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Optimization of SO_2-Catalyzed Steam Pretreatment of Corn Fiber for Ethanol Production

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Abstract

A batch reactor was employed to steam explode corn fiber at various degrees of severity to evaluate the potential of using this feedstock as part of an enzymatically mediated cellulose-to-ethanol process. Severity was controlled by altering temperature (150–230°C), residence time (1–9 min), and SO_2 concentration (0–6% [w/w] dry matter). The effects of varying the different parameters were assessed by response surface modeling. The results indicated that maximum sugar yields (hemicellulose-derived water soluble, and cellulose-derived following enzymatic hydrolysis) were recovered from corn fiber pretreated at 190°C for 5 minutes after exposure to 3% SO_2. Sequential SO_2-catalyzed steam explosion and enzymatic hydrolysis resulted in a conversion efficiency of 81% of the combined original hemicellulose and cellulose in the corn fiber to monomeric sugars. An additional posthydrolysis step performed on water soluble hemicellulose stream increased the concentration of sugars available for fermentation by 10%, resulting in the high conversion efficiency of 91%. *Saccharomyces cerevisiae* was able to ferment the resultant corn fiber hydrolysates, perhydrolysate, and liquid fraction from the posthydrolysis steps to 89, 94, and 85% of theoretical ethanol conversion, respectively. It was apparent that all of the parameters investigated during the steam explosion pretreatment had a significant effect on sugar recovery, inhibitory formation, enzymatic conversion efficiency, and fermentation capacity of the yeast.

Index Entries: Corn fiber; steam pretreatment; enzymatic hydrolysis; posthydrolysis; fermentation; ethanol.

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Introduction

Corn fiber is a very attractive lignocellulosic substrate for the bioconversion to ethanol process because it has a high carbohydrate content, it is present in large quantities in most ethanol- and starch-processing plants, and it has few competing uses. Corn fiber is a mixture of corn hulls and residual starch not extracted during the wet-milling process and comprises up to 11% of the dry weight of the corn kernel *(1)*. It has been predicted that the utilization of the corn fiber fraction in the conversion process could potentially increase the overall ethanol yield by an additional 10% *(2)*.

For efficient production of ethanol from lignocellulosic biomass by sugar hydrolysis and fermentation, the lignocellulosic substrate should be pretreated to more effectively recover the hemicellulose and, concurrently, to make the cellulose more accessible to enzymatic hydrolysis. To be fully sufficient, the pretreatment should provide effective recovery of the majority of the hemicellulose-derived sugars, lignin, and cellulosic component that can be readily hydrolyzed by enzymes and, later on, fermented. Several pretreatment options for corn fiber have been evaluated for their efficacy in solubilizing, fractionating, hydrolyzing, and separating these major compositional constituents. Dilute–sulfuric acid hydrolysis of corn fiber at 140–160°C and pH 1.5–2.0 for 10–30 min followed by partial neutralization and enzymatic saccharification of polysaccharides gave a yield of 81–87% of total carbohydrates as free sugars *(2)*. Although this process showed that enzymatic hydrolysis achieved a high conversion of all polysaccharides in the corn fiber, it was also apparent that significant inhibitor formation occurred at all pretreatment conditions tested between 140 and 160°C *(2)*. Other pretreatments used with corn fiber (ammonia fiber explosion [AFEX] treatment and hot water), although effectively preparing cellulose for enzymatic hydrolysis, did not completely hydrolyze the xylan *(3,4)*. Although many corn fiber pretreatment methods have been proposed, none have been proven to be fully effective. Previously, we analyzed SO_2-catalyzed steam explosion of corn fiber in the temperature range of 170–200°C, time of 1.5–5 min, and SO_2 concentration of 0–6% *(5)*. Although, the results indicated that maximum sugar yield (soluble and following enzymatic hydrolysis) was recovered from corn fiber pretreated at 190°C for 5 min with 6% SO_2, the effects of varying the different parameters were not assessed by response surface modeling. We also did not analyze oligomeric sugars, or the potential fermentability of water soluble fractions obtained from each of the pretreatment, posthydrolysis, and enzymatic hydrolysis.

The purpose of the present study was to investigate the influence of steam explosion pretreatment parameters (time, temperature, and pH) on the subsequent enzymatic saccharification and fermentation of corn fiber and, as a result, to establish optimum pretreatment conditions for the feedstock by using standard response surface technique. This statistical model involved fitting an empirical model to the experimental data and identify-

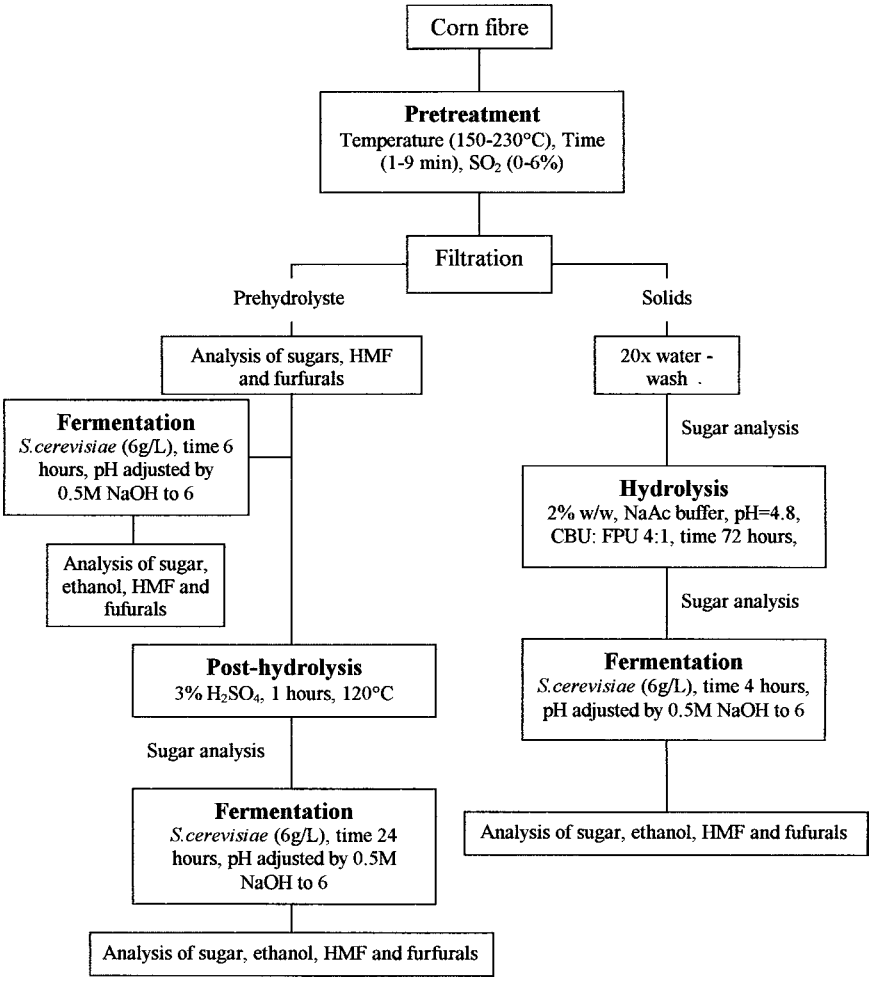


Fig. 1. Experimental procedure to SO_2-catalyzed steam explosion of corn fiber and consequent posthydrolysis, fermentation, and enzymatic hydrolysis.

ing the optimal residence time, temperature, and SO_2-impregnation level in the pretreatment stage.

Materials and Methods

Substrate Pretreatment

The experimental procedure used in this study is shown schematically in Fig. 1. Corn fiber (60.0% moisture content) was obtained from the National Center for Agricultural Utilization Research (Peoria, IL) and stored at –20°C until use. Samples of 300 g (dry wt) were impregnated overnight with anhydrous SO_2 in plastic bags. The uptake of SO_2, expressed as a percentage of the

Table 1
Conditions for SO_2-Catalyzed Steam Pretreatment of Corn Fiber and Shot Yields Expressed as Percentage of Original Oven Dried Corn Fiber After Steam Explosion

Experiment	Severity of pretreatment (log R_o)	Temperature (°C)	Time (min)	SO_2 (%)	Shot yield (%)
1	2.06	170	1	3	97
2	2.17	150	5	3	85
3	2.76	170	5	0	89
4	2.76	170	5	6	92
5	3.02	170	9	3	89
6	3.35	190	5	3	87[a]
7	3.35	190	5	3	91[a]
8	3.35	190	5	3	86[a]
9	3.58	210	2.2	1	89
10	3.58	210	2.2	5	86
11	4.13	210	7.8	1	79
12	4.13	210	7.8	5	81
13	4.53	230	5	3	74
14	4.35	190	5	0	91
15	3.35	190	5	6	86

[a]Average shot yield: 88 ± 2.6 %.

oven-dried corn fiber, was measured by weighing the corn fiber before and after SO_2 addition. The samples were then loaded, in 50-g batches, into a preheated 2-L Stake Tech II batch reactor (Stake Tech-Norvall, Ontario, Canada) and exploded at different severities (temperature ranging from 150–230°C; time from 1.5 to 5 min; SO_2 concentration from 0 to 6% weight/oven-dried weight of fiber) (Table 1). The severity of the steam explosion pretreatment was represented by the severity factor as defined by Overend and Chornet *(6)*. This severity factor (R_o) combines the effects of time and temperature, as follows:

$$R_o = t \times e^{(T_r - T_b)/14.75} \quad (1)$$

in which t is the residence time (min), T_r is the reaction temperature (°C), and T_b is a reference temperature (100 °C).

Following steam explosion, the concentration of sugars in the water soluble fraction was quantified by high-performance liquid chromatography (HPLC), while the water-insoluble fraction (water-washed) was collected and adjusted to 2% (w/w) dry matter content and subsequently used for enzymatic hydrolysis. Shot yields (%) were determined by dividing the dry weight of the steam-exploded sample by the dry weight of the nonpretreated sample (300 g) (Table 1).

Characterization of Substrate

The chemical composition of the original starting material and the steam-exploded solids was determined using a modified Klason lignin method derived from the TAPPI Standard method T222 om-98 *(7)*. Briefly, 0.2 g of sample was incubated at 20°C with 3 mL of 72% H_2SO_4 for 2 h with mixing every 10 min. The reaction was then diluted with 112 mL of deionized water (final acid concentration of 4% H_2SO_4) and transferred to a serum bottle. The solution was subjected to autoclaving at 121°C for 1 h and filtered through a medium coarseness sintered-glass filter for gravimetric determination of acid-insoluble lignin. Each experiment was run in triplicate. The concentration of sugars in both the filtrate and the stream pretreatment hydolyzate, as well as inhibitors such as 5-hydroxymethylfurfurals (HMFs), were determined by HPLC analysis. The HPLC system (Dionex DX-500; Dionex, Sunnyvale, CA) was equipped with an ion-exchange PA1 (Dionex) column, a pulsed amperometric detector with a gold electrode, and a Spectra AS 3500 autoinjector (Spectra-Physics, Oroville, CA). Prior to injection, samples were filtered through 0.45-mm HV filters (Millipore, Bedford, MA) and a volume of 20 µL was loaded. The column was equilibrated with 250 m*M* NaOH and eluted with deionized water at a flow rate of 1.0 mL/min.

Ethanol and furfural concentration were determined using a Hewlett-Packard 5890 gas chromatograph equipped with a 6890 autoinjector, and a flame ionization detector. Components were separated using a 30-m Stabilwax-DA column supplemented with a 5-m deactivated guard column (Restek).

Posthydrolysis

Duplicate samples containing 27 mL of the water soluble fraction were posthydrolyzed after adding concentrated H_2SO_4 to achieve a final concentration of 3% acid. Posthydrolysis was performed by heating the solution at 121°C for 1 h in an autoclave. A batch of sugar standards was also autoclaved under the same conditions, to estimate any hydrolysis loss. Sugar concentrations were detected by HPLC as described previously.

Enzymes

A complete *Trichoderma reesei* cellulase (Celluclast®, Novo Nordisk, Franklinton, NC) was used in combination with a commercial β-glucosidase (Novozym 188®, Novo Nordisk) for cellulose degradation, while gluco-amylase (200 U/mL) and α-amylase (300 U/mL) (Sigma, St. Louis, MO) were used to ensure complete hydrolysis of the starch. The Celluclast preparation contained 49 mg of protein/mL as measured by the Bio-Rad protein assay (Bio-Rad, Hercules, CA) and had the following hydrolytic activities: 80 filter paper units (FPU)/mL (filter paper activity), 52 IU/mL of carboxymethylcellulase (CMCase), 226 IU/mL of xylanase, and 50 IU/mL of β-glucosidase. The protein content and activities of Novozym 188 were as follows: 44 mg/mL, 792 cellobiose units (CBU)/mL, 5 FPU/mL,

34 IU/mL of CMCase, 94 IU/mL of xylanase, and 500 IU/mL of β-glucosidase. The enzyme activities were measured as described by Ghose *(8)*.

Enzymatic Hydrolysis

Water-washed pretreated solids obtained during steam explosion were hydrolyzed at a 2% (w/v) solid concentration in 50 m*M* sodium acetate buffer (pH 4.8) at 45°C with continuous agitation (200 rpm) for 72 h. Each flask was inoculated with enzyme based on the amount of FPU of cellulase and CBU (Novozyme 188) added at a CBU:FPU ratio of 4:1, with excess glucoamylase and α-amylase as described by Grohmann and Bothast *(2)*. Aliquots of 200 μL were aseptically removed at different reaction intervals and boiled for 5 min to inactivate the enzymes. The sugar concentration was then determined by HPLC. Each experiment was run in duplicate. The fermentability of sugars obtained after enzymatic hydrolysis (liquid fraction) was tested using *Saccharomyces cerevisiae*.

Fermentation Microorganisms

A spent sulfite liquor–adapted strain of *S. cerevisiae* was generously provided by Tembec (Quebec, Canada), and used for all fermentations. The yeast was maintained at 4°C on YPD medium (2% glucose, 1% yeast extract, 2% peptone, and 1.8% agar). For each fermentation, *S. cerevisiae* was pregrown in 500 mL of YP medium (1% glucose, 1% yeast extract, and 1% peptone) at 30°C for 3 d, and then harvested after 24 h, and resuspended in fresh YP medium. After extensive washing, the inoculum cell concentration was adjusted with sterile deionized water to provide the final cell concentration of 6 g/L (oven-dried cell weight).

Fermentation of Corn Fiber Hydrolysate

Fermentation of liquid sugar fractions (prehydrolysate, post-hydrolyzed prehydrolysate, and liquid fraction obtained during enzymatic hydrolysis) was conducted in 50-mL beakers by preadjusting liquid broth to pH 6.0 with 0.5 *M* sodium hydroxide and supplementation with 0.125 mL of 2 *M* $(NH_4)_3PO_4$. Each 50-mL beaker contained 20 mL of the water soluble sugar source and 1 mL of *S. cerevisiae* inoculum (6 g/L of oven-dried cell weight). The fermentation vessels were maintained at 30°C with continuous agitation (200 rpm). Sugars, ethanol, HMF, and furfurals were determined periodically from the aliquot culture samples during the course of the fermentation. The relative ethanol yield, Y_{EtOH}/Y^{ref}_{EtOH}, was defined as the ratio of the ethanol yield of the filtrate and the reference fermentation. Each experiment was run in duplicate.

Response Surface Methodology

A response surface methodology was used to study the effects of temperature, time, and SO_2 concentration on hemicellulose recovery, enzymatic digestibility, and fermentability of corn fiber after steam explosion

Table 2
Fitted Coefficients in Eq. 1 for Empirical Models and Corresponding R^2 Values

Experiment	Monomer sugar recovery in prehydrolysate	Sugar hydrolysability	Prehydrolysate fermentability
Intercept	26.47	80.47	94.33
C_1	2.88	1.59	0
C_2	5.06	1.65	–2.38
C_3	2.51	2.91	0
C_4	–1.55	–3.61	0
C_5	0	0	0.13
C_6	0	0	0
C_7	0	0	–2.56
C_8	–3.03	–6.79	–5.83
C_9	0	–1.41	–2.94
R^2	0.99	0.99	0.97

(9). A full three-level factorial design consisted of 13 sets of experimental conditions, including the center-point experiment, which was chosen based on the results of our previous finding *(5)*, and was performed in triplicate (Table 1). In addition, to test the influence of SO_2 impregnation during pretreatment, two additional steam explosion conditions were included. Statistical analysis was performed by fitting Eq. 2 to experimental response data (Y) by multiple linear regression, using Microsoft Excel:

$$Y = \text{Intercept} + C_1 \times T + C_2 \times q + C_3 \times S + C_4 \times Tq + C_5 \times TS + C_6 \times qS + C_7 \times T^2 + C_8 \times q^2 + C_9 \times S^2 \quad (2)$$

in which T-time (min), q is temperature (°C), and S is SO_2 (% [w/w]). Terms that were statistically insignificant (at the 95% confidence level) were eliminated from Eq. 2 to give an empirical model for each dependent variable in terms of time, temperature, and SO_2 concentration. The coefficient of determination and R^2 are reported in Table 2 to indicate the adequacy of fit for each empirical model. Means and variances were calculated for three repeats of the center-point conditions.

Results and Discussion

Shot Yield

As mentioned in the Introduction, it is important that pretreatment conditions that ensure good hydrolysis of the water-insoluble cellulose stream but that are not so severe that the overall yield of recovered carbohydrate is unacceptably low are defined. When we first assessed a range of conditions for pretreating corn fiber it was apparent that the severity had

a substantial effect on total recovery of solids after steam explosion with the shot yield ranging from 74 to 97% (Table 1). Clearly, increased pretreatment severity reduced the yield recovery of the original corn fiber. The best yields were obtained when corn fiber was treated at lower temperatures (150–170°C), while lowest shot yield was obtained at 235°C. At the same temperature (170 and 210°C), longer reaction times led to lower shot yield. It was apparent that the more severe steam explosion conditions significantly decreased the shot yield for the recovery of solid material. It was probable that the reduction in the amount of fibrous material with increasing severity could be attributed to the solubilization of the hemicellulose, since similar results have been reported during SO_2-catalyzed steam explosion of *Pinus radiata (10)*, *Pseudotsuga menziezii (11)*, and mixture of *Picea abies* and *Pinus silvestris (12)*.

Total Sugar Yield

Monomer Sugar Yield

Once we had established conditions that provided optimum recovery of the hemicellulose and cellulose fractions, we next wished to assess the nature and concentration of sugars in the water-soluble, hemicellulose-rich fraction. When comparing the chemical composition of the original corn fiber (Table 3), it was apparent that following steam explosion, a high percentage of all monomeric hemicellulose-derived sugars was recovered at temperatures ranging from 170 to 210°C (Table 4). As the pretreatment severity increased, the concentration of monomers in the prehydrolysate increased. At each of the pretreatment temperatures used (170, 190, and 210°C), increased pretreatment times and SO_2 concentrations yielded higher recovery of monomeric hemicellulose-derived sugars (Table 4 and Fig. 2A,B). Maximum recoveries of the hemicellulose-derived monomers (34%) were obtained at temperatures between 190 and 220°C and pretreatment times of 6–8 min (Fig. 2A). Although temperature and time appeared to be the main factors influencing recovery optimization, interaction between temperature and SO_2 levels also appeared to influence hemicellulose recovery. Maximum recovery of the hemicellulose-derived monomers (37%) was obtained at temperatures between 190 and 220°C and SO_2 concentrations between 4 and 6% (Fig. 2B). Increasing SO_2 concentration increased monomer sugar yield but reached a plateau at roughly 3%. The impregnation of additional SO_2 prior to pretreatment did not seem to significantly improve monomer sugar recovery, although it did result in a decrease in the concentration of total sugars. Similar findings were previously reported by Clark and Mackie *(10)*, in which the effect of SO_2 loading on total sugar recovery of steam-exploded *Pinus radiata* was significant up to approx 3% (w/w) dry matter. These findings imply that the observed decrease in prehydrolysate oligomer recovery with increased SO_2 concentration results from the lower pH, which facilitates the complete depolymerization of oligomeric hemicellulose.

Table 3
Composition of Original Corn Fiber (% wt)

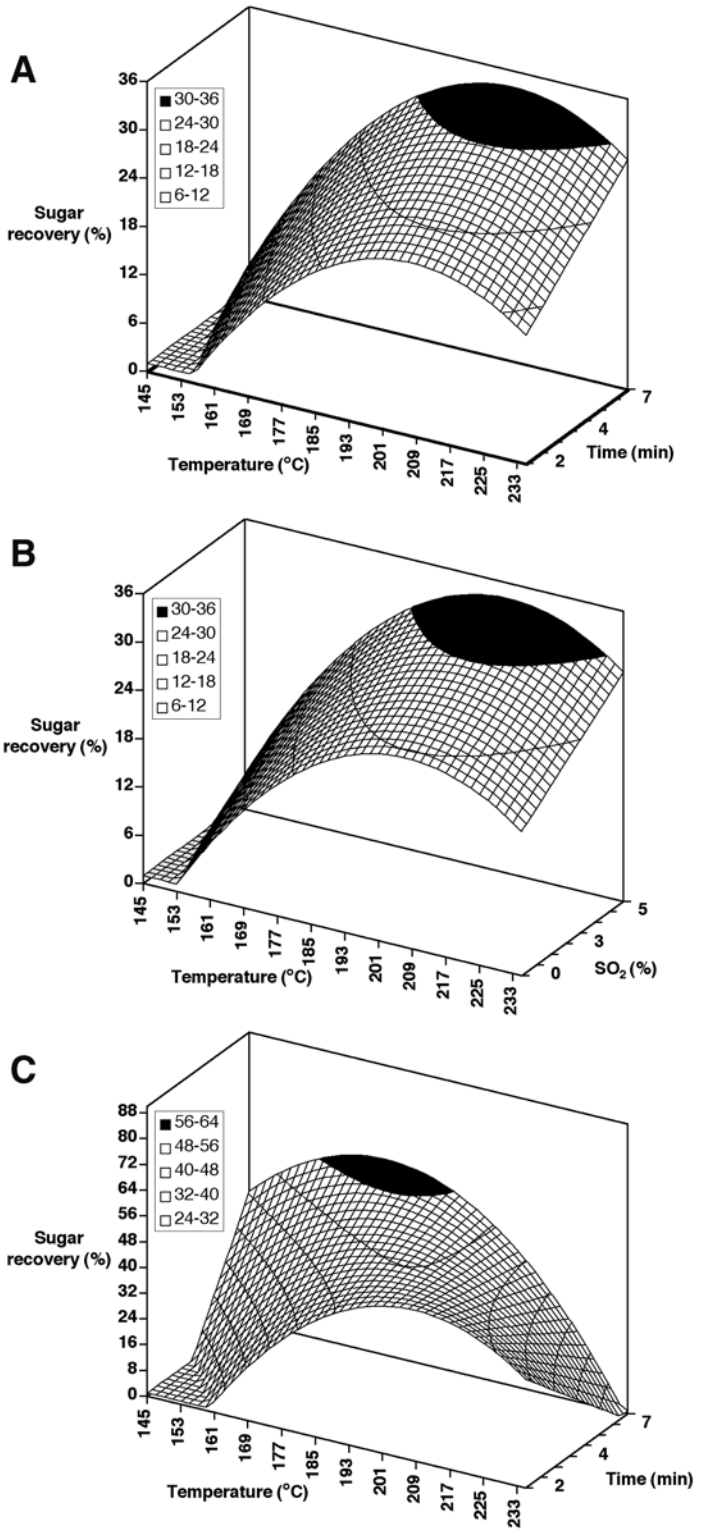
Ara	Gal	Glu	Xyl	Man	Total sugars	Klason lignin	Ash
12.11 ± 0.31	2.70 ± 0.16	42.75 ± 1.08	18.03 ± 0.56	0.58 ± 0.03	76.17 ± 2.08	8.72 ± 0.21	0.65 ± 0.02

Table 4
Yield of Hemicellulose-Derived Sugars (Excluding Glucose)
in Prehydrolysate After Steam Explosion (Monomers)
and Steam Explosion and Posthydrolysis (Oligomers and Total Sugars)
Expressed as G/100 g of Hemicellulose in Original Corn Fiber

Treatment	Monomers (%)	Oligomers (%)	Total sugars (%)
170°C, 1 min, 3%	6.1	10.4	16.5
150 °C, 5 min, 3%	5.7	7.1	12.8
170°C, 5 min, 0%	10.9	18.6	29.5
170°C, 5 min, 6%	18.5	24.5	43.0
170°C, 9 min, 3%	23.8	31.0	54.8
190°C, 5 min, 3%	26.5% ± 1.0	23.8% ± 1.6	50.3% ± 0.8
210°C, 2.2 min, 1%	21.5	19.2	40.8
210°C, 2.2 min, 5%	27.6	6.3	33.9
210°C, 7.8 min, 1%	22.5	12.5	35.0
210°C, 7.8 min, 5%	24.1	5.9	30.0
230°C, 5 min, 3%	23.0	4.1	27.1
190°C, 5 min, 0%	15.4	30.6	46.0
190°C, 5 min, 6%	27.8	19.2	46.9

Oligomer and Total Sugar Yield

It was apparent that posthydrolysis of the recovered prehydrolysate using 3% H_2SO_4 (120°C, 1 h) effectively depolymerized the solubilized oligomeric hemicellulose-derived sugars. The percentage of oligomers present in the prehydrolysate ranged from 4 to 31% of the total soluble sugars (Table 4). Time and SO_2 concentration had different effects on oligomer and total sugar recovery, depending on the pretreatment temperature. At the lower temperatures (170°C), longer times and higher SO_2 concentrations led to better oligomer and total sugar recoveries. Conversely, at higher temperatures (210°C), longer times and higher SO_2 concentrations led to decreased oligomer and total sugar recoveries. Similar effects were reported by San Martin et al. *(13)* in which the water soluble fraction obtained from steam-exploded *P. radiata* at 220°C and 30 s contained 50% oligomers, while the samples steam exploded at the same temperature for 2 min contained only 15% oligomers. However, there was no statistically significant correlation among time, temperature, and SO_2 concentration regarding oligo-


A
30-36
24-30
18-24
12-18
6-12
Sugar recovery (%)
0
6
12
18
24
30
36
145
153
161
169
177
185
193
201
209
217
225
233
Temperature (°C)
2
4
7
Time (min)
B
30-36
24-30
18-24
12-18
6-12
Sugar recovery (%)
0
6
12
18
24
30
36
145
153
161
169
177
185
193
201
209
217
225
233
Temperature (°C)
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3
5
SO2 (%)
C
56-64
48-56
40-48
32-40
24-32
Sugar recovery (%)
0
8
16
24
32
40
48
56
64
72
80
88
145
153
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169
177
185
193
201
209
217
225
233
Temperature (°C)
2
4
7
Time (min)

mer recovery. This can be attributed to the degradation of sugars during posthydrolysis, as shown previously by Shevchenko et al. *(14)*.

There was an interaction between temperature and time in terms of total sugar recoveries, which was illustrated in the response surface contour (Fig. 2C). Maximum recoveries of total hemicellulose sugars (64%) were obtained at a temperature ranging from 160 to 190°C and times from 6–8 min. Previous research has indicated that hemicellulose-derived sugars are less amenable to inhibitory byproduct generation at temperatures lower than or near 200°C for softwood and hardwood residues *(10,15)*. Low concentrations of monomeric hemicellulose-derived sugars and high concentrations of oligomers have also been observed during the pretreatment of corn fiber by hot water and steam fractionation *(4)* or by ammonia fiber explosion system *(3,16)*. It is likely that the production of oligosaccharides and consequently the incomplete hydrolysis of xylans is partially owing to their structure. As observed by Hespell et al. *(16)*, during enzymatic hydrolysis of AFEX-treated corn fiber, starch and cellulose components were converted solely to glucose. However, oligosaccharides represented 30–40% of the xylan degradation products. In the case of hot water and steam fractionation, the majority of the solubilized pentosans generated after both pretreatments existed as oligomers *(4)*. Corn fiber xylan is poorly degraded and structural analyzes suggest that > 70% of the xylose backbone residues have one or more arabinose, 4-*O*-methylglucouronic acid, or other side chains. As a result, there are few unsubstituted regions in corn fiber xylan *(17)*.

Enzymatic Hydrolysis

Once we had established conditions that would allow maximum recovery of the hemicellulose sugars (while it was hoped minimizing sugar decomposition products), we next had to assess whether the resultant water-insoluble cellulosic fraction was readily hydrolyzed. Recovered pretreated, water-washed corn fiber solids were subjected to enzymatic hydrolysis for 72 h with a combination of cellulases and amylases supplemented with an excess of β-glucosidase. The results indicated that the hydrolysability of the solids improved as pretreatment conditions became more severe, reaching a maximum at 190°C, 5 min, and 3% SO_2, as was previously shown *(5)*. At higher severities (3.6, 4.1, and 4.5) a significant decrease in total sugar yield was observed (Table 5). This trend had previously been observed in softwood residues, in which optimal enzymatic hydrolysis of the cellulosic component occurred at higher pretreatment severities *(18)*. The sequential steam explosion pretreatment

Fig. 2. *(opposite page)* Response surface for monomeric hemicellulose recovery in prehydrolysate with respect to **(A)** temperature and time, **(B)** temperature and SO_2 concentration, and **(C)** total hemicellulose recovery in prehydrolysate regarding temperature and time.

Table 5
Percentage of Monomeric Sugars in Water Soluble Fraction of Steam Exploded Corn Fiber Following Different Pretreatment Conditions, and Hydrolysates Following Enzymatic Hydrolysis

Treatment	Monomers (%)	Monomers after enzyme hydrolysis (%)
170°C, 1 min, 3%	2.3	57.4
150 °C, 5 min, 3%	2.1	46.8
170°C, 5 min, 0%	4.0	57.5
170°C, 5 min, 6%	7.0	65.5
170°C, 9 min, 3%	9.4	71.1
190°C, 5 min, 3%	11.4 ± 0.5	69.1% ± 2.1
210°C, 2.2 min, 1%	9.3	62.9
210°C, 2.2 min, 5%	13.6	61.2
210°C, 7.8 min, 1%	13.3	47.1
210°C, 7.8 min, 5%	22.2	53.0
230°C, 5 min, 3%	16.2	41.6
190°C, 5 min, 0%	5.9	70.7
190°C, 5 min, 6%	12.8	61.4

followed by enzymatic hydrolysis of corn fiber in the current study showed an 81% conversion of all original polysaccharides to monomeric sugars, which is comparable with that of the work of Grohmann and Bothast *(2)*, who successfully converted 85% of the total available carbohydrate using dilute-acid treatment in combination with enzyme hydrolysis. The lower yield in the current study is owing to higher concentrations of oligomers (xylose) present in the prehydrolysate after steam explosion. According to the model, the optimum in yield of fermentable sugars after pretreatment and enzymatic hydrolysis was obtained at 170–200°C and 4–7 min (Fig. 3A). SO_2 concentrations also influenced the recovery of monomeric sugars after pretreatment and hydrolysis. Maximum recovery of monomeric sugars (85%) was obtained at temperatures between 185 and 205°C and SO_2 concentrations between 3 and 6% (Fig. 3B). In addition, the influence of SO_2 impregnation at a given temperature on the monomer recovery after pretreatment and enzymatic hydrolysis was evident at 190°C, 5 min at SO_2 concentrations ranging from 0 to 6% (Table 4). Similar to the findings by Clark et al. *(19)*, the enzymatic digestibility of the water-insoluble corn fiber and monomer sugar yield were substantially improved over the situation in which no SO_2 impregnation was employed. However, above 3% SO_2 concentration, there were no further observable improvements.

Fermentation of Corn Fiber Hydrolyzate

The water soluble fractions obtained from each of the pretreatment, posthydrolysis, and enzymatic hydrolysis steps were next assessed for their

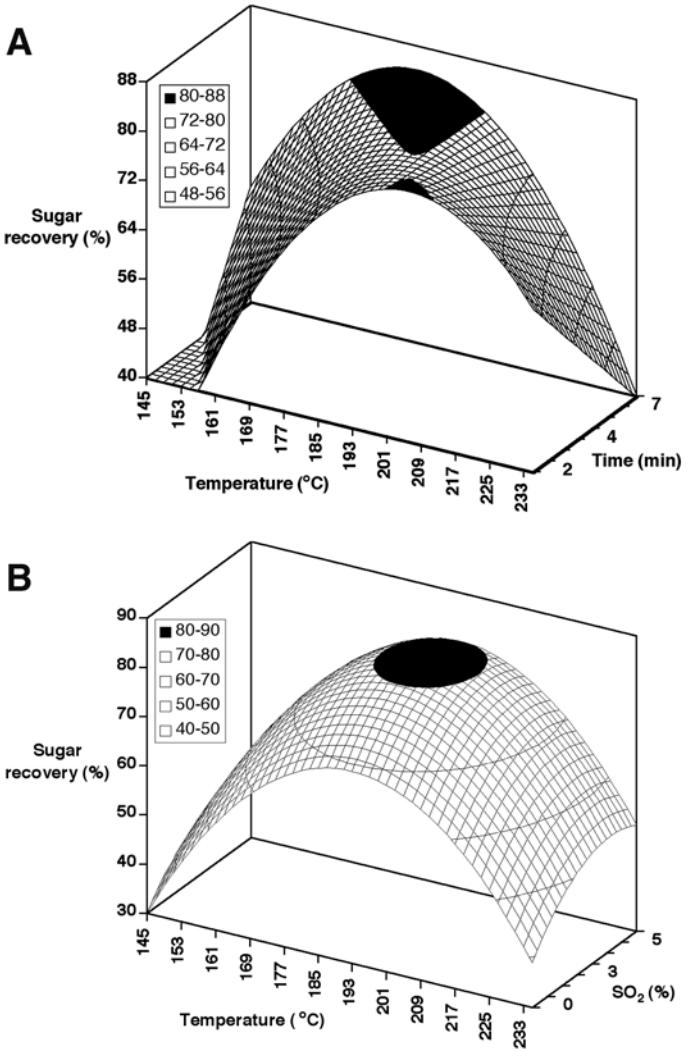

Fig. 3. Response surface for monomeric sugar recovery after pretreatment and enzymatic hydrolysis regarding **(A)** temperature and time, and **(B)** temperature and SO_2 concentration.

efficiency of fermentation to ethanol, without employing any detoxification steps. The average ethanol yield in the fermentation of sugars present in the liquid fractions obtained after pretreatment, posthydrolysis, and enzymatic hydrolysis were determined (Table 6). The relative ethanol yield, Y_{EtOH}/Y^{ref}_{EtOH} was defined as the ratio of the ethanol yield of the filtrate and the reference fermentation. There was a clear relationship between the fermentability of the prehydrolysate obtained after steam explosion and severity. As expected, after 6 h, only the hexose sugars glucose and mannose, liberated from the corn fiber in the prehydrolysate, were effectively

Table 6
Relative Ethanol Yield for Liquid Fraction Obtained After Pretreatment (Prehydrolysate) (6 h), Enzymatic Hydrolysis (2 h), Posthydrolysis (24 h), and Concentration of HMF and Furfurals in Prehydrolysate (Original Sugar %)

	Prehydrolysate			Enzymatic hydrolysis	Posthydrolysis
Treatment	HMF (%)	Furfurals (%)	Y_{EtOH}/Y^{ref}_{EtOH} (%)	Y_{EtOH}/Y^{ref}_{EtOH} (%)	Y_{EtOH}/Y^{ref}_{EtOH} (%)
170°C, 1 min, 3%	0.01	0.57	79	85	80
150 °C, 5 min, 3%	0.01	0.18	85	100	83
170°C, 5 min, 0%	0.03	0.34	79	83	81
170°C, 5 min, 6%	0.07	0.87	82	81	89
170°C, 9 min, 3%	0.12	1.10	85	100	83
190°C, 5 min, 3%	0.24 ± 0.01	1.17 ± 0.07	94 ± 1	89 ± 1	85 ± 4
210°C, 2.2 min, 1%	0.29	0.97	79	81	86
210°C, 2.2 min, 5%	0.45	1.03	81	87	84
210°C, 7.8 min, 1%	1.07	1.05	66	92	84
210°C, 7.8 min, 5%	1.47	1.38	69	95	82
230°C, 5 min, 3%	1.73	1.93	69	96	81
190°C, 5 min, 0%	0.23	1.01	91	86	80
190°C, 5 min, 6%	0.26	1.20	95	89	89

consumed by *S. cerevisiae*. Conversions to ethanol increased with increased severity of the pretreatment, reaching a maximum at 190°C, 5 min, and 3% SO_2, demonstrating a yield of 94% of theoretical (Table 6). The model predicted a range of optimum pretreatment conditions regarding time, temperature, and SO_2 concentrations (Fig. 4 A,B) and was in agreement with experimental data. According to the model, the optimum relative ethanol yield (96%) of fermentable sugars after pretreatment was obtained at 181–193°C, 4–5.5 min, and SO_2 concentrations from 2.2 to 3.6% (Figs. 4A,B). However, at severities higher than 3.4, a distinct decrease in fermentability was observed. It is well established that as steam explosion severity increases, so does the degradation of monomeric sugars, by dehydration and condensation reactions *(20)*. As shown in Table 6, the amount of HMF and furfurals present increased with the severity of pretreatment conditions. However, the low concentration of inhibitors generated at low temperatures (lower than 190°C) and the poor fermentability of prehydrolysate obtained at these conditions suggests that other byproducts may be acting as inhibitors. It has been shown that sugar degradation products such as HMF and furfurals are less inhibitory than lignin-derived compounds such as vanillin or syringylaldehyde on ethanol fermentation by *S. cerevisiae (18)*.

There was no clear relationship between the fermentability of the liquid fractions obtained after enzymatic hydrolysis and posthydrolysis and

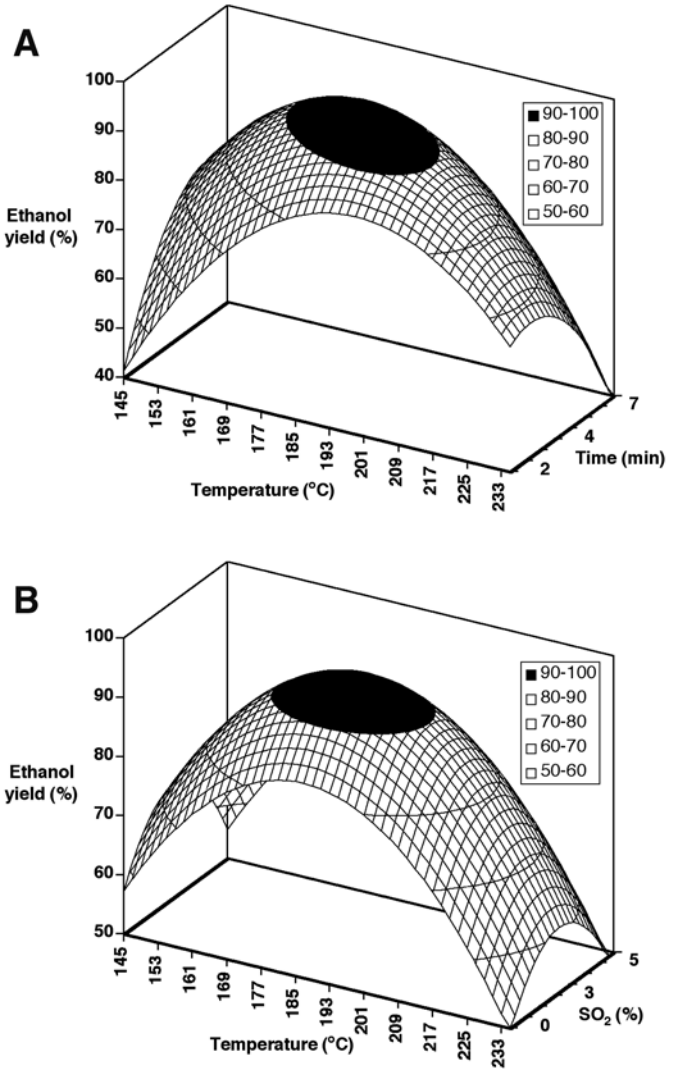

Fig. 4. Response surface for relative ethanol yield for liquid fraction obtained after pretreatment respect to **(A)** temperature and time, and **(B)** temperature and SO_2 concentration.

pretreatment severity (Table 6). Relative ethanol yields were high, ranging from 81 to 100%, and 80 to 89% for sugars obtained after enzymatic hydrolysis and posthydrolysis, respectively. The relatively high ethanol yields obtained during 2 h of fermentation of the liquid fraction collected after enzymatic hydrolysis were comparable with the results obtained by Allen et al. *(4)*. After simultaneous saccharification and fermentation of pretreated corn fiber by hot water and steam explosion, 86 and 90% conversion of glucan to ethanol by *S. cerevisiae* was observed, respectively.

Posthydrolysis, using the H_2SO_4 catalyzed aqueous solution obtained from SO_2-catalyzed steam explosion of corn fiber depolymerized the soluble oligomeric hemicelluloses and released most of the available carbohydrates in a fermentable form. At optimal pretreatment conditions, posthydrolysis increased the concentration of sugars available for fermentation by 10%. These results were comparable to work done by Schevchenko et al. *(14)*, who reported 100% conversion rates for hexoses present in the post-hydrolysates of SO_2-catalyzed steam exploded Douglas-fir.

Conclusion

Each of the parameters investigated during steam pretreatment by response surface modeling (temperature, time, and SO_2 concentration) had an effect on the hemicellulose monomer recovery, its subsequent fermentability, and enzymatic hydrolysis. Although the wide range of pretreatment conditions investigated precluded an exact determination of optimal conditions, the predicted optimal pretreatment conditions were in agreement with the optimum conditions observed for maximum sugar yield obtained after pretreatment and enzymatic hydrolysis, and fermentability of the monomeric sugars. The results show that there is a need for SO_2 impregnation of corn fiber prior to steam explosion.

A two-stage treatment for corn fiber processing, comprising SO_2-catalyzed steam explosion and hydrolysis by a mixture of cellulolytic and amylolytic enzymes, proved to be a very effective method for converting the available polysaccharides in the residue to monomeric sugars, as evidenced by the 81% conversion observed without the need for a detoxification step. Maximum sugar yields (soluble and following enzymatic hydrolysis) were recovered from corn fiber pretreated at 190°C for 5 min with 3% SO_2. Posthydrolysis of the aqueous solution from SO_2-catalyzed steam explosion effectively depolymerized the soluble oligomeric hemicellulose and increased the concentration of carbohydrates in a fermentable form by 10% at optimum pretreatment conditions. Subsequently, *S. cerevisiae* was able to convert the resultant corn fiber hydrolysates, prehydrolysate and liquid fraction from posthydrolysis to ethanol efficiently, respectively yielding 89, 94, and 85% of theoretical conversion.

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