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Industrial Scale-Up of pH-Controlled Liquid Hot Water Pretreatment of Corn Fiber for Fuel Ethanol Production

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Abstract

The pretreatment of cellulose in corn fiber by liquid hot water at 160°C and a pH above 4.0 dissolved 50% of the fiber in 20 min. The pretreatment also enabled the subsequent complete enzymatic hydrolysis of the remaining polysaccharides to monosaccharides. The carbohydrates dissolved by the pretreatment were 80% soluble oligosaccharides and 20% monosaccharides with <1% of the carbohydrates lost to degradation products. Only a minimal amount of protein was dissolved, thus enriching the protein content of the undissolved material. Replication of laboratory results in an industrial trial at 43 gallons per minute (163 L/min) of fiber slurry with a residence time of 20 min illustrates the utility and practicality of this approach for pretreating corn fiber. The added costs owing to pretreatment, fiber, and hydrolysis are equivalent to less than $0.84/gal of ethanol produced from the fiber. Minimizing monosaccharide formation during pretreatment minimized the formation of degradation products; hence, the resulting sugars were readily fermentable to ethanol by the recombinant hexose and by

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pentose-fermenting *Saccharomyces cerevisiae* 424A(LNH-ST) and ethanologenic *Escherichia coli* at yields >90% of theoretical based on the starting fiber. This cooperative effort and first successful trial opens the door for examining the robustness of the pretreatment system under extended run conditions as well as pretreatment of other cellulose-containing materials using water at controlled pH.

Index Entries: Corn fiber; cellulose; ethanol; stillage; degradation products; pretreatment.

Introduction

The growth of fuel ethanol production in the United States from corn has resulted in the proliferation of fiber-rich byproduct. This fiber is largely in the kernel pericarp and is a major component of the byproduct of fuel ethanol production, variously called distillers' grains or corn gluten feed, depending on whether it is a product of dry or wet milling. These fiber-rich byproducts are sold as medium-protein animal feeds (18–30% protein) with a relatively low value of approx $75–$85/dry US ton *(1)*. Although of relatively low value, this byproduct has a significant impact on the profitability of corn to ethanol production *(2)*. As the industrial production capacity of fuel ethanol has increased, there has not been a concurrent increase in the demand for the animal feed byproduct to match the increased supply. This has resulted in a downward pressure on the selling price of this byproduct. These factors have been the focus of attention in research into alternative uses or value-added products that could be produced from corn fiber *(3–6)*. One possible use for corn fiber is as a source of fermentable sugars by hydrolyzing the cellulose fraction to glucose *(2)*.

Wet milling currently accounts for approx 35% of the annual US production of fuel ethanol. Corn fiber derived from wet milling is approx 65% carbohydrates in the form of residual starch, cellulose, and hemicellulose (Table 1). Cellulose represents a potential source of fermentable glucose, if hydrolyzed, that would increase the yield of ethanol while also decreasing the amount of fiber byproduct per bushel of processed corn.

Cellulose is a highly recalcitrant, crystalline polymer that is resistant to hydrolysis by mineral acids or enzymes *(7–9)*. Pretreatment is required to disrupt the plant cell wall structure and nanoscale structure of cellulose to improve hydrolysis yields and efficiency *(10–12)*. Liquid hot water has been shown to effectively pretreat lignocellulosic biomass by partially hydrolyzing the hemicellulose and disrupting the lignin and cellulose structures *(13–17)*. By controlling the pH of the aqueous phase during pretreatment, the formation of monomeric sugars by hydrolysis of the soluble hemicellulose oligosaccharides is restricted *(10,14,18)*. Thus, further sugar degradation reactions that produce furfural and 5-hydroxymethylfurfural (HMF) and other products that are toxic to microbial fermentations are limited *(19–21)*. Generally, concentrations of furfural or HMF >1 mg/mL cause significant inhibition of fermentation. Optimized liquid hot water pretreatments can produce pretreatment liquors below this threshold.

Table 1
Composition for Corn Fiber and Centrifuge Cake Undissolved Solids From 16% (w/v) Solids Loading

	Corn fiber (feed) (% dry basis)[a]	Centrifuge cake (product) (% dry basis)
Glucan		
Starch	24	0.5
Cellulose	14	33
Xylan/galactan	17	22
Arabinan	11	5
Protein	12	22
Crude fat	n/m	7
Klason lignin	8	7
Acetyl	n/m	4
Ash	0.4	0.4

[a]n/m, not measured.

Liquors from the pilot-scale pretreatment of corn fiber contained concentrations of furfural and HMF of <0.3 and <0.5 mg/mL, respectively. This represents <1% degradation of the total sugars.

Description of Pretreatment Process

The patented *(18)* corn fiber pretreatment process is illustrated in Fig. 1 with the approximate mass flows as given in Table 2. The values in Table 2 are calculated estimates based on measured total flow rate through the pretreatment coil (streams 1 + 2), estimated steam flow rate, and measured moisture and solids content of the centrifuge streams. Fiber was obtained from a Vetter press. This fiber entered the pretreatment process at 60% moisture (total weight basis) and was mixed with stillage with a composition of 11% dissolved solids and 2% undissolved solids. Stillage is the bottoms from the distillation of the ethanol from the fermentation beer. Stillage was chosen as the pretreatment liquid because it does not add additional water to the overall plant process. Additional water dilutes the plant throughput and increases the energy costs of drying and distillation as well as the load on the plant wastewater treatment facility. Stillage was found to be beneficial for the pretreatment process because protein and lactic acid in the stillage will buffer the slurry to pH 4.0 during pretreatment. Prior research has shown that maintaining the pH above 4.0 limits hydrolysis of polysaccharides *(13–15)*. Because monosaccharide degradation products, such as furfural and HMF, inhibit the ability of bacteria or yeast to ferment sugars to ethanol, limiting hydrolysis of the polysaccharides to monosaccharides during pretreatment prevents the formation of these toxins. At this time, there are few data on the effect of pretreatment on the quality of the protein in the residual fiber, which would be sold as animal feed.

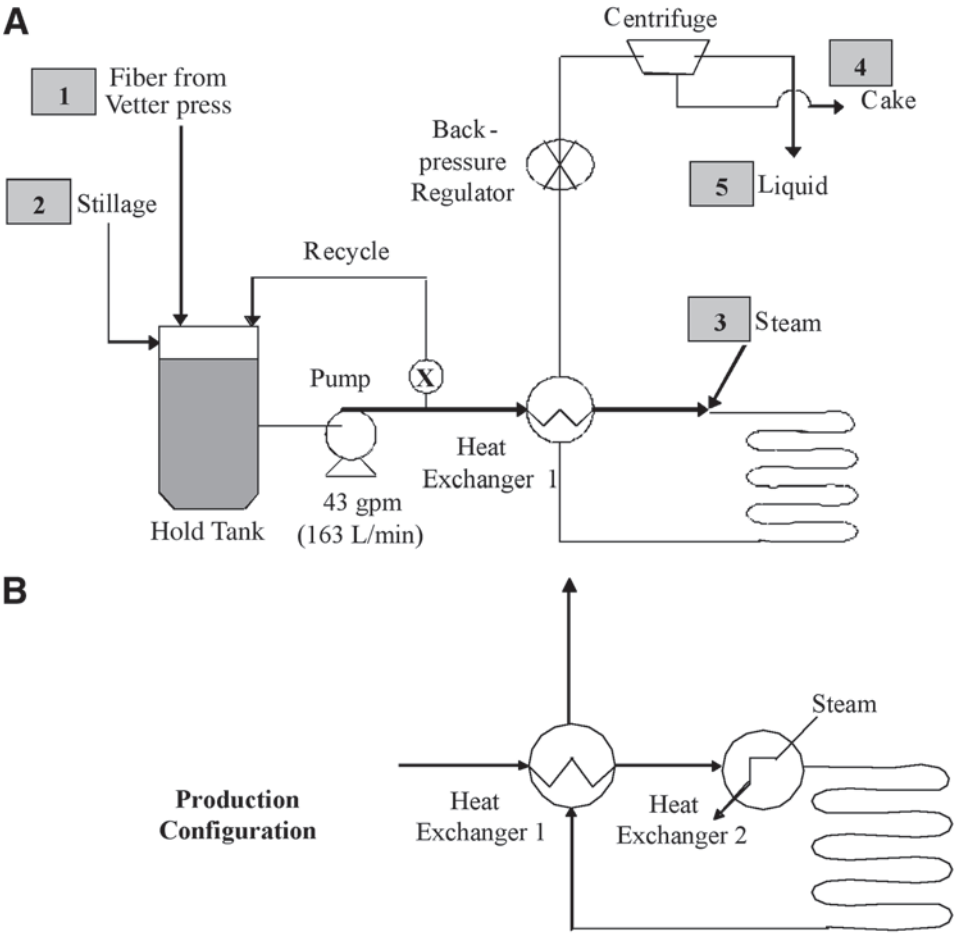

Fig. 1. Process flow diagram. **(A)** Process configuration with heat supplied by the direct injection of steam flow (flow 3). **(B)** Process configuration where direct injection of steam is replaced by a heat exchanger. Alternate configuration replaces steam injection with heat exchanger. Fiber (stream 1) and stillage (stream 2) are mixed in the hold tank. Heat from the slurry exiting the pretreatment coil is recovered to heat the incoming stream in heat exchanger 1. Trim heat to make up for losses is supplied by direct injection of steam (stream 3) or by heat exchanger (production configuration). The pretreated slurry is separated by the centrifuge into solids cake (stream 4) and liquid (stream 5). Approximate flow rates, compositions, and material and energy balances corresponding to the various sampling points are given in Tables 2–4, respectively.

The slurry entering the pretreatment coil was 7.8% (w/v) dry corn fiber. The total undissolved solids entering the pretreatment coil was 9.5% (w/v) (including undissolved solids from stillage), and the dissolved solids was 8.7%. This gives a total solids (dissolved and undissolved) loading rate of 18.2%. The slurry was heated by crossflow contact with the outlet from the coil through the heat exchanger. For purposes of this test, steam was directly injected into the slurry at point 3 to supply heat for the process start-up and for trim heat during steady-state operation at a rate of about

Table 2
Nominal Mass Flow Rates
in Pretreatment Pilot-Scale Process Corresponding to Fig. 1A

Stream 1		Stream 2	
Approximate flow rate		Approximate flow rate	
Total flow	9 gpm	Total flow	34 gpm
Solids	27 lbm/min	Dissolved solids	32 lbm/min
Water	41 lbm/min	Undissolved solids	7 lbm/min
	Water	258 lbm/min	
Stream 1 + 2		**Stream 3**	
Approximate flow rate		Approximate flow rate	
Total flow	43 gpm	Water	25 lbm/min
Dissolved solids	32 lbm/min	Pressure	150 psi
Undissolved solids	33 lbm/min		
Water	299 lbm/min		
Stream 4		**Stream 5**	
Approximate flow rate		Approximate flow rate	
Total flow	115 lbm/min	Total flow	275 lbm/min
Dissolved solids	10 lbm/min	Dissolved solids	36 lbm/min
Undissolved solids	20 lbm/min	Undissolved solids	0 lbm/min
Water	85 lbm/min	Water	239 lbm/min

25 pounds mass (lbm) (12 kg) of steam/min. Steam injection was used to avoid the need to purchase or rent an additional heat exchanger for purposes of this test. However, at production scale, a second heat exchanger will replace steam injection for the purposes of preserving the water balance, avoiding dilution of the slurry, and reducing energy consumption. The impact of substituting steam with a heat exchanger is discussed as part of the economic analysis (*see* Economic Evaluation). Steam at 25 lbm/min (12 kg/min) adds 7.5% to the total volume of the slurry, thereby reducing fiber solids from 8.5 to 7.8%. The resulting flows are given in Table 2.

The slurry enters the coil, where it experiences an average retention time of 20 min at 320°F (160°C). This residence time is optimized residence time as determined by batch pretreatment experiments carried out at the laboratory scale using capped stainless steal tubes heated in a fluidized sand bath as described in Materials and Methods. The slurry leaving the pretreatment coil is cooled to 212°F (100°C) by the incoming slurry that enters the heat exchanger at 207°F (97°C). The pretreated slurry then passes through a Centrisys centrifuge (Kenosha, WI), resulting in a solids cake with 74% moisture (Table 2) and a clear liquid with the composition shown in Table 3. Approximately 18% of the solubilized glucan found in the liquid was hydrolyzed to glucose with the balance of glucans present as soluble oligosaccharides.

Table 3
Composition of Dissolved Solids in Stillage and Centrifuge Liquor

	Stillage (mg/mL)	Centrifuge liquor (mg/mL)[a]
Glucan	7.1	20.2
Glucose	0.3	5.8
Xylan/galactan	0.9	8.5
Xylose/galatose	1.1	1.1
Arabinan	0.0	1.8
Arabinose	1.0	2.7
Acetic acid	0.0	0.4
Lactic acid	10.4	12.2
Glycerol	15.5	12.6
HMF	0.0	0.4
Furfural	0.0	0.3
2,3-Butanediol	3.4	n/m

[a]n/m, not measured.

Materials and Methods

Laboratory-Scale Pretreatment

Laboratory-scale scoping runs were carried out to narrow preferred temperature/hold time for optimal pretreatment (i.e., maximum solubilization/minimum hydrolysis). Reactors were constructed from stainless teel tubes having a length of 4.5 in., an outside diameter of 1.0 in., and a measured internal volume of 45 mL. These were filled with 33.75 mL of slurry and heated to pretreatment temperatures in a fluidized sand bath. Swagelok 316 stainless steel caps were fitted onto each end of the tubes. The standard working volume for each tube was 33.75 mL to allow a 25% headspace above the sample to permit thermal expansion of the liquid, which was kept in a liquid state. Fiber loadings varied between 2.6 (7.8% loading) and 5.4 (16% loading) dry g/tube. Fiber was assumed to have a specific gravity of 1.00 for the purposes of calculating the volume of stillage required to fill each tube to 33.75 mL. Heat-up and temperature control of the reactors was achieved using a Tecam® SBL-1 fluidized sand bath and controller (Cole-Parmer, Vernon Hills, IL). The time required to heat the contents of the reactor tubes to the desired temperature was determined by measuring the internal temperature of a water-filled reactor tube fitted with a thermocouple. A heat-up time of 5 min was required for the contents of the tube to reach 320°F (160°C). The hold time was measured from the time that the temperature plateaued. After the hold time at the desired temperature was attained, the tubes were removed and quenched in room temperature water. Internal reactor temperature dropped from 320°F (160°C) to below 212°F (100°C) in <20 s when the tubes were submersed in 10 L of water.

Pilot-Scale Pretreatment

The scale-up runs were performed at Aventine Renewable Energy (Pekin, IL). Corn fiber and stillage were diverted from plant processing lines for use during the trial. Carbohydrate analyses of the various solid and liquid streams followed the laboratory analytical procedures developed by the National Renewable Energy Laboratory (NREL) as described below *(22)*.

Collection and Comparison of Samples

Samples of the centrifuge cake, liquor, and uncentrifuged pretreatment slurry were collected while the pilot-scale pretreatment system operated at steady state. These samples were then analyzed and compared against laboratory-scale pretreatment samples for composition, enzyme digestibility, and fermentability of the resulting sugar-rich liquor.

Carbohydrate Analysis

Total glucan analysis of corn fiber was performed by the procedure developed by Saha and Bothast *(23)*. Corn fiber was treated in 4% sulfuric acid at 120°C for 1 h in an autoclave. The low lignin content of corn fiber eliminates the need for the strong acid pretreatment of the NREL method (72% sulfuric acid for 2 h at 30°C), which actually can result in substantial loss of labile carbohydrates to degradation products *(23)*. The dilute-acid-autoclaved fiber was adjusted to pH 5.0 using sodium hydroxide. Using a 1:1 ratio mixture of Celluclast 1.5L and Novozyme 188 (Novozymes, Franklinton, NC) at a loading of 10 filter paper units (FPU)/g of dry fiber solids, the solids were hydrolyzed at 50°C in a shaker at 350 rpm for 120 h. The hydrolysate was analyzed by high-performance liquid chromatography (HPLC) utilizing an HPX-87H organic acid column (Bio-Rad, Hercules, CA). This analytical column allows the concurrent analysis of liquid samples for the presence of acetic and lactic acids as well as sugar degradation products. Standardization curves were developed for all compounds using pure chemicals obtained from Sigma-Aldrich (St. Louis, MO). The glucan analyzed by this procedure represents the sum of starch and cellulose in the corn fiber. A second procedure using amylase was necessary to determine the starch content independently in order to account for starch and cellulose separately.

Starch Analysis

Starch content of the solids was determined by hydrolysis of the fiber or the pretreated fiber with α-amylase (Sigma a45551) and amyloglucosidase (Sigma a7420). A dry fiber solids mass of 5 g was mixed with 70 mL of 50 m*M* Tris buffer (pH 7.0), α-amylase (6300 U/g of dry fiber solids), and amyloglucosidase (80 U/g of dry solids). The slurry was then incubated in a shaker at 37°C, 150 rpm agitation for 119 h. The resulting measured glucose in the supernatant was converted into the equivalent

mass of starch. Cellulose content was calculated by difference in which the starch analysis was subtracted from the total glucan determined by acid pretreatment/enzyme hydrolysis described in the previous section.

Starch Hydrolysis and Fermentation

For *Saccharomyces cerevisiae* starch fermentations, 100 g of pretreated hydrolysate, which was sterilized by autoclaving at 121°C for 15 min, was placed in a 125-mL Erlenmeyer flask and mixed with 8 mL of YP 10X stock (100 g/L of yeast extract and 200 g/L of peptone) and 10–100 µL of filter-sterilized glucoamylase (AMG300L from Novozymes, Bioindustrial A/S, Denmark; density of 1.2 g/mL, reported activity of 300 amyloglucosidase units/mL). The flask was inoculated with *S. cerevisiae* Y-2034 (ARS Culture Collection, Peoria, IL) to a beginning OD_{600nm} of 0.1 and capped with a rubber stopper that had been pierced with a 22-gage needle to allow for CO_2 exhaust. The culture was incubated at 32°C with agitation (120 rpm). The seed culture was grown for 18 h in YP medium supplemented with 50 g/L of glucose and resuspended in basal YP medium prior to inoculation. The culture was sampled periodically for glucose and ethanol. Total available glucose was determined by digesting samples with 2 *N* trifluoroacetic acid (Sigma) at 100°C for 1 h followed by HPLC analysis of sugars.

Enzymatic Digestibility of Cellulose

Corn fiber before and after pretreatment was hydrolyzed using a blend of commercially available cellulase preparations. Into a 250-mL polypropylene bottle was placed 33.75 mL of corn fiber and stillage slurry with deionized water to a total liquid volume of 100 mL. To this diluted slurry was added a 1:1 mixture of Novozyme 188 and Celluclast 1.5L (Novozymes, Bioindustrial A/S) to give an enzyme loading of 10 FPU/g of fiber (dry basis), equivalent to 70 FPU/g of cellulose. Hydrolysis was carried out in a New Brunswick Scientific model G24 Environmental Incubator Shaker at 50°C and 250 rpm.

Fermentation Using Xylose-Fermenting S. cerevisiae 424A(LNH-ST)

For ethanol production, 8 mL of seed culture *S. cerevisiae* 424A(LNH-ST) *(24–26)* was used to inoculate 100 mL of YEPD (YEP plus 2% glucose) in a 300-mL baffled Erlenmeyer flask equipped with a side arm. The cultures were incubated in a shaker at 30°C and 200 rpm and grown aerobically overnight (OD = 350–400 KU). The yeast was harvested by centrifuging (J-21 Beckman centrifuge) at 3000*g* for 5 min at room temperature. The supernatant was discarded, and the cells were transferred into a 300-mL baffled Erlenmeyer flask containing 100 mL of hydrolysate supplemented with 10 mL of 10% yeast extract. The initial cell mass concentration prior to fermentation in each experiment was 8.5–9 g of dry wt/L. The flasks were then sealed with Saran wrap to allow fermentation to be carried out under largely anaerobic conditions. The cultures were placed in a shaker

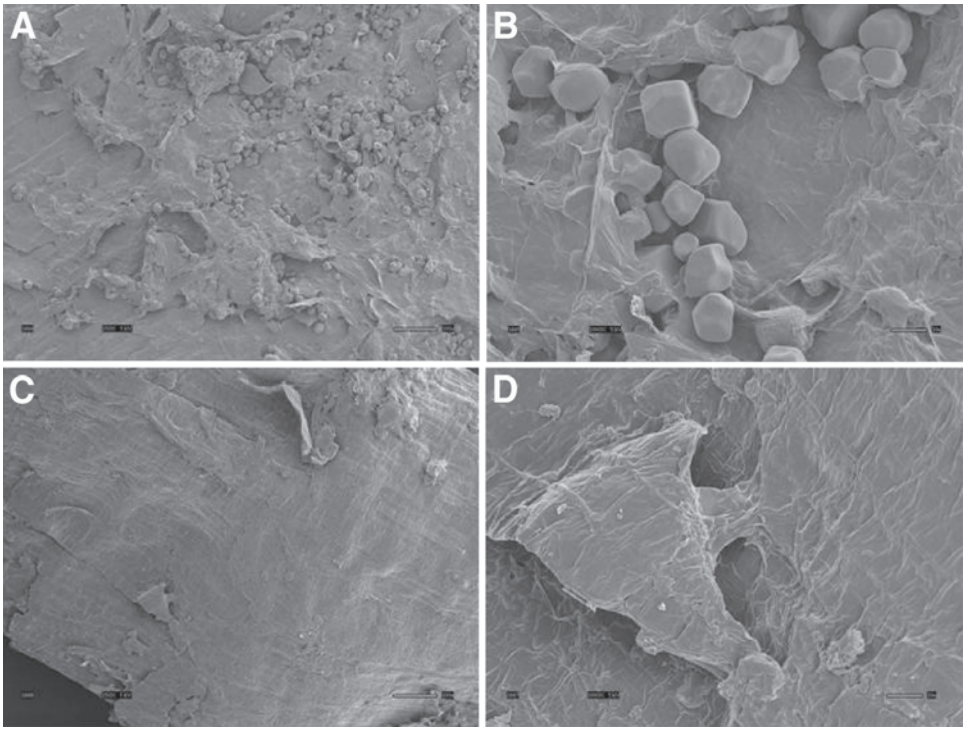

Fig. 2. Scanning electron micrographs of **(A,B)** untreated corn fiber and **(C,D)** pretreated corn fiber. Note that no starch granules were observed on the pretreated corn fiber. Magnification: (A) ×150; (B) ×1000; (C) ×150; (D) ×1000.

and incubated at 30 or 37°C. One milliliter of the fermentation mixture was removed at proper intervals to serve as a sample for monitoring the fermentation.

Scanning Electron Microscopy

Samples of corn fiber before and after pretreatment were mounted on stubs and sputter-coated with AuPd prior to imaging with a JEOL JSM-840 scanning electron microscope using 5 kV of accelerating voltage. Digital images were captured using 1280 × 960 resolution and a 160-s dwell time.

Results and Discussion

Extraction and Saccharification of Starch

Nearly all of the insoluble starch on the fiber surface was dissolved into soluble maltodextrans by pretreatment at 160°C, as indicated by starch analysis (Table 1) and scanning electron micrographs of the corn fiber before and after pretreatment (Fig. 2). Nearly 50% of the fiber solids was dissolved during pretreatment. The addition of a cellulase mixture increased solubilization to 70% in 2 h and nearly 80% within 24 h (Fig. 3). Glucose concentra-

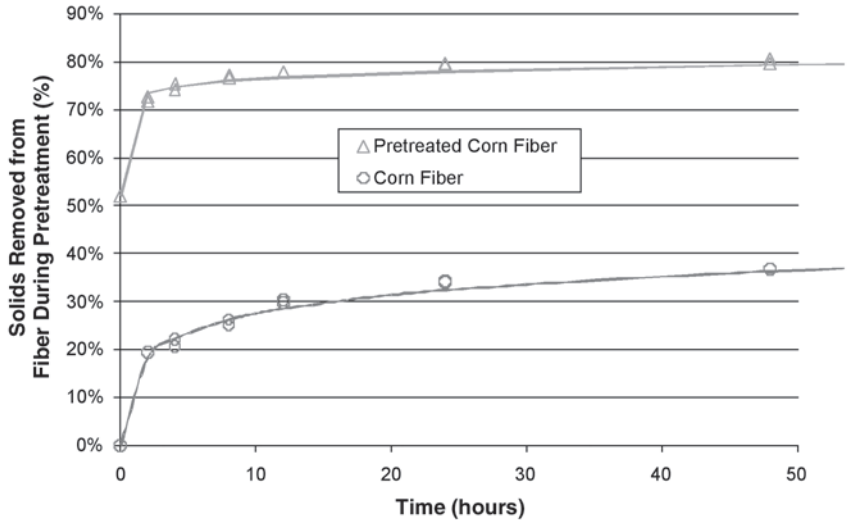

Fig. 3. Solubilization of pretreated and untreated corn fiber by cellulase (Novozyme 188 and Celluclast 1.5L in a 1:1 ratio, 10 FPU/g of dry corn fiber) at 50°C. Replicate runs at each condition are shown.

tion plateaued at 50 mg/mL (data not shown) and corresponded to 80% solids loss (Fig. 3).

Hydrolysis of corn fiber using amylase at conditions used for starch analysis resulted in a yield of 20 to 24% of the total solids as soluble glucose. α-Amylase and glucoamylase hydrolysis of pretreated corn fiber from the centrifuge cake that was washed with distilled, deionized water resulted in a yield of 0.5% of the total solids as soluble glucose (data not shown). By comparison, hydrolysis of the unwashed centrifuge cake with amylase resulted in 8.8% of the total solids being hydrolyzed to glucose. The difference between glucose hydrolyzed from the pretreated, unwashed cake and the washed cake showed that soluble maltodextrans were present in the liquid from the centrifuge cake because washing was easily able to remove them. This conclusion is further confirmed by scanning electron microscopy of the fiber surfaces. Figure 2A,B shows starch particles on the surface of untreated fiber. After pretreatment the surface was smooth and devoid of starch particles (Fig. 2C,D).

Fermentation of Solubilized Starch to Ethanol

The solubilized starch was fermented to ethanol using *S. cerevisiae* Y-2034. Simultaneous saccharification and fermentation was conducted at glucoamylase loadings of 0, 0.12, 0.24, 0.60, and 1.2 mg of enzyme preparation/g of substrate. The fermentations were completed within 32 h and the yields varied from 93 to 103% based on available noncellulosic glucose; the higher than theoretical yield could be accounted for by the fermentation of galactose. The starch dissolved from the corn fiber surface was shown to be readily fermented to ethanol.

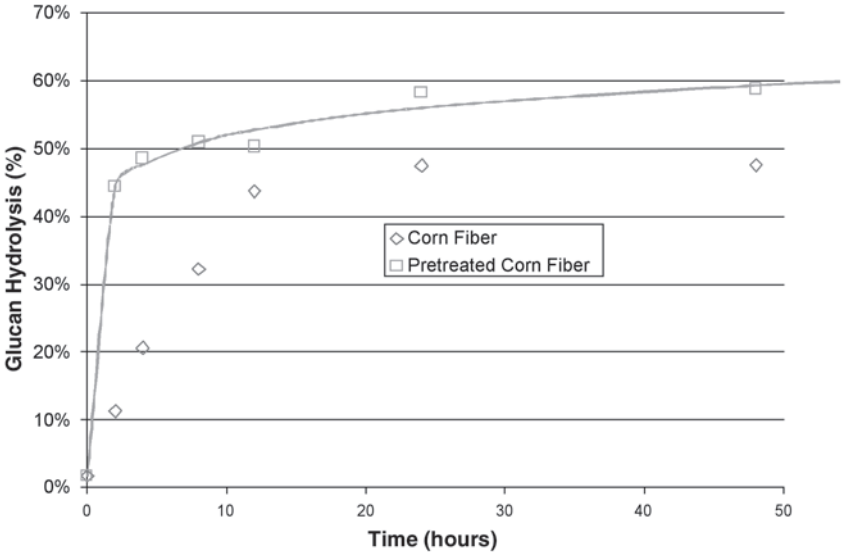

Fig. 4. Cellulase (Novozyme 188 and Celluclast 1.5L in a 1:1 ratio, 10 FPU/g of dry corn fiber) hydrolysis of laboratory-pretreated corn fiber at 50°C.

Cellulose Digestion

The hydrolysis of corn fiber pretreated in the laboratory reaction tubes showed that the initial rate of cellulose hydrolysis was 1.86×10^{-2} g of glucan/h/FPU of enzyme/g of total pretreated solids compared with 0.93×10^{-2} g of glucan/h/FPU of enzyme/g of untreated solids during the first 5 h (Fig. 4). After 24 h, the extent of cellulose conversion was 47% for untreated fiber compared with 58% for pretreated fiber. Pretreatment effectively reduced hydrolysis time for equivalent enzyme loadings. Similar extents of hydrolysis of the pretreated fiber occurred in half the time required to achieve the same results as for untreated fiber.

The dissolved carbohydrates in the liquid from pretreated samples included a significant fraction of oligosaccharides from hemicellulose. The supernatant of cellulase-digested pretreated solids contained only trace amounts of monomeric xylose. However, 4% sulfuric acid hydrolysis *(27)* of this liquid gave 10.8 g/L of xylose, showing that the liquid contained significant amounts of soluble xylooligosaccharides. After 72 h of cellulase hydrolysis, approx 70% of the remaining insoluble xylan in the pretreated corn fiber became soluble oligosaccharides, as determined by comparing the results of the 4% acid hydrolysis of the supernatant from the cellulase-hydrolyzed pretreated solids *(27)* to the compositional analysis of the pretreated solids *(27)*. By contrast, much lower concentrations of soluble xylan oligosaccharides were found in the supernatant of the untreated fiber solids digested with cellulase. These results suggest that the pretreatment also pretreats the hemicellulose. It is possible that the pretreated hemicellulose is more easily hydrolyzed to soluble xylan oligomers by the xylanase activ-

ity in the enzyme mixture. The dissolution of hemicellulose allows rapid saccharification of the insoluble cellulose fraction by cellulases following pretreatment. The reduction in undissolved solids improves the soluble glucan recovery from the undissolved solids when pretreated solids are centrifuged to remove free liquid owing to the reduced mass of solids that will trap residual liquid.

Fermentation of Cellulase Hydrolysate to Ethanol

Pretreated fiber was hydrolyzed by cellulase enzymes and then fermented using xylose-fermenting *S. cerevisiae* 424A(LNH-ST) and ethanologenic *Escherichia coli* FBR16. The results from the xylose-fermenting *S. cerevisiae* 426A(LNH-87) are shown in Fig. 5. The pretreated slurry prior to centrifugation was hydrolyzed with cellulase for 96 h at 50°C, followed by fermentation for 50 h at 30°C by yeast. A 90% or better theoretical yield of ethanol from all glucans (starch and cellulose) in the starting material was achieved with 20 g/L of ethanol concentration formed. The combined results show that furfural and HMF present in the pretreated and hydrolyzed material (<0.3 and <0.5 mg/mL, respectively) did not significantly inhibit the yeast fermentation. Although this strain of recombinant yeast is able to ferment both xylose and glucose, xylose concentrations in the pretreatment liquid were at the minimum levels required for fermentation (Fig. 5A). Spiking of the pretreatment liquid with xylose and fermentation with yeast (424A[LNH-ST]) showed that xylose was fermented as well as glucose (Fig. 5B), with a higher ethanol yield and more rapid conversion occurring at pH 5.7 compared with pH 4.4 (Fig. 5B). However, extant xylanase activity present in the enzyme mixture was not directly accounted for.

Corn fiber hydrolysate was also evaluated for fermentation using *E. coli* strain FBR16. Xylan oligomers were completely hydrolyzed by treating the hydrolysate with 3% (w/v) H_2SO_4 for 1 h at 121°C prior to fermentation. The mixture was neutralized to pH 6.5 with $Ca(OH)_2$ and fermented with FBR16 as previously described *(28)* in pH-controlled minibioreactors. The fermentation was completed within 36 h, and the ethanol yield was 84% of theoretical based on initial free arabinose, glucose, and xylose concentrations (data not shown). The ethanol yield was improved to 97% of theoretical by treating the hydrolysate with XAD4 resin as described by Weil et al. *(29)* to remove furfural and other inhibitory compounds. These results illustrate that the low concentration of furfural and HMF will significantly inhibit fermentation by recombinant *E. coli*.

Effect of Fiber Loading on Soluble Glucan Recovery

The corn fiber pretreatment process (see Fig. 1) separates liquid containing solubilized starch (i.e., glucan) from the undissolved solids by centrifugation. The fermentation system at, Aventine Renewable Energy, the wet-milling facility, was designed to remove undissolved solids before the sugars are fermented. Because centrifugation is not 100% efficient, some portion of the liquid will be entrapped in the solids cake (stream 4 in Fig. 1).

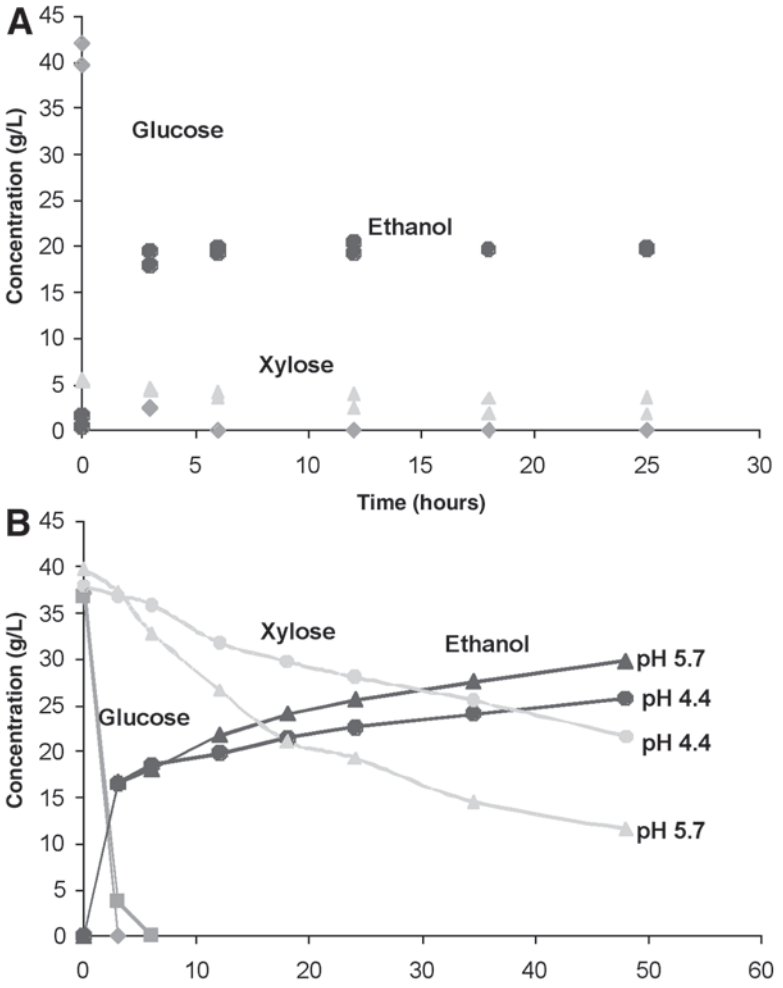

Fig. 5. Fermentation of cellulase-treated centrifuge cake (9% dry mass in liquid) and pretreatment liquid. Centrifuge cake in pretreatment liquid was treated with an equal mixture of Celluclast 1.5L and Novozyme 188 (50 FPU/mL) at a loading of 10 FPU/g of dry solids at 50°C for 96 h. After saccharification, the liquid was fermented with xylose-fermenting recombinant yeast (424A[LNH-ST]) at 30°C. Samples were collected every 3 h and analyzed by HPLC. **(A)** Two replicates of fermentation of liquid after saccharification; **(B)** fermentation of liquid after saccharification (pH 4.4) spiked with xylose to fermentable levels and liquid after saccharification spiked with xylose and pH adjusted to 5.7.

Hence, the impact of fiber loading and centrifuge efficiency on liquid recovery was calculated. Although tuning the centrifuge to produce a drier cake may result in some solids exiting with the centrate (liquid), solids carryover into the supernate from the centrifuge was insignificant under the conditions used in our study.

Figure 6 illustrates the impact of fiber loading in the pretreatment step on soluble glucan concentration recovered in the liquid stream from the

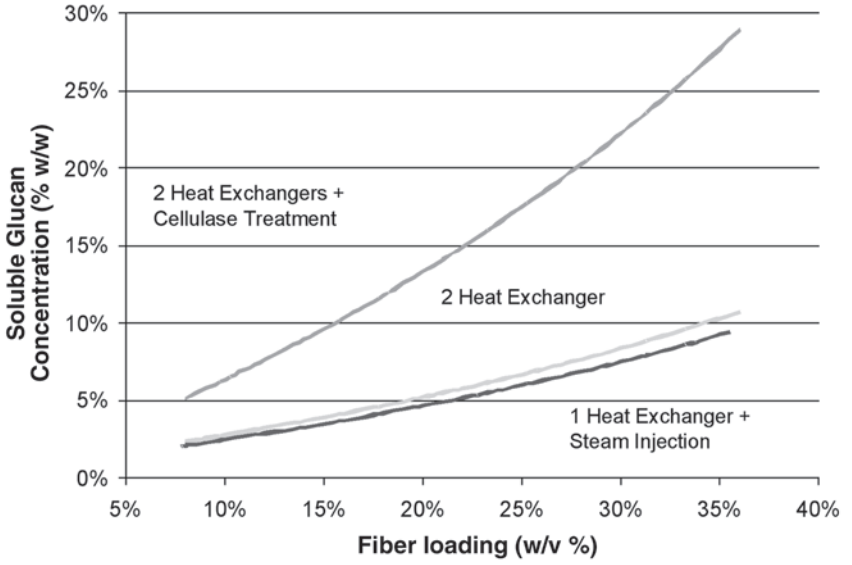

Fig. 6. Concentration of soluble glucan (starch) in pretreatment liquid as function of initial fiber loading expressed as weight/volume × 100. Curves for steam injection for trim heat, two heat exchangers, and two heat exchangers plus cellulase treatment are shown.

centrifuge. The soluble glucan concentration corresponding to the trial run (one heat exchanger + steam injection at 7.8% fiber) matches the analytical results (glucan was 26.39 mg/mL = 2.6% in stream 5; Fig. 1). Steam injection for makeup heat slightly dilutes the glucan concentration. Hydrolyzing the pretreated material with cellulase increases the glucan concentration (two heat exchangers + enzyme case in Fig. 6). The bases for these calculations are as follows:

1. The concentration of soluble solids in the liquid entrapped in the solids cake (stream 4) was equivalent to the concentrations in the liquid stream (stream 5). HPLC analysis of materials generated by the pilot run confirm that this assumption is reasonable by using glycerol as an internal standard for liquid from the centrifuge and liquid washed from the solids cake (Table 3). Glycerol concentrations in the two streams are similar.
2. All of the undissolved starch granules on the corn fiber were completely solubilized through pretreatment for all fiber loadings. This assumption adheres to the results from laboratory tests for loadings up to 16% fiber (Table 1 and Fig. 2).
3. Total undissolved solids were reduced by 50% through pretreatment for all fiber loadings. This assumption adheres to the results from laboratory tests for loadings up to 16% fiber. This assumption might not hold at higher loadings.

4. All soluble glucans in the liquid stream (stream 5) were enzymatically hydrolyzed in postprocessing of the liquid. This includes cases in which amylases and/or cellulases were used.
5. Glucose from soluble glucan hydrolysis was fermented to ethanol at 95% of theoretical yield. No other monosaccharides were assumed to be fermented, for purposes of evaluation of the economics of the process presented in the next section.
6. Unfermented dissolved solids were carried through distillation in the stillage stream to be mixed with the pretreated solids, dried, and sold as animal feed.

Three simulations were run for fiber loadings between 8 and 28%. The first simulation was based on the system as operated in the pilot test: one heat exchanger for heat recovery followed by direct injection of steam (*see* schematic diagram in Fig. 1A). The second simulation was conducted for a system with two heat exchangers to eliminate the diluting effect of direct injection of steam. The second heat exchanger was assumed to be supplied with 150 psi of saturated steam for makeup or trim heat. The effectiveness of this heat exchanger was assumed to be 85%; that is, 85% of the total enthalpy transferred from the steam to the pretreatment stream. The third simulation assumes that the pretreatment stream is treated with cellulase, which solubilizes an additional 45% of the remaining undissolved solids before centrifugation (Fig. 3); however, the percentage of solubilization might not be constant. This assumption is based on the laboratory data shown in Fig. 3. The additional 45% solubilization (72% total solubilization of the inlet corn fiber) occurs at 2 h of cellulase treatment.

The results of increased fiber loading are illustrated in Fig. 7A, which plots glucan recovery as a function of fiber loading for the two-heat-exchanger process configuration with and without cellulase hydrolysis prior to centrifugation. Both curves assume that the resulting centrifuge cake has a moisture content of 60%. Higher fiber loading translates into a larger percentage of exit solids because both the amount of fiber and the liquid it holds become larger (stream 4 in Fig. 1A). The higher the solids the higher percentage of the total liquid entrapped in that solids cake. It is in this context that enzyme hydrolysis of cellulose (solids) will reduce the mass of undissolved solids and result in higher recovery of soluble glucans in the liquid stream. Thus, a higher fiber loading must be followed by enzyme treatment of unhydrolyzed pretreated solids.

Figure 7B shows the calculation of the mass of soluble maltodextran recovered vs the heat energy required to bring the pretreatment slurry to process temperature for a two-heat-exchanger process configuration (no exogenous water is added). These data show clear optimal conditions for each centrifuge cake moisture content if fermentable sugar recovery is to be maximized and energy consumption minimized. For the 74% moisture content case, the optimum is approx 22% (w/v) fiber loading; for the 70% case, nearly 25%; and for the 60% case, 35%.

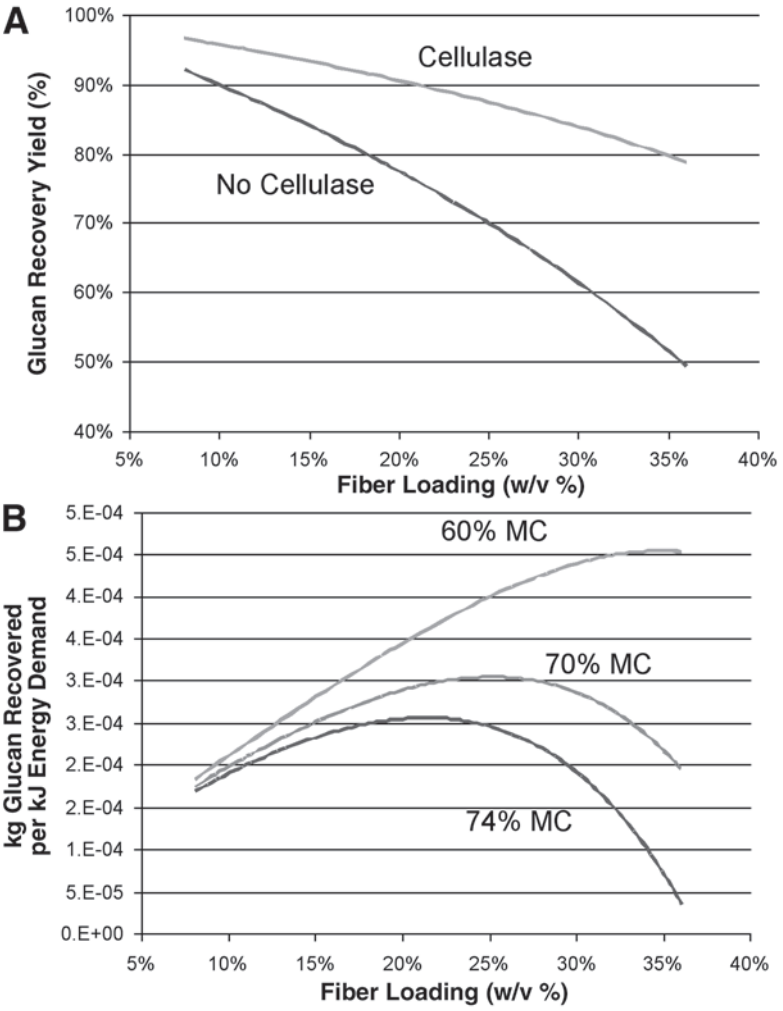

Fig. 7. Calculated effect of fiber loading on the two-heat-exchanger pretreatment process for **(A)** recovery of soluble glucan from the centrifuge in the liquid (stream 5) with 60% moisture cake with and without cellulase hydrolysis prior to centrifugation and **(B)** mass of glucans recovered per kilojoule of heat supplied for pretreatment for various centrifuge cake moisture contents (MC).

The optimal economic fiber loading balances the decreased steam demand and increased sugar concentration against decreased sugar recovery for increasing fiber loading. Enzyme treatment prior to centrifugation offers a possible way to shift the optimum toward higher fiber loading. However, the economic benefit of this approach will be at least partially offset by increased capital costs (holding tanks for saccharification) and operational costs (enzyme).

Economic Evaluation

The simulation results for three different fiber loadings (w/v), including the fiber loading used for the pilot trial, were used to calculate and evaluate the economics of this pretreatment process for the production of fuel ethanol. The full-scale economic evaluation was based around a pretreatment line that could be integrated into the process at Aventine Renewable Energy. Such a process handles 200 gpm (767 L/min) of slurry, which is equivalent to 10,000 lbm/h (4500 kg/h) of corn fiber on a dry basis. Equipment costs were subjected to straight-line depreciation over a 10-yr time frame. The cost of capital equipment and installation for the pretreatment process was estimated as $1.5 million based on information provided by Aventine that was obtained from equipment manufacturers.

Operational costs consist of fiber, stillage, steam, electricity, labor, and maintenance. Fiber and stillage input costs are expressed as a composite number based on total solids (dry basis). The value of these solids was calculated from the opportunity cost of these solids sold as corn gluten feed (dry basis). Unit costs are expressed in terms of cents per gallon of ethanol produced from the glucose and soluble glucans present in the liquid stream from the centrifuge assuming 95% fermentation efficiency. Credits are calculated on the same basis, for which solids are defined as any component that is not fermented and sold as corn gluten feed of industry-standard quality. The electrical, maintenance, and labor costs were estimated using a lumped sum of $0.191/gal of ethanol produced.

It was of interest to calculate the effect of increasing the ratio of fiber to stillage on the economics of the process. Three fiber loadings were examined (case 1: 7.8% [w/v]; case 2: 16% [w/v]; and case 3: 28% [w/v]) with and without applying cellulase immediately prior to centrifugation. Treatment using cellulase is expected to increase the efficiency of the centrifuge by reducing the undissolved solids in the entering stream. Based on the laboratory enzyme digestion results shown in Fig. 3, it was assumed that treating with cellulase reduced the undissolved solids by 45%.

The amount of dissolved and undissolved solids consumed to form fermentation ethanol was calculated by difference based on the total dissolved solids removed with the liquid (stream 5). All other remaining solids are prorated at a value (cost) equivalent to $65/t, average value for corn gluten feed (dry basis). Consequently, solids remaining after fermentation are being credited at $65/t against the cost of dissolved (in stillage) and undissolved (fiber) solids entering the process. Hence, the solids are the combination of material in stream 4 and solids in steam 5 that remains after fermentation.

An additional operational cost of cellulase enzyme at 10¢/gal of ethanol produced was assumed and included in the net cost of production for the cases in which cellulases were used. Downstream processing costs (distillation, ethanol drying, and so on) were not included. The fiber-loading simulation was used to estimate the energy required in the form of 150 psi

Table 4
Predicted Steam Demand for Three Case Types[a]

	Case 1 (7.8% [w/v] fiber)		Case 2 (16% [w/v] fiber)		Case 3 (28% [w/v] fiber)	
	A	A&C	A	A&C	A	A&C
Stillage mass (lbm/lbm fiber)	10.37	10.37	3.75	3.75	1.07	1.07
Total mass (lbm/lbm fiber)	12.87	12.87	6.25	6.25	3.57	3.57
Ethanol production (gal/lbm fiber)	0.017	0.037	0.013	0.037	0.009	0.033
Steam demand (lbm/lbm fiber)[b]	0.83	0.98	0.41	0.48	0.23	0.27

[a]Case A = glucan derived from starch, and case B = glucan derived from starch and cellulose. A, amylase alone; A&C, amylase + cellulase.
[b]Assumes 85% recovery of thermal energy.

of saturated steam for heating the corn fiber/stillage slurry to the processing temperature. An energy cost of $5/10^6$ BTU of energy was assumed. The amount of steam required to process 1 lbm of fiber shown on the bottom line of Table 4 is directly related to the solids loading into the pretreatment coil and the assumption that 85% of the energy is transferred from the "cold" side of the heat exchanger to the "hot" side. The cost of energy per gallon of ethanol produced drops as the loading increases simply because there is less water to be heated per lbm of fiber processed at higher loadings. The addition of cellulases increases the yield of fermentable glucose per lbm of fiber processed, thus leading to the significantly lower energy cost per gallon of ethanol in these cases when compared to the same loading without added cellulase (Table 4).

Table 5 shows the operational costs of the pretreatment process expressed as cents per gallon of ethanol for three cases. The operational costs include the opportunity cost of the fiber as feedstock for additional ethanol, energy cost (steam) for the pretreatment, and a fixed labor and overhead cost of 19.10¢/gal of ethanol. For case studies in which cellulase is added, an additional operational cost of 10¢/gal of ethanol is added. The cost of amylase for hydrolysis of the maltodextran fraction will add several tens of cents per gallon of ethanol but are not shown in Table 5. These costs do not include capital costs or the energy savings for the reduced mass of fiber in the corn gluten feed driers. The operational costs for the pretreatment of corn fiber in a wet-milling plant is less than $1.00/gal of ethanol produced from the sugars derived from the pretreated corn fiber. Increasing the fiber loading from 8 to 16% decreases the operational expenses by more than 7¢/gal of ethanol when cellulase hydrolysis is not included in the process. Further increasing the fiber loading from 16 to 28% reduces the operational expenses by only approx 2.5¢/gal owing to the loss of liquid with the solids from the centrifuge.

Table 5
Operational Expenses Based on Simulation
of Corn Fiber Pretreatment at 200-gpm Scale (757 L/min)[a]

Operational expense	Case 1 (7.8% [w/v] fiber)		Case 2 (16% [w/v] fiber)		Case 3 (28% [w/v] fiber)	
	A (¢/gal ethanol)	A&C (¢/gal ethanol)	A (¢/gal ethanol)	A&C (¢/gal ethanol)	A (¢/gal ethanol)	A&C (¢/gal ethanol)
Net solids	44.5	44.5	44.5	44.5	44.5	44.5
Steam heat @ $5/$10^6$ BTU	20.0	9.4	12.6	5.3	10.2	3.5
Cellulase	N/A	10.0	N/A	10.0	N/A	10.0
Electric, maintenance, and labor	19.1	19.1	19.1	19.1	19.1	19.1
Total	$0.84	$0.83	$0.76	$0.79	$0.74	$0.77

[a]A, amylase alone; A&C, amylase + cellulase. N/A, not applicable.

The net cost of solids per gallon of ethanol produced is constant at 44.5¢/gal for all cases, because the same mass of sugar is required to produce 1 gal of ethanol in all cases. The major savings in operational cost as the solids loading is increased is in the energy required for pretreatment, which drops from 20¢/gal in the 7.8% (w/v) solids loading case to 12.6¢/gal in the 16% (w/v) solids loading case and then increases to 3.5¢/gal in the 28% (w/v) loading case. However, owing to the increasing solids load in the centrifuge as the solids loading increases, the yields of glucose in the liquid stream drop from 83% in the 16% (w/v) loading case to 65% in the 28% (w/v) loading case (Fig. 7B; no cellulase).

Although the amount of ethanol produced per pounds mass of fiber is significantly increased when the glucose from cellulase-digested cellulose is included (Table 5), the assumed cost of the enzyme at 10¢/gal of ethanol causes the operational expenses for these cases to be nearly the same as the cases in which no cellulase was added for the same fiber loading.

The experimental results for cellulase digestion of the pretreated fiber suggest that lower enzyme loadings may result in significant improvements in fermentable glucose generation and recovery for less than 10¢/gal of ethanol produced. Further examination of the effect of enzyme loading on hydrolysis yields and rates is needed to optimize the process to generate the lowest operational and capital costs for cellulase hydrolysis of pretreated corn fiber for the production of fermentable sugars.

Conclusion

The pretreatment of corn fiber at a pH maintained at 4.0 was demonstrated at a pilot plant scale of 43 gal (163 L) of slurry (containing 18.2% dissolved and insoluble solids)/min. The pretreatment results confirm

laboratory data that this pretreatment process solubilizes nearly all of the residual starch from the corn fiber surface while also producing residual undissolved solids that are completely digested by commercially available cellulase preparations at a rate that is two times more rapid than untreated fiber at the same enzyme loading of 10 FPU/g of fiber (dry basis). The effect of increasing the percentage of corn fiber in the slurry shows that higher loading increases the soluble glucan concentration and decreases the energy required per mass unit of corn fiber that is processed. However, recovery of the soluble products through centrifugation becomes more difficult because a finite amount of the liquid will remain trapped in the undissolved solids fraction. However, hydrolysis of the cellulose addresses this disadvantage by decreasing the total solids that are processed in the centrifuge. Preliminary economic analysis shows that the operational cost of this pretreatment process is less than $0.84/gal of ethanol produced. Further work is required to demonstrate longer-term operability of the pretreatment process as well as to determine applicable enzyme loading and additional capital costs to include cellulase hydrolysis in the system and to determine its likely impact on the economics.

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