Chemical Pretreatments of Corn Stover for Enhancing Enzymatic Digestibility

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Abstract

Corn stover, the most abundant agricultural residue in Hungary, is a potential raw material for the production of fuel ethanol as a result of its high content of carbohydrates, but a pretreatment is required for its efficient hydrolysis. In this article, we describe the results using various chemicals such as dilute H_2SO_4, HCl, and NaOH separately as well as consecutively under relative mild conditions (120°C, 1 h). Pretreatment with 5% H_2SO_4 or 5% HCl solubilized 85% of the hemicellulose fraction, but the enzymatic conversion of pretreated materials increased only two times compared to the untreated corn stover. Applying acidic pretreatment following a 1-d soaking in base achieved enzymatic conversion that was nearly the theoretical maximum (95.7%). Pretreatment with 10% NaOH decreased the lignin fraction >95%, increased the enzymatic conversion more than four times, and gave a 79.4% enzymatic conversion. However, by increasing the reaction time, the enzymatic degradability could also be increased significantly, using a less concentrated base. When the time of pretreatment was increased three times (0.5% NaOH at 120°C), the amount of total released sugars was 47.9 g from 100 g (dry matter) of untreated corn stover.

Index Entries: Biomass pretreatment; corn stover; enzymatic hydrolysis.

Introduction

A promising scenario to reduce the negative consequences of the greenhouse effect is to produce fuel from biomass. Lignocellulosic materials have especially great potential since they are ubiquitous and can serve as substrates for bioethanol production. In fact, cellulose comprises more than half of all organic carbon in the biosphere: every year 10^{15} kg of cellulose are synthesized and degraded *(1)*.

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Table 1
Amount of Hungarian Agricultural Byproducts from 1986 to 1999

Years	Wheat Straw (x10⁶t)	Barley Straw (x10⁶t)	Corn Stover (x10⁶t)
1985–1990[a]	6.3	1.1	11.4
1991–1995[a]	4.4	1.5	9.1
1998	4.9	1.3	10.8
1999	2.6	1.0	12.6

[a]In average.

However, to gain commercial status, the production cost must be reduced to that for fossil fuels. Since the cost of the raw material represents the major fraction of the total ethanol production cost, a sufficient amount of cheap and readily available raw material is a necessity *(2,3)*. Corn stover is the most abundant agricultural residue in Hungary (Table 1) and, therefore, has potential as an industrial fermentation substrate. Only 10% of the total amount of corn stover is needed as animal feed, and the utilization of surplus material has not been solved yet *(4)*.

In corn stover (and plants in general), cellulose is associated with hemicellulose and other structural polysaccharides and is also surrounded by lignin sheath. This substance around the cellulose microfibrils is partly covalently associated with hemicellulose; thus, it covers some region of cellulose from access of hydrolytic enzymes and acids *(5,6)*. The highly organized, crystalline structure of cellulose itself poses another obstacle in hydrolysis *(7)*. To enhance the enzymatic susceptibility of these carbohydrates, specific pretreatment processes are essential. The goal of the pretreatment is to disrupt the lignocellulosic matrix to make the substrate more accessible to the enzymes *(5)*. Pretreatment can generally be carried out in three different ways: physical, biological, or chemical treatment with acidic or basic catalysts.

Among physical treatments, high-energy radiation and ball milling are reported to be ineffective, energy-intensive, and therefore expensive *(5,7,8)*. On the other hand, some reviews indicated that milling alone can result in yields near to the theoretical maximum from some sources of lignocellulose (e.g., straw, wood) *(9,10)*.

The category of biologic pretreatments comprises the techniques of applying lignin-solubilizing microorganisms to render lignocellulosic materials amenable to enzyme digestion. These methods appear to have the advantages of no extra chemical requirement and low-energy input; however, these environmentally friendly and energy-saving methods are relatively slow, and most of the lignin-solubilizing microorganisms also solubilize or consume hemicellulose and cellulose *(11–13)*.

Chemical pretreatments can be simple; such as soaking the biomass in NaOH at room temperature, or as complicated as treating the material with high-temperature steam (acidic) or ammonia explosion *(14–16)*. Previous studies of pretreatment of enzymatic hydrolysis of corn stover have shown

ambivalent results. Wilke et al. *(17)* obtained only 40% conversion of cellulose to glucose and 60% conversion of the theoretical maximum for all polysaccharides by enzymatic hydrolysis, following dilute H_2SO_4 pretreatment. However, dilute acids, mainly H_2SO_4, are efficiently used for saccharification of hemicellulose *(18)*.

Compared to acid processes, alkaline pretreatment results in less degradation of sugars. Sodium, potassium, calcium, ammonium, and ammonium hydroxide are appropriate chemicals for pretreatment. MacDonald et al. *(19)* obtained 77.5% overall conversion applying dilute NaOH pretreatment at high temperature. Elshafei et al. *(20)* achieved nearly the theoretical conversion maximum for cellulose by soaking the corn stover in 1.0 *M* NaOH for 24 h at room temperature. Karr and Holtzapple *(21)* reported 88.0% conversion of cellulose to glucose using slake lime as pretreatment for 4 h at 120°C. Moreover, lime makes it possible to recover calcium quite easily, as an insoluble calcium carbonate.

Corn stover is the most promising alternative fermentation substrate in Hungary, but without efficient pretreatment, the alcohol yield after fermentation would not be high enough to be economical. According to the literature, chemical pretreatment could be an easy and successful pretreatment method for corn stover. The results presented herein originated form an investigation to determine the best chemicals to efficiently break down the intimate association between carbohydrates and lignin in corn stover, and to make the substrates more accessible for the cellulases. Therefore, corn stover was treated with 1 and 5% (w/w) acids (H_2SO_4 and HCl) and base (NaOH, Ca[OH]$_2$) separately, and consecutively, as well. Sodium hydroxide seemed to be the most efficient pretreating agent; thus, it was used for further experiment in which the effect of the time of pretreatment was studied.

Materials and Methods

Raw Material

Corn stover, which is the corn stalk and leaves without root and corncob, was grown in South Hungary and harvested in October 1999, following the harvest of the mature crop. The material was air-dried and ground to approx 3-mm particle size. The composition of the raw material and the solid fraction obtained after pretreatment was analyzed using a modi-fication of Hägglund's method *(22)*. The air-dried (92.8% dry matter content) material was dried at 105°C for 24 h, then ground in a coffee mill. The fine-ground corn stover was hydrolyzed with 72% (w/w) H_2SO_4 for 4 h at room temperature, and then the hydrolysate was diluted 11 times with distilled water in two steps and was boiled for 6 h at 100°C using reflux cooler. The solution was filtered through a G4 glass filter funnel, and the lignin fraction was gravimetrically determined, as the weight of dried filter cake. For the analysis of the mono- and disaccharides including glucose, xylose, cellobiose, and arabinose in the supernatant, an Aminex HPX-87H liquid-chromatography

Table 2
Applied Chemicals and Concentrations During Pretreatments

Acidic Pretreatment ([w/w] %)	Alkaline Pretreatment ([w/w] %)	Two-Step Pretreatments	
		Soaking Base ([w/w] %)	Acid ([w/w] %)
1% H_2SO_4	1% NaOH	1% NaOH	1% HCl
5% H_2SO_4	5% NaOH	5% NaOH	1% HCl
1% HCl	10% NaOH	1% $Ca(OH)_2$	1% HCl
5% HCl		1% NaOH	1% H_2SO_4

column was used at 65°C. The eluent was 5 mM H_2SO_4 at a flow rate of 0.5 mL/min with detection by a refractometric detection.

To measure ash content, approx 0.5-g sample of corn stover was placed in a crucible and ignited in a muffle furnace at 550°C for 3 h, cooled in a desiccator and weighed.

Pretreatments

Alkaline and Acidic Pretreatments

The ground corn stover was treated with different chemicals under the same conditions. Table 2 gives the applied chemicals and their concentration. Pretreatments were carried out in 1-L round-bottomed flasks, which were placed in an autoclave and boiled for 1 h under pressure at 120°C. Thirty grams of raw corn stover (27.8 g of dry matter) was pretreated in each case, and the concentration of the substrate was 10% (w/w) dry matter; thus, the total amount of the slurry was 300 g. After pretreatment, the slurry was separated into liquid and solid fractions, which were thoroughly washed with distilled water. Both the solid and liquid fraction and also the wash water were analyzed using Hägglund's method combined with high-performance liquid chromatography (HPCL) sugar analysis.

In other cases, the solids were not separated form the liquid phase after acidic pretreatment, but the whole slurry was hydrolyzed after the pH was adjusted to 4.8 with 20% NaOH. The purpose of this experiment was to determine whether the enzymes were inhibited when the whole slurry was sued by hydrolysis.

Two-Step Pretreatments

In the two-step pretreatments, the corn stover was first soaked in base for 1 d at room temperature. Then, it was filtered carefully, washed, and finally boiled with acid as described earlier. The sugar components of the liquid fraction were analyzed by HPCL.

Pretreatments with 0.5% (w/w) NaOH

After the impressive results of the first series of experiments with alkali, dilute NaOH was also used, as a pretreatment agent. In these pre-

treatments, 30 g of air-dried corn stover was boiled with 270 mL of 0.5% (w/w) NaOH for 30, 60, and 90 min, at 100 and 120°C in autoclave. After pretreatment, the slurry was also separated into liquid and solid fractions, and the solid fraction was thoroughly washed with distilled water. The sugar components of the liquid fraction were analyzed by HPLC.

Enzymatic Hydrolysis

The pretreated material was enzymatically hydrolyzed to determine the degree of cellulose conversion. The pretreated corn stover was diluted to 5% dry matter using 0.05 *M* acetate buffer (pH 4.8). Hydrolysis flasks containing 80 g of suspension were maintained at 50°C during the hydrolysis. Hydrolysis was performed in 100-mL shake flasks, placed in an incubated shaker (New Brunswick G24). The speed of agitation was 150 rpm.

Commercial enzyme solution, Celluclast 1.5 L cellulase, and Novozym 188 β-glucosidase, kindly supplied by Novosymes (A/S, Bagsvaerd, Denmark), were used in the enzymatic hydrolysis. In all cases, the hydrolysis was performed in duplicate, and the results are presented as the mean value. The enzyme loading was 25 filter paper units (FPU)/g of dry matter. The reaction time was 72 h, and 3-mL samples were taken at the start and after 2, 4, 6, 24, 48, and 72 h. The samples were centrifuged at 10,000 rpm for 5 min. The reducing sugar concentration was analyzed by dinitrosalicylic acid assay *(23)*. The rest of the samples were kept frozen until ready for analysis by HPCL.

Results and Discussion

Effect of Alkaline Pretreatment on Corn Stover

Table 3 summarizes the influence of the various pretreatments on the components of corn stover. The acid-solubilized lignin fraction, the proteins, and other non-cell-wall components were not measured in the experiments, which would explain why the summations are under 100%.

In excess of 50% of the total material was solubilized during the alkaline pretreatment (Table 4). The concentration of the base, which was used in the pretreatment, and the quantity of the solubilized solid material correlates, although this correlation is valid only for a certain range of alkaline concentration. The solubilization of both the lignin and hemicellulose were the highest when the most concentrated base (10%) was applied. Following this strong alkaline pretreatment, the amount of the lignin in the solid fraction decreased from the original 22.1 to 0.9 g; thus, reduction of the lignin content was 95.9%. The decrease in the hemicellulose content was also considerable, 88.2% of the original amount of hemicellulose solubilized. The solubilization of cellulose was also highest at 10% NaOH, with more than half (53.3%) of the original cellulose solubilized. There was a significant modification in the composition of the solid fraction of the pretreated corn stover compared with the untreated.

Table 3
Composition of Untreated Stover and Pretreated Solids [a]

Type of Pretreatment[a]	Lignin	Ash	Cellulose	Hemicellulose	Total Carbohydrate	Summation
Untreated corn stover	22.1	6.3	41.3	27.9	69.2	97.6
1% NaOH	4.3	2.2	65.4	21.7	87.2	93.6
5% NaOH	3.8	12.7	63.6	15.3	78.9	95.4
10% NaOH	2.9	18.8	58.9	10.0	68.9	90.6
1% H_2SO_4	29.5	3.0	50.6	11.9	62.4	95.0
5% H_2SO_4	30.3	2.9	56.0	7.3	63.3	96.5
1% HCl	27.9	3.0	52.4	13.5	65.9	96.7
5% HCl	31.2	4.0	54.3	6.4	60.8	95.9
TS1	15.1	3.0	63.3	14.3	77.7	95.7
TS2	13.3	1.1	74.2	6.2	80.5	94.9
TS3	14.7	2.7	64.0	15.6	79.6	97.1
TS4	26.7	1.0	55.9	12.2	68.0	95.7

[a]Values are indicated as percentage of dry matter.
[b]Two-step (TS) pretreatments were as follows: TS1, 1% NAOH and 1% HCl; TS2, 5% NaOH and 1% HCl; TS3, 1% Ca(OH)$_2$ and 1% HCl; TS4, 1% NaOH and 1% H_2SO_4.

Table 4
Mass Balance of Alkaline Pretreatment from 100 g (DM) of Untreated Corn Stover

Type of Pretreatment	Amount of Filter Cake (g) (DM)[a]	Liquid Fraction (g)		Solid Fraction (g)			
		Cellulose	Hemicellulose	Lignin	Ash	Cellulose	Hemicellulose
Untreated corn stover	100.0	—	—	22.1	6.3	41.3	27.9
1% NaOH	43.8	12.0	17.5	1.9	0.9	28.6	9.5
5% NaOH	37.7	15.4	21.7	1.4	4.8	24.0	5.8
10% NaOH	32.7	19.6	24.4	0.9	6.1	19.3	3.3

[a]DM, dry matter.

During the alkaline pretreatments, >90% of the original lignin content and >60% of the original hemicellulose content were solubilized. This caused increase in the cellulose content of the remaining solids ranging from 58.9 to 65.4% of the total pretreated solids compared with 41.3% cellulose for the untreated corn stover. A further increase in the base concentration resulted in higher amounts of solubilized cellulose; thus, the cellulose content of the pretreated solid was lower than when using less concentrated base. Table 4 shows the amounts of solubilized and remaining components from 100 g dry matter of untreated corn stover following alkaline pretreatment. After the pretreatment, the alkaline-solubilized lignin fraction was not measured; only the lignin fraction in the remaining solid was subjected for further experiments.

Effect of Acidic Pretreatments on Corn Stover

During the acidic prehydrolysis, 40.8–48.9% of the total material was solubilized (Table 5). Here was a significant difference in the quantity of solubilized hemicellulose when more concentrated acid was used, but the amount of the solubilized hemicellulose seems to depend on the type of applied acid. The highest amount (87.5%) of the solubilized hemicellulose was achieved when 5% HCl was used as a pretreatment agent.

With the solubilization of hemicellulose, the acidic pretreatment of the corn stover caused an increase in the cellulose content of the remaining solids ranging from 50.6 to 56% of the total pretreated solids compared to the 41.3% cellulose content of the untreated material. The mass balance of the acidic pretreatments is summarized in Table 5.

Effect of Two-Step Pretreatments on Corn Stover

Using acidic prehydrolysis, after a 1-d alkaline soaking the results depended on the applied chemicals. More than 51% of the total material was solubilized during the two-step pretreatments when 1% NaOH was used as a swelling agent (Table 6). The $Ca(OH)_2$ pretreatment resulted in 40% solubilization to the total material. The amount of the solubilized lignin and also the hemicellulose were the highest (77.7 and 88.3%, respectively) when 1% HCl was applied following soaking with 1% NaOH. In general, in these two-step pretreatments, the solubilization of lignin was higher than it was in the acidic pretreatments, but it was lower than in the alkaline pretreatments. The amount of solubilized hemicellulose was similar, but the amount of solubilized cellulose was lower than it was in the acidic prehydrolysis. Solubilized cellulose was extremely low (7.2%), when corn stover was treated with $Ca(OH)_2$ and HCl consecutively (TS3).

Enzymatic Hydrolysis

The enzymatic degradability of the cellulose in the pretreated solid material is a critical point in the production of bioethanol. The enzymatic conversion of cellulose to glucose gives valuable information about the

Table 5
Mass Balance of Acidic Pretreatment from 100 g (DM) of Untreated Corn Stover

Type of Pretreatment	Weight of Filter Cake (g) (DM)[a]	Liquid Fraction (g)		Solid Fraction (g)			
		Cellulose	Hemicellulose	Lignin	Ash	Cellulose	Hemicellulose
Untreated corn stover	100.0	—	—	22.1	6.3	41.3	27.9
1% H_2SO_4	51.1	14.9	21.2	15.1	1.5	25.8	6.1
5% H_2SO_4	52.7	11.6	23.3	16.0	1.5	29.5	3.9
1% HCl	59.2	9.3	19.1	16.5	1.8	31.0	8.0
5% HCl	54.0	10.7	23.9	16.8	2.1	29.3	3.5

[a]DM, dry matter.

Table 6
Mass Balance of Two-Step Pretreatment from100 g (DM) of Untreated Corn Stover

Type of Pretreatment[a]	Weight of Filter Cake (g) (DM)[b]	Liquid Fraction (g)		Solid Fraction (g)			
		Cellulose	Hemicellulose	Lignin	Ash	Cellulose	Hemicellulose
Untreated corn stover	100.0	—	—	22.1	6.3	41.3	27.9
TS1	49.0	10.1	20.4	7.4	1.5	31.0	7.0
TS2	37.0	13.2	24.6	4.9	0.4	27.4	2.3
TS3	59.3	3.0	17.8	8.7	1.6	38.0	9.2
TS4	34.5	20.7	22.9	9.2	0.3	19.3	4.2

[a]Two-step (TS) pretreatments were as follows: TS1, 1% NaOH and 1% HCl; TS2, 5% NaOH and 1% HCl; TS3, 1% Ca(OH)$_2$ and 1% HCl; TS4, 1% NaOH and 1% H_2SO_4.
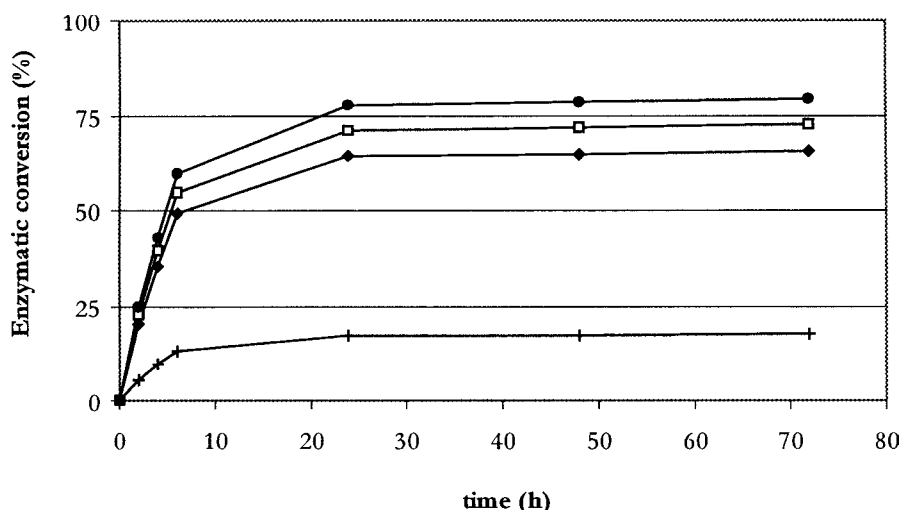[b]DM, dry matter

Fig. 1. Enzymatic conversion (%) of alkaline pretreated corn stover during enzymatic hydrolysis at 50°C, with 25 FPU/g of dry matter. The pretreating agents were as follows: 1% (◆), 5% (□), and 10% NaOH (●); untreated corn stover (+).

efficiency of pretreatments. Figure 1 shows the efficiency of enzymatic conversions of the solid fraction following alkaline pretreatment. The enzymatic conversion was calculated as follows:

$$\text{Conversion (\%)} = 100 \times \frac{\text{glucose (g) after enzymatic hydrolysis}}{\text{cellulose (g) before enzymatic hydrolysis} \times 1.1}$$

The 1.1 factor corrects the amount of glucose to "monocellulose."

The alkaline pretreatment (Fig. 1) increased the enzymatic hydrolysis of corn stover four times compared with untreated corn stover. The conversion increased from 17.6 to 65.6, 72.8, and 79.4% using 1, 5, and 10% NaOH, respectively. Although the highest conversion rate (79.4%) was achieved, applying 10% NaOH, this pretreatment released the least glucose. After 48 h of enzymatic hydrolysis, the amount of released glucose was 20.7, 19.2, and 16.8 g from 100 g dry matter of untreated material, respectively, using 1, 5, and 10% NaOH.

As Fig. 2 shows, the enzymatic conversion of acidic pretreated carbohydrates in the remaining solids was between 32.4% (using 1% HCl) and 46.2% (using 5% H_2SO_4) of the theoretical limit, which was only 2–2.7 times higher, compared with the untreated control. The amount of the released glucose was not significantly higher than the untreated corn stover. Using more concentrated H_2SO_4 and HCl, the amount of hydrolysis-released glucose was 13.1 and 14.5 g, respectively. Using less concentrated acid, these values were 11.1 and 12.1 g from 100 g dry matter of untreated stover.

The enzymatic conversion of the two-step pretreated carbohydrates in the remaining solids, presented in Fig. 3, gave different results according to the different composition of the pretreated material. Under the two-step
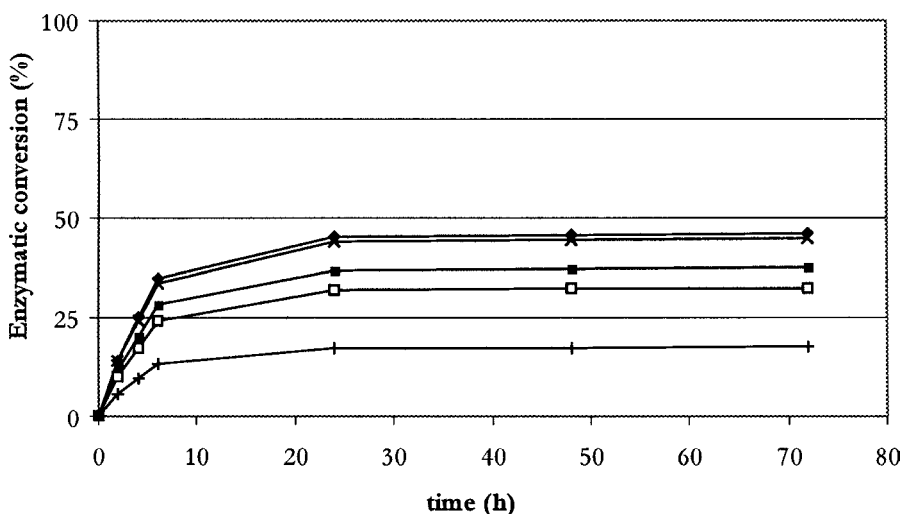
Fig. 2. Enzymatic conversion (%) of acidic pretreated corn stover during enzymatic hydrolysis at 50°C, with 25 FPU/g of dry matter. The pretreating agents were as follows: 1% (■) and 5% (◆); 1% (□) and 5% HCl (×); untreated corn stover (+).
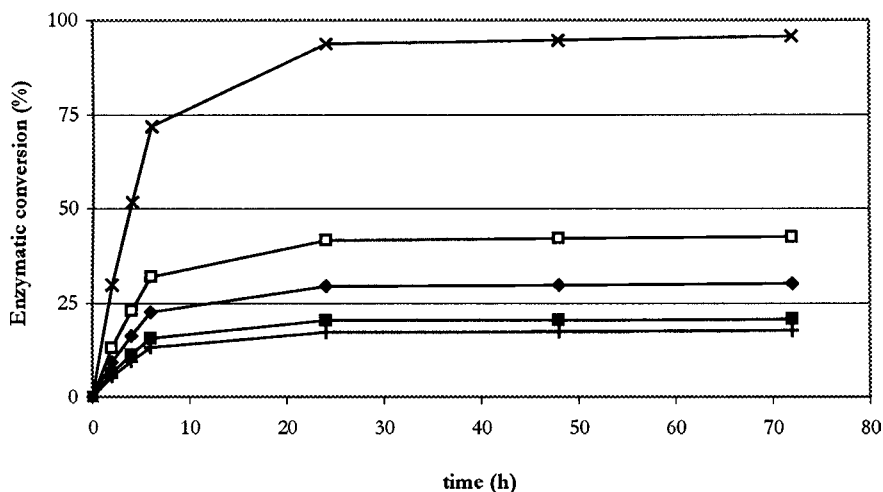

Fig. 3. Enzymatic conversion (%) of two-step pretreated corn stover during enzymatic hydrolysis at 50°C, with 25 FPU/g of dry matter. The pretreating agents were as follows: TS1 (◆), TS2 (■), TS3 (□), TS4 (×), untreated corn stover (+). TS1, 1% NaOH and 1% HCl; TS2, 5% NaOH and 1% HCl, TS3, 1% Ca(OH)$_2$ and 1% HCl; TS4, 1% NaOH and 1% H$_2$SO$_4$.

pretreatments, TS2 pretreatment caused the most significant modification in the composition of corn stover (Table 6), but it seems that this pretreatment did not increase the degree of the enzymatic degradability at all. The best enzymatic conversion (95.7%) was achieved following the TS4 pretreatment. This pretreatment decreased the lignin content form the original 22.1 g to 9.2 g. This means that there was only 58.3% delignification,

compared to 77.7%, which was seen after the TS2 pretreatment. Both pretreatments decreased the hemicellulose content significantly; more than 85% of the original hemicellulose was solubilized.

The enzymatic reaction time was 72 h, but the difference between the enzymatic conversions after 24 h was 3% less in each experiment compared with the conversion after 3 d. The hydrolysis of corn stover seems to be quite fast at 50°C; thus, 1 d appears to be enough for hydrolysis.

To get a real view of the possible use of the pretreated material, an overall conversion was defined and determined as the total production of monosaccharides via enzymatic hydrolysis from the remaining solids and the solubilized sugars in the liquid supernatant, based on the quantity of carbohydrates in the untreated corn stover. The highest overall conversion achieved was from the two-step pretreatment (TS4), which gave 89.7% conversion of monosaccharides form carbohydrates, resulting in 68.3 g of released sugars from 100 g dry matter of untreated corn stover. Pretreatment with NaOH also provided favorable results; the amounts of total released sugar were 57.0, 60.9, and 63.9 g, using 1, 5, and 10% NaOH, respectively. The disadvantage of applying concentrated bases is that the large part of the liberated sugars solubilized during the pretreatment, not in the hydrolysis, which could make its utilization problematic. In Fig. 4 and Table 7, the amounts of liberated sugars from 100 g of untreated corn stover following different pretreatments are summarized.

Although the enzymatic conversions following acidic hydrolysis were not really high, the overall conversions using 5% H_2SO_4 of 5% HCl were >65%, resulting in 52.2 and 50.8 g of released sugars, respectively, from 100 g dry matter of untreated corn stover. The acidic pretreatments have the advantage that 60–80% of the solubilized sugars is hemicellulose and during the enzymatic hydrolysis released sugars is mainly (95%) glucose. In some experiments, following dilute H_2SO_4 pretreatment instead of filtering and washing, the whole pretreated slurry was hydrolyzed after the pH was adjusted to 4.8 with 20% NaOH. In these cases, the total amount of released sugars was between 30.2 and 41.1 g, which was not significantly higher than the amount of sugar that was released during the pretreatment. According to previous results (data not shown), there is no product inhibition until glucose concentration of 15 g/L; thus, the high salt concentration of the liberated inhibitors might have had a negative effect on enzymatic hydrolysis.

Based on the results of our investigation, it appears that NaOH is a good pretreating chemical for corn stover. Although the highest overall conversation was achieved using 10% NaOH, considering both economical and environmental aspects diluted NaOH was used for further investigations.

The corn stover was treated with 0.5% NaOH for 30, 60, and 90 min at 100 and 120°C. Table 8 shows the results following 48 h of enzymatic hydrolysis. Both the highest enzymatic (80.1%) and overall conversion (83.3%) were achieved following pretreatment at 120°C for 90 min. These values were very attractive, higher than conversions after pretreatment
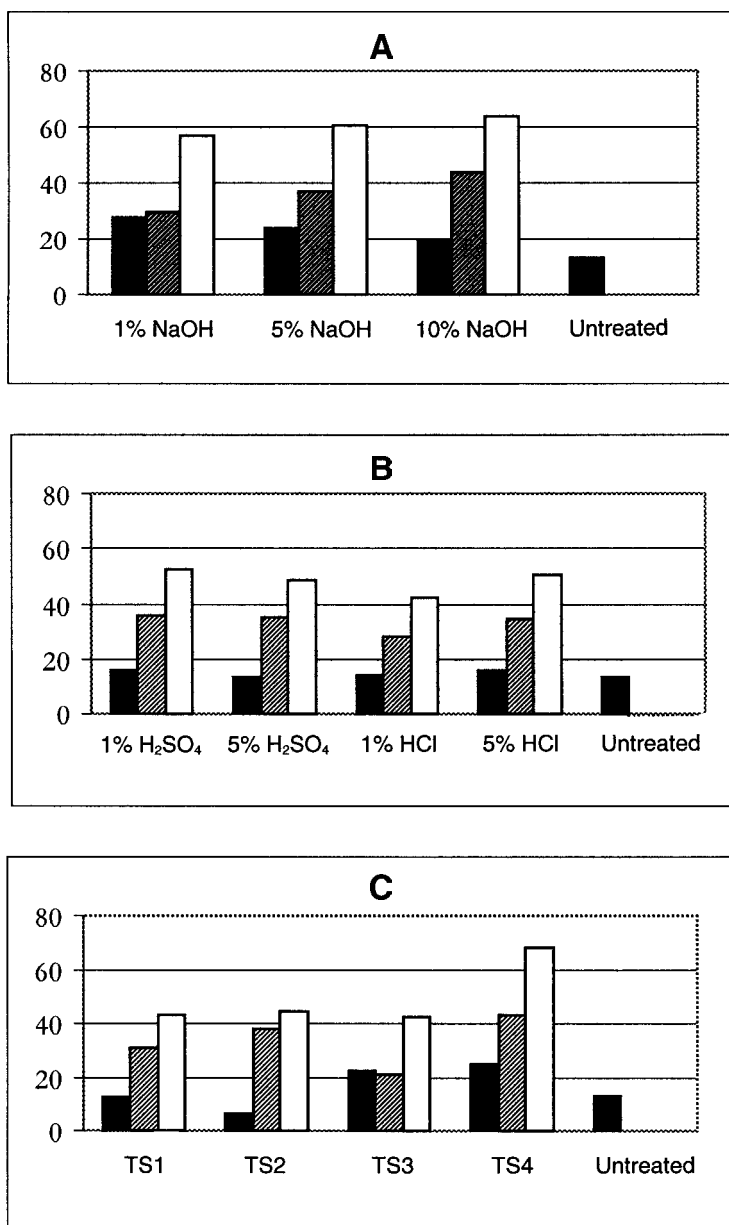
Fig. 4. Weight of released sugars (g) from 100 g of untreated corn stover. **(A)** Alkaline pretreatment; **(B)** acidic pretreatment; **(C)** two-step pretreatment. Weight of sugars (g) from hydrolysis (■), from pretreatment (▨), and total amount of liberated sugars (□).

with a more concentrated base for 60 min. It seems that the longer pretreatment time increased the accessibility of the corn stover and gave 50.5, 65.8, and 80.1% enzymatic conversion after pretreatment for 30, 60, and 90 min respectively.

Table 7
Amount of Released Sugars (g) from 100 g of Untreated Corn Stover
During Different Pretreatment and Enzymatic Hydrolysis Conditions
(after 48 h, at 50°C, 25 FPU/g of dry matter)
and Amount of Total Released Sugars

	Released sugars from hydrolysis (g)	Released sugars from pretreatment (g)	Total released sugars (g)
Untreated corn stover	13.4	0.0	13.4
1% NaOH	27.5	29.5	57.0
5% NaOH	23.8	37.1	60.3
10% NaOH	19.7	44.0	63.7
1% H_2SO_4	16.2	36.1	52.2
5% H_2SO_4	13.7	34.9	48.7
1% HCl	13.9	28.5	42.4
5% HCl	16.2	34.7	50.8
TS1	12.6	30.5	43.1
TS2	6.8	37.8	44.6
TS3	22.0	20.8	42.8
TS4	24.7	43.6	68.3

[a]Two-step (TS) pretreatments were as follows: TS1, 1% NaOH and 1% HCl; TS2, 5% NaOH and 1% HCl; TS3, 1% $Ca(OH)_2$ and 1% HCl; TS4, 1% NaOH and 1% H_2SO_4.

Table 8
Enzymatic and Overall Conversion Percentage After 48 h of
Enzymatic Hydrolysis at 50°C, 25 FPU/g of dry matter,
Following Pretreatment with 0.5% NaOH

	Time of pretreatment with 0.5% NaOH		
	30 min	60 min	90 min
Enzymatic conversion (%)			
100°C	46.3	62.5	67.0
120°C	50.5	65.8	80.1
Overall conversion (%)			
100°C	54.8	58.3	71.6
120°C	55.6	72.7	83.3

Conclusion

It appears that corn stover would be a valuable substrate for ethanol production in Hungary, considering both its vast amount and its degradability. The aim of the present investigation was to determine what kind of chemicals are the most efficient in breaking down the tight association between carbohydrates and lignin, and to enhance enzymatic digestibility.

Nearly theoretical maximum enzymatic conversion (95.7%) was achieved following two-step pretreatment, in which dilute H_2SO_4 was used

after 1-d alkaline soaking. To obtain this attractive result, the delignification of the corn stover was not necessary, and 24 h seemed to be enough to achieve enzymatic hydrolysis of corn stover. Using only dilute acids for the pretreatment, the enzymatic conversions were quite low, but such pretreatment appears to have the advantage that mainly the hemicellulose fraction is solubilized during the pretreatment process, not the cellulose. The best overall conversion, 89.7%, was also achieved by applying 1% H_2SO_4 after 1-d soaking in 1% NaOH. However, by increasing the reaction time, the enzymatic degradability could also be increased significantly, using less concentrated base. By increasing the reaction time threefold in the pretreatment with 0.5% NaOH at 120°C, the overall conversion increased from 55.8 to 83.3%. Considering economical and environmental aspects, diluted NaOH would also be a much more suitable pretreating chemical agent than a more concentrated base. Finding the optimal conditions of the alkaline pretreatment could lead to conversions of corn stover polysaccharide approaching 100%.

The success of the saccharification after pretreatments depends on the enzyme loading, which is also a critical point from an economical view. Future work will focus on optimization of alkaline pretreatment and investigation of the possibility of decreasing the enzyme loading.

Acknowledgments

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