April 2003 • NREL/SR-510-33894

Subcontractor Report

Building a Bridge to the Ethanol Industry—Follow-Up Project

Period of Performance: February 22, 2001–December 31, 2002

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Overview

The first trial of the corn fiber pretreatment process has been completed. The data and operating experience for the pump, heat exchanger, coil, and centrifuge show the pretreatment of corn fiber in water is technically achievable and economically feasible. The goals of this trial include showing that the pretreatment process is scaleable to fit the existing process at Williams Bioenergy, that pilot-size equipment achieves the goals of pretreatment – at least 75% recovery of starch from the corn fiber, and testing the performance of pilot-scale equipment at or near operational conditions. These goals were met. Cellulose conversion was also significantly enhanced by the pretreatment process.

The first trial of the pretreatment process occurred over 5 days of operation and troubleshooting. The longest continuously-maintained steady-state operation of the trial system was approximately 4 hours from which material samples were collected for analysis. A second trial consisting of an extended run of 10 days with 90% (72,000 lb/day fiber) operating efficiency is still needed to prove robustness of the pretreatment system and equipment. Once robustness is demonstrated, a continuously operating production pilot unit in the Williams plant will be the next step.

The trial system consisted of a mixing tank, centrifugal pump, spiral heat exchanger, steam injector, coil, and centrifuge integrated into a portion of the existing Williams Bioenergy wet-milling process. The system is designed to process up to 80,000 lbs/day fiber (dry basis). The concentration of total solids (dissolved and undissolved) entering the pretreatment coil was 18.5%, of which 7.8% was corn fiber. The balance of undissolved and dissolved solids came from the stillage to which the fiber was added. The pretreatment was carried out at a hold (residence) time of 20 min. at 160°C. The fiber was processed at a rate of 28.5 lbs/min or 41,000 lbs/day (dry basis), which is 50% of design capacity. The next trial will have the goal of doubling fiber loading, and thereby throughput, as well as achieving extended operation over a 10 day period.

The results from this trial showed that the carbohydrates in the pretreated liquid and solid streams are readily hydrolyzed by enzymes and easily fermentable to ethanol by yeast. Starch was nearly 100% solubilized from the corn fiber by pretreatment, and total starch recovery from the solids by centrifugation was 85%. Fermentation inhibitors were not formed by the pretreatment process, and nearly theoretical fermentation yields are achieved. Hydrolysis of washed, pretreated fiber with amylase gave less than 0.5% glucose per g fiber (dry basis) while hydrolysis with cellulases gave 41%. Economic analyses, based on results of this trial, indicate that a discounted rate of return of 25% or higher is attainable if an ethanol selling price of $1.00 is assumed. This calculation is based on a fiber process for which a solids loading of 16%, an energy cost of $5 per million BTU, and cellulase cost of 10¢/gallon ethanol is attained. The final report summarizes process operating characteristics, ethanol production, and costs for different scenarios. These results are presented in the context of plans for modifying the system for the next test.

Summary of Results from September/October, 2002, Pretreatment Trial

The pretreatment of fiber involves the processing steps illustrated in Figure 1. Photographs of the corresponding key equipment are shown in Figure 2. Fiber is obtained from a Vetter press and mixed with stillage. The fiber from the Vetter press enters the pretreatment process at 59% moisture, and is mixed with 295 lbs of stillage with a composition of 10.8% dissolved solids and 2.2% undissolved solids. The amount of fiber that enters the pretreatment coil is at 7.8% solids (Figure 2(c)). The total undissolved solids entering the pretreatment coil are 9.5% (includes undissolved solids from stillage), while the dissolved solids are 8.7%. At steady state, the slurry is heated by cross-flow contact with the outlet from the coil through heat exchanger 1 (Figure 2(b)). Start-up, and heating to temperature was to be handled by a second heat exchanger. However, for purposes of this test, steam was directly injected into the slurry at point 3, at a rate of about 25 lbm/min. A full-scale process will replace steam injection with a heat exchanger for purposes of preserving the water balance, and avoiding dilution of the slurry, as is later shown in the calculation of costs. In this case, 25 lbs/min of steam adds 7.5% to the total volume of the slurry, thereby reducing fiber solids from 8.5% to 7.8%. The approximate flows are shown as part of Figure 1.

The slurry then enters the coil (Figure 2(c)) where it is retained for 20 min at 320°F (160°C) before being cooled by the incoming slurry that enters the heat exchanger at 207°F (97°C). This passes through a Centrisys centrifuge (Figure 2(d)). The compositions leaving the heat exchanger are shown in the tables associated with numbers (4) and (5) in the continuation of Figure 1. A cake with 74% moisture was produced by the centrifuge with 52% of the fiber solids dissolved. There was very little protein detected in the outlet liquid stream 5, with the composition of dissolved solids in the liquid being as shown in the extended legend for Figure 1. Approximately 18% of the glucan appears as glucose, with the rest being soluble oligosaccharides as confirmed by hydrolysis of the liquid stream.

Hydrolysis of the unwashed cake with amylase resulted in 8.8% of the total solids being recovered as glucose. When cellulase was used instead, 39.2% of the cake was hydrolyzed to glucose. When the cake was washed, the amount of glucose released upon amylase treatment was less than 0.5%, while cellulase treatment gave 41.2% (Table 1). The difference between glucose hydrolyzed from the cake and the washed cake show that soluble maltodextrans are in the liquid entrained in the centrifuge cake because washing is able to remove them from the solids. These data are consistent with starch being solubilized and removed from the fiber.

Table 1. Enzyme Treatments of Centrifuge Cake.

Glucose (% per g dry mass) by enzyme treatment of centrifuge cake

	Amylase[1]	Cellulase[2]
Cake[3]	8.8%	39.2%
Washed Cake[3]	0.4%	41.2%

[1] 80 units of amyloglucosidase + 6310 units of alpha-amylase per gram dry biomass.

[2] 10.2 FPU per gram dry biomass (equal parts Celluclast 1.5L and Novozyme 188).

[3] hydrolyzed glucose % per dry gram of solids

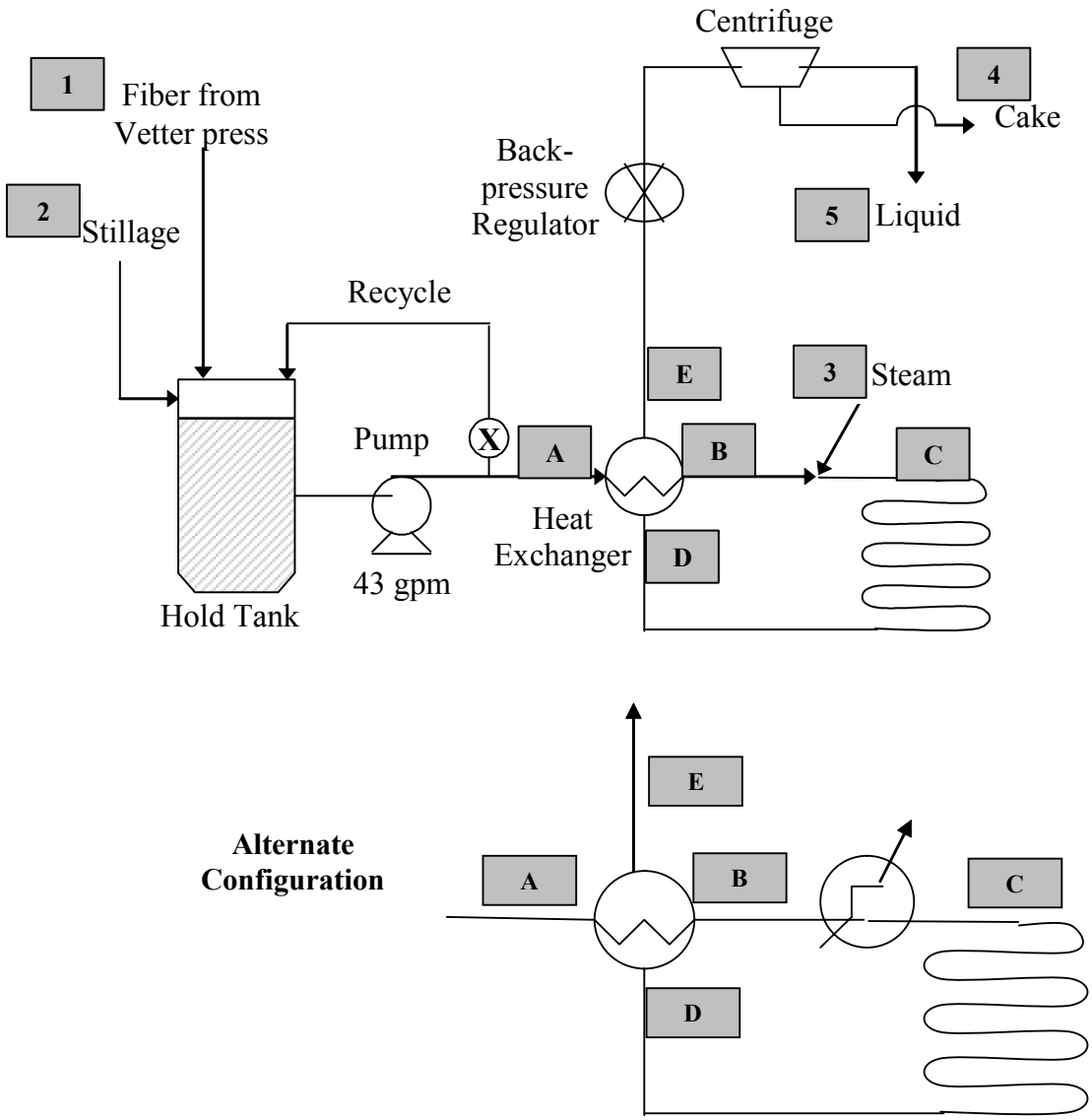

Figure 1. Process flow diagram with material compositions and mass flow rates (numbers), and enthalpies (letters). Alternate configuration replaces steam injection with heat exchanger. Compositions and material and energy balances that correspond with the various sampling points are given on the next two pages.

1

Approximate Flow Rate

Total Flow	8.8	GPM
Solids	28.53	lbm/min (dry basis)
Water	40.97	lbm/min

Composition of Undissolved Solids

	% (dry basis)	Approximate Mass Flow Rate (lbm/min)
Glucan		
starch	23.7%	6.76
cellulose	14.3%	4.08
Xylan/Galactan	16.8%	4.79
Arabinan	10.8%	3.08
Protein	11.8%	3.37
Klason Lignin	8.4%	2.40
Ash	0.4%	0.11
Total	86.2%	24.59

2

Approximate Flow Rate

Total Flow	34.2	GPM
Dissolved Solids	31.87	lbm/min (dry basis)
Undissolved Solids	6.51	lbm/min
Water	257.54	lbm/min

Composition of Total Solids

	mg/mL	Approximate Mass Flow Rate (lbm/min)
Glucan	7.13	1.84
Glucose	0.31	0.08
Xylan/Galactan	0.92	0.24
Xylose/Galatose	1.13	0.29
Arabinose	0.99	0.25
Lactic Acid	10.40	2.68
Glycerol	15.52	4.00
2,3 Butanediol	3.36	0.87
Water	92.72	% of total
Dissolved Solids	1.52	% of total
Undissolved Solids	5.76	% of total

3

Approximate Flow Rate

Total Flow	3.30	GPM
Water	25	lbm/min
Pressure	150	psi
Enthalpy of Condensation	901	BTU/lbm

Figure 1 (continued). Process flow diagram with material compositions.

4

Approximate Flow Rate

	6.15	GPM
Solids	13.57	lbm/min (dry basis)
Liquid	52.434	lbm/min

Composition of Undissolved Solids

	% (dry basis)	Approximate Mass Flow Rate (lbm/min)
Glucan		
starch	0.0%	
cellulose	33.1%	4.49
Xylan/Galactan	21.6%	2.93
Arabinan	5.1%	0.69
Protein	21.5%	2.91
Crude Fat	6.7%	0.91
Klason Lignin	7.0%	0.95
Acetyl	4.1%	0.56
Ash	0.4%	0.05
Total	99.4%	13.48

Composition of Dissolved Solids

	mg/mL	Approximate Mass Flow Rate (lbm/min)
Glucan	26.39	1.38
Glucose	5.83	0.31
Xylan/Galactan	10.24	0.54
Xylose/Galatose	1.11	0.06
Arabinan	1.84	0.10
Arabinose	2.73	0.14
Acetic Acid	0.38	0.02
Lactic Acid	12.80	0.67
Glycerol	12.64	0.66
HMF	0.40	0.02
Furfural	0.26	0.01

5

Approximate Flow Rate

	41.53	GPM
Dissolved Solids	15.64	lbm/min
Water	318.03	lbm/min

Composition of Dissolved Solids

	mg/mL	Approximate Mass Flow Rate (lbm/min)
Glucan	26.39	8.39
Glucose	5.83	1.85
Xylan/Galactan	10.24	3.26
Xylose/Galatose	1.11	0.35
Arabinan	1.84	0.58
Arabinose	2.73	0.87
Acetic Acid	0.38	0.12
Lactic Acid	12.80	4.07
Glycerol	12.64	4.02
HMF	0.40	0.13
Furfural	0.26	0.08

A

"Cold" Inlet to Heat Exchanger

Mass Flow Rate	365	lbm/min
Temperature	207°F	(97°C)
Enthalpy	175	btu/lbm
Enthalpy Flow	63950	btu/min

B

"Cold" Outlet from Heat Exchanger

Mass Flow Rate	365	lbm/min
Temperature	263	(128°C)
Enthalpy	233	btu/lbm
Enthalpy Flow	85140	btu/min

C

Inlet to Snake-coil

Mass Flow Rate	400	lbm/min
Temperature	322°F	(161°C)
Enthalpy	292	btu/lbm
Enthalpy Flow	116920	btu/min

D

"Hot" Inlet to Heat Exchanger

Mass Flow Rate	400	lbm/min
Temperature	315°F	(157°C)
Enthalpy	285	btu/lbm
Enthalpy Flow	114160	btu/min

E

"Hot" Outlet from Heat Exchanger

Mass Flow Rate	400	lbm/min
Temperature	231°F	(111°C)
Enthalpy	199	btu/lbm
Enthalpy Flow	79684	btu/min

Enthalpy Balance Around Heat Exchanger

ΔH Cold Side	21190	btu/min
ΔH Hot Side	34476	btu/min
Enthalpy Loss	13286	btu/min

Figure 1 (continued). Process flow diagram with material compositions and mass flow rates (numbers), and enthalpies (letters).

Figure 2(a). Pump.

Figure 2(b). Centrifuge

Figure 2(c). Photographs of the pretreatment coil.

Figure 2(d). Centrisys centrifuge.

Figure 2(c). Mixing tank (on left).

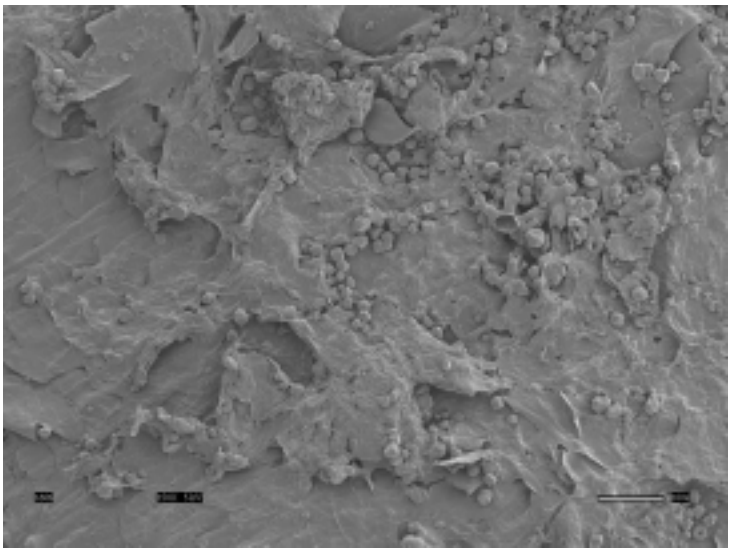

Figure 3(a). SEM of corn fiber, 150x magnification.

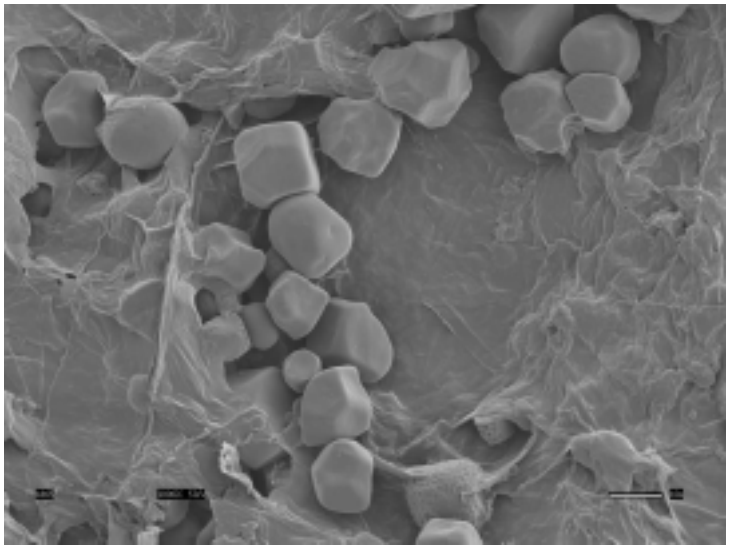

Figure 3(b). SEM of corn fiber, 1000x magnification. Starch granules covering surface.

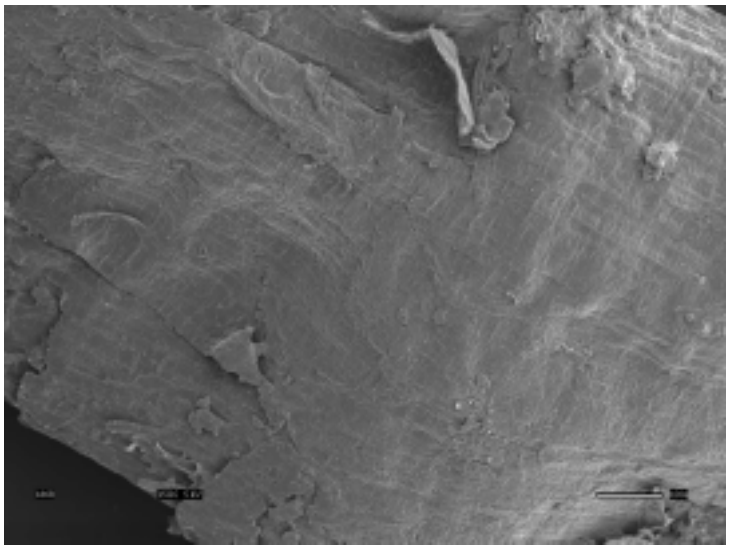

Figure 3(c). SEM of pretreated corn fiber, 150x magnification. No starch granules present.

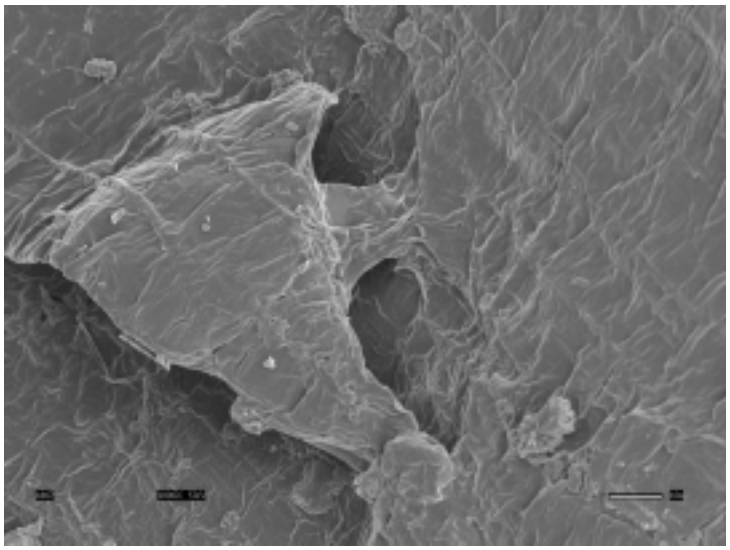

Figure 3(c). SEM of pretreated corn fiber, 1000x magnification. No starch granules found on surface.

This is further confirmed by scanning electron micrographs of the fiber before and after pretreatment. Figures 3(a) and 3(b) show starch particles on the surface of untreated fiber. After pretreatment the surface is smooth and devoid of particle (Figures 3(c) and 3(d)). Subsequent simultaneous saccharification and fermentation of the pretreatment liquid using only glucoamylase with little or no cellulase activity resulted in approximately 100% theoretical yield to ethanol, thus indicating the balance of the glucans in the pretreatment liquid are maltodextrans as shown by Bruce Dien of the USDA NCAUR laboratory (Table 2).

Table 2. Simultaneous saccharification and fermentation of liquid stream from centrifuge.

Glucoamylase[1] μL	Ethanol Concentration (g/L) 24 hr	48 hr	average	% theoretical yield[2]
10	8.41	8.54	8.48	89%
20	8.49	9.11	8.80	92%
50	9.17	9.76	9.47	99%
100	9.47	10.05	9.76	100%

1 - amount Alltech's Glucoamylase added per 100 g of liquid
2 - yield based a 0.51 g/g theoretical yield. Note: this analysis does not take in account galactose or cellulose.

When the solids were recombined with liquid and hydrolyzed with cellulase for 96 hours at 50°C followed by fermentation, 90% or better theoretical yield of ethanol from all glucans (starch and cellulose) was achieved, with 2% ethanol concentration formed. The combined results are not an indicator of practical ethanol yields. Rather, they show that fermentation inhibitors were not formed by the pretreatment process. This was verified by HPLC analysis.

Equipment Observations and Issues that Still Need to be Addressed

The centrifugal pump performed well during the initial test run. However, the feed line to the pump was prone to plugging. Replacement of the centrifugal pump with a Moyno pump is being considered for several reasons. (1) Mixing of the fiber with stillage occurred in a tank (Figure 2(b)), which takes up floor space. A Moyno pump may enable fiber and stillage to be mixed at the inlet of the pump, thereby removing the need for a tank. (2) The next test will increase fiber, as a percent of the stillage, to 16% solids. In this case, the centrifugal pump may still be workable. However, a higher solids loading would require a Moyno pump. (3) The stillage enters the system at close to boiling. The pressurized operation of the pump may help to minimize flashing at the inlet of the pump.

The spiral heat exchanger (from Alfa-Laval) also performed according to expectations. (Specifications are given in prior monthly reports). The unsteady state nature of the first set of tests resulted in less than optimal operating conditions with exposure of heat exchange surfaces to vapors or flashing during start-up and shut-downs of the system. At the end of the first test, the heat exchanger was disassembled and examined. "Scale" consisting of protein had formed in the heat transfer surfaces, and had to be cleaned off. An extended test will give some indication of the severity of this problem at steady-state conditions. Based on this observation, however, the design for a pilot-scale (production) unit will likely be based on three heat exchangers. Two

would be sequenced with steam injection replaced with the second heat exchanger. The third heat exchanger would be cycled in and out of service, so the other heat exchanger can be cleaned-in-place while the process is in operation. This requires that all three heat exchangers be equivalent in size. These costs are factored into the economic analyses presented below.

Fermentability

The liquid from the pretreated fiber, as well as combined fiber/liquid stream, were fermented using recombinant yeast (426A (LNH-87)) at 37°C in order to probe for presence of fermentation inhibitors. The fermentations were carried out in LORRE by Dr. Ho's group and by Bruce Dien of the USDA NCAUR laboratory. In addition to yeast, Dr. Bruce Dien also tested fermentability with respect to *E. coli*. However, the presence of lactic acid from the stillage, which is carried over from the steeping process, has a known inhibitory effect, hence the results were not meaningful and therefore not shown. Xylose concentrations in the pretreatment liquid were at the minimum levels required for fermentation. Spiking of the pretreatment liquid with xylose and fermentation with yeast (426A (LNH-87)) showed xylose was fermented as well as glucose. The results for 426A are shown in Figure 6.

Enzyme Formulation and Usage

The optimal pretreatment conditions for this specific pretreatment system correspond to ones where hydrolysis is minimal. This goal and rationale differentiate this type of pretreatment from others, where hydrolysis occurs by choice, or by the conditions selected for pretreatment (acid, base, steam explosion, etc.) In the case of corn fiber, glucans (hexosans) and xylans (pentosans) are dissolved while resulting in minimal formation of monosaccharides (glucose or xylose). Consequently, fermentation requires addition of enzyme to hydrolyze oligosaccharides in the liquid and/or pretreated solids that are more susceptible to hydrolysis, but are not dissolved.

Three cases were tested by Dr. Bruce Dien: cellulase alone, glucoamylase alone and a mixture of glucoamylase and cellulase. These three cases were examined with respect to yeast fermentation of the pretreated (but not centrifuged) fiber stream (stream E in Figure 1) for purposes of assessing fermentability. This should be viewed as an assay procedure rather than an optimized fermentation. All three cases gave fermentability with 90% of the maximal yield being achieved in 48 hours or less at 32°C when the mixtures were inoculated with yeast at 10^7 cells/mL of a hexose only fermenting organism. This inoculum volume gave an effective viable yeast cell count of 10^5 cells/mL at the beginning of the fermentation.

These results (Table 3) show that significant amounts of starch/maltodextrans are present in the liquid fraction requiring the presence of glucoamylase for hydrolysis. While commercially available cellulase mixtures show some activity toward maltodextrans, both cellulases and glucoamylase are required to completely covert all glucans (dissolved maltodextrans and undissolved cellulose) to fermentable sugars in the cases where the pretreated, but undissolved, solids are not separated from the liquid (compare samples 5 and 10).

The effect of pretreatment on solids reduction and glucan hydrolysis by commercial cellulase is illustrated in Figures 4 and 5. Nearly 50% of the undissolved solids are dissolved during pretreatment. Addition of a cellulase mixture increased the solubilization to 70% in 2 hours and nearly 80% within 24 hours. Curves (a) and (b) in Figure 4.

While untreated corn fiber is hydrolyzed as indicated by the solids reduction in Figure 4 hydrolysis occurs much more rapidly after pretreatment. The yields in Figure 5 are low due to glucose inhibition of the enzymes in the undiluted corn fiber – stillage mixture (corn fiber at 16% loading) which limited glucan hydrolysis to 60%. Glucose concentrations in these hydrolysis experiments plateaued at nearly 5% (50 mg/mL).

The effect of pretreatment on solubilization is not completely accounted for by the glucose formed (i.e., cellulose digestibility). The dissolved corn fiber in the pretreated samples that is not dissolved starch is largely hemicellulose. The cellulase mixture has little xylanase activity (less than 20% hydrolysis in the un-pretreated corn fiber digestion, 26% in the pretreated corn fiber digestion). Since the pretreatment reduces the hemicellulose degree of polymerization, soluble xylans are released into solution as the cellulose is hydrolyzed by the enzymes in the pretreated corn fiber. While little free xylose was detected in the liquid of the enzyme digested pretreated solids, 4% sulfuric acid hydrolysis (NREL LAP-014) of this liquid showed that the large oligosaccharide peak observed in the liquid chromatogram was xylan. The 4% acid catalyzed the hydrolysis of the xylan to xylose. This result was used to estimate the amount of xylan that became soluble after cellulase enzyme was used to hydrolyze pretreated corn fiber for 72 hours. About 70% of the xylan became soluble after the enzyme was contacted with the solid material.

Although solubilization of hemicellulose was not a goal of this particular project, the dissolution of hemicellulose allows rapid saccharification of the insoluble cellulose fraction by cellulase enzymes following pretreatment. Additionally, the greater reduction in solids improves the glucose recovery in the liquid stream from the centrifuge. This effect is illustrated in greater detail below.

Table 3. Effect of enzyme loading on simultaneous saccharification and fermentation.

Sample No.	Substrate	Cellulase IU / g dw	Gluco-amylase IU/g dw	Maximum Ethanol g/l
1	whole	0	0	2.9
2	whole	0.6	0	5.0
3	whole	1.1	0	5.4
4	whole	2.8	0	6.5
5	whole	5.5	0	7.5
6	whole	0	0.3	8.4
7	whole	0	0.7	8.8
8	whole	0	1.4	9.3
9	whole	0	3.3	9.8
10	whole	1.1	0.7	11.8
11	liquid	0	0	2.7
12	liquid	0.6	0	4.6
13	liquid	1.1	0	4.9
14	liquid	2.8	0	5.5
15	liquid	5.5	0	6.0
16	liquid	0	0.3	8.8
17	liquid	0	0.7	9.0
18	liquid	0	1.4	9.6
19	liquid	0	3.3	10.1
20	liquid	1.1	0.7	11.2

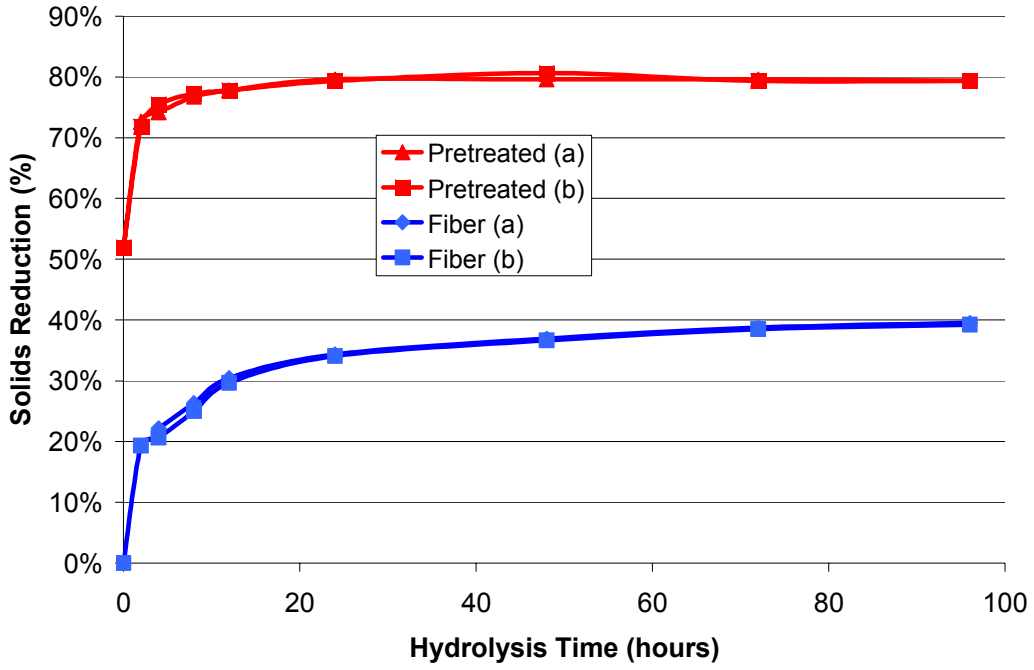

Figure 4. Solubilization of corn fiber and laboratory pretreated corn fiber by cellulase enzymes (Novozyme 188 and Celluclast 1.5L in 1:1 ratio, 10 FPU/g dry corn fiber) at 50°C.

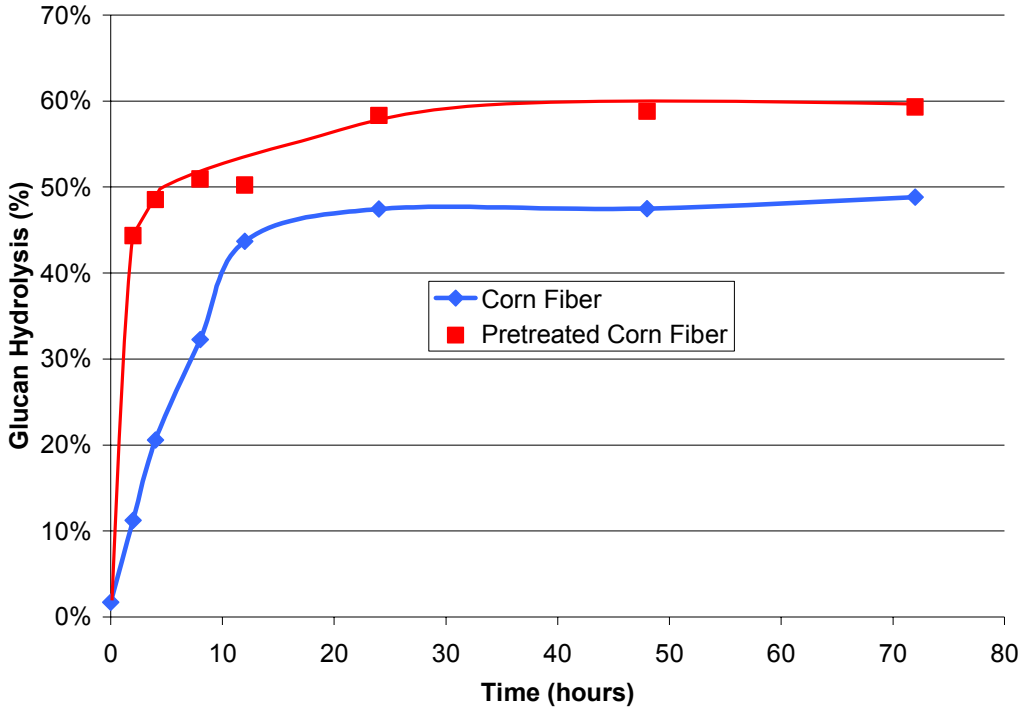

Figure 5. Hydrolysis of glucans to glucose of corn fiber and laboratory pretreated corn fiber by cellulase enzyme mixture (Novozyme 188 and Celluclast 1.5L in 1:1 ratio, 10 FPU/g dry corn fiber) at 50°C.

Basis for Calculation of Costs

The basis for full-scale economical evaluation is based around a single pretreatment "line" that could be integrated into the process at Williams BioEnergy. One line processes pretreatment slurry at 200 gpm (equivalent to 10,000 lbm/hour of corn fiber, dry basis). Equipment costs will be subject to straight-line depreciation over a 10-year timeframe. The calculation of capital cost of the equipment for the pretreatment process is based on information provided by Williams as obtained from equipment suppliers. The equipment costs for a 200 gpm capacity are summarized in Table 4.

Table 4. Equipment costs for proposed 200 gpm (10,000 lb/hr) pretreatment line (as of Nov., 2002).

Item	Purchase Price	Installation Factor	Installed Cost
Moyno Pump	68,330	1.5	102,500
Centrifuge	340,000	1.5	510,000
Pair of Spiral Heat Exchangers	200,000	1.5	300,000
Coil	----	----	300,000
Instrumentation (Automated Valves, Temperature, Pressure)	----	----	100,000
Conveyers (2)	100,000	1.5	150,000
		TOTAL	1,462,500

The cost for equipment to be used in the next trial is based on the same nominal capacity of 60 gpm as was specified for the trial that was completed in October, 2002 (Table 5). The first heat exchanger (spiral type) and centrifuge (Centrisys Co.) were rented for purposes of this test, and installed in the Williams plant. Hence, purchase prices for these items are not given here.

Table 5. Equipment costs for proposed 60 gpm (72,000 lb/day = 3000 lb/hr) test run.

Equipment Item	Cost	Installation Factor	Installed Cost
Moyno Pump[1]	40,000	1.5	60,000
Spiral Heat Exchanger[2]	50,000	1.5	75,000
Coil	----	----	90,000
Conveyer	(this was redirected from an existing fiber line)		
Back Pressure Valve	----	----	15,000
Flow Meter	----	----	4,000
Temperature, Pressure Monitors	----	----	4,000
		TOTAL	248,000

[1]This type of pump is needed to replace centrifugal pump to achieve higher solids loading
[2]Current system (Sept.-Oct., 2002 test) had one heat exchanger with steam injection in place of second heat exchanger, since a second spiral heat exchanger was not available for rental.

Operational costs consist of fiber, stillage, steam, electricity, labor and maintenance. Fiber and stillage costs are expressed on a single line as dissolved and un-dissolved solids (solids denote a dry basis). Unit costs are expressed in terms of ¢/lbm of corn fiber and ¢/gal of ethanol produced from the glucose and soluble glucans present in the liquid stream from the centrifuge. Credits are calculated on the same basis, where solids are defined as any component that is not fermented. The unit costs are as provided by Williams. The electrical, maintenance, and labor costs were provided by Williams as a lumped sum of $0.191/gal ethanol produced.

Three cases are presented.

Case 1(a): Test case as run in the Williams plant. Specifications include: 7.8% fiber loading; 74% moisture for cake leaving in stream 4; no enzyme hydrolysis or cellulase enzyme added; 1 heat exchanger; and steam injection. Results are shown in Table 6.

Case 1(b): Same as case 1(a), except that a mixture of cellulase is added to stream ***E***, and hydrolysis reduces the volume of the cake (un-dissolved solids) by about 45%, and the weight of solids also by about 45%. This results in significantly higher recovery of liquid containing fermentable sugars, or oligosaccharides that are hydrolyzed to fermentable sugars.

Case 2(a): This represents the proposed test where fiber loading is 16%, and no cellulase is used. However, we assume the centrifuge is tuned to give 60% moisture in the remaining cake, although a moisture content as low as 50% may be possible according to engineers from the centrifuge manufacturer.

Case 2(b): This is the same as case 2(a) except enzyme hydrolysis reduces un-dissolved solids by 45%.

Case 3(a) is the same as 2(a) except the fiber loading is now at 28%.

Case 3(b) is the same as 3(a) except that cellulase enzyme is used and reduces un-dissolved solids by 45%.

The amount of dissolved and un-dissolved solids consumed to form fermentation ethanol is calculated by difference based on the total dissolved solids removed with the liquid stream (5). All other remaining solids are pro-rated at a value (cost) equivalent to $65/ton. Consequently, solids remaining after fermentation are being credited against the cost of dissolved (in stillage) and un-dissolved (fiber) solids entering the process. Hence the solids are the combination of material in stream (4) and solid in steam (5) that remains after fermentation. While the test shows a significantly enhanced protein content in the solids after pretreatment and fermentation, additional credit is <u>not</u> given due to higher protein content, for purposes of the cost and pro-forma calculations.

The current animal feed market is weak and not expected to improve as the fuel ethanol industry continues to expand. Additionally, less solids at the end of the process translate into less energy demand for drying. As long as the additional revenue of additional ethanol production surpasses the lost revenue of animal feed, the economics of the pretreatment process looks favorable.

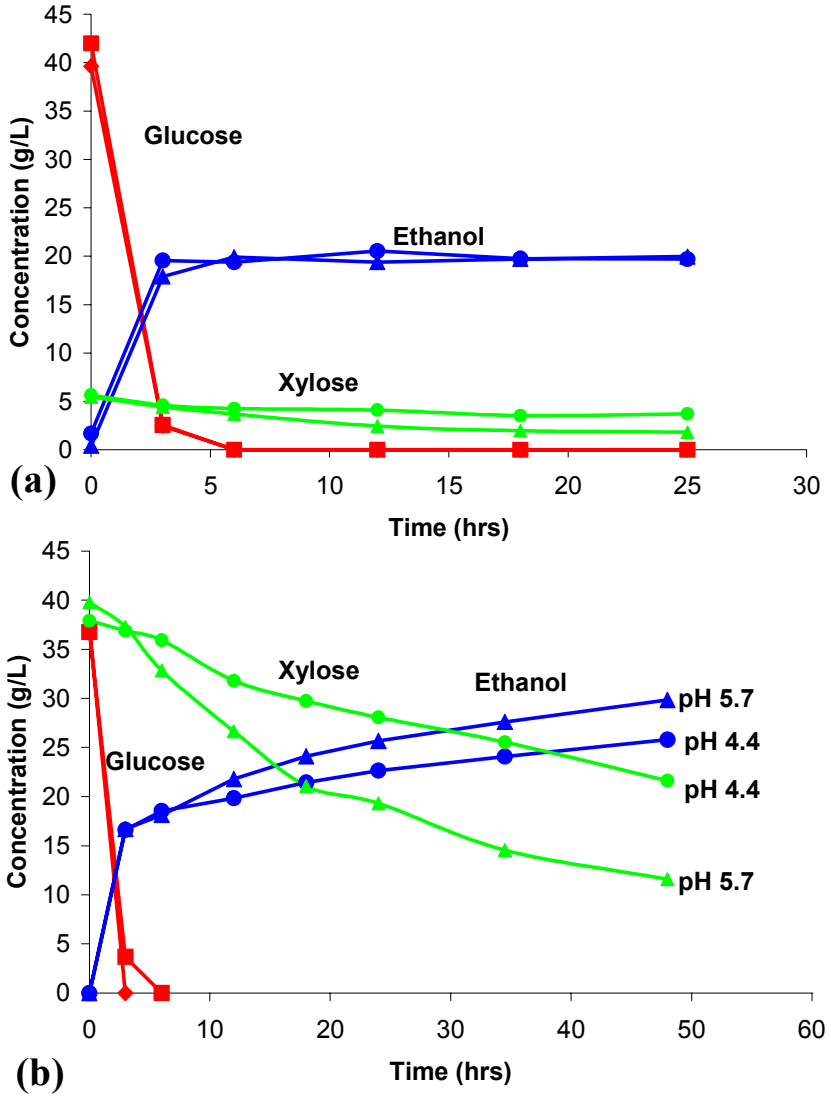

Figure 6. Fermentation of cellulase treated centrifuge cake (9% dry mass in liquid) and pretreatment liquid. Centrifuge cake in pretreatment liquid (9% dry solids loading) corresponding to fiber loading (15% dry solids with 60% solubilization by pretreatment) was treated with an equal mixture of Celluclast 1.5 L and Novozyme 188 (50 FPU/mL) at a loading of 10 FPU/g dry solids at 50°C for 96 hours. After saccharification, liquid was fermented with xylose-fermenting recombinant yeast (426A (LNH-ST)) at 37°C. Samples were collected every three hours and analyzed by HPLC. (a) liquid after saccharification. (b) 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.

Cost Analyses for Pretreatment Trial at Williams BioEnergy

Effect of Fiber Loading on Soluble Glucan Recovery

The corn fiber pretreatment process, reproduced in Figure 7 for the reader's convenience, separates liquid containing solubilized starch, i.e. glucan, from the undissolved solids by centrifugation. The fermentation system at a wet-milling facility, such as Williams Bioenergy, is unable to handle undissolved solids, therefore making such a separation step necessary before the fermentable sugars can be fermented. Because centrifugation is not 100% efficient, i.e. some portion of the liquid will be entrapped in the solids cake (stream 4), the effect of fiber loading and centrifuge efficiency was explored through simulation. In the trial run, no significant solids were found in the liquid fraction from the centrifuge. While tuning the centrifuge to produce a drier cake may result in some solids exiting with the centrate (liquid), this was not included in the simulation. The simulation was constructed using Microsoft Excel. The following assumptions were made.

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 valid by using glycerol as an internal standard for liquid from the centrifuge and liquid washed from the solids cake (Figure 1).

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 conducted at LORRE (Table 1 and Figure 3).

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 conducted at LORRE (Figures 4 and 5).

4. All soluble glucans in the liquid stream are enzymatically hydrolyzed in post processing of the liquid (stream 5). This includes cases where amylases and/or cellulases are used.

5. Glucose from soluble glucan hydrolysis is fermented to ethanol at 95% of theoretical yield. No other monosaccharides were assumed to be fermented, for purposes of these pro-forma cost calculations.

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 upon the system as operated in the pilot test – 1 heat exchanger for heat recovery and direct injection of steam. The second simulation was for the proposed pilot test system with 2 heat exchangers. The second heat exchanger used 150 pound steam for makeup or trim heat.

The effectiveness of this heat exchanger was assumed to be 85%, i.e.85% of the total ΔH transferred from the steam to the pretreatment stream. The third simulation assumes that the pretreatment stream is treated with cellulase that solubilizes an additional 45% of the remaining undissolved solids before centrifugation (Figure 4). This assumption is based upon laboratory data described above where a mixture of pretreated solids and liquid were treated with cellulase (10 FPU per gram of dry undissolved solids) at 50°C. The additional 45% solubilization (72% total solubilization) occurs at 2 hours of enzyme treatment.

Results from this simulation for optimizing the pretreatment process are based on experimental measurements and different assumed solids loadings. The resulting (a) concentration of soluble glucan (starch) in solution (b) soluble glucan recovery yield in liquid from centrifuge (stream 5) (c) steam demand and (d) ethanol yield in gallons per lbm of fiber (dry mass) processed are summarized below (Table 6). Steam demand and ethanol yield were used for the economic analysis described in the proceeding section.

Figure 8 illustrates the direct relationship between fiber loading and soluble glucan concentration in solution. Note that the soluble glucan concentration corresponding to the trial run (1 heat exchanger + steam injection at 7.8%) matches the analytical results (glucan is 26.39 mg/ml = 2.6% in stream 5, Figure1). Steam injection for makeup heat slightly dilutes the glucan concentration. Hydrolyzing the pretreated material with cellulase greatly increases the glucan concentration (2 heat exchangers + enzyme case in Figure 8). The non-obvious results of increased fiber loading are illustrated in Figure 9 which plots glucan recovery as a function of fiber loading for several different scenarios. Higher fiber loading translates into a larger percentage of the exit stream as solids (stream 4) with higher percentage of the total liquid entrapped in that solids cake.

Three different centrifuge efficiencies are shown in Figure 9. The bottom curve represents the case where the centrifuge produces a solids cake with 74% moisture content. This represents the actual centrifuge cake moisture content and centrifuge efficiency from the pilot trial of September/October 2002. Representatives from the centrifuge manufacture were confident that with equipment tuning and higher fiber loading, the centrifuge could produce a cake with moisture content near 60%, representing the best case in Figure 9(a) and (b). The cellulase treated solids from the laboratory have changed from flakes to a fine particulate slurry that resembles sludge due to its high protein content. Because of the change in physical properties, a best case of 50% moisture content of the cake from cellulase treated material was examined.

The results from these simulations are summarized to show that 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 that is offset by increased capital (holding tanks for saccharification) and operational (enzyme) costs.

Economic Analysis

Table 6 summarizes the key results from the simulation for each of the above cases. The simulation used one unit dry mass of corn fiber as the basis or analysis. "Total solids" represents

the one unit mass of corn fiber plus the dissolved and undissolved solids from the stillage per one unit dry mass of corn fiber into the pretreatment system.

The operational costs for the pretreatment process are shown in Table 7. Operational costs are shown in ¢ per lbm of fiber processed and ¢ per gallon of ethanol produced. The cost of total solids was $65 per dry ton (3.25¢ per lbm), as provided by Williams Bioenergy. Results from the materials obtained from the pilot trial showed that the protein content, and thus potential selling value, was higher after pretreatment. However, the total unfermented solids were credited back after the processing for the same price as the untreated solids at $65 per dry ton. Each case was evaluated at a high steam energy costs at $5 per million BTU of enthalpy and a low steam energy cost at $2.5 per million BTU of enthalpy.

No downstream processing costs (distillation, ethanol drying, etc.) are included. Sufficient capacity exists to accommodate the incremental increase in ethanol production (i.e. no additional capital expenditures needed) and the incremental increased operational costs of ethanol processing are assumed to be offset by operational savings from the reduced volume of solids that require processing into feed.

The operational cost per gallon of ethanol was determined by multiplication of the cost per lbm corn fiber by the ethanol yield per lbm corn fiber as shown in Table 6. A lump sum of 19.1¢ per gallon of ethanol produced was used to account for electricity, labor, and maintenance. The sum of operational costs per gallon of ethanol produces "cash cost of ethanol" for the high and the low energy costs for each case listed above. 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 capital costs for a 200 gpm pretreatment unit are listed in Table 8. Both the capital and installed cost are shown. Numbers are based upon quotes from equipment manufacturers obtained by Williams Bioenergy.

From the numbers in Tables 6, 7, and 8, discounted cash flows over 10 years were generated for each case at both the high and low steam energy costs ($5 and $2.5 per million BTU). Table 9 is an example of discounted cash flows for cases 2(a) and (b). The annual cash income of ethanol sold was determined by subtracting the cash cost of ethanol produced from an assumed selling price of $1.00 per gallon. A straight line depreciation curve for the capital costs over the 10-year period was used. The capital equipment was assumed to have no salvage value at the end of the 10-year period. A tax rate of 38% was used to determine after-tax income from incremental ethanol produced by the pretreatment process. An iterative process was used to find the discount rate that generates a net present value of zero. This discount rate represents the return on capital investment (ROI) over the 10 year period.

For the (b) cases where cellulase is used to hydrolyze the pretreated solids to reduce solids and increase fermentable sugars, initial capital expense was increased by 50% to $2.19MM to cover addition capital equipment (tanks, etc.) required for the residence time of saccharification. An additional operational cost of cellulase enzyme at 10¢ per gallon of ethanol produced was included in the cash cost of ethanol (Table 7).

A summary of these returns on capital investment (ROR) are shown in Table 10. As predicted, increased fiber loading improves the process economics only to a point. The lesser influence of energy cost on the ethanol cash cost for the higher loadings (Table 7) made differences in ROR between the high and low energy cost for cases 2 and 3 much less significant than case 1. Additionally, Case 2(a), 16% fiber loading, generates a higher return than case 3(a), 23% fiber loading. For all cases, the hydrolysis of the pretreated solids using cellulase before centrifugation offers significantly higher returns on investment. This is true even for the conservative estimate on additional capital requirements and the added cost of enzyme.

The rates of return increase dramatically between the (a) and (b) cases given in Table 10. This arises from the higher glucose yields obtained in the (b) cases due to the use of both amylase and cellulase to hydrolyze pretreated corn fiber. Comparison of the case studies in Table 6 show higher yields for the (b) cases, which reflects not only the added impact of enzyme hydrolysis of the cellulose fraction, but also enhanced solids/liquid separation since the enzyme hydrolysis reduces the mass of solid left, and therefore reduces water retained by the centrifuge cake.

Sensitivity of rate of return to ethanol selling price is shown by comparing the various cases where the selling price of ethanol is $0.90 per gallon (Table 11) and $1.10 per gallon (Table 12) to the base case of $1.00 per gallon (Table 10). Values 0.0% in Table 11 represent cases that do not break even after the 10 year analysis. Ethanol selling price has a very strong influence on overall rate of return. A change in selling price of 10% translates into a change in rate of return of nearly 10% (compare case 2(b) 29.3% at $1.00/gal, 18.6% at $0.90/gal, and 39.2% at $1.10/gal).

The rates of return for the (b) cases where cellulase are used are not very sensitive to large changes in enzyme cost (Tables 13 and 14). The effect of halving or doubling enzyme cost only affects the rate of return at the tenths of a percent (compare case 3(b) 27.3% at 10¢/gal versus 27.2% at 20¢/gal). This is unsurprising since enzyme cost is only between 6-8% of the total cash cost of ethanol for the (b) cases (Table 7).

Further work will be required 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. For the conservative estimates of these factors, the promising economics warrant this further study.

Table 6. Results from Simulation for Economic Case Studies. "(a)" Cases are for glucan derived from starch, while "(b)" cases are for glucan derived from starch and cellulose. Enzyme used in "(a)" cases is amylase only; while in "(b)" cases is amylase and cellulase combined.

Case Study Summaries

Case 1(a) - as complete
Steam Injection - 74% MC cake

7.8% fiber loading	w/w to dry fiber	
Total Solids	2.348	
Glucan Used	0.236	
Soluble Glucan	2.04%	w/w % in solution
Glucan Yield	87.12%	
Ethanol (95% yield)	0.1144	w/w
	0.1440	L/kg fiber
	0.0173	Gal per lbm fiber

Case 1(b) - as complete
Steam Injection - 74% MC cake after cellulase

7.8% fiber loading	w/w to dry fiber	
Total Solids	2.204	
Glucan Used	0.503	
Soluble Glucan	4.87%	w/w % in solution
Glucan Yield	85.80%	
Ethanol (95% yield)	0.2437	w/w
	0.3069	L/kg fiber
	0.0368	Gal per lbm fiber

Case 2(a)
2 Heat Exchangers - 60% MC cake

16% fiber loading	w/w to dry fiber	
Total Solids	1.683	
Glucan Used	0.181	
Soluble Glucan	4.16%	w/w % in solution
Glucan Yield	82.86%	
Ethanol (95% yield)	0.0878	w/w
	0.1105	L/kg fiber
	0.0132	Gal per lbm fiber

Case 2(b)
2 Heat Exchangers - 60% MC cake after cellulase

16% fiber loading	w/w to dry fiber	
Total Solids	1.683	
Glucan Used	0.504	
Soluble Glucan	10.34%	w/w % in solution
Glucan Yield	92.86%	
Ethanol (95% yield)	0.2441	w/w
	0.3075	L/kg fiber
	0.0368	Gal per lbm fiber

Case 3(a)

2 Heat Exchanges - 60% MC cake

28% fiber loading	w/w to dry fiber	
Total Solids	1.334	
Glucan Used	0.128	
Soluble Glucan	7.68%	w/w % in solution
Glucan Yield	65.00%	
Ethanol (95% yield)	0.0622	w/w
	0.0783	L/kg fiber
	0.0094	Gal per lbm fiber

Case 3(b)

2 Heat Exchanges - 60% MC cake after cellulase

28% fiber loading	w/w to dry fiber	
Total Solids	1.334	
Glucan Used	0.445	
Soluble Glucan	20.28%	w/w % in solution
Glucan Yield	85.42%	
Ethanol (95% yield)	0.2158	w/w
	0.2718	L/kg fiber
	0.0326	Gal per lbm fiber

	Case 1(a)	Case 1(b)	Case 2(a)	Case 2(b)	Case 3(a)	Case 3(b)
Water in Fiber (kg/kg fiber)	1.50	1.50	1.50	1.50	1.50	1.50
Stillage Mass (kg/kg fiber)	10.37	10.37	3.75	3.75	1.07	1.07
Total Mass (kg/kg fiber)	12.87	12.87	6.25	6.25	3.57	3.57
Enthalpy Demand BTU/lbm fiber	754.06	754.06	366.13	366.13	209.21	209.21
Steam Demand lbm/lbm fiber	0.83450	0.98177	0.40518	0.47669	0.23153	0.27239

Table 7. Operational Expenses for Corn Fiber Pretreatment (200 gpm scale)

				Case 1(a)		Case 1(b)		Case 2(a)		Case 2(b)		Case 3(a)		Case 3(b)	
Operational Expenses		**Capital Cost**	**Installed Cost**	**¢/lbm fiber (dry)**	**¢/gal ethanol**	**¢/lbm fiber (dry)**	**¢/gal ethanol**	**¢/lbm fiber (dry)**	**¢/gal ethanol**	**¢/lbm fiber (dry)**	**¢/gal ethanol**	**¢/lbm fiber (dry)**	**¢/gal ethanol**	**¢/lbm fiber (dry)**	**¢/gal ethanol**
	Total Solids			7.63	442.18	7.16	194.81	5.47	412.84	5.47	148.42	4.34	462.07	4.34	133.15
	Steam Heat @ $\$5/10^6$ BTU			0.34	19.97	0.34	9.37	0.17	12.63	0.20	5.34	0.10	10.19	0.11	3.45
	@ $\$2.5/10^6$ BTU			0.17	9.98	0.17	4.69	0.08	6.32	0.10	2.67	0.05	5.09	0.06	1.73
	Cellulase				0.00		0.10		0.00		0.10		0.00		0.10
	Electric., Maint., & Labor			N/A	19.10	N/A	19.10	N/A	19.10	N/A	19.10	N/A	19.10	N/A	19.10
Credits	Pretreated Solids			(6.86)	(397.73)	(5.53)	(150.36)	(4.88)	(368.39)	(3.83)	(103.97)	(3.92)	(417.62)	(2.89)	(88.70)
Cash Cost of Ethanol	@ $\$5/10^6$ BTU				$0.835		$0.730		$0.762		$0.690		$0.737		$0.671
	@ $\$2.5/10^6$ BTU				$0.735		$0.683		$0.699		$0.663		$0.686		$0.654

Table 8. Capital Expenses for Corn Fiber Pretreatment (200 gpm scale)

	Capital Cost	**Installed Cost**
Heat Exchanger	$200,000	$300,000
Coil	$200,000	$300,000
Centrifuge	$340,000	$510,000
Pump	$68,330	$102,495
Conveyers (2)	$100,000	$150,000
Instrumention and Controls	$66,667	$100,000
Total	$974,997	$1,462,495

Table 9. Cash Flows for Case 2(a) & (b) – 16% fiber loading, 2 heat exchangers, ethanol selling price is $1.00 per gallon.

Case 2(a) $5/MM BTU

Before Tax					After Tax				
Year	Annual Ethanol Cash Income	Annual Depreciation (straight line)	Taxable Income	Tax (38%)	Annual Capital Expenditure	Net Annual Cash Flow	Discount Factor (f_d)	Annual Discounted Cash Flow	Net Present Value
0	$ -	$ -	$ -	$ -	$ 1,462,495	$ (1,462,495)	1.0000	$ (1,462,495)	$ (1,462,495)
1	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.9202	$ 206,650	$ (1,255,845)
2	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.8468	$ 190,163	$ (1,065,682)
3	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.7792	$ 174,991	$ (890,691)
4	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.7171	$ 161,030	$ (729,661)
5	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.6599	$ 148,182	$ (581,478)
6	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.6072	$ 136,360	$ (445,118)
7	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.5588	$ 125,481	$ (319,637)
8	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.5142	$ 115,470	$ (204,168)
9	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.4732	$ 106,257	$ (97,911)
10	$ 272,567	$ 146,250	$ 126,318	$ 48,001	$ -	$ 224,567	0.4354	$ 97,780	$ (131)
						Discount Rate	8.67%		

Case 2(b) $5/MM BTU

Before Tax					After Tax				
Year	Annual Ethanol Cash Income	Annual Depreciation (straight line)	Taxable Income	Tax (38%)	Annual Capital Expenditure	Net Annual Cash Flow	Discount Factor (f_d)	Annual Discounted Cash Flow	Net Present Value
0	$ -	$ -	$ -	$ -	$ 2,193,743	$ (2,193,743)	1.0000	$ (2,193,743)	$ (2,193,743)
1	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.7736	$ 537,905	$ (1,655,837)
2	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.5985	$ 416,142	$ (1,239,695)
3	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.4630	$ 321,942	$ (917,753)
4	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.3582	$ 249,065	$ (668,688)
5	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.2771	$ 192,686	$ (476,002)
6	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.2144	$ 149,068	$ (326,934)
7	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.1659	$ 115,324	$ (211,610)
8	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.1283	$ 89,219	$ (122,391)
9	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.0993	$ 69,023	$ (53,368)
10	$ 986,991	$ 219,374	$ 767,616	$ 291,694	$ -	$ 695,296	0.0768	$ 53,398	$ 31
					Discount Rate (NPV=0)		29.26%		

Table 10. Return on Capital Investment (ROR) for All Cases, ethanol selling price is $1.00 per gallon, capital + 50% and enzyme costs $0.10 per gallon of ethanol produced for (b) cases. Refer to Table 6 for glucan balances and ethanol yield for (a) (amylase only) and (b) (amylase and cellulase combined) for the two cases.

Case	Capital Expense	$5/MM BTU	$2.5/MM BTU
1(a)	$ 1,462,495	7.0%	15.8%
1(b)	$ 2,193,743	25.0%	29.9%
2(a)	$ 1,462,495	8.7%	13.0%
2(b)	$ 2,193,743	29.3%	32.0%
3(a)	$ 1,462,495	4.8%	7.5%
3(b)	$ 2,193,743	27.3%	28.9%

Table 11. Return on Capital Investment (ROR) for All Cases, ethanol selling price is $0.90 per gallon, capital + 50% and enzyme costs $0.10 per gallon of ethanol produced for (b) cases.

Case	Capital Expense	$5/MM BTU	$2.5/MM BTU
1(a)	$ 1,462,495	0.0%	7.0%
1(b)	$ 2,193,743	13.8%	19.3%
2(a)	$ 1,462,495	0.9%	6.0%
2(b)	$ 2,193,743	18.6%	21.5%
3(a)	$ 1,462,495	0.0%	2.0%
3(b)	$ 2,193,743	17.7%	19.4%

Table 12. Return on Capital Investment (ROR) for All Cases, ethanol selling price is $1.10 per gallon, capital + 50% and enzyme costs $0.10 per gallon of ethanol produced for (b) cases.

Case	Capital Expense	$5/MM BTU	$2.5/MM BTU
1(a)	$ 1,462,495	15.8%	23.6%
1(b)	$ 2,193,743	35.2%	39.8%
2(a)	$ 1,462,495	15.4%	19.3%
2(b)	$ 2,193,743	39.2%	41.8%
3(a)	$ 1,462,495	10.0%	12.4%
3(b)	$ 2,193,743	36.2%	37.7%

Table 13. Return on Capital Investment (ROR) for All Cases, ethanol selling price is $1.00 per gallon, capital + 50% enzyme costs $0.05 per gallon of ethanol produced for (b) cases.

Case	Capital Expense	$5/MM BTU	$2.5/MM BTU
1(a)	$ 1,462,495	7.0%	15.8%
1(b)	$ 2,193,743	25.1%	29.9%
2(a)	$ 1,462,495	8.7%	13.0%
2(b)	$ 2,193,743	29.3%	32.0%
3(a)	$ 1,462,495	4.8%	7.5%
3(b)	$ 2,193,743	27.3%	28.9%

Table 14. Return on Capital Investment (ROR) for All Cases, ethanol selling price is $1.00 per gallon, capital + 50% enzyme costs $0.20 per gallon of ethanol produced for (b) cases.

Case	Capital Expense	$5/MM BTU	$2.5/MM BTU
1(a)	$ 1,462,495	7.0%	15.8%
1(b)	$ 2,193,743	24.9%	29.8%
2(a)	$ 1,462,495	8.7%	13.0%
2(b)	$ 2,193,743	29.2%	31.9%
3(a)	$ 1,462,495	4.8%	7.5%
3(b)	$ 2,193,743	27.2%	28.8%

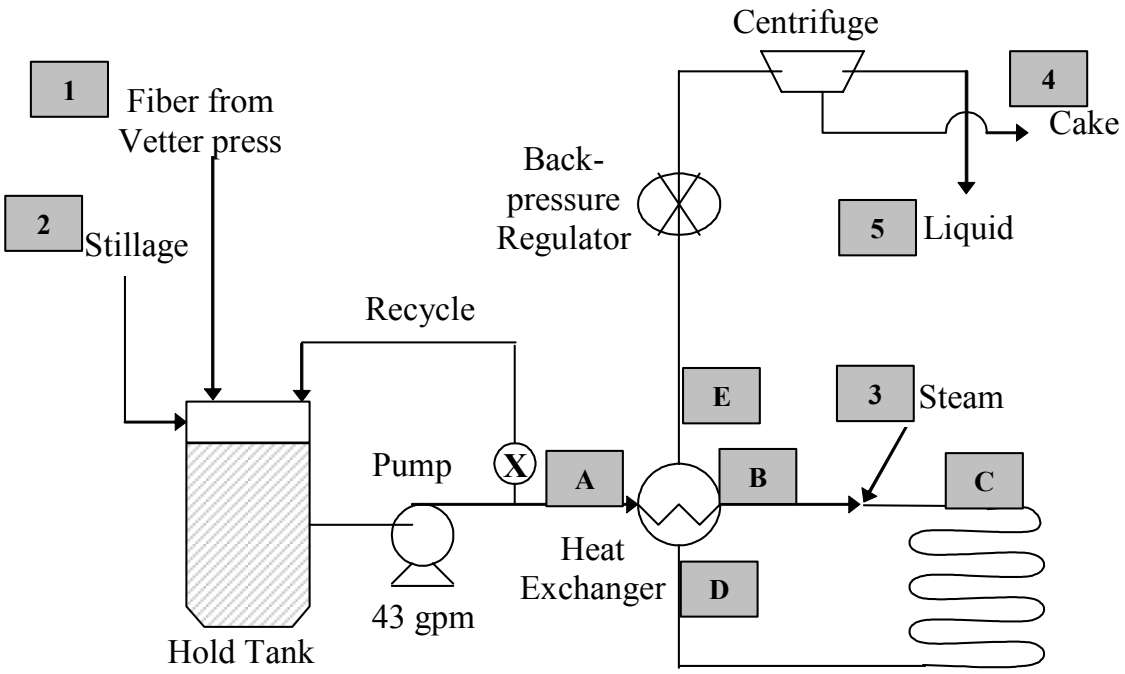

Figure 7. Process flow diagram.

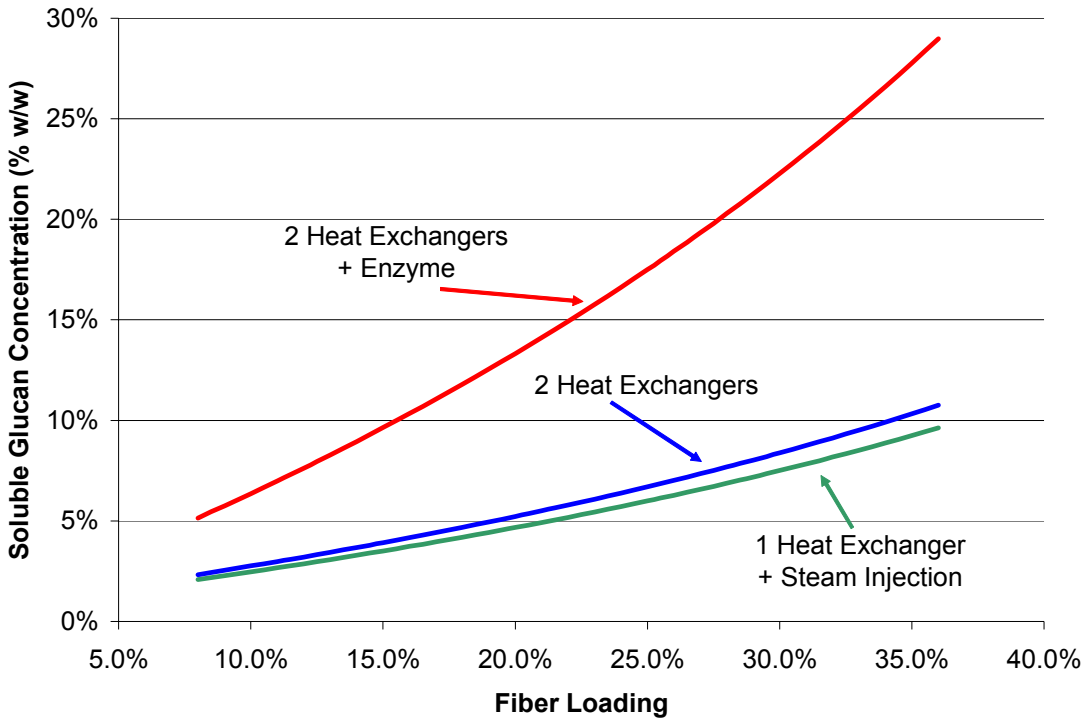

Figure 8. Concentration of soluble glucan (starch) in liquid.

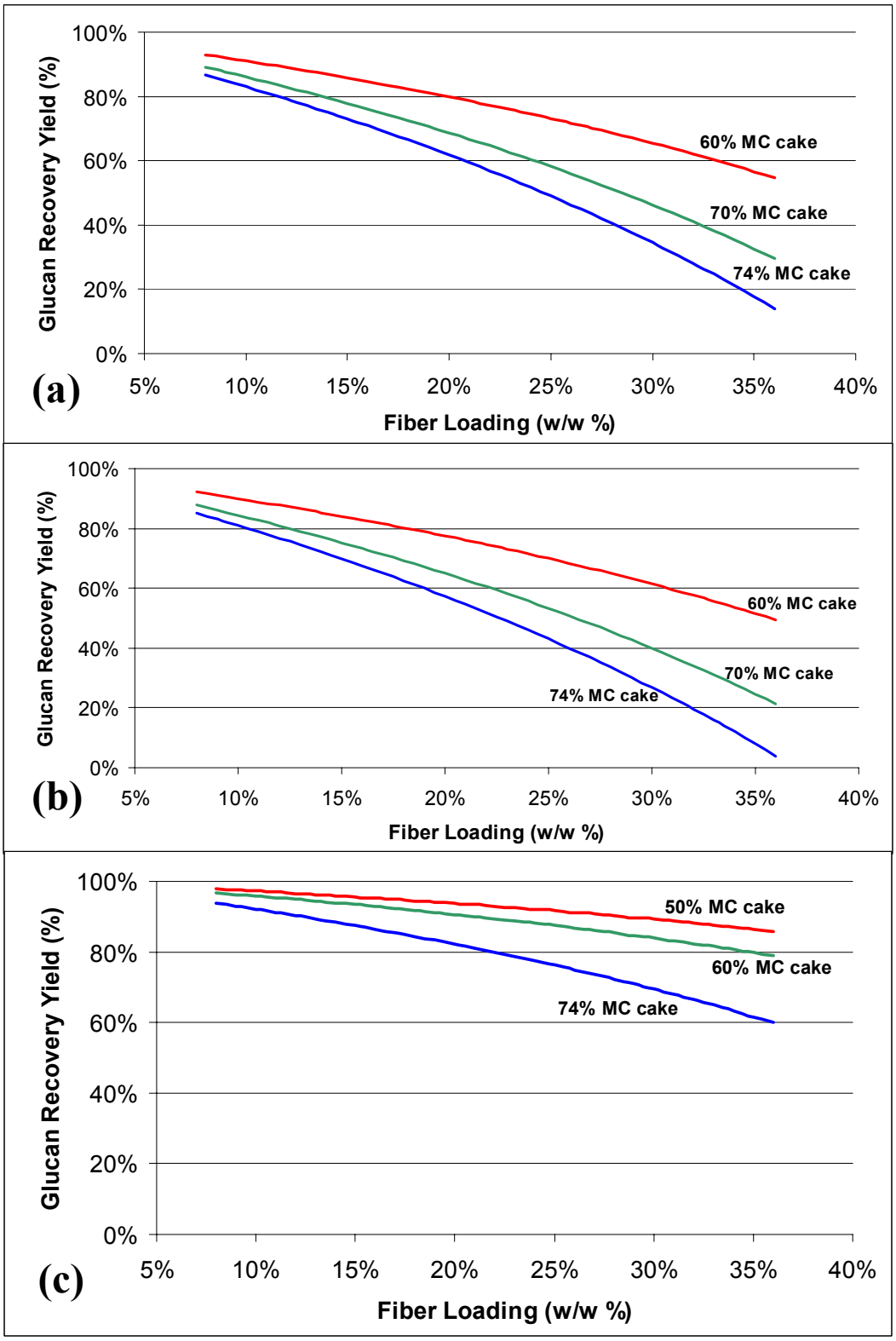

Figure 9. Recovery of soluble glucan from the centrifuge in the liquid (stream 5) for three different centrifuge efficiencies given (a) 1 heat exchanger + steam injection, (b) 2 heat exchangers, and (c) 2 heat exchangers + cellulase treatment.

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