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# Effects of Temperature and Moisture on Dilute-Acid Steam Explosion Pretreatment of Corn Stover and Cellulase Enzyme Digestibility

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## Abstract

Corn stover is emerging as a viable feedstock for producing bioethanol from renewable resources. Dilute-acid pretreatment of corn stover can solubilize a significant portion of the hemicellulosic component and enhance the enzymatic digestibility of the remaining cellulose for fermentation into ethanol. In this study, dilute H<sub>2</sub>SO<sub>4</sub> pretreatment of corn stover was performed in a steam explosion reactor at 160°C, 180°C, and 190°C, approx 1 wt % H<sub>2</sub>SO<sub>4</sub>, and 70-s to 840-s residence times. The combined severity (Log<sub>10</sub> [R<sub>0</sub>] – pH), an expression relating pH, temperature, and residence time of pretreatment, ranged from 1.8 to 2.4. Soluble xylose yields varied from 63 to 77% of theoretical from pretreatments of corn stover at 160 and 180°C. However, yields >90% of theoretical were found with dilute-acid pretreatments at 190°C. A narrower range of higher combined severities was required for pretreatment to obtain high soluble xylose yields when the moisture content of the acid-impregnated feedstock was increased from 55 to 63 wt%. Simultaneous saccharification and fermentation (SSF) of washed solids from corn stover pretreated at 190°C, using an enzyme loading of 15 filter paper units (FPU)/g of cellulose, gave ethanol yields in excess of 85%. Similar SSF ethanol yields were found using washed solid residues from 160 and 180°C pretreatments at similar combined severities but required a higher enzyme loading of approx 25 FPU/g of cellulose.

**Index Entries:** Pretreatment; dilute-acid; acid hydrolysis; corn stover; enzymatic hydrolysis.

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## Introduction

Enzymatic utilization of the cellulose in lignocellulosic feedstocks requires effective pretreatment to make the recalcitrant cellulose more accessible to cellulase enzymes (1–8). Dilute-acid pretreatment can improve enzyme accessibility to cellulose in pretreated lignocellulosic feedstocks and solubilize a significant portion of the hemicellulosic component under pretreatment temperatures ranging from 140 to 180°C (9,10). In general, higher pretreatment temperatures and shorter reactor residence times result in higher soluble xylose recovery yields and enzymatic cellulose digestibility (11).

A preliminary series of pretreatment experiments based on the literature was carried out using a 4-L steam explosion reactor at 160 and 180°C, 1% H<sub>2</sub>SO<sub>4</sub>, and 2 to 14-min reactor residence times. These conditions established baseline total soluble xylose recovery yield for this reactor and could be readily scaled up to the National Renewable Energy Laboratory's (NREL's) Pilot Development Unit (PDU) vertical Sunds Hydrolyzer (12,13). However, the maximum total xylose yield obtained in this series of experiments was 77% of theoretical value, well below our goal of >85%. In an attempt to increase soluble xylose recovery yield, the temperature range was increased to 190°C with 1.0–1.1% (w/w) H<sub>2</sub>SO<sub>4</sub>, and 70-s to 4-min reactor residence time. The combined severity factor ( $\text{Log}_{10} [R_o] - \text{pH}$ ) (14,15) for these higher temperature pretreatment conditions was in the 1.9–2.2 range. These higher-temperature conditions were based on modeling results reported in the literature, which suggest that 90% of the corn stover xylan can be solubilized in 1 min, using H<sub>2</sub>SO<sub>4</sub> concentrations >0.9% (w/w) and pretreatment temperatures >190°C (9). In addition, the effects of dewatering methods and moisture content of input corn stover on pretreatment xylose yields were also investigated (16).

An important goal of pretreatment is to increase the enzymatic digestibility of cellulose remaining in pretreated biomass residues. Higher-temperature dilute-acid pretreatment has been shown to increase cellulose digestibility of pretreated residues (8). The present study compares the digestibility of cellulose remaining in corn stover residues pretreated at 160, 180, and 190°C using a simultaneous saccharification and fermentation (SSF) assay (17). The SSF assay was chosen over the standard enzymatic saccharification assay because of the lower enzyme loading, reaction temperatures, and removal of enzymatic end-product inhibition.

## Materials and Methods

### *Preparation of Feedstock*

Corn stover harvested from the Harlan, Iowa, area (B/MAP, Harlan, IA) during two separate years (1997 and 1998) was coarsely ground at the source and delivered to NREL. The particles in the separate ground batches from each harvest year varied from fines to a fraction (approx 10 wt% of

input mass) containing particles as large as 2–6 in. (5–15 cm) long and 0.5–0.8 in. (1.3–2 cm) thick. Typically, a tote (4 ft [1.2 m] on each side) containing approx 70 kg of dry corn stover (93 wt% solids content) from each harvest year was washed in the NREL PDU wash tank (Sunds Defibrator, Sundsvall, Sweden) with approx 1500 L of room-temperature city water for 45 min. The wash tank consisted of an inverted truncated stainless steel cone (90 in. [2.3 m] diameter  $\times$  80 in. [2 m] high) equipped with an agitator powered by a 60-hp (45-kW) electric motor. The washed corn stover was drained into a stainless steel wire mesh basket overnight to approx 18 wt% solids content with a wet solids recovery of approx 276 kg (76% recovery of input dry solids). The mesh size was approx 0.25 in. (6.4 mm), consisting of 0.125 in. (3.2 mm) wire with an approximate opening size of 0.125 in. (3.2 mm). The difference in nonrecoverable biomass lost in the washing step is attributable to fines, dirt, rocks, tramp metal, and other material suspended or dissolved in the wash water. The washed and drained corn stover was air-dried to approx 45 wt% solid content by spreading on a large rubber-coated tarpaulin and blowing air across it with large fans for several days. The corn stover was mixed and spread with rakes approx once each hour during the workday to promote even air-drying.

### Acid Impregnation

Acid impregnation was carried out in batches of approx 20 kg in Hastelloy<sup>®</sup> C-276 wire mesh (100 mesh) baskets using a recirculating bath. The bath recirculated 1% (w/w) H<sub>2</sub>SO<sub>4</sub> (titrated at 0.98% using NIST traceable NaOH solution) at 50°C evenly through the baskets for 3 or 4 h. Each batch was removed from the hot acid, covered with a plastic bag to minimize evaporation, drained overnight, and mixed by coning and quartering a minimum of three times. The combined batches were dewatered by pressing to approx 50 wt% solids using a hydraulic press (the cylinder mold measured 25 cm in diameter by 30.5 cm high) at an internal pressure within the mold of approx 600 psi. The removal of pressate was assisted by vacuum (approx 22 in. Hg). The pressed solid cakes were broken up by hand and cone/quarter mixed a minimum of three times prior to weighing the mixed solids into sealed Ziplock<sup>®</sup> bags. Each bag to be loaded into the reactor contained 600  $\pm$  0.1 g of processed solids. Moisture loss during mixing resulted in an increase in the acid concentration in the liquid within the corn stover from 0.98 to 1.067%. The biomass titration procedure consisted of taking a random sample of 200  $\pm$  0.1 g of the acid-impregnated, pressed, and mixed feedstock, leaching the acid into 1000  $\pm$  0.1 g of deionized water with constant stirring overnight at 45°C. This was followed by titration with NIST traceable standard NaOH solution (J. T. Baker, Phillipsburg, NJ) in triplicate 50- or 100-mL samples of the leachate.

A second large batch of washed corn stover from the 1998 harvest was soaked with hot 1.2% (w/w) H<sub>2</sub>SO<sub>4</sub> solution as just described using the recirculating bath. The final acid concentration within the corn stover feedstock was determined to be 1.0% (w/w) by leaching and titration. The

difference in pH is attributable to neutralization by some of the ash present in the feedstock. Two aliquots of the acid-soaked corn stover from this batch were pressed using the hydraulic press to produce a batch of 37 to 38 wt% solids content and a second batch of 47 wt% solids content.

In addition, two 5.5-kg batches of washed Harlan corn stover (1997 harvest) were acid-impregnated with 0.36% (w/w)  $H_2SO_4$  at 50°C for 4 h using the recirculating bath. The acid-impregnated and drained corn stover batches were air-dried to approx 46 wt% solids content. The corn stover was mixed every 30 min and the weight of the drying batch monitored until the target evaporative weight loss was achieved. This gave an  $H_2SO_4$  concentration inside the corn stover particles of 1.1% as measured by titration using the leaching method described earlier.

### *Pretreatment*

Pretreatment was carried out using a 4-L steam explosion reactor (NREL Digester) as described earlier (18). The steam pressure was adjusted to achieve the desired reaction temperature. Residence times were varied for each experiment. Each pretreatment experiment used 600 g of input feedstock. The pretreated solids blown from the reactor for each experiment were collected separately in 55-gal nylon Hotfill® or polyethylene drum liners ("bags") sealed in a cooled flash tank connected to the NREL Digester. The bags were removed from the sealed flash tank following either a single or second "shot," and then sealed and cooled to 4°C overnight to condense any steam and volatile compounds (i.e., furfural). The pretreated materials were transferred from the sealed bags to sealed plastic containers, and mixed, and 500-g random samples of each were pressed in the hydraulic press at 600 psi for 8 min. The liquors (pressates) were analyzed for monomeric sugars, total sugars, and organic acids according to established methods using high-performance liquid chromatography (HPLC) (19). The pressed solid cakes were washed exhaustively by suspending in hot water for 30 min and filtering on a Buchner funnel (five times with 40°C tap water and once with 60°C deionized water) before submitting samples for solids compositional analysis by outside laboratories and near infrared (NIR) spectroscopy, and use in SSF enzymatic assays. The results of the liquor and solids compositional analyses were used in a mass balance spreadsheet to determine yield.

### *Cellulose Digestibility of Pretreated Corn Stover Using SSF*

Enzymatic digestibility of the extensively washed pretreated solids was determined using an NREL standard SSF assay (17), that measures the ethanol yield from the fermentation of digested cellulose from the washed residues. These assays help eliminate the end-product inhibition commonly associated with digestibility assays using enzymes alone. The glucan value of pretreated washed solids was initially determined by a rapid NIR method (20) for predicting the cellulose content of each residue for adjustment of enzyme loading. Since the NIR method was in the early

stages of development at this time, the predicted cellulose contents were later confirmed or adjusted using wet chemical analysis results. Aliquots of the exhaustively washed pretreated solid residues were placed in 250-mL baffled Erlenmeyer flasks with water bubble trap stoppers and sterilized at 121°C for 30 min. The sterilized washed solids were diluted to a level of 6 wt% cellulose (approx 10 wt% insoluble solids) by adding sterile components to give a final level: yeast extract (1 wt%), peptone (2 wt%), and citrate buffer (50 mM, pH 5.2). Iogen (Ottawa, Canada) cellulase enzyme (lot no. BRC 191095) was added to give varying enzyme loading levels of approx 5, 15, and 25 filter paper units (FPU)/g of cellulose based on cellulose content in the residues.

A control flask without pretreated solids was used to determine ethanol produced from the media and enzyme components alone. The flasks were inoculated with a cocktail containing both the enzyme level needed and the yeast *Saccharomyces cerevisiae* D<sub>5</sub>A, at an initial optical density of 0.5. Samples were taken at 7, 24, 48, 72, 120, 144, and 168 h. A YSI model 25 glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH) was used to monitor glucose concentrations in each timed sample. HPLC using an Aminex HPX-87H column (Bio-Rad, Richmond, CA), operating at 65°C and a flow rate of 0.6 mL/min with 0.005 M H<sub>2</sub>SO<sub>4</sub> as eluent, and a Hewlett-Packard model 1047 refractive index detector was used to measure ethanol and byproduct concentrations. Ethanol yields were calculated based on the theoretical ethanol concentration from cellulose after subtracting out ethanol produced from the no-solids control flask containing only media and enzyme. These SSF assays are expected to closely mimic the results achievable in a larger-scale SSF fermentation with pretreated corn stover residues at a level of approx 10 wt% solids and lower enzyme loading and fermentation temperatures.

## Results and Discussion

As shown in Fig. 1, the highest total soluble xylose yields (>90%) were achieved with experiments performed at 190°C with a combined severity of approx 1.95. The gravimetric material balances for the pretreatment experiments at 190°C were between 94 and 100%. Therefore, the high xylose yield at 96.3% is not owing to problems with mass balance closure. Yields >90% were found at 190°C even though washed corn stover feedstocks from two different harvest years, 1997 and 1998, were utilized in the pretreatment experiments several months apart. A drop in xylose yield performance was experienced when the temperature was reduced to either 180 or 160°C, even though similar ranges of pretreatment severities were utilized. Material balances dropped from near 100 to 90% under pretreatment conditions with combined severities >2.2. This suggests losses owing to production of volatile degradation components under more severe pretreatment conditions. A maximum soluble xylose yield of approx 77% occurred at 180°C at a combined severity of approx 2.05. The effects of dewatering methods can be seen in Fig. 1 by comparing

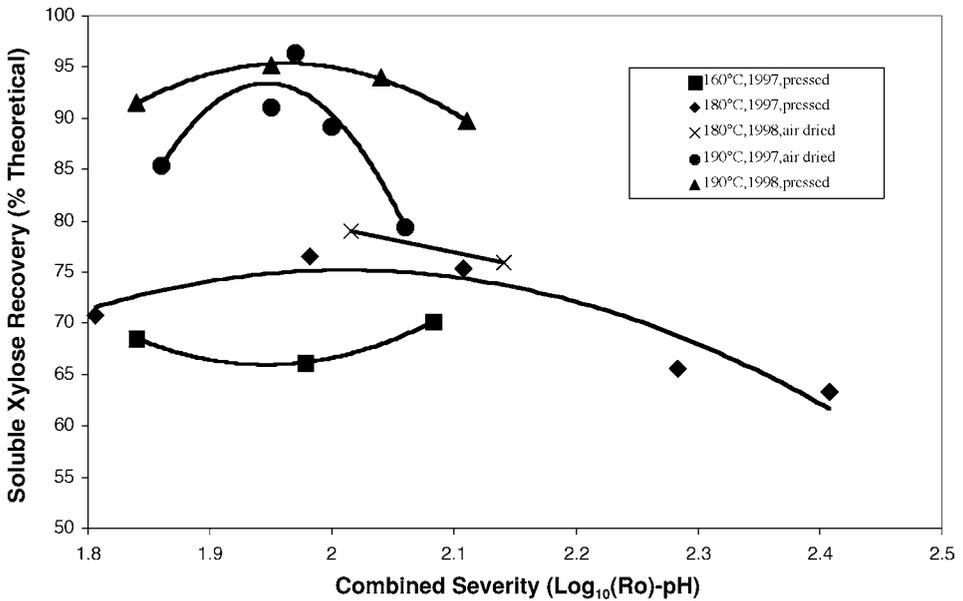


Fig. 1. Total soluble xylose yields from pretreatment experiments using acid-impregnated corn stover at various temperatures, combined severities, harvest years, and dewatering methods after acid soaking. (■) Pretreatment at 160°C using 1997 feedstock pressed to approx 50 wt% solids; (◆) pretreatment at 180°C using 1997 feedstock pressed to approx 50 wt%; (×) pretreatment at 180°C using 1998 feedstock partially air-dried to 47wt%; (●) pretreatments at 190°C using 1997 feedstock partially air-dried to 46-wt%; (▲) pretreatments at 190°C using 1998 feedstock pressed to approx 47 wt% solids.

the two sets of experiments performed at 180 and 190°C, in which the soluble xylose yields are comparable between pressed and partially air-dried acid-impregnated feedstocks.

The effects of increased moisture in the acid-impregnated feedstock on soluble xylose yields for pretreatments at 190°C are shown in Fig. 2. As the solids content of the acid-impregnated feedstock decreased, the soluble xylose yields at lower severities decreased. The effect is similar to decreasing the pretreatment temperature to 180°C. However, as the severity of pretreatment was increased, the soluble xylose yield increased above 90% theoretical, then rapidly dropped off as the conditions became more severe. The peak (combined severity approx 2.13) is rather abrupt, and the optimal condition would be difficult to maintain in a large-scale pretreatment reactor where a few seconds' change in either direction for residence time would significantly alter yield. The much broader maximum associated with the drier, higher-solids-content acid-impregnated feedstock would allow a large-scale pretreatment reactor to maintain conditions necessary for maximum yield even though process conditions varied somewhat during operation. The higher acid loading associated with

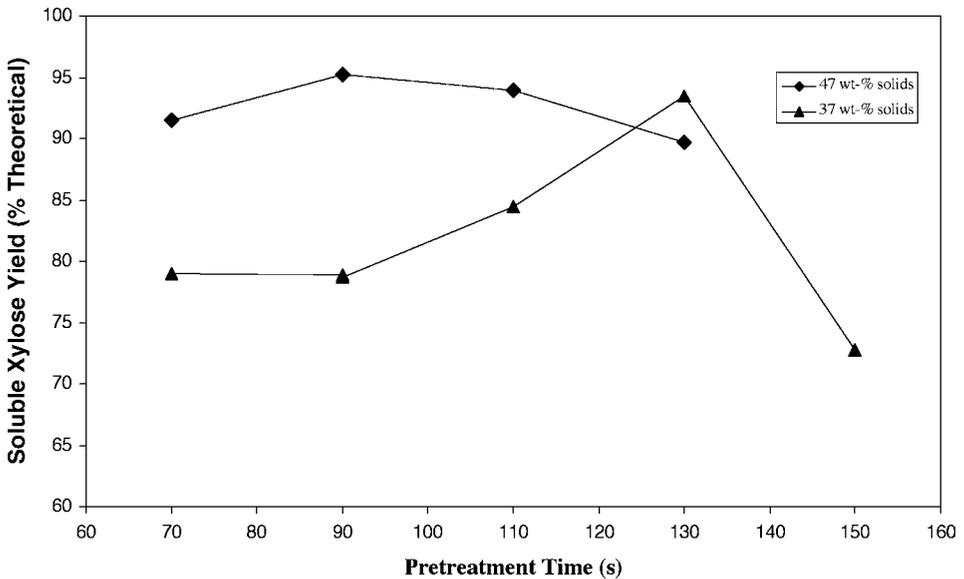


Fig. 2. Effects of moisture level in acid-impregnated corn stover feedstocks on pretreatments at 190°C. (▲) Pretreatments using feedstock harvested in 1998 and pressed to 37–38 wt% solids content; (◆) pretreatments using feedstocks harvested in 1998 and pressed to 47 wt% solids content.

wetter input feedstocks (g acid/g dry biomass) is not beneficial in the pretreatment, since the higher liquid content slows heating of the biomass particles as the result of higher heat capacity, resulting in lower soluble hemicellulose yields. The higher acid loading is less desirable because it would require more neutralizing base (lime) in the overall process, producing more gypsum, and increase downstream separation and disposal costs. The effects of going to lower solids in the reactor (i.e., 10–25%) are being investigated.

Table 1 summarizes key pretreatment conditions harvest year time, temperature, acid concentration, solids content going into the reactor, and calculated combined severities and key results (pH, solids content, and total soluble xylose yields) from each pretreatment. The measured pH values listed were used in the calculation for combined severity (15) reported in this table.

Figure 3 shows the effects of pretreatment temperature on the SSF ethanol yields on four exhaustively washed solid residues from pretreatment experiments carried out at 160, 180, and 190°C for 8 and 14 min, 2 min and 90 s, respectively. Essentially, 70–92% of the cellulose was converted to ethanol. The enzymatic digestibility of corn stover residues pretreated at 190° was higher than at 160 or 180°C under these assay conditions using an enzyme loading of approx 25 FPU/g of cellulose, 32°C, approx 6 wt% cellulose loading, and the yeast *S. cerevisiae*.

Table 1  
Summary of Corn Stover Pretreatment Conditions and Results

Run no.	Input feedstock				Pretreatment conditions			After pretreatment		
	Harvest year	Dewatering method	Solids content (wt%)	Acid conc. (% [w/w])	Temperature (°C)	Time (s)	Combined severity	Solids content (wt%)	pH of liquor	Total soluble xylose yield (% theoretical)
1	1997	Pressing	50	1.07	160	480	1.84	38.3	1.37	68.5
2	1997	Pressing	50	1.07	160	660	1.98	36.5	1.44	66.2
3	1997	Pressing	50	1.07	160	840	2.08	35.7	1.49	70.2
4	1997	Pressing	50	1.07	180	120	1.81	27.1	1.56	70.8
5	1997	Pressing	50	1.07	180	180	1.98	37.4	1.87	76.5
6	1997	Pressing	50	1.07	180	240	2.11	36.6	1.90	75.4
7	1997	Pressing	50	1.07	180	360	2.28	32.9	1.93	65.6
8	1997	Pressing	50	1.07	180	480	2.41	33.0	1.95	63.3
9	1997	Drying	46	1.06	190	70	1.86	25.2	1.1	85.4
10	1997	Drying	46	1.06	190	85	1.95	26.9	1.1	92.9
11	1997	Drying	46	1.06	190	90	1.97	25.5	1.0	96.3
12	1997	Drying	46	1.06	190	95	2.00	25.5	1.1	89.1
13	1997	Drying	46	1.06	190	110	2.06	27.6	1.0	79.4
14	1998	Pressing	47	1.0	190	70	1.84	26.9	1.2	91.5
15	1998	Pressing	47	1.0	190	90	1.95	29.1	1.3	95.2
16	1998	Pressing	47	1.0	190	110	2.04	27.1	1.3	94.0
17	1998	Pressing	47	1.0	190	130	2.11	25.5	1.4	89.7
18	1998	Pressing	38	1.0	190	70	1.86	18.4	1.3	79.0
19	1998	Pressing	38	1.0	190	90	1.97	20.8	1.1	78.9
20	1998	Pressing	38	1.0	190	90	1.97	25.3	1.0	78.7
21	1998	Pressing	38	1.0	190	110	2.05	23.6	1.1	84.5
22	1998	Pressing	37	1.0	190	130	2.13	22.3	1.2	93.5
23	1998	Pressing	37	1.0	190	150	2.09	29.2	1.1	72.8

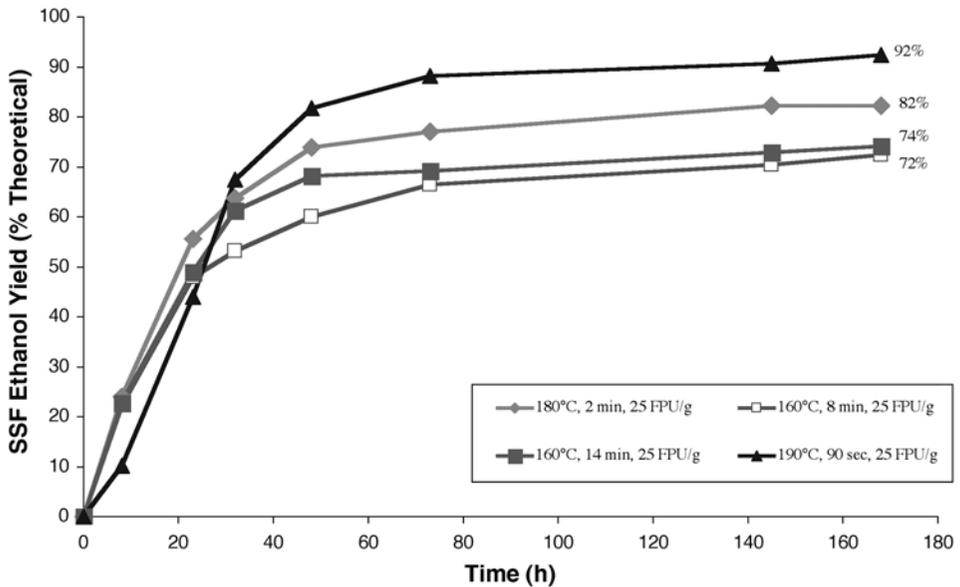


Fig. 3. Effects of pretreatment temperature on SSF ethanol yield from exhaustively washed pretreated residues. Pretreatment was carried out in a 4-L steam explosion reactor with approx 1.1 wt%  $H_2SO_4$ .

The effects of enzyme loading on initial rates and final extent of reaction for converting cellulose to ethanol from corn stover pretreated at 160°C are shown in Fig. 4. Pretreatment of corn stover was carried out in a 4-L steam explosion reactor at 160°C for 14 min, with 1.07%  $H_2SO_4$ . The SSF assays were carried out with *S. cerevisiae* D<sub>5</sub>A yeast with enzyme loadings of approx 5, 15, and 25 FPU/g of cellulose. Solids were loaded to give final cellulose content of 6 wt% based on NIR analysis of the 160°C pretreated residue.

Figure 5 shows the effects of enzyme loading on ethanol yields in SSF fermentations at 32°C for an exhaustively washed residue resulting from pretreatment of corn stover at 190°C. The cellulose loading was adjusted to give 6 wt% based on wet chemical analysis of the residue. Ethanol yield is based on the total expected from the amount of cellulose loaded into each fermentation flask.

The preceding results show that >90% soluble xylose recovery yield and >90% SSF cellulose digestibility can be obtained from corn stover using dilute-acid steam explosion pretreatment at 1%  $H_2SO_4$  (before steam addition), 190°, and short residence times (90–110 s). Lowering the pretreatment temperature to 160 or 180°C, and increasing the residence times to obtain a combined severity factor similar to 190° pretreatments, resulted in lower xylose recovery yield and lower cellulose digestibility.

There are several possible causes for the lower yields at lower pretreatment temperatures. First, high temperature and short residence times

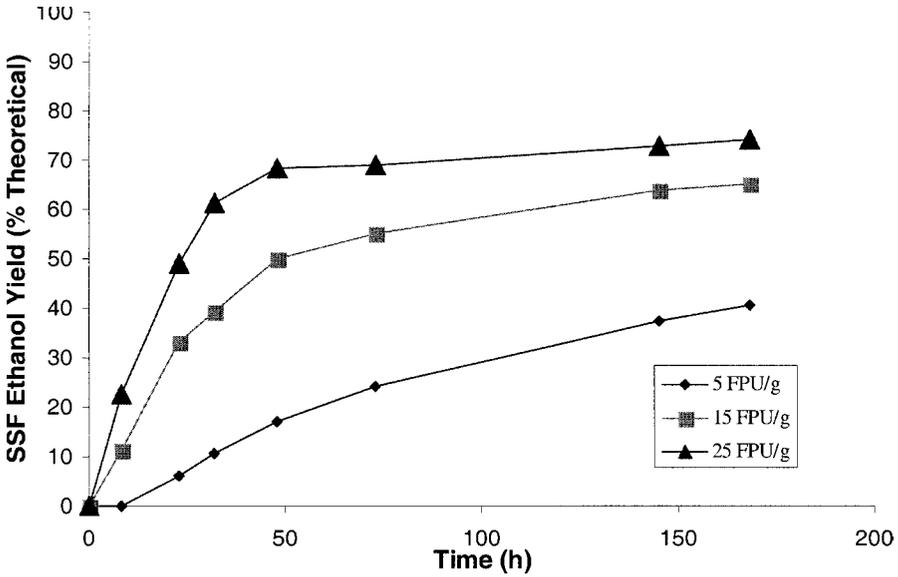


Fig. 4. Effects of enzyme loading on initial rates and final extent of reaction for converting cellulose to ethanol. Pretreatment of corn stover was carried out in a 4-L steam explosion reactor at 160°C for 14 min with 1.07%  $H_2SO_4$ .

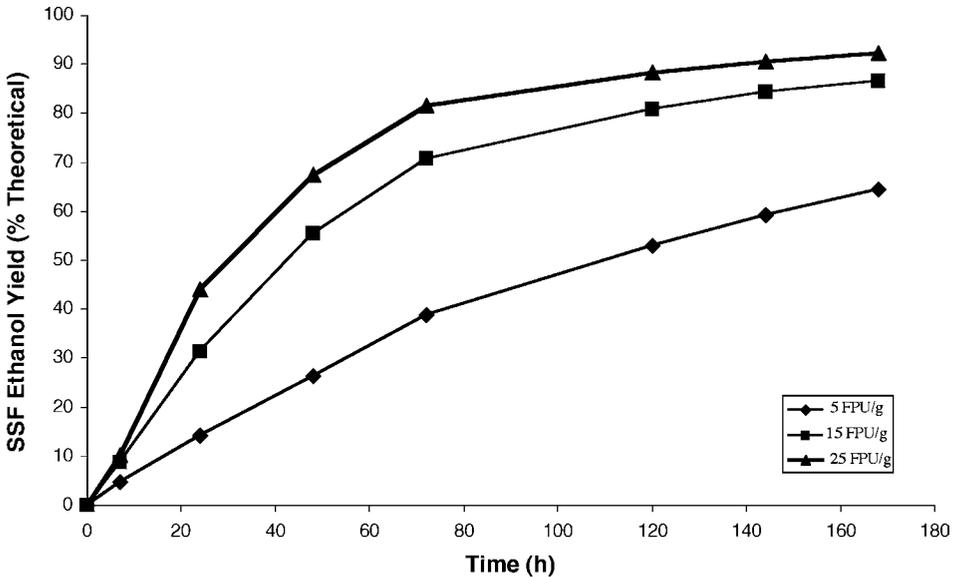


Fig. 5. Effects of enzyme loading on initial rates and final extent of reaction of converting cellulose to ethanol. Pretreatment of corn stover was carried out in a 4-L steam explosion reactor at 190°C for 90 s with 1.06%  $H_2SO_4$ .

favor maximum xylose recovery yields because of differences in activation energy for xylan hydrolysis and xylose degradation reactions. The activation energy of xylan hydrolysis is slightly higher than that of xylose degradation into intermediate products, thus, raising reaction temperatures increases the potential xylose yield during the initial reaction time when xylose concentrations are low. Second, corn stalks contain approx 28% nodes, which have higher density and lignin content (20% lignin in nodes compared with 16.7% in whole stalks, 14.6% in leaves, and 13.3% in pith) than nearby tissues (21). The high-lignin content and dense nodes may require higher temperature to achieve sufficient breakdown of the lignin-carbohydrate complex and increase xylose yields.

The yield of enzymatic digestion of cellulose in pretreated biomass has been reported to increase with removal of xylan. Higher-temperature pretreatments giving the same extent of xylan removal as lower-temperature pretreatments have been reported to give higher cellulose digestibility (1). The lower xylan removal from the 160 and 180°C pretreated samples was a result of incomplete hydrolysis. Therefore, one would expect lower cellulose digestibility.

The effect of starting total solids content in the range studied (37–47%) did not appear to have a significant impact on peak xylose yield at 190°C. Although the peak xylose yield is about the same as that obtained from drier material, the residence time for obtaining high xylose yield for wet corn stover shifts from about 90 to about 130 s (38% solids content) (Fig. 2). The longer residence time is presumably required because of greater heat capacity and slower heat transfer throughout the wetter biomass. The higher-solids feedstocks appear to have a broader range of pretreatment reactor residence time for achieving maximum xylose yields and thus are more desirable from a process control viewpoint. Furthermore, lower-solids feedstocks lead to higher steam requirements (for pretreatment and product recovery) and possibly larger downstream equipment to process more dilute streams.

Unlike pretreatment of softwood, pressing with the hydraulic press before pretreatment did not seem to cause any negative effects (compared to air-drying) on sugar yield from corn stover. This is probably because of the spongy and elastic characteristics of corn stover structure (21).

The SSF cellulose digestibility assays (using an enzyme loading of approx 25 FPU/g of cellulose) of 160, 180, and 190°C pretreated corn stover materials were in the 70, 80, and 90% ranges, respectively. Lowering the cellulase loading to 15 FPU/g of cellulose lowered the SSF cellulose digestibility to 65 and >85% for washed pretreated residues from 160 and 190°C, respectively. The SSF conditions of enzyme loading, temperature, and yeast fermentation are more representative of large-scale process conditions than those found in the typical enzyme digestibility assays in which strong feedback inhibition occurs and high enzyme loadings are typically used.

Acid-catalyzed steam explosion pretreatment of corn stover (pre-impregnated with 1% H<sub>2</sub>SO<sub>4</sub>) was shown to produce digestible residues

and solubilize significant amounts of the hemicellulosic fraction. Soluble xylose yields varied from 63 to 77% of theoretical from pretreatments of corn stover at 160 and 180°C. However, soluble xylose yields >90% of theoretical were found with dilute-acid pretreatments at 190°C. This suggests that a transition occurs between 180 and 190°C for dilute-acid steam explosion pretreatment of corn stover for both hemicellulose hydrolysis and effects on the enzymatic digestibility of cellulose remaining in the residue. A similar transition was found for dilute-acid steam explosion pretreatment of softwood forest thinnings (15).

## Conclusion

Acid-catalyzed steam explosion pretreatment of corn stover (preimpregnated with 1% H<sub>2</sub>SO<sub>4</sub>) produced digestible residues and solubilized significant amounts of the hemicellulosic fraction. Soluble xylose yields varied from 63 to 77% of theoretical from pretreatments of corn stover at 160 and 180°C. However, soluble xylose yields >90% of theoretical were found with dilute-acid pretreatments at 190°C.

The effect of starting total solids content in the range studied (37–47%) did not have a significant impact on peak xylose yield at 190°C; however, the peak xylose yield shifted to longer residence times, from about 90 to about 130 s. The longer residence time is presumably required because of greater heat capacity and slower heat transfer throughout the wetter biomass. The higher-solids feedstocks had a broader range of pretreatment reactor residence time for achieving maximum xylose yields and thus are more desirable from a process control viewpoint.

SSF of washed solids from corn stover pretreated at 190°C, using an enzyme loading of 15 FPU/g of cellulose, gave ethanol yields in excess of 85%. Similar SSF ethanol yields were found using washed solid residues from 160 and 180°C pretreatments at similar combined severities but required a higher enzyme loading of approx 25 FPU/g of cellulose.

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