations was based on the mass balance (Tables 1 and 2).

Recovery of cellulose (%) =

$$\frac{\left[ \text{cellulose in solid residue (g)} + \left( \text{released glucose in filtrate (g)} / 1.11 \right) \right]}{\text{cellulose in untreated corn stover (g)}} \quad (2)$$

Table 3  
Recovery (%) of Cellulose and Hemicellulose

Pretreatment conditions			Recovery %		
Temperature (°C)	Acid concentration (%)	Time (min)	Cellulose	Hemicellulose	All sugars
190	0.5	2	88.8	83.8	86.6
		5	83.4	80.6	86.5
	2	2	89.7	75.7	79.7
200	0.5	5	83.1	72.4	82.5
		2	89.7	73.5	82.8
	2	5	85.3	66.5	77.2
210	0.5	2	76.9	65.9	72.2
		5	74.2	64.1	69.9
	2	2	83.2	66.5	76.1
210	2	5	84.2	69.2	77.7
		2	73.5	58.0	66.8
	—	5	73.1	55.9	65.7
210	—	5	95.9	93.8	97.2

Recovery of HC (%) =

$$\frac{\left[ \text{HC in solid residue (g)} + \left( \text{released xylose + arabinose in filtrate (g)} / 1.14 \right) \right]}{\text{HC in untreated corn stover (g)}} \quad (3)$$

in which HC is hemicellulose. The amount of HC in the solid residue was calculated from the amount of xylose and arabinose in the filtrate following Hägglund's hydrolysis.

Table 3 shows that mainly the hemicellulose was converted and/or degraded in the steam pretreatment. About 60% of the original hemicellulose could be recovered, except when 2%  $\text{H}_2\text{SO}_4$  was combined with high reaction temperature (210°C). In this case, the hemicellulose recovery was only 55.9%. This relatively low recovery could be explained by the hemicellulose degradation to other byproducts during steam pretreatment.

The maximal recovery of hemicellulose (83.8%) was obtained following the mildest pretreatment at 190°C for 2 min using 0.5%  $\text{H}_2\text{SO}_4$ . The harsher the conditions, the lower the hemicellulose recovery was reached. The maximum cellulose recovery was 89.7%, following steam pretreatment at 200°C, but the recovery was sufficient, about 75%, even at 210°C. Cellulose and hemicellulose exhibited maximum recovery at different conditions, which was also found in other studies (24,25). However, in the case of softwood, the hemicellulose solubilization needed harsher pretreatment conditions than corn stover, and the maximum mannose yield from wood was obtained at between 200 and 210°C (26).

The concentration of  $\text{H}_2\text{SO}_4$  affected the recovery of cellulose significantly. Using less concentrated  $\text{H}_2\text{SO}_4$  (0.5%), the recovery of cellulose was approx 10% higher than using 2%  $\text{H}_2\text{SO}_4$  at the same temperature and time.

The overall recovery of all sugars varied from 79 to 86% at 190°C and from 65 to 77% at 210°C, which was a bit lower than achieved with corn stover using wet oxidation (27).

### Enzymatic Hydrolysis

The pretreated solid residue was enzymatically hydrolyzed to determine the degree of cellulose conversion to glucose (ECC%), which is valuable information about the effectiveness of the pretreatment. Hydrolysis at 50°C with the applied relative high enzyme loading was completed after 24 h, as was also found in a previous study (27). The achieved conversion after 48 h of enzymatic hydrolysis is presented in Table 4. The enzymatic conversion of pretreated cellulose was between 31 and 83% compared with 27% obtained with enzymatic hydrolysis of untreated corn stover. To verify the pretreatment effect of the freezing-thawing period, a portion of the raw material was also frozen and then hydrolyzed. The achieved enzymatic conversion was not significantly higher (29.2%) in this case.

Considering that the pretreatment without addition of  $\text{H}_2\text{SO}_4$  only slightly modified the composition of corn stover (Table 1), it is not sur-

Table 4  
ECC% Conversion After 48 h of Enzymatic Hydrolysis and Amounts of Released Glucose (g)  
from 100 g of Untreated Material (DM) During Pretreatment and Hydrolysis

Pretreatment conditions			During the hydrolysis			During hydrolysis and pretreatment	
Temperature (°C)	Acid conc. (%) <sup>a</sup>	Time (min)	ECC (%)	Released glucose (g)	Released xylose (g)	Total released glucose (g)	Total sugars (g)
Raw material Frozen raw material 190	0.5	2	27.3	12.5	0.2	12.5	12.7
			29.2	13.4	0.2	13.5	13.9
		5	47.4	17.4	0.3	20.7	31.9
	2	2	52.2	17.0	0.2	23.2	35.8
		5	71.8	19.2	0.2	29.1	49.1
200	0.5	2	81.1	21.6	0.5	34.3	56.1
			58.4	21.1	0.5	25.0	39.2
		5	65.7	23.6	0.5	26.0	38.8
	2	2	65.8	17.4	0.5	24.9	41.4
		5	83.6	18.4	0.3	31.3	50.5
210	0.5	2	67.2	22.7	0.3	26.3	41.0
			74.8	24.8	0.4	29.3	45.6
		5	74.0	14.2	0.3	27.0	43.3
	2	2	78.8	12.0	0.3	28.3	45.3
		5	31.1	13.2	0.4	14.2	21.2

<sup>a</sup> Conc., concentration.

prising that the ECC after this pretreatment was quite poor (31.1%). The highest conversion (83.6%) was achieved with a pretreatment at 200°C for 5 min with 2% H<sub>2</sub>SO<sub>4</sub>. The ECC conversions in general were higher following pretreatment using 2% H<sub>2</sub>SO<sub>4</sub>, than 0.5% H<sub>2</sub>SO<sub>4</sub>, but the amounts of released glucose by enzymatic hydrolysis were still higher using less concentrated H<sub>2</sub>SO<sub>4</sub>. This could be explained by the higher solubilization of cellulose during pretreatment. The highest amount of total released glucose (34.3 g/100 g of DM) was achieved following pretreatment at 190°C for 5 min with 2% H<sub>2</sub>SO<sub>4</sub>.

Although most cellulase enzyme complexes, including Celluclast, contain xylanase activity (data not shown), the amount of released xylose after enzymatic hydrolysis was negligible, especially compared with the amount of released glucose after pretreatment.

The highest overall sugar yield after both pretreatment and enzymatic hydrolysis, 56.1 g/100 g of DM, was also obtained at 190°C, for 5 min with 2% H<sub>2</sub>SO<sub>4</sub>.

### *Fermentability Test*

Ethanol production depends not only on the sugar yield, but also on the fermentability of the solution. To investigate the fermentability of the pretreated corn stover, fermentations were performed with baker's yeast. Baker's yeast has often been proposed as the best organism for the fermentation of lignocellulosic hydrolysates (28,29) and has the advantages that it is quite robust and was found to be less sensitive to inhibitors than cultivated yeast (30,31).

Table 5 shows the achieved ethanol concentrations after 24 h of fermentation and the calculated ethanol yield as percent of theoretical. After 1 d of fermentation the ethanol concentrations varied from 12.1 to 13.8 g/L, corresponding to 78.4 and 90.3% of the theoretical yield. Glucose was totally consumed after 6 h of fermentation following pretreatment with 0.5% H<sub>2</sub>SO<sub>4</sub>. The maximum ethanol concentration was also reached after 6 h in these cases, but when 2% H<sub>2</sub>SO<sub>4</sub> was used in pretreatment, glucose was consumed more slowly and the formation of ethanol needed more time. However, even in these cases the achieved ethanol yield was about 85% of the theoretical. These results are quite similar to those achieved after fermentation of wet oxidized wheat straw in a previous study (17).

### **Conclusion**

This study shows that steam pretreatment is an efficient method to increase the enzymatic accessibility of the water-insoluble, cellulose-rich component in corn stover. After pretreatment, the enzymatic conversion from cellulose to glucose increased nearly four times, compared to the untreated corn stover.

The best pretreatment conditions for obtaining high conversion of cellulose to glucose were 200°C for 5 min after swelling the fibers with 2%

Table 5  
Ethanol Yield (%) of Theoretical and Achieved Ethanol  
Concentration (g/L) After 24 h of Fermentation at 30°C  
with Baker's Yeast from 30 g/L of Glucose Solution

Pretreatment Conditions				
Temperature (°C)	Acid concentration (%)	Time (min)	EtOH concentration (g/L)	Ethanol yield (%)
Control			12.9	84.0
190	0.5	2	13.2	86.0
		5	13.4	87.5
	2	2	13.3	87.3
		5	12.1	78.4
200	0.5	2	13.4	87.8
		5	13.7	89.7
	2	2	13.0	85.2
		5	13.7	89.4
210	0.5	2	13.8	90.3
		5	13.8	90.1
	2	2	13.5	88.4
		5	13.1	85.6
210	—	5	13.6	89.2

H<sub>2</sub>SO<sub>4</sub>. Most of the hemicellulose was dissolved during the pretreatment, and approx 60% of the original hemicellulose could be recovered. Enzymatic hydrolysis at 50°C was completed after 24 h, and the highest enzymatic conversion from cellulose to glucose was above 80%, corresponding to 18 g of glucose/100 g of untreated solid material. However, the highest overall yield of sugars was 56.1 g from 100 g of untreated material DM, corresponding to 73% of the theoretical, which was achieved following steam pretreatment at 190°C for 5 min with 2% H<sub>2</sub>SO<sub>4</sub>.

The fermentability of the solution gave good results. The achieved ethanol yields were about 90%, slightly above those obtained with a reference sugar solution, showing that baker's yeast could adapt to the pretreated liquor and ferment the glucose to ethanol without problems.

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