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Pretreatment and Enzymatic Saccharification of Corn Fiber†

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Abstract

Corn fiber consists of about 20% starch, 14% cellulose, and 35% hemicellulose, and has the potential to serve as a low-cost feedstock for production of fuel ethanol. Several pretreatments (hot water, alkali, and dilute acid) and enzymatic saccharification procedures were evaluated for the conversion of corn fiber starch, cellulose, and hemicellulose to monomeric sugars. Hot water pretreatment (121°C, 1 h) facilitated the enzymatic saccharification of starch and cellulose but not hemicellulose. Hydrolysis of corn fiber pretreated with alkali under similar conditions by enzymatic means gave similar results. Hemicellulose and starch components were converted to monomeric sugars by dilute H_2SO_4 pretreatment (0.5–1.0%, v/v) at 121°C. Based on these findings, a method for pretreatment and enzymatic saccharification of corn fiber is presented. It involves the pretreatment of corn fiber (15% solid, w/v) with dilute acid (0.5% H_2SO_4, v/v) at 121°C for 1 h, neutralization to pH 5.0, then saccharification of the pretreated corn fiber material with commercial cellulase and β-glucosidase preparations. The yield of monomeric sugars from corn fiber was typically 85–100% of the theoretical yield.

Index Entries: Corn fiber; pretreatment; enzymatic saccharification; fuel ethanol.

Introduction

In the United States, about 1.3 billion gal of ethanol are produced annually, with approx 95% derived from corn starch *(1)*. Corn fiber repre-

†Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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sents a renewable resource that is available in significant quantities from the corn wet-milling industries. It is a mixture of corn hulls and residual starch not extracted during the milling process and makes up 11% of the dry weight of the corn kernel *(2)*. Corn fiber contains about 70% fermentable sugars, of which approx 20, 14, and 35% come from starch, cellulose, and hemicellulose, respectively. Typically, 32 lb of starch is obtained from a bushel of corn (56 lb), which yields about 2.5 gal of ethanol in industrial practice. From the same bushel of corn, about 4.5 lb of corn fiber is obtained, which can be converted to about 3.15 lb of fermentable sugars. These fermentable sugars will theoretically yield about 0.3 gal of ethanol *(3)*. The low cost and high carbohydrate content of corn fiber makes it an attractive feedstock for conversion to fuel ethanol. Gulati et al. *(4)* estimated that a corn wet-milling facility that currently produces 100 million gal of ethanol per year from starch would generate an additional $4–8 million of annual income if the sugar components of corn fiber were converted into ethanol. Currently, the utilization of corn fiber to produce fuel ethanol presents significant technical and economic challenges, and its success depends largely on the development of environmentally friendly pretreatment procedures, highly effective enzyme systems for conversion of pretreated corn fiber substrate to fermentable sugars, and efficient microorganisms to ferment mixed sugars to ethanol. In this article, we report on several pretreatments (hot water, alkali, and dilute acid) and enzymatic saccharification of corn fiber.

Materials and Methods

Wet corn fiber (~60% moisture, w/w) was obtained from Pekin Energy (Pekin, IL). It was dried in a forced-air oven at 55°C for 24 h and milled in a hammer mill to pass through a 1.27-mm screen. The dried corn fiber was stored at room temperature. Various commercial enzyme samples were obtained from different manufacturers: Diazyme L-200 from Solvay Enzymes (Elkhart, IN); Celluclast 1.5 L and Novozym 188 from Novo-Nordisk Bioindustrials (Danbury, CT); and Econase CE, Econase HC, and Enzeco Pectinase liquid from Enzyme Development (New York, NY). Glucose, xylose, L-arabinose, furfural, hydroxy methyl furfural (HMF) and levulinic acid were purchased from Sigma (St. Louis, MO).

Pretreatment and Enzymatic Saccharification

Dried corn fiber was slurried in water, dilute acid, or alkali (15% solid, w/v, unless otherwise stated) and pretreated in an autoclave at 121°C. The pretreated corn fiber was neutralized to pH 5.0 with 10*M* NaOH or 10*M* HCl before enzymatic treatment. The enzyme treatment was performed at 45°C and pH 5.0.

Enzyme Assays

Amylase, xylanase, and carboxymethyl cellulase (CMCase) activities were assayed in a reaction mixture (0.5 mL) containing 1% (w/v) boiled soluble starch, 1% (w/v) oat spelt xylan, and 1% (w/v) carboxy methyl cellulose, respectively, 50 m*M* acetate buffer, pH 5.0, and appropriately diluted enzyme solutions. After 30 min of incubation at 50°C, the reducing sugar liberated in the reaction mixture was measured by the dinitrosalicylic acid method *(5)*. One unit of each enzyme activity is defined as the amount of enzyme that produces 1 µmol of reducing sugar as glucose (xylose in the case of xylanase) in the reaction mixture per minute under the previously specified conditions.

β-Glucosidase, β-xylosidase, and α-L-arabinofuranosidase activities were assayed in the reaction mixture (1 mL) containing 4 m*M p*-nitrophenyl-β-D-glucoside, 2 m*M p*-nitrophenyl-β-D-xyloside and 1 m*M p*-nitrophenyl-α-L-arabinofuranoside, respectively, 50 m*M* acetate buffer, pH 5.0, and appropriately diluted enzyme solutions. After incubation at 50°C for 30 min, the reaction was stopped by adding 1 mL of ice-cold 0.5*M* Na_2CO_3, and the color that developed as a result of *p*-nitrophenol liberation was measured at 405 nm. One unit of each enzyme activity is defined as the amount of enzyme that releases 1 µmol of *p*-nitrophenol per minute in the reaction mixture under these assay conditions.

Analytical Procedures

Sugars, furfural, HMF, and levulinic acid were analyzed by high-performance liquid chromatography (HPLC). The separation system consisted of a multisolvent delivery system (SP8800 ternary pump, Spectra-Physics, San Jose, CA) equipped with an autosampler (717, Waters Chromatography Division, Millipore, Milford, MA), a refractive index detector (410 differential refractometer, Waters), and an integrator (ChromJet, Spectra-Physics). An ion-moderated partition chromatography column (Aminex HPX-87P) fitted with a Carbo-P guard cartridge was used. The column was maintained at 85°C, and the sugars were eluted with Milli-Q filtered water at a flow rate of 0.6 mL/min. Peaks were detected by refractive index and were identified and quantified by comparison to retention times of authentic standards (glucose, xylose, galactose, L-arabinose, furfural, HMF, and levulinic acid).

Results and Discussion

Corn Fiber Composition

The same corn fiber sample was used throughout this study. It had the following composition (per gram) as reported by Grohmann and Bothast *(2)*: 372 ± 19 mg glucose, 176 ± 18 mg xylose, 36 ± 3 mg galactose, and 113 ± 15 mg L-arabinose (697 ± 55 mg total sugars). The starch content was 197 ± 9 mg, which accounts for the noncellulosic glucose

content of the corn fiber. The other components (per gram) were 141 ± 10 mg crude fiber, 110 ± 5 mg protein, 25 ± 3 mg crude fat, 17 ± 1 mg acetyl group, 78 ± 7 mg klason lignin, 6 ± 1 mg ash, and 68 mg unknown compounds. Osborn and Chen *(6)* reported similar values for corn hull composition (per gram): 320 mg glucose, 187 mg xylose, 105 mg L-arabinose, and 118 mg protein.

Hydrolysis of Corn Fiber by Treatment with Hot Water and Enzymes

Corn fiber (15% solid, w/v) slurry in water was treated at 121°C for 1 h. After neutralization to pH 5.0, it was then treated with a combination of enzymes (Diazyme L-200, Econase CE, Novozym 188, Econase HC, and Enzeco Pectinase liquid) at a dose level of 2% for each enzyme preparation (2 mL/100 g of solid). Table 1 shows the various commercial enzyme preparations used and the activity level of each assayed enzyme present in these preparations. The hot water treatment did not release any glucose, xylose, or galactose in the reaction mixture. Only 20 ± 0 mg of L-arabinose/g of corn fiber was detected in the reaction mixture by HPLC analysis. After enzymatic saccharification of the hot water–treated corn fiber for 87 h, 323 ± 18 mg of glucose and 64 ± 4 mg of L-arabinose were obtained per gram of corn fiber by HPLC analysis. No xylose and galactose peaks were detected. The hot water pretreatment did not facilitate the enzymatic breakdown of corn fiber xylan into xylose at all. The starch present in the corn fiber sample was gelatinized by the hot water treatment and converted to glucose by glucoamylase. Also, the cellulosic portion of the pretreated corn fiber was hydrolyzed significantly by the enzymes used. This indicates that hot water pretreatment at 121°C for 1 h may be sufficient for enzymatic hydrolysis of the starch and cellulose portion of the corn fiber.

Hydrolysis of Corn Fiber by Treatment with Alkali and Enzymes

Corn fiber and $Ca(OH)_2$ at a ratio of 10:1 (w/w) were slurried in water (5% solid, w/v; pH ~12.6) and treated at 100°C for 1 h. After neutralization to pH 5.0 with HCl, it was treated with an enzyme mixture containing Diazyme L-200, Econase CE, Econase HC, and Enzeco Pectinase liquid at the dose level of 2 mL of each enzyme preparation per 100 g of solid for 40 h. About 268 ± 5 mg of glucose and 30 ± 0 mg of L-arabinose/g of corn fiber were released in the reaction mixture. No xylose and galactose were detected. One hour of alkali pretreatment of corn fiber at 121°C and subsequent enzymatic saccharification for 24 h failed to release any xylose in the reaction mixture. However, after alkali pretreatment at 121°C for 3 h, enzymatic (Diazyme L-200, Celluclast 1.5 L, Novozym 188, Econase HC, and Enzeco Pectinase—each at 2% level; 2 mL/100 g solid) saccharification yielded 343 ± 9 mg of glucose, 23 ± 7 mg of xylose, and 31 ± 2 mg L-arabinose/g of corn fiber in 24 h. After 90 h of enzyme treatment, about 355 ± 2 mg of glucose, 31 ± 14 mg of xylose, and 42 ± 1 mg of L-arabinose were detected in the reaction mixture per gram of corn fiber.

Table 1
Commercial Enzymes Used in Corn Fiber Saccharification

Enzyme preparation	Activity (U/mL)[a]					
	Diazyme L-200	Econase CE	Econase HC	Enzeco Pectinase	Celluclast 1.5 L	Novozym 188
Glucoamylase	12,674	167	128	161	7	7070
Xylanase	46	707	8323	591	424	557
CMCase	4	378	189	11	1688	27
β-Glucosidase	0.6	37	19	19	28	500
β-Xylosidase	0.1	1	5	8	5	5
α-L-Arabinofuranosidase	0.4	65	25	112	3	23

[a]At pH 5.0 and 50°C

In another experiment, corn fiber was first treated with 88% isopropanol and NaOH (100:400:1, w/w) to extract zein *(7)*. It was then treated with $Ca(OH)_2$ (10:1, w/w) for 1 h at 121°C. After neutralization, the resultant material was treated with a combination of enzymes (Diazyme L-200, Econase HC, Econase CE, Novozym, Enzeco Pectinase liquid—each at 2% level; 2 mL/100 g of corn fiber). No xylose and galactose were detected in the enzymatic hydrolyzate after 48 h. About 198 ± 13 mg of glucose and 21 ± 0 mg of L-arabinose/g of corn fiber were detected in the reaction mixture. This result indicates that removing zein from corn fiber prior to alkali treatment did not facilitate the enzymatic breakdown of the corn fiber hemicellulose. High doses of xylanase (Econase HC), the addition of more accessory enzymes (Enzeco Pectinase liquid) and sequential enzyme treatments (the substrate was first treated with xylanase and then with accessory enzymes or the substrate was first treated with accessory enzymes and then with xylanase) of the corn fiber arabinoxylan did not enhance the release of xylose. All these results clearly indicate that corn fiber arabinoxylan is resistant to enzymatic hydrolysis. By contrast, the starch and cellulose portion of the alkali-pretreated corn fiber were hydrolyzed well by enzymes.

Hespell et al. *(8)* performed ammonia fiber explosion treatment of corn fiber to increase degradability, and then enzymatically digested the pretreated corn fiber with a combined mixture of commercial amylase, xylanase, and cellulase enzyme preparations. Whereas the starch and cellulose components were converted solely to glucose, oligosaccharides represented 30–40% of the xylan degradation products. The incomplete degradation of the corn fiber hemicellulose may be at least in part owing to its structural complexity. Structural analysis of corn fiber arabinoxylan suggests that more than 70% of the xylose backbone residues have one or more L-arabinose, 4-*O*-methylglucuronic acid, or other sidechains *(9–12)*. Saulnier et al. *(11)* predicted that the highly branched nature of the corn fiber heteroxylan, diferulic acid bridges between heteroxylan chains, and protein polysaccharide linkages may be responsible for the extreme resistance of xylan to enzymatic attack.

Hydrolysis of Corn Fiber by Treatment with Dilute Acid

The presence of sugar degradation products (furfural and HMF) in lignocellulosic hydrolyzate can inhibit fermentation of the sugars in the hydrolyzate to ethanol *(13–16)*. Grohmann and Bothast *(2)* reported that the formation of inhibitory compounds became readily apparent for all acid pretreatments of corn fiber performed at 140 and 160°C. Lee et al. *(17)* reported that a large portion of the xylose fraction was degraded to furfural and that glucose was degraded to HMF when the dilute acid (0.5–2.0%, v/v) pretreatments of lignocellulosic materials were performed at higher temperatures (160–170°C). Thus, the acid pretreatments of corn fiber were performed with dilute acid (0.5–1.0% H_2SO_4, v/v) at a comparatively low temperature (121°C) in order to minimize the formation of inhibitory com-

pounds. Figure 1A–D shows the effects of treatment times (15–60 min) on the release of glucose, xylose, L-arabinose, and total sugars (glucose, xylose, L-arabinose, and galactose) from corn fiber by dilute acid pretreatment at three acid levels (0.5, 0.75, and 1.0% H_2SO_4, v/v). Longer time and higher acid level were found to be favorable for maximum release of the component reducing sugars. L-Arabinose was easily released and was not dependent on the duration and concentration of acid. The liberation of xylose was dependent on the duration of treatment but was not very dependent on the concentration of acid. The formation of glucose, on the other hand, depended on both the duration and acid concentration. These results indicate that the hemicellulose component of corn fiber, although very resistant to enzymatic hydrolysis after hot water and alkali pretreatments, can be easily converted to monomeric sugars by dilute acid hydrolysis. As expected, the starch portion of corn fiber was converted to glucose by the dilute acid pretreatment. No detectable peaks of furfural, HMF, and levulinic acid were obtained, even in the undiluted corn fiber acid hydrolyzate (15% solid, w/v; 0.5% H_2SO_4, v/v; 121°C; 1 h).

It is desirable to establish a relationship that simultaneously takes into account the effects of acid concentration and the time of pretreatment on each individual sugar yield. The following model equation was used:

$$\text{Sugar yield (\%)} = \text{Constant} + \beta 1\ (\text{Time}) + \beta_2\ (\text{Acid}) + \beta_3\ (\text{Time} \times \text{Acid})$$

Each sugar yield was fit to acid and time by linear regression methods. A preliminary analysis found no significant ($p > 0.05$) effects of an Acid × Time interaction. Table 2 presents the empirical model equations obtained. Glucose release was significantly correlated with xylose ($r = 0.94$), galactose ($r = 0.53$), and L-arabinose ($r = 0.45$) where r is simple correlation.

Hydrolysis of Dilute Acid–Pretreated Corn Fiber by Enzymes

Corn fiber (15% solid, w/v) was treated with 0.25% H_2SO_4, v/v, at 121°C for 1 h (pH 1.3 ± 0.0), neutralized to pH 5.0, then treated with an enzyme mixture containing Econase CE, Novozym 188, Econase HC, and Enzeco Pectinase liquid (each at 2% level; 2 mL of each enzyme/100 g of solid). Acid-pretreated samples contained 23 ± 1 mg of glucose, 75 ± 1 mg of xylose, and 88 ± 1 mg of L-arabinose/g of corn fiber. After enzyme treatment for 87 h, glucose, xylose, and L-arabinose contents increased to 358 ± 2, 132 ± 2, and 127 ± 1 mg/g of corn fiber, respectively. Thus, treatment of corn fiber with 0.25% H_2SO_4, v/v, at 121°C for 1 h is not sufficient for the release of xylose although the acid pretreatment significantly helped the enzymatic release of xylose (an increase from 75 ± 1 to 132 ± 2 mg/g of corn fiber) from pretreated corn fiber.

Based on the results shown in Fig. 1A–D, we have chosen to pretreat corn fiber with 0.5% H_2SO_4, v/v, at 121°C for 1 h and then to treat with only commercial cellulase (Celluclast 1.5 L) and β-glucosidase (Novozym 188) preparations in order to saccharify the cellulose component (14%) of the

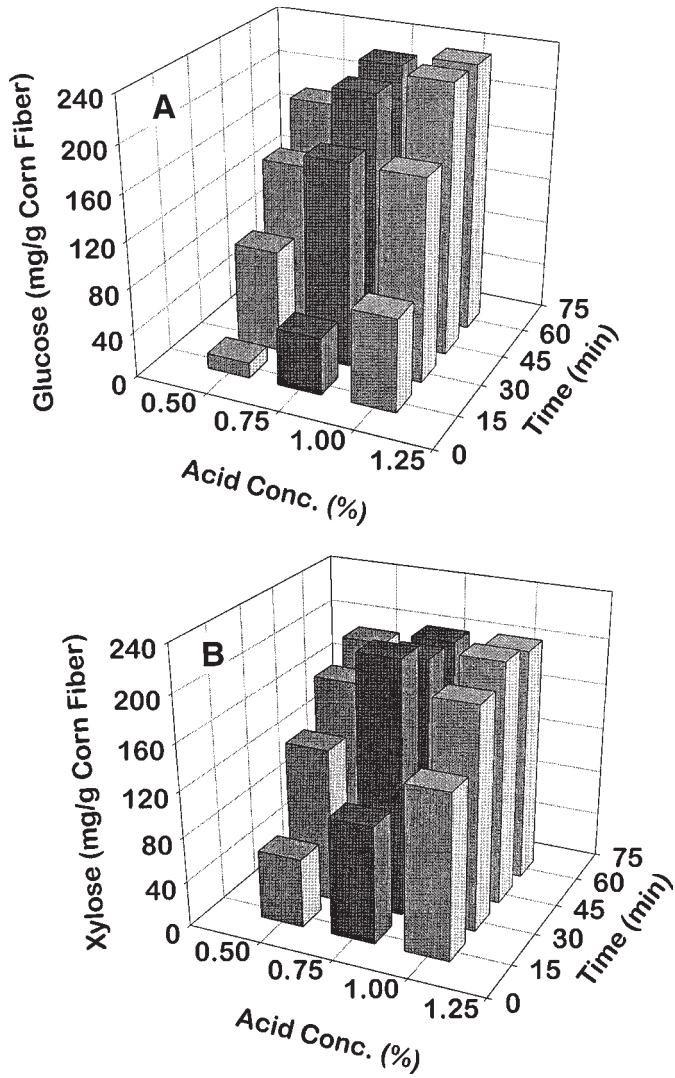

Fig. 1. Effect of treatment time (15–60 min) on the release of glucose **(A)**, xylose **(B)**, L-arabinose **(C)**, and total sugars (glucose, xylose, L-arabinose, and galactose) **(D)** from corn fiber (15%, w/v solid) by dilute acid pretreatment at three acid levels (0.5, 0.75, and 1.0%, v/v; H_2SO_4, v/v) at 121°C.

corn fiber. Table 3 gives the results of the enzymatic saccharification of the pretreated corn fiber (0.5% H_2SO_4, v/v; 121°C; 1 h) after 72 h at four dose levels of enzyme loading. It is evident that loading of Celluclast 1.5 L and Novozym 188 at the 1% (1 mL/100 g of corn fiber) level is suitable for saccharification of the cellulose portion of the acid-pretreated corn fiber as further loading of enzymes did not increase the glucose yield significantly. As shown in Table 3, after enzymatic treatment of the dilute acid–pretreated corn fiber for 72 h, the glucose yield increased from 154 ± 5 to 336 ± 15 mg, the xylose yield increased from 148 ± 2 to 200 ± 10 mg, the L-arabi-

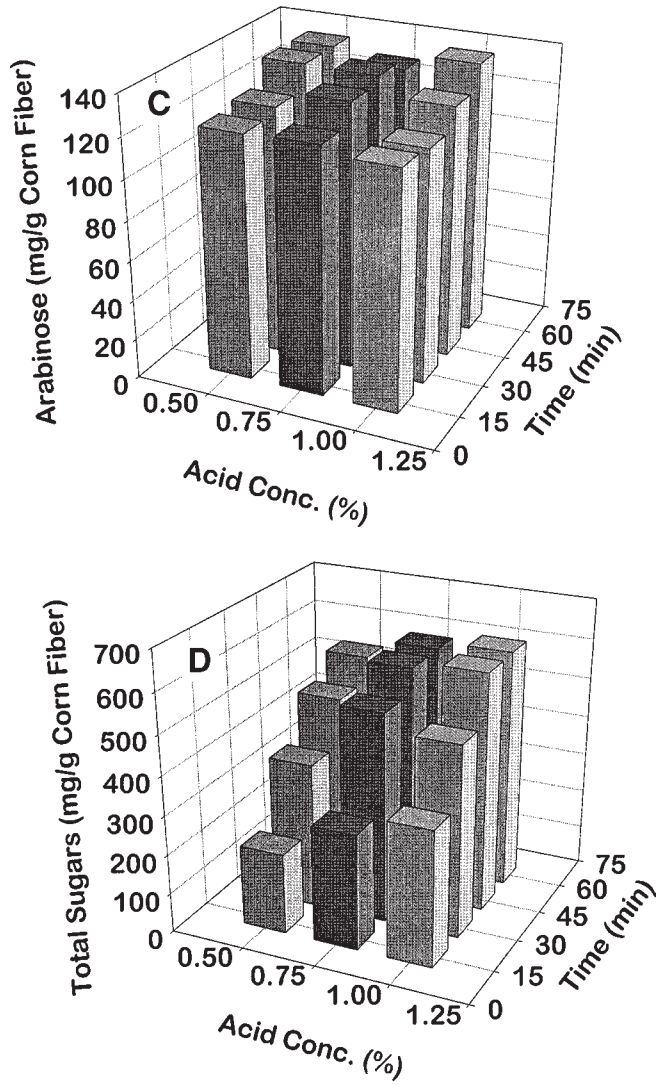

Fig. 1. *(continued)*.

nose yield increased from 105 ± 1 to 138 ± 8 mg, and the galactose yield increased from 20 ± 0 to 29 ± 0 mg/g of corn fiber. The yield of total sugars increased from 427 ± 8 to 703 ± 33 mg/g of corn fiber. These results indicate that enzymatic treatment of the dilute acid–pretreated corn fiber had a significant effect not only on glucose yield but also on xylose, L-arabinose, and galactose yields.

Pretreatment of corn fiber with 0.5% H_2SO_4 at 121°C for 1 h is not sufficient to hydrolyze the starch and hemicellulose components completely. Both commercial cellulase and β-glucosidase preparations had significant xylanase activities, and the β-glucosidase preparation had good glucoamylase activity (Table 1). The pretreatment, however, facilitated the

Table 2
Empirical Model Equations for Quantification of the Effects of Time (15–60 min) and Concentration of Dilute Acid (0.5–1.0% H_2SO_4, v/v) Pretreatment at 121°C on Monomeric Sugar Yield from Corn Fiber (15% solid, w/v)

Sugar	Equation: Yield (%) = Constant + β_1 (Time) + β_2 (Acid)	$R^{2\,a}$
Glucose	Yield (mg/g corn fiber) = –96.44 + 3.65 (Time in min) + 149.25 (Acid %, v/v)	0.87
Xylose	Yield (mg/g corn fiber) = 22.38 + 2.02 (Time in min) + 96.50 (Acid %, v/v)	0.65
L-Arabinose	Yield (mg/g corn fiber) = 123.44 + 0.31 (Time in min) – 9.25 (Acid %, v/v)	0.32
Galactose	Yield (mg/g corn fiber) = 4.72 + 0.60 (Time in min) – 10.75 (Acid %, v/v)	0.60
Total sugars	Yield (mg/g corn fiber) = 54.10 + 6.58 (Time in min) + 225.75 (Acid %, v/v)	0.82

[a]R^2 is coefficient of determination.

Table 3
Effect of Enzyme Dose on Saccharification of Dilute Acid (0.5% H_2SO_4, v/v; 121°C; 1 h)–Pretreated Corn Fiber

		Sugar yield (mg/g corn fiber)[b]				
Enzyme dose[a]	Time (h)	Glucose	Xylose	Galactose	L-Arabinose	Total
A	0	150 ± 1	148 ± 1	19 ± 0	105 ± 1	422 ± 3
	72	278 ± 12	184 ± 10	28 ± 1	122 ± 6	612 ± 29
B	0	154 ± 5	148 ± 2	20 ± 0	105 ± 1	427 ± 8
	72	336 ± 15	200 ± 10	29 ± 0	138 ± 8	703 ± 33
C	0	156 ± 1	147 ± 1	21 ± 0	105 ± 1	429 ± 3
	72	347 ± 14	190 ± 8	28 ± 1	131 ± 7	696 ± 30
D	0	160 ± 1	149 ± 2	22 ± 1	108 ± 0	439 ± 4
	72	337 ± 6	183 ± 7	27 ± 2	126 ± 6	673 ± 21

[a]Enzymes used (mL/100 g corn fiber): A, 0.5 Celluclast and 0.5 Novozym 188; B, 1.0 Celluclast and 1.0 Novozym 188; C, 1.5 Celluclast and 1.5 Novozym 188; D, 2.0 Celluclast and 2.0 Novozym 188.
[b]The enzyme treatment was performed at 45°C and pH 5.0.

commercial enzymes to work well to hydrolyze the remaining starch and hemicellulose components. The effects of enzyme loading and time were determined by analysis of variance. The dose level of enzymes affected the glucose yield ($p < 0.05$) as expected. Pretreatment of southern mixed hardwood with 5% (v/v) acid at 120°C and subsequent enzymatic hydrolysis of the residues with cellulase at a 1% cellulose level gave only 54.6% conversion in 168 h *(17)*. This indicates that corn fiber is easily convertible to fermentable sugars in comparison to the hardwood substrate However, the cost of cellulase enzymes at the current price is ~80 cents/gal of theoretical ethanol produced from corn fiber hydrolyzate, and its long incubation time is a major constraint for the conversion of corn fiber cellulose to monomeric sugars.

The pretreatment of corn fiber is crucial before enzymatic hydrolysis *(18)*. The results indicate that there are no suitable commercial hemicellulase preparations or pretreatment procedures (other than dilute acid) that can hydrolyze corn fiber hemicellulose to monomeric sugars efficiently. Dilute acid pretreatment at a relatively low temperature to minimize the formation of inhibitory compounds, followed by enzymatic saccharification of the cellulosic portion, may be a workable process for generating fermentable sugars from corn fiber. The overall goal of dilute acid pretreatment of corn fiber is to achieve a high yield of monomeric sugars (xylose, L-arabinose, and galactose) from the hemicellulosic portion, while minimizing the breakdown of sugars to inhibitory products such as furfural and HMF. By using the enzymatic treatment after dilute acid (0.5%, v/v; 121°C, 1 h) pretreatment of corn fiber, we were able to increase the yield of each individual sugar significantly (Table 2).

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