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Pretreatment of Corn Stover Using Wet Oxidation to Enhance Enzymatic Digestibility

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Abstract

Corn stover is an abundant, promising raw material for fuel ethanol production. Although it has a high cellulose content, without pretreatment it resists enzymatic hydrolysis, like most lignocellulosic materials. Wet oxidation (water, oxygen, mild alkali or acid, elevated temperature and pressure) was investigated to enhance the enzymatic digestibility of corn stover. Six different combinations of reaction temperature, time, and pH were applied. The best conditions (60 g/L of corn stover, 195°C, 15 min, 12 bar O_2, 2 g/L of Na_2CO_3) increased the enzymatic conversion of corn stover four times, compared to untreated material. Under these conditions 60% of hemicellulose and 30% of lignin were solubilized, whereas 90% of cellulose remained in the solid fraction. After 24-h hydrolysis at 50°C using 25 filter paper units (FPU)/g of drymatter (DM) biomass, the achieved conversion of cellulose to glucose was about 85%. Decreasing the hydrolysis temperature to 40°C increased hydrolysis time from 24 to 72 h. Decreasing the enzyme loading to 5 FPU/g of DM biomass slightly decreased the enzymatic conversion from 83.4 to 71%. Thus, enzyme loading can be reduced without significantly affecting the efficiency of hydrolysis, an important economical aspect.

Index Entries: Corn stover; wet oxidation; slurry; filter cakes; enzymatic convertible cellulose; enzymatic digestibility.

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Introduction

Lignocellulosic materials, such as agricultural residues, are abundant renewable resources for bioconversion to sugars, which can then be fermented to fuel ethanol. The most important benefit of fuel ethanol production from biomass is reduced CO_2 emissions, thus reducing the greenhouse effect *(1)*.

The cost of raw material dominates the cost of total ethanol production. To attain commercial interest, the costs of bioethanol production must be reduced, and a sufficient amount of cheap and readily available raw material is a necessity *(2,3)*. Corn stover is an ubiquitous agricultural byproduct in Hungary, comprising more than 10 million tonnes every year *(4)*, and, therefore, it has potential as an industrial fermentation substrate. Currently, only 10% of corn stover is used for animal feeding, whereas an efficient use for the remaining 90% has not been found yet.

In corn stover (and plants in general), cellulose is associated with hemicellulose and other structural polysaccharides, surrounded by a lignin sheath. The lignin, which is a complex three-dimensional polyaromatic matrix, is partly covalently associated with hemicellulose, thus preventing hydrolytic enzymes and acids from accessing some regions of the holocellulose *(5,6)*. The highly ordered, crystalline structure of cellulose itself poses another obstacle to hydrolysis *(7)*. To enhance the enzymatic susceptibility of cellulose, applying a specific pretreatment process is essential. The goal of the pretreatment is to disrupt the lignocellulosic matrix to make the substrate more accessible to the enzymes *(5)*.

A number of pretreatment processes have been developed to break down the lignin structure and open the crystalline structure in cellulose. Physical pretreatments, including milling and grinding, pyrolysis, and ionizing radiation are usually reported to be quite ineffective *(5,6,8)*. A size reduction step is required before most chemical and thermochemical pretreatment processes.

Chemical pretreatments with acidic or basic catalysis, especially at high temperature (including steam explosion), are effective methods. During these processes, hemicellulose and lignin may be hydrolyzed to their monomeric constituents and lignin-cellulose-hemicellulose interactions are partially disrupted, thus increasing the enzymatic digestibility of cellulose *(9–11)*.

Biologic pretreatments apply lignin-solubilizing microorganisms to soften lignocellulosic materials suitable for enzymatic digestion. These methods are environmentally friendly and energy saving but are relatively slow, and most lignin-solubilizing microorganisms also solubilize or consume hemicellulose and cellulose to grow *(12–14)*.

Wet oxidation, a reaction involving oxygen and water at elevated temperature and pressure, was presented in the early 1980s to pretreat lignocellulose (wood) as an alternative to the well-studied steam explosion *(15)*. Compared to other pretreatment processes, wet oxidation has been proven

to be more efficient for treating some lignocellulosic materials, because the crystalline structure of cellulose is opened during the process. Organic molecules, including lignin, decompose to CO_2, H_2O, and simpler and more oxidized organic compounds, mainly to low-molecular-weight carboxylic acids *(16)*. Wet oxidation appears to have the advantage of producing fewer byproducts, such as furfural and hydroxymethylfurfural *(17,18)*. Under the conditions of wet oxidation, aliphatic aldehydes and saturated carbon bonds are very reactive; hence, the sugar degradation products, which are known inhibitors of microbial growth *(19)*, are not expected to be produced at high concentration.

In the present study, the wet oxidation process was applied to corn stover and the influence of temperature, time, and pH were studied. To determine the efficiency of the treatment, both the remaining solid fraction after the pretreatment and the unseparated slurry were enzymatically hydrolyzed for 24 h. The time required for total hydrolysis at 50°C and the possibility of reducing enzyme loading and temperature during hydrolysis were also investigated.

Materials and Methods

Raw Material

Corn stover was grown in South Hungary and harvested in 1999. Materials were air-dried and then ground to a 3-mm particle size. The composition of the dried raw material (approx 95% dry matter [DM] content) and the solid fraction remaining after pretreatment were analyzed using a modified gravimetric method described by Goering and Van Soest *(20)*. This method employs neutral detergent washing to remove soluble components (lipids, protein, free sugars, and water-soluble minerals); acidic detergent washing to remove hemicellulose; and oxidation with potassium permanganate to remove lignin, leaving the cellulose and some ash. To measure the total ash content, approx 0.5 g of corn stover was placed in a crucible, ignited at 550°C for 3 h, cooled in a desiccator, and weighed.

Wet Oxidation Pretreatment

Wet oxidation was performed in a specially designed loop autoclave constructed at the Risø National Laboratory *(17)*. The reactor was made of Sandvik Sanicro 28 (27% Cr, 31% Ni, 3.5% Mo, 1% Cu) and mounted on a rack facilitating the control of temperature by immersing the reactor in an appropriate heating or cooling bath. Because of the excellent heat-transfer conditions, the heating and cooling times were very short (about 2 min), which made the pretreatment much more controllable and reproducible.

The wet oxidation condition was selected based on previous pretreatment studies on wheat straw *(21,22)*. Sixty grams (DM) of corn stover was mixed with 1 L of water. A statistical partial factorial design was used to determine the importance of different pretreatment parameters. Three variable factors were selected: pH, reaction temperature, and reaction time.

Table 1
Pretreatment Conditions

	A	B	C	D	E	F
Temperature (°C)	185	185	185	195	195	195
Time (min)	5	15	5	15	5	15
pH before pretreatment	9.2	7.3	3.5	9.2	7.3	3.5
pH after pretreatment	5.7	3.5	3.4	3.9	4.0	2.7

The oxygen pressure and concentration of corn stover were kept constant. The pH was adjusted with 2 g of Na_2CO_3 in the alkaline pretreatment and with 1.9 mL of 36.5% (w/w) H_2SO_4 in the acidic pretreatment; and for neutral pH nothing was added. The conditions of various pretreatments are presented in Table 1. The applied chemicals were added before closing the autoclave and applying oxygen pressure. All pretreatments were run within 1 wk by a single operator to minimize block effects. Each experiment was performed in duplicate and the order of experiments was randomized. After the pretreatment, half of each sample was separated into a liquid and a solid fraction, and both fractions were analyzed and compared to the untreated (only ground) corn stover. The filter cakes were dried in a climate cabinet at 20°C and 65% relative humidity. The filtrate and the nonseparated slurries were stored frozen (–20°C) for further analysis including enzymatic hydrolysis.

Poly- and Monosaccharide Analysis of Liquid Fraction by High-Performance Liquid Chromatography

Samples were hydrolyzed with 4% (w/w) H_2SO_4 at 121°C for 10 min. Sulfate anions were precipitated by 0.5 g of $Ba(OH)_2$. The supernatant was diluted 1:1 with eluent. Average recovery in this purification procedure for glucose, xylose, and arabinose was 86, 83, and 86%, respectively. The purified samples were analyzed by high-performance liquid chromatography (HPLC), using an Aminex HPX-87H column with a matching precolumn (Bio-Rad, Hercules, CA) at 63°C. The eluent was 4 m*M* H_2SO_4 at a flow rate of 0.6 mL/min with detection by refractive index. The amount of the monosaccharides released by the pretreatment was also analyzed by HPLC.

Enzymatic Hydrolysis

The pretreated solid materials were enzymatically hydrolyzed to determine the efficiency of cellulose conversion. The fibrous material was diluted to 2% (DM) using 0.2 *M* citrate buffer (pH 4.8). Hydrolysis was performed in 10-mL test tubes, placed in a heating bath, for agitation with magnetic stirrers. Commercially available enzyme solutions, Celluclast 1.5L and Cellubrix L (cellulase from *Trichoderma reesei*) and Novozym 188 (β-glucosidase; Novozymes A/S, Bagsværd, Denmark), were applied in the enzymatic hydrolysis. Hydrolysis of the filter cake was performed in

duplicate, whereas hydrolysis of slurries was performed in triplicate. The tubes contained 2 g of suspension with an enzyme loading of 25 filter paper units (FPU)/g of DM, and the reaction time was 24 h at 50°C. For the hydrolysis of the slurries, samples were diluted with an equivalent amount of 0.2 *M* citrate buffer, pH 4.8.

For the enzyme-loading study, cellulase loadings were 5, 10, 15, and 25 FPU/g of DM, and the hydrolysis was conducted at 40 and 50°C. To determine the time required for total hydrolysis, samples were hydrolyzed after 72 h.

After hydrolysis, the samples were centrifuged at 8000 rpm for 10 min for removal of residual fibers. The reducing sugar concentration was analyzed by dinitrosalicylic acid (DNS) assay *(23)* and the monosaccharide composition by HPLC. The percentage of cellulose enzymatically converted to glucose (enzymatic convertible cellulose [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:

$$\mathrm{ECC} = \frac{c \cdot V}{m \cdot 1.11} \cdot 100\%$$

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 1.11 factor 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 × 6 cm strip of a 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. The samples were incubated for 1 h at 50°C. Reducing sugars were determined after stopping the hydrolysis by adding 3 mL of DNS solution followed by boiling for 5 min. After cooling, 20.0 mL of distilled water was added and the absorbance read at 540 nm *(24)*.

β-Glucosidase activity was measured by incubating the enzyme solution with 10 µ*M* *p*-nitrophenyl-β-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_2CO_3 and the liberated *p*-nitrophenol measured spectrophotometrically at 400 nm. One unit of activity was defined as the release of 1 µmol of *p*-nitrophenol/min *(25)*.

Results and Discussion

Effects of Pretreatments on Corn Stover

The wet oxidation process was investigated to fractionate corn stover to solubilize the hemicellulose fraction, enhance the enzymatic digestibility of cellulose to glucose, and maximize the recovery of both polysaccharides.

The influence of the six various pretreatment conditions on the composition of corn stover are summarized in Table 1. At high reaction temperature and longer reaction time, the solid fraction was enriched in cellulose. The relative cellulose content ranged from 50.5 to 71.8% compared with 41% cellulose in untreated stover, the higher concentration resulting from the removal of lignin and especially hemicellulose. At 195°C and acidic conditions, the solubilization of hemicellulose was most effective with 95% of the hemicellulose fraction dissolved. However, this pretreatment decreased the lignin by only 18.3%, from 8.7 to 7.1 g/100 g of raw biomass (Table 2). At 195°C, alkaline conditions were most efficient for delignification, in which 59.7% of the original lignin was removed. In addition, this treatment solubilized 57.8% of the hemicellulose. Pretreatments at lower temperatures resulted in less modification of the composition of the solid portion of the pretreated corn stover (Table 2). At 185°C under alkaline or acidic conditions, the lignin content was lowered by approx 30%, but solubilization of hemicellulose was more significant at pH 3.5 than at pH 9.3.

Reaction temperature, time, and the amount of applied chemicals are all economically and environmentally important factors in the overall process; therefore, it is promising that lower temperature (185°C) combined with longer reaction time (15 min) without adding chemicals resulted in a significant modification of the corn stover composition. Delignification was 49.4%, which is nearly as high as at 195°C with alkali addition. The solubilization of hemicellulose was also high (about 42%). At 195°C and neutral pH, similar degrees of delignification and hemicellulose solubilization were achieved, but, unfortunately, without adding acid or base the reaction temperature was difficult to control during the process; thus, this pretreatment might be less reproducible. Figure 1 shows the cellulose and hemicellulose content for each condition.

The pretreatment process enlarges the inner surface area partly by hemicellulose solubilization and lignin degradation. The maximal recovery of hemicellulose and cellulose sugars is important for an ideal pretreatment. The recovery of cellulose and hemicellulose was calculated to estimate their losses during wet oxidation at the different conditions (Table 3). Calculation was based on the mass balance. The following equations show the calculation for the recovery of cellulose and hemicellulose:

$$\text{Recovery of cellulose (\% [w/w])} = \frac{\text{[Cellulose in filter cake (g)]} + \text{[Glucose in filtrate (g)/1.11]}}{\text{Cellulose in untreated corn stover (g)}}$$

$$\text{Recovery of hemicellulose (\% [w/w])} = \frac{\text{[Hemicellulose in filter cake (g)]} + \text{[Xylose and arabinose in filtrate (g)/1.14]}}{\text{Hemicellulose in untreated corn stover (g)}}$$

In wet oxidation, mainly the hemicellulose was converted and/or degraded. About 60% of the original hemicellulose could be recovered in all experiments. This relatively low recovery was probably owing to

Table 2
Material Balances for Each Pretreatment Condition (g/100 g raw material)

	Native corn stover	A	B	C	D	E	F
Solid fraction							
DM (g)	100.0	76.4 (100%)	53.3 (100%)	65.1 (100%)	48.6 (100%)	60.0 (100%)	53.7 (100%)
NCWM (g)[a]	11.7	8.5 (11.1%)	6.1 (11.4%)	8.2 (12.6%)	5.7 (11.7%)	7.3 (12.2%)	7.4 (13.8%)
Hemicellulose (g)	33.7	21.6 (28.3%)	3.3 (6.2%)	9.2 (14.1%)	2.6 (5.3%)	6.9 (11.5%)	0.9 (1.7%)
Cellulose (g)	41.0	38.7 (50.7%)	37.8 (70.9%)	39.8 (61.1%)	34.9 (71.8%)	39.2 (65.3%)	36.2 (67.4%)
Lignin (g)	8.7	5.8 (7.6%)	4.4 (8.3%)	5.9 (9.1%)	3.5 (7.2%)	5.0 (8.3%)	7.1 (13.2%)
Ash (g)	4.9	2.1 (2.7%)	1.5 (2.8%)	2.1 (3.2%)	1.9 (3.9%)	1.6 (2.7%)	2.1 (3.9%)
Liquid fraction							
Glucose (g)	—	1.6	2.8	1.1	3.4	1.5	2.3
Xylose (g)	—	3.2	16.4	14.3	16.5	12.5	17.1
Arabinose (g)	—	1.2	3.0	1.3	3.0	3.2	3.2

[a]Non–cell wall material: water-soluble substances and extractives such as pectin and protein.

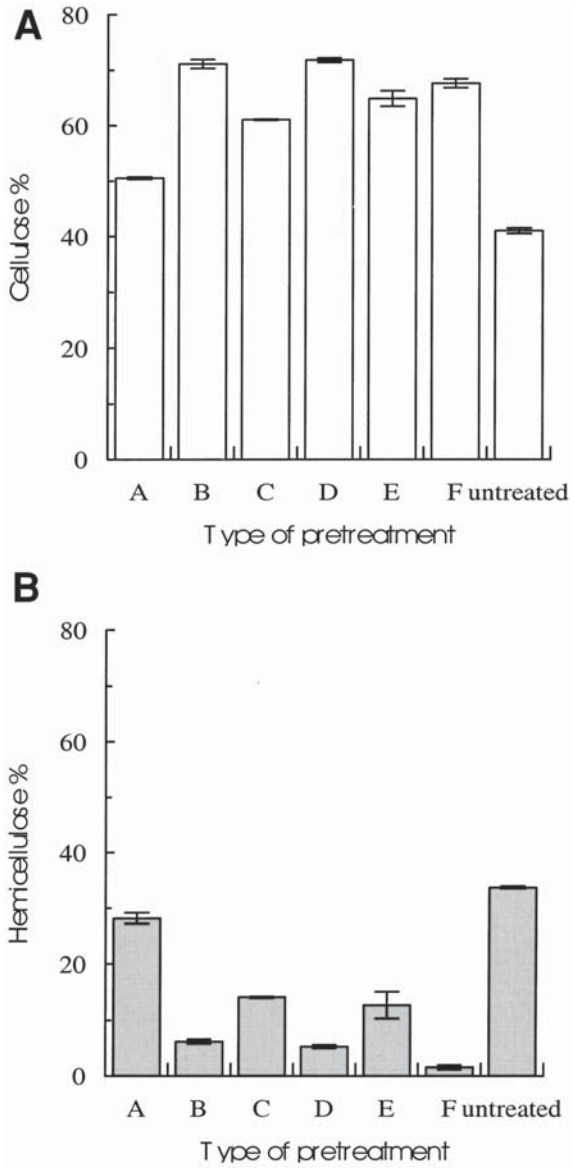

Fig. 1. **(A)** Cellulose and **(B)** hemicellulose content (% [w/w]) after different pretreatments.

Table 3
Recovery (% [w/w]) of Different Components for Each Pretreatment Condition

	A	B	C	D	E	F
Hemicellulose	76.0	60.3	69.5	60.1	64.9	57.2
Cellulose	98.0	98.7	99.6	92.6	98.3	93.4
Overall recovery	88.1	81.4	86.0	78.0	83.2	77.1

hemicellulose oxidation to other products, such as carboxylic acids, CO_2, and H_2O. The recovery of cellulose at 185°C was nearly 100%, whereas at 195°C it was still above 90%. An overall recovery of 80% carbohydrates was obtained, which is similar to the results achieved with wet oxidation of wheat straw *(21)*.

Enzymatic Hydrolysis

Enzymatic accessibility of the cellulose in the pretreated solid material is one of the most important factors for producing bioethanol. Conversion of cellulose to glucose by enzymatic hydrolysis provides valuable information about the efficiency of the wet oxidation pretreatment. Table 4 shows the percentage of ECC of the filter cakes after 24 h of hydrolysis at 50°C.

Enzymatic conversion of pretreated cellulose in the remaining solid was between 52 and 83% compared with 18% for the native corn stover (Table 4). The highest conversion (83.1%) was achieved with a pretreatment at 195°C for 15 min at alkaline pH. This was expected because this pretreatment most significantly modified the composition of the solid portion of pretreated corn stover compared with the untreated corn stover. The pretreatments at 195°C more effectively increased the ECC than pretreatments at 185°C. In particular, pretreatments at alkaline or acidic pH gave significantly higher conversions. This could be explained either by lignin removal (at high pH) or by decreased crystallinity of cellulose during the more harsh pretreatment catalyzed by hydrolysis. Although pretreatment at 195°C at neutral pH gave ECC similar to pretreatment at 185°C (62 and 63%, respectively), the conversions of the replicate samples showed wide variance (63 and 72%, respectively) (Fig. 2). This large variance also occurred in their compositions (Fig. 1). At 185°C with alkali addition, the pH decreased only slightly. In this case, both the hemicellulose and lignin contents changed the least, giving the poorest ECC value, of about 50%.

In a high-temperature pretreatment, furfurals (e.g., hydroxymethylfurfural) and other byproducts can be produced, which are known inhibitors of microorganisms and adversely affect the action of cellulases. To investigate the inhibitory effects of these byproducts, the whole slurry was also hydrolyzed. Hydrolysis yields based on the DNS total reducing sugar analysis are presented as a saccharification yield in Table 4.

Saccharification yield (%) ($Yield_{DNS}$, %) based on the total reducing sugar measured by DNS was calculated as follows:

$$Yield_{DNS} = \frac{c_{DNS} \cdot V}{m_{cellulose} \cdot 1.11 + m_{hemicellulose} \cdot 1.14} \cdot 100\%$$

in which c_{DNS} is the reducing sugar (g/L) measured by DNS; V is the total volume (L); and $m_{cellulose}$ and $m_{hemicellulose}$ are the weights of cellulose and hemicellulose, respectively, before enzymatic hydrolysis (g). The 1.11 and the 1.14 factors convert the polymer concentrations to the equivalent monomer concentrations.

Table 4
Cellulose Conversions (% ECC) and Saccharification Yields (% of theoretical) After Hydrolysis (24 h at 50°C, 25 FPU/g DM) of Filter Cakes and Slurries

	Untreated corn stover	185°C, 5 min, pH 9.2	185°C, 15 min, pH 7.3	185°C, 5 min, pH 3.5	195°C, 15 min, pH 9.2	195°C, 5 min, pH 7.3	195°C, 15 min, pH 3.5
Saccharification yield							
Filter cake at 50°C	18.9	52.2	73.9	70.3	93.0	68.3	78.7
Slurry at 50°C	—	50.5	61.6	66.1	71.8	70.0	61.4
Slurry at 40°C	—	38.1	50.7	48.2	64.4	52.2	52.7
Cellulose conversion							
Filter cake at 50°C	18.1	52.0	62.0	61.7	83.1	63.0	73.7
Slurry at 50°C	—	55.9	59.1	61.5	63.5	60.3	47.2
Slurry at 40°C	—	39.5	44.8	47.0	55.2	43.6	46.2

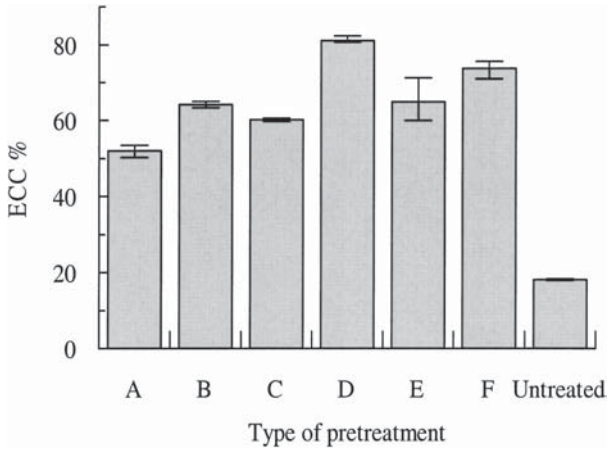

Fig. 2. Conversion of cellulose (% ECC) of remaining solids following 24-h enzymatic hydrolysis at 50°C.

Similar to hydrolysis of the filter cake, 195°C and alkaline pH resulted in the highest ECC (about 63%) and the highest saccharification yield (71.8%) for the hydrolysis of slurries at 50°C, although these values were lower than achieved by filter cake. The ECC conversions of slurries following pretreatment at 185°C were similar to the results at 195°C, except at acidic conditions. The ECC of filter cake was 73.7%, compared with 47.2% in the case of slurry.

The steps following pretreatment (i.e., hydrolysis and fermentation) can be run separately (separate hydrolysis and fermentation) or simultaneously (simultaneous saccharification and fermentation [SSF]). The main benefit of SSF is that the yeast immediately consumes the produced glucose and, thus, the strong inhibitory effect of the glucose in hydrolysis can be avoided. Using thermotolerant yeast (*Klyveromyces marxianus*) *(26)*, the temperature maximum of the SSF is 40°C; hence, hydrolysis of the slurries was also performed at 40°C.

Hydrolysis at 40°C after 24 h gave about 20% lower conversions than at 50°C (Fig. 3). Hydrolysis at 50°C seemed to be completed after 24 h, but at 40°C cellulose conversion increased even after 48 h. At 50°C, the enzymatic reaction was faster, whereas after 48 h the enzyme activities decreased by 25%. However at 40°C, the activity remained after 72 h (Fig. 2). Optimal temperature for enzymatic hydrolysis using *T. reesei* cellulase is usually considered to be 50°C but is for short reaction times, such as filter paper assay. However, for a longer hydrolysis, denaturation and inactivation of the enzymes should be considered, which has also been reported by Kaar and Holtzapple *(27)*.

To enhance the commercial competitiveness of bioethanol, its production cost must be reduced. When considering the cost of hydrolysis, the enzymes are the greater part. For that reason, it is important to use enzymes efficiently by creating a favorable environment in the hydrolysis. The effects

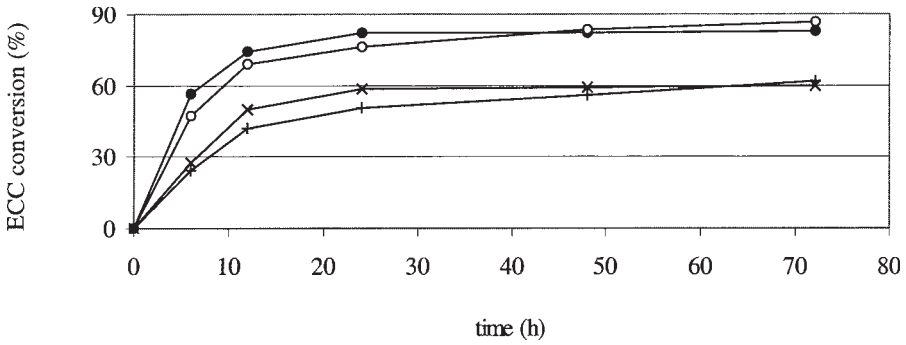

Fig. 3. Time curve of hydrolysis of filter cake (pretreated at 195°C for 15 min at pH 9.2) at 40°C (○) and 50°C (●), and time curves of hydrolysis of slurry at 40°C (+) and 50°C (×).

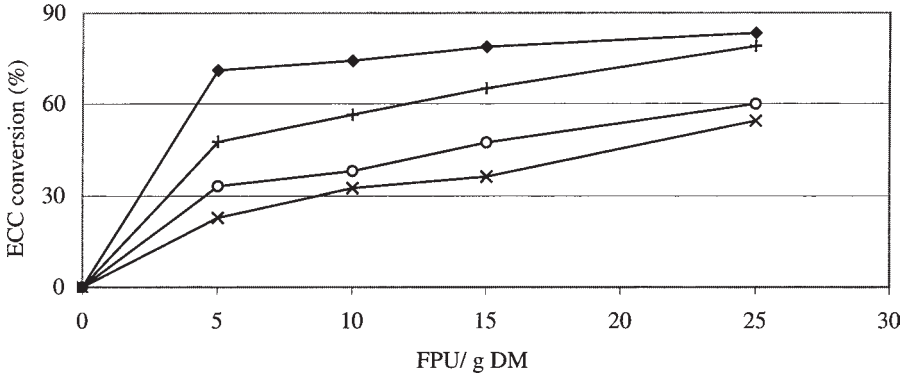

Fig. 4. Cellulose conversion for different enzyme loading after 24-h hydrolysis of filter cake at 50°C (◆) and 40°C (+), and slurry at 50°C (○) and 40°C (×). Pretreatment conditions: 195°C for 15 min at pH 9.2.

of enzyme loading at 40 and 50°C are shown in Fig. 4. The highest enzymatic conversion was achieved when the highest filter paper units were applied, both for hydrolysis of slurries and for filter cakes. However, in the hydrolysis of filter cakes at 50°C, conversion was only 15% lower when enzyme loading was decreased from 25 to 5 FPU/g of dry biomass. In hydrolysis of slurry, decreasing the enzyme loading by fivefold decreased cellulose conversion by 50%.

Cellubrix L, another cellulase enzyme solution from Novozymes A/S, was also tested in the hydrolysis experiments. The filter paper activity of this enzyme solution was 96 FPU/mL, which is about 25% higher than the filter paper activity of Celluclast 1.5 L. Cellubrix L also has some β-glucosidase activity, about 50 IU/mL, which is 10 times lower than the β-glucosidase activity of Novozym 188. This new enzyme solution degraded cellulose as well as the commonly used Celluclast L, but complementation with Novozyme 188 was necessary to reach an appropriate β-glucosidase

Table 5
Enzymatic Cellulose Conversions (% ECC) and Saccharification Yields (% of theoretical) After Hydrolysis with Cellubrix L with and Without β-Glucosidase Complementation[a]

	Cellubrix L	Cellubrix L and Novozym 188	Celluclast L 1.5 and Novozym 188
DNS reducing sugars (g/L)	14.9	15.2	16.2
Glucose (g/L)	9.4	12.7	13.2
Xylose (g/L)	1.8	1.7	2.1
Arabinose (g/L)	1.3	1.2	0.7
Saccharification yield (%)	83.6	86.3	91.3
ECC (%)	57.8	78.5	81.2

[a]Conditions of hydrolysis were 24 h, 50°C, and 25 FPU/g of DM of filter cake following pretreatment at 195°C for 15 min at pH 9.3.

activity for hydrolysis. Without β-glucosidase complementation, the cellulose conversion (ECC) was quite poor (Table 5).

Conclusion

Our study shows that considering its abundance and high cellulose content corn stover could be an excellent substrate for ethanol production in Hungary. Wet oxidation was an efficient process for breaking the tight association between lignin and polysaccharides in corn stover. After the best pretreatment, the enzymatic accessibility of the cellulose in corn stover increased four times. The best conditions for obtaining high convertible cellulose were 195°C for 15 min with added Na_2CO_3, giving about 85% conversion to glucose with 25 FPU/g of cellulase loading. Most of the hemicellulose was dissolved, and approx 36–45% of the original hemicellulose could be identified as saccharides in the wet oxidation filtrate. Enzymatic hydrolysis at 50°C was completed after 24 h, and enzyme activity decreased by 50%. A temperature of 40°C needed longer time for hydrolysis, but after 72 h even better results of cellulose conversion were obtained compared to the 1-d conversions at 50°C after 24 h. Enzyme loading could be decreased from 25 to 5 FPU/g of DM efficiently by the hydrolysis of filter cakes, which is important to make the hydrolysis process more economical.

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