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A bioethanol process development unit: initial operating experiences and results with a corn fiber feedstock

Daniel J. Schell [a,*], Cynthia J. Riley [a], Nancy Dowe [a], Jody Farmer [a], Kelly N. Ibsen [a], Mark F. Ruth [a], Susan T. Toon [a], Robert E. Lumpkin [b]

[a] National Renewable Energy Laboratory (NREL), National Bioenergy Center, 1617 Cole Boulevard, Golden, CO 80401, USA
[b] SWAN Biomass Co. 17W755 Butterfield Rd, Oak Brook Terrace, IL 60181, USA

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Abstract

Interest in bioethanol production from lignocellulosic feedstocks for use as an alternative fuel is increasing, but near-term commercialization will require a low cost feedstock. One such feedstock, corn fiber, was tested in the US Department of Energy (DOE)/National Renewable Energy Laboratory (NREL) bioethanol pilot plant for the purpose of testing integrated equipment operation and generating performance data. During initial runs in 1995, the plant was operated for two runs lasting 10 and 15 days each and utilized unit operations for feedstock handling, pretreatment by dilute sulfuric-acid hydrolysis, yeast inoculum production, and simultaneous saccharification and fermentation using a commercially available cellulase enzyme. Although significant operational problems were encountered, as would be expected with the startup of any new plant, operating experience was gained and preliminary data were generated on corn fiber pretreatment and subsequent fermentation of the pretreated material. Bacterial contamination was a significant problem during these fermentations.

Keywords: Corn fiber; Cellulose; Pilot plant; Pretreatment; Fermentation; Dilute-acid hydrolysis; Cellulase; Ethanol; Contamination

1. Introduction

Ethanol from lignocellulosic biomass is widely recognized as an environmentally friendly and acceptable substitute for gasoline or as an additive to gasoline (Lugar and Woolsey, 1999; Wyman, 1999). Along with this acceptance has come increased interest in commercializing technology for production of ethanol from potentially inexpensive cellulosic feedstocks. BC International (BCI) Corporation and Masada Resources Group, L.L.C. are currently planning construction of first-of-a-kind biomass-to-ethanol plants, while Iogen is now operating a 50 ton/week pilot plant.

Besides needing cost effective technology, two additional requirements necessary for commercialization are a low cost feedstock and process data at a sufficient scale to obtain engineering and process guarantees. While some of the companies mentioned above are pursuing opportunities with low cost feedstocks such as bagasse (BCI) and municipal solid waste (Masada), the US Department of Energy (DOE) and the National Renewable Energy Laboratory (NREL) have built a bioethanol process development unit (PDU) designed to produce the important process data (Nguyen et al., 1996).

A feedstock that has generated considerable interest as a possible substrate for ethanol production is corn fiber (Gulati et al., 1996; Dale et al., 1996; Grohmann and Bothast, 1997; Saha et al., 1998). This material, a by-product of the corn wet-milling industry, consists of corn hulls and residual starch not extracted by the milling process. Conversion of the starch along with the lignocellulosic components in the corn fiber would increase ethanol yields from a corn wet mill by 13% (Grohmann and Bothast, 1997) and is promising if the value of the corn fiber as an animal feed product is not severely affected.

In 1995 as part of a Cooperative Research and Development Agreement between Amoco Corporation (Naperville, IL) and DOE/NREL, the PDU was operated using a corn fiber feedstock. The purpose of this

* Corresponding author. Fax: +1-303-384-6227.
E-mail address: dan_schell@nrel.gov (D.J. Schell).

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work was to test equipment operation and generate performance data. This was an initial effort in a longer-term project to operate the PDU using corn fiber and was the first time the plant was operated in a continuous and integrated fashion employing unit operations for feedstock handling, biomass pretreatment, seed or inoculum production, and fermentation. Corn fiber was pretreated by high-temperature, dilute sulfuric-acid hydrolysis and the cellulose was converted to ethanol using the simultaneous saccharification and fermentation process. Cellulase was purchased from a commercial supplier and yeast was grown in the PDUs seed production equipment.

2. Pilot plant description

A process flow diagram for the PDU is shown in Fig. 1 and elaborates on information presented in a previous paper (Nguyen et al., 1996). The plant was designed to continuously process a lignocellulosic feedstock at a nominal feed rate of 900 dry kg/day.

Plant automation and control is provided by a data acquisition and control system (DACS) that is a relatively inexpensive and easily reconfigurable system designed to handle a reasonable number of input/output (I/O) signals (≈900 for this plant). The hardware components consist of an Opto 22 (Temecula, CA, USA) Mistic control system consisting of microprocessor-controlled I/O modules and Mistic Controllers that provides communications between I/O modules and a host computer. The host computer is a Sun (Palo Alto, CA, USA) SPARC 20 server in addition to three Sun SPARC X terminals that serve as operator control stations. Opto's Cyrano configuration program (later changed by the manufacturer to Opto Control) is a combination ladder logic and flow-chart package used to configure the Mistic Controllers, define I/O points, and configure control loops and logic for the I/O modules. Gensym's (Cambridge, MA, USA) G2 is a knowledge-based expert system used to configure operator screens for process observation and control as well as for data acquisition and storage.

2.1. Feedstock handling

If required, feedstock can be washed in a 6000-l conical tank with bottom agitation. Approximately 2 m^3 of feedstock is dumped into the water-filled, open-top tank. Agitation washes dirt and other contaminants from the feedstock and allows heavy materials (rocks and/or tramp metal) to sink to the bottom of the tank. Feedstock is discharged from the side of the tank into stainless steel, wire-mesh totes that allow water to drain.

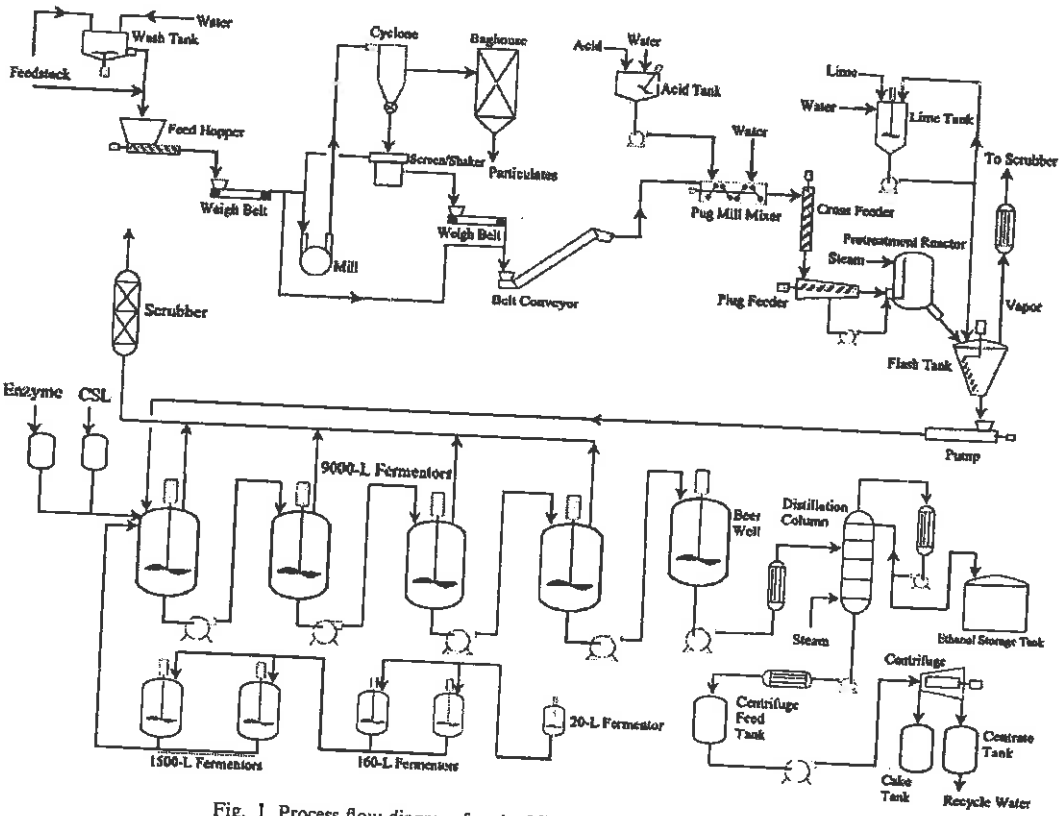

Fig. 1. Process flow diagram for the NREL/DOE bioethanol PDU.

Heavy materials are discharged from the bottom of the tank after removing the feedstock.

Continuous operation of the plant begins at the feed hopper. Washed or as-received feedstock (some materials are not always washed, e.g., wood chips) in totes is dumped into the feed hopper. Feedstock is continuously metered from the hopper by a screw onto a weigh belt. A weight measurement from the weigh belt provides feedback control of screw speed for control of weigh belt loading. The feedstock subsequently exits the weigh belt onto a belt conveyor that delivers it to the pretreatment section of the plant or alternatively, if required, the feedstock is delivered to a mill for size reduction.

An air-swept impact mill (#13 Imp Mill System, ABB Raymond, Lisle, IL, USA, see Schell and Harwood, 1994) is used to reduce feedstock size to approximately 0.002 m (shortest dimension). Feedstock exiting from the weigh belt is pneumatically conveyed through the mill and extracted from the air stream by a cyclone. Milled feedstock is discharged from the bottom of the cyclone through an airlock feeder onto a vibrating shaker. Dust-sized particles remaining in the air stream are removed in a baghouse. A course screen (≈0.01 m) in the shaker catches and returns larger material to the mill, while the rest of the feedstock exits the shaker onto another weigh belt. The second weigh belt measures feedstock mass flow rate after milling and feeds material onto the belt conveyor for delivery to the pretreatment section of the plant.

2.2. Pretreatment

The continuous pretreatment system consists of acid and lime (for acid neutralization) supply tanks; a biomass mixer; a high-temperature, high-pressure reactor system; and a flash tank. The pretreatment reactor system is a vertical pulp digestor supplied by Sunds Defibrator, Inc. (now Metso Paper USA, Inc. Norcross, GA, USA) and includes the reactor and material feed (plug feeder) and discharge (reciprocating popet values, not shown) systems. The acid and lime delivery systems consist of two fiberglass-reinforced plastic tanks for each system (feeding from one tank at a time) and associated pumps. Acid is diluted to 5–10% (w/w) in the acid tank and lime is mixed with water to approximately 25% (w/w) in the lime tank and continually circulated by a centrifugal pump to prevent settling of lime particulates.

Feedstock from the belt conveyor enters a pug mill mixer and is mixed with dilute acid and water. Water is added as needed to adjust the solids concentration in the pretreatment reactor. The wetted feedstock is screw conveyed to a plug feeder that compresses the material into an impermeable plug that is then forced into the pretreatment reactor. Liquid expressed from the material by the plug feeder is pumped into the pretreatment reactor. The feedstock enters through the side of the reactor and is conveyed to the top by twin screws overflowing a weir and entering the main reactor body. There is no mechanical mixing (e.g., agitator in the reactor) and the material moves by gravity flow to the discharge port at the bottom of the reactor and is directed into the flash tank. Since the consistency of the material is like "damp sawdust", no mixing occurs and hydrolysis of the starch and hemicellulose components are unlikely to reduce the consistency enough at the high solids concentration to promote mixing. A rotating scraper at the bottom of the reactor facilitates movement of material to the discharge port. The reactor is heated by steam to achieve the desired temperature and residence times from 3 to 20 min are achieved by controlling material level in the reactor.

The flash tank, which receives the hot pretreated slurry, is a conical screw mixer also used to blend the lime slurry with the pretreated feedstock. Vapor from the flashing mixture exits the top of the tank and is sent to a condenser, while the remaining non-condensable fraction is sent to a scrubber. Pretreated feedstock then exits the bottom of the flash tank and is pumped to the first 9000-l fermentor.

2.3. Fermentation and seed production

Fermentations are performed in four 9000-l vessels each containing complete instrumentation and controls for aerobic or anaerobic operation. Weight measurements from load cells on each of the vessels are used for level control. In addition, there are two 1500-l fermentors, two 160-l fermentors, and one 20-l fermentor. All fermentors were obtained from Associated Bio-Engineers and Consultants (Allentown, PA, USA), except for the 20-l fermentor (Bioflo IV, New Brunswick Scientific Co., Edison, NJ, USA). The fermentors are piped to allow sterile transfer of seed culture to the next larger fermentor. Seed culture from the final seed fermentor (1500-l vessel) is transferred to seed hold tanks (not shown) or directly to the first 9000-l fermentor. Dual tanks allow parallel seed production trains if required.

Pretreated feedstock as well as the soluble sugars generated during pretreatment are continuously fed to the first 9000-l fermentor along with appropriate amounts of seed culture, nutrients, and enzyme. Nutrients (typically corn steep liquor) and purchased enzyme are stored in tanks and pumped to the first fermentor at the required flow rates. When the first fermentor reaches the desired level (or residence time, typically 24 h), broth is pumped to the next 9000-l fermentor in the train and level is controlled. Broth from the fourth 9000-l fermentor is continuously pumped to a 9000-l beer well.

2.4. Distillation and solid/liquid separation

The beer well serves as a surge tank for the distillation system, which can process one day's worth of

production in 5–6 h. APV Crepaco, Inc. (Tonawanda, NY, USA) supplied the direct-steam-injected distillation system consisting of dual preheaters; a 0.4-m diameter by 10-m high, 19-plate, sieve-tray column; overhead condenser; vent condenser; and bottoms cooler. The distillation system was designed to strip ethanol from the fermentation broth and produces a 50% (w/w) ethanol stream that is sent to a 22,680-l storage tank. The stream from the bottom of the column is cooled and pumped to the centrifuge feed tank.

Material from the centrifuge feed tank is continuously pumped to a solid-bowl decanting centrifuge (Sharples Model P3000, Alfa-Laval Separations, Inc. Warminster, PA, USA). Dewatered solids are collected in the cake tank and can be pumped into containers or drums for disposal or further work. The liquid fraction is collected in the centrate tank and is either disposed of or used as recycle water.

Table 1
Run 1 operating conditions for feedstock handling and pretreatment systems for the first two days of operation (first value) and for the rest of the run (second value)

Equipment	Operating condition	Value
Feedstock handling	Feedstock flow rate (dry kg/h)	38, 38
Acid tanks	Acid concentration (wt%)	5, 5
Lime tanks	Lime concentration (wt%)	25, 25
Sunds reactor	Temperature (°C)	170, 160
	Residence time (min)	15, 10
	Acid concentration (%)[a]	0.7, 0.7
	Solids concentration (%)[b]	25, 25
Flash tank	Solids concentration (%)[b]	25, 15
	pH[c]	5, 5

[a] Weight % sulfuric acid in the liquid phase (g acid/g acid + g water).
[b] Weight % dry solids based on dry feedstock flow rate (actual solids concentration is slightly lower due to conversion of some of the solids to volatile compounds during pretreatment).
[c] pH in the tank was not measured, but the lime addition rate was varied to achieve the desired pH value in samples withdrawn from the tank.

3. Methods

3.1. Corn fiber feedstock

Corn fiber was obtained from a local corn wet mill (Golden Technologies, Inc. Johnstown, CO) and stored in a refrigerated trailer for no longer than a month. Usually multiple shipments (or lots) were required to supply the pilot plant with enough feedstock for operation during an extended run. The corn fiber moisture content was 55–60% (w/w) as received.

3.2. Microorganism

The glucose-fermenting microorganism used in this work was *Saccharomyces cerevisiae* strain L1400, although this is the host for a recombinant glucose/xylose-fermenting microorganism (Toon et al., 1997), the recombinant was not used for the work reported in this paper. Cells were grown on 1% (w/v) yeast extract, 2% (w/v) peptone, and 2% (w/v) glucose and mixed with 20% (w/w) glycerol and stored in vials at −70 °C.

3.3. Pilot plant operation

During initial shakedown operation of the PDU two runs were performed. We operated the PDU for 15 days during the first run (Run 1) and for 10 days during the second run (Run 2). In Run 1 the feed handling system, pretreatment reactor, seed production train, and 9000-l fermentors were used and operation was as described in Section 2. Operating conditions for the feed handling and pretreatment equipment during Run 1 are described in Table 1 and were changed to new values after 2 days of operation (second set of numbers). For many lignocellulosic feedstocks, a conditioning or detoxification step is needed after pretreatment to remove fermentation inhibitors (McMillan, 1994), however, a conditioning step was not needed for this work as relatively small amounts of inhibitors are produced from corn fiber.

A proprietary pretreatment device supplied by Amoco Corporation and fed corn fiber from a different feed hopper was used in Run 2. The feed rate was still approximately 38 dry kg/h. Pretreated corn fiber from this device was fed directly to the first 9000-l fermentor. This arrangement by-passed the PDUs feed handling and pretreatment equipment, but the 9000-l fermentor train and seed production train were still operated as described in Section 2, except that the lime addition point was moved to the first 9000-l fermentor. However, when lime began plugging pipes in the lime transfer system, lime addition was eliminated and 50% (w/w) NaOH was used to neutralize sulfuric acid and adjust pH in the 9000-l fermentors. Only three of the 9000-l fermentors were used during Run 2.

For both runs, inoculum was produced by inoculating a 250-ml baffled shake flask (100 ml culture volume) with 1.5 ml of frozen L1400 cells, the contents of this flask were subsequently transferred to a 2-l baffled shake flask (1 l culture volume). Cell mass was further increased by successive transfers into the larger PDU fermentors. The operating conditions and medium requirements for the seed production train and 9000-l fermentors are shown in Table 2. pH was not controlled in the shake flasks and 3.0 M NaOH was used for pH control in the 20- and 160-l fermentors. pH in the 1500-l fermentors was controlled with 50% (w/w) NaOH. Inoculum from the 1500-l fermentors was transferred to the seed hold tanks to await addition to the first 9000-l fermentor. The seed tanks were agitated at 50 rpm, maintained at a pressure of 133

Table 2
Seed production train and main fermentor operating conditions and medium requirements

	Flask #1	Flask #2	20-l	160-l	1500-l	9000-l
Working volume (l)	0.1	1.0	10	100	1000	[a]
Operating conditions						
Temperature (°C)	30	30	30	30	30	30
Pressure (kPa)	–[b]	–	133	133	133	133
Air flow rate (l/min)	–	–	5	50	250	30[c]
Agitation rate (rpm)	150[d]	150[d]	150	100	75	50
pH	–	–	5.0	5.0	5.0	5.0
Residence time (h)	8	8	8	12	12	24
Medium						
Glucose (%, v/w)	2	2	2	2	2	–
Peptone (%, v/w)	2	–	–	–	–	–
Yeast extract (%, v/w)	1	–	–	–	–	–
CSL (%, v/w)	–	1	1	1	1	1, 0[e]
Corn oil (ml/l)	–	–	0.5	0.5	0.5	–
Cellulase loading (FPU/g cellulose)	–	–	–	–	–	25, 10[e]

[a] Level was controlled to maintain a 24 h residence time.
[b] (–) not applicable.
[c] Small amount of air was used to maintain a positive pressure in the vessel.
[d] In a laboratory shaking incubator.
[e] Value in Run 2.

kPa, and cooled to about 15–20 °C. Seed culture was pneumatically transferred to the first 9000-l fermentor at a feed rate of approximately 0.4 l/min. Seed culture was held in the seed hold tanks for a period of 1–2 days. Although cell viability decreased during the hold period, there was no noticeable drop in cell viability in the 9000-l fermentors after steady operation was achieved (see Fig. 4). It was not clear that continuous inoculation was necessary to maintain a stable cell population in the 9000-l fermentors.

The initial batch of seed culture was produced as described above, however, usually 10% (v/v) of the contents of the 1500-l fermentor was left in the vessel and the fermentation was restarted after addition of fresh medium. This avoided continual operation of the smaller seed production fermentors. However, a new batch of seed culture was started from frozen cells every 5–6 days.

Fermentation conditions in the 9000-l fermentors are also presented in Table 2. Corn steep liquor (CSL) obtained in drums from Grain Processing Corporation (Muscatine, IA, USA) supplied nutrients for the first run, but CSL was not used in the second run. We determined from shake flask testing that pretreated corn fiber contained enough nutrients to support fermentation without the use of CSL. pH was controlled with 50% (w/w) NaOH in all of the 9000-l fermentors. Cellulase obtained from Iogen Corporation (Ottawa, ON, Canada) was added continuously at a loading of 25 FPU/g cellulose (Run 1) or 10 FPU/g cellulose (Run 2) based on the cellulose content of the raw corn fiber. During the first run, it was initially difficult to mix the contents of the first 9000-l fermentor because solids settled while filling the vessel before the level was high enough to begin agitation. For the second run, the first 9000-l fermentor was started with 1250 l of sterile water in the fermentor, which thinned the broth enough to allow adequate mixing. Thereafter, the total solids concentration was controlled at 25% (w/w, based on dry corn fiber fed to pretreatment ignoring volatile compounds generated and loss during pretreatment) in the first 9000-l fermentor.

Pretreated corn fiber samples were taken from the flash tank ever 3–4 h and the 9000-l fermentors were sampled every 8 h. Data from plant sensors (e.g., temperature, pH) were logged by the DACS every 20 min.

3.4. Analytical methods

Procedures for measuring both the raw and pretreated feedstock compositions (ash, lignin, and carbohydrate components except starch) used methods reported previously (Nguyen et al., 1998). Protein content was calculated as 5.2 times the nitrogen content. The conversion factor (5.2) for corn fiber was determined from an amino acid analysis performed by an outside laboratory (University of Virginia School of Medicine, Charlottesville, VA). Nitrogen content was measured with a Perkin-Elmer Series 2400 CHNS/O Elemental Analyzer (Norwalk, CT, USA).

Corn fiber starch content was determined by enzymatic digestion of the starch to glucose (YSI Incorporated, 1994). Corn fiber samples were first incubated in 0.6 N NaOH for 20 min at 90 °C to destroy any free sugars contained in the sample and to make the material more amenable to enzymatic digestion. The samples

were then incubated in a pH 4.2 acetate buffer at 40 °C for 1 h with 6 units of amyloglucosidase activity/ml. The reaction was quenched by addition of trichloracetic acid and glucose was measured using a YSI Model 2700 Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Amylopectin samples were run in parallel to correct for any starch losses.

Monomeric sugar concentrations (glucose, galactose, mannose, xylose, and arabinose) were measured by high performance liquid chromatography (HPLC) using a Biorad HPX-87P column (Biorad Laboratories, Hercules, CA) operating at 85 °C with water (0.6 ml/min) as the mobile phase. Total sugars (oligomeric and monomeric) were determined by hydrolysis of a liquor sample in 4% (w/w) sulfuric acid at 121 °C for 1 h followed by measurement of monomeric sugars using the HPX-87P column. Corrections were made for sugars lost from degradation reactions during the procedure. Oligomeric sugars were calculated as the difference between total and monomeric sugars. Organic acids, furfural, hydroxymethylfurfural (HMF), and ethanol were measured by HPLC using a Biorad HPX-87H column operating at 65 °C and 0.01 N sulfuric acid (0.6 ml/min) was the mobile phase.

Measurements of component concentrations by HPLC were not duplicated (one measurement only). However, after calibration of the instrument and during routine operation a sample of known concentration, a quality control standard (QCS) containing all measured components, was analyzed every 10th sample and a deviation of any measured component in the QCS of 3% from the known value caused rejection of all previously analyzed samples. These rejected samples were subsequently reanalyzed. However, the measured values of the QCS were typically within ±1% of the known value.

The insoluble solids content of pretreated corn fiber was determined by repeated washing of a sample until all of the dissolved solids were removed. Typically, a 10-g sample was diluted with 100 ml of deionized water and after centrifugation the liquid was discarded and the solid was again diluted with water. This procedure was repeated at least three times and the remaining solids were dried and weighed.

Viable yeast cell counts were performed by serial dilution of a 1 ml corn fiber fermentation broth samples. The samples were serially dilute in a 1% NaCl solution, plated on YPD (1% yeast extract, 2% peptone and 2% dextrose) and incubated at 37 °C for 2 days.

3.5. Statistical analysis

Composition of untreated corn fiber is reported as the average of at least two, and sometimes more, independent samples taken from the same lot of material. The average and standard deviation values were calculated using the respective functions (AVERAGE, STDEV) available in Microsoft® Excel. The composition of pretreated corn fiber was an average of two separate determinations of composition of the same sample. Averages and standard deviations were again calculated using Microsoft® Excel.

4. Results

4.1. Corn fiber composition

Composition of three different corn fiber lots used during this work along with values reported in the literature are presented in Table 3. In Run 1 one lot of corn fiber was used, while two different lots were used during Run 2. All lots were obtained from the same corn wet mill, but on different days and obvious compositional differences existed between the different lots used during Runs 1 and 2. The starch content of the Run 2 material is greater than material from Run 1 and was probably due to process variability at the corn mill. The compositional values obtained by Grohmann and Bothast (1997) compare well with our values except for cellulose. Protein content would also be similar if 5.2 instead of 6.25 as used by Grohmann and Bothast

Table 3
Corn fiber composition (weight % dry basis) and standard deviations (in parenthesis) based on duplicate analysis except as noted

Run/lot	Cellulose[a]	Xylan	Galactan	Arabinan	Lignin	ASL[b]	Ash	Starch	Protein
1	14.4 (0.8)	20.8 (0.3)	3.5 (0.1)	13.6 (0.3)	6.5 (0.4)	3.4 (0.1)	1.0 (0.1)	15.7 (0.3)	8.7 (0.3)
2/1[c]	12.6 (1.2)	18.6 (0.1)	6.9 (0.3)	11.2 (0.2)	7.8 (1.2)	7.8 (0.6)	0.9 (0.1)	24.9 (0.7)	8.7 (n.d.[d])
2/2[c]	10.3 (1.5)	18.8 (0.1)	6.8 (0.2)	10.7 (0.5)	8.5 (0.3)	8.1 (1.4)	0.9 (0.1)	25.6 (0.3)	9.7 (n.d.)
e	17.5 (2.1)	17.6 (1.8)	3.6 (0.3)	11.3 (1.5)	7.8 (0.7)	n.d.	0.6 (0.1)	19.7 (0.9)	11.0 (0.5)
f	12.4	30.8[g]	n.d.	n.d.	1.4	n.d.	0.8	n.d.	n.d.

[a] Calculated as difference between total glucan and starch.
[b] Acid soluble lignin.
[c] Based on four samples.
[d] n.d.: not determined.
[e] Source: Grohmann and Bothast (1997), number of replicates not reported.
[f] Source: Dale et al. (1996), standard deviations not reported.
[g] Reported as hemicellulose.

(1997) was used to convert nitrogen content to protein content.

4.2. Run 1 results

Our initial attempt at integrated plant operation was plagued with many equipment and operational problems. For example, little ethanol was produced during the initial two days of the fermentation. Pretreatment conditions may have been too severe producing high levels of fermentation inhibitors, thus we changed pretreatment conditions (see Table 1). However, confounding this problem was the high solids concentration in the first 9000-l fermentor that led to the mixing difficulties previously mentioned (Section 3.3). The high solids level interfered with both pH and temperature probe readings (stagnant broth insulated the probe from conditions in the bulk material) and actual operating conditions in the fermentor were unknown. Problems encountered with the pretreatment reactor limited its operating time to approximately five days out of the total of 15 days of continuous operation. Problems with lubrication system and seals on the reactor's rotating scraper caused a five days shutdown. When the pretreatment reactor was not operating, the fermentation was sustained by the direct addition of glucose to each 9000-l fermentor. Although during reactor operation data was obtained on the pretreatment process, but operating problems limited usefulness of the fermentation results.

Monomeric sugar, acetic acid, and furfural concentrations in the pretreated corn fiber liquor taken from the flash tank after dilution to 15% (w/w) total solids are shown in Fig. 2. This was for a period of 44 h near the end of the run when we achieved reliable and steady operation at the second set of pretreatment conditions shown in Table 1. Sugar concentrations are relatively stable and the variability was probably caused by slight changes in operating conditions or measurement errors.

At two time points during this period of operation (separated by 20 h and identified on Fig. 2 as Points 1 and 2), the composition of the solids fraction of the pretreated corn fiber was analyzed allowing detailed mass balance calculations to be performed. The composition of the pretreated corn fiber is shown in Table 4. Since the analysis could not distinguish between cellulose and starch, the value for glucan included both components. The total mass did not add up to 100% because protein and other unmeasured components (e.g., ash) were not reported.

The mass balance methodology followed the procedures presented by Hatzis et al. (1996). Obtaining nearly complete carbon mass balance closure provides reasonable confidence in the accuracy of the underlying data. Mass balance closure is defined as the mass of a component (e.g., xylan) in the output stream comprising the solid (e.g., xylan in the residual pretreated solids),

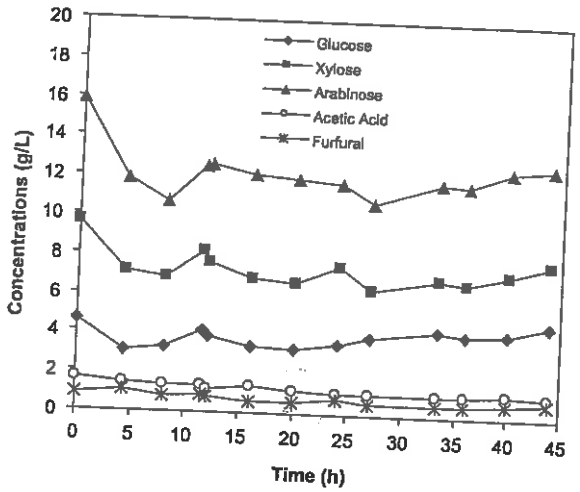

Fig. 2. Concentrations of various components in the pretreated corn fiber liquor taken from the flash tank during 44 h of operation near the end of Run 1. Operating conditions were 160 °C, 0.7% (w/w) acid concentration, and 10 min residence time. Only monomeric sugar concentrations are shown.

Table 4
Composition of pretreated corn fiber[a] (% dry basis) at the two time points (see Fig. 2)

Component	Point 1	Point 2
Glucan	36.7 (1.1)	36.2 (0.6)
Galactan	1.7 (0.1)	1.9 (0.1)
Xylan	11.3 (0.5)	13.5 (0.2)
Arabinan	4.9 (0.7)	4.7 (0.3)
Lignin	28.0 (1.9)	26.1 (1.6)

[a] Standard deviations given in parenthesis based on duplicates.

liquor, and vent streams (e.g., as xylose or by-product furfural) divided by the mass of the component in the raw corn fiber. These results, along with individual sugar yields, are presented in Table 5. Lignin (as Klason and acid-soluble) was only measured in the solid materials (i.e., raw and pretreated corn fiber) and any lignin solubilized during pretreatment and contained in the pretreatment liquor is not accounted for, thus, the material balance closure for lignin was probably low. Yield of furfural from xylan is also shown, although we assumed all furfural originated from xylan to simplify the calculation, since degradation of arabinose could also have contributed furfural.

As expected, the results were similar for the two points because the operating conditions were similar. However, the rather low yield for xylose and arabinose as well as for by-product furfural indicated a low severity pretreatment. At least 75% conversion of xylan to monomeric xylose is desired. The glucose yield was consistent with a large fraction (92%) of the starch being converted to glucose, making the reasonable assumption that no cellulose is converted to glucose. The mass balance closure for glucose, xylose, and arabinose was

Table 5
Pretreatment performance data at two different times (see Fig. 2) during Run 1

Component	Point 1			Point 2		
	Yield[a] (%)	Monomer yield (%)	MBC[b] (%)	Yield[a] (%)	Monomer yield (%)	MBC[b] (%)
Glucose[c]	48.0	7.7	98.5	53.4	7.7	99.4
Galactose	90.3	46.7	110.5	83.7	38.7	104.5
Xylose	67.2	22.5	99.9[d]	58.3	18.0	86.6[d]
Arabinose	63.3	50.4	91.8	58.5	45.4	83.1
Furfural[e]	–[f]	3.9	–	–	2.5	–
Lignin	–	–	117.4	–	–	100.9

[a] Percentage of component converted to oligomeric plus monomeric sugar (total sugar).
[b] Mass balance closure.
[c] Includes both starch and cellulose.
[d] Includes furfural that is assumed contributed only by degradation of xylose.
[e] Yield of furfural from xylan only.
[f] (–) not applicable.

nearly complete. The lignin mass balance closure was high and could be higher since solubilized lignin is not accounted for in the mass balance. This probably indicated a problem with measuring lignin content of the pretreated feedstock. Several authors (Bouchard et al., 1989; Nguyen et al., 1999) have alluded to the difficulties with measuring the composition of lignocellulosics altered by acid hydrolysis.

4.3. Run 2 results

Nearly continuous operation of the proprietary pretreatment reactor was maintained for nine days during Run 2. There were minor problems that took the reactor off-line for short periods of time, nevertheless, valuable operational experience and performance data was obtained on the fermentation process.

Concentration profiles of various components in the 9000-l fermentors are shown in Fig. 3 where time zero is defined as the time of inoculation of the first 9000-l fermentor. Glucose concentrations usually remained low (Fig. 3a) because yeast readily consumed this sugar. A glucose concentration spike occurred at 120 h because a power failure caused loss of cooling water flow to the fermentors, which increased the temperature in the first fermentor to above 40 °C for about 5 h killing most of the yeast and stopping glucose consumption. Addition of glucose contained in the pretreated feedstock and continuing enzymatic saccharification of cellulose then rapidly increased glucose concentrations in the fermentors. Once proper temperature control was reestablished, the yeast rapidly metabolized the glucose. As was previously noted yeast was continually being fed to the first fermentor replenishing yeast that was lost earlier. The smaller spike at 180 h was caused by an accidental overaddition of enzyme producing a rapid increase in the cellulose hydrolysis rate.

Ethanol production occurred primarily in the first fermentor (Fig. 3b). At 48 h into the run, ethanol concentration declined corresponding to an increase in both acetic and lactic acid concentrations (Fig. 3e and f, respectively). Contaminating bacteria produced these acids and microscopic observations revealed rapid proliferation of the bacteria. This microorganism was able to out compete the yeast for glucose producing the subsequent drop in ethanol concentration. The temperature spike at 120 h killed most of the bacteria and so organic acid concentrations decreased. However, near the end of the run both organic acid concentrations and bacterial cell density increased.

During the same time period arabinose concentration decreased (Fig. 3c) and was likely consumed by the bacteria, since the yeast cannot utilize arabinose. However, there was no noticeable decrease in xylose concentration (Fig. 3d). We suspected the decrease in both xylose and arabinose concentration in the first fermentor near the end of the run was due to a change in pretreatment severity that decreased sugar concentrations and not metabolism of the sugars by a microorganism.

Viable yeast counts in the three 9000-l fermentors are shown in Fig. 4 when the data were available. After steady operation was achieved, time constraints eliminated further measurements of cell concentrations. After inoculation of the first 9000-l fermentor, cell counts in this vessel rapidly increased because of rapid consumption of the glucose initially present in the pretreated corn fiber. The pretreated material contains significant quantities of glucose from hydrolysis of the starch. After yeast concentrations peak near 24 h, cell concentrations decreased and appeared to achieve steady value after 72 h into the run. Contaminant bacterial cell populations were not enumerated.

5. Discussion

The advantages of operating a plant of this size are the opportunities to discover and troubleshoot real

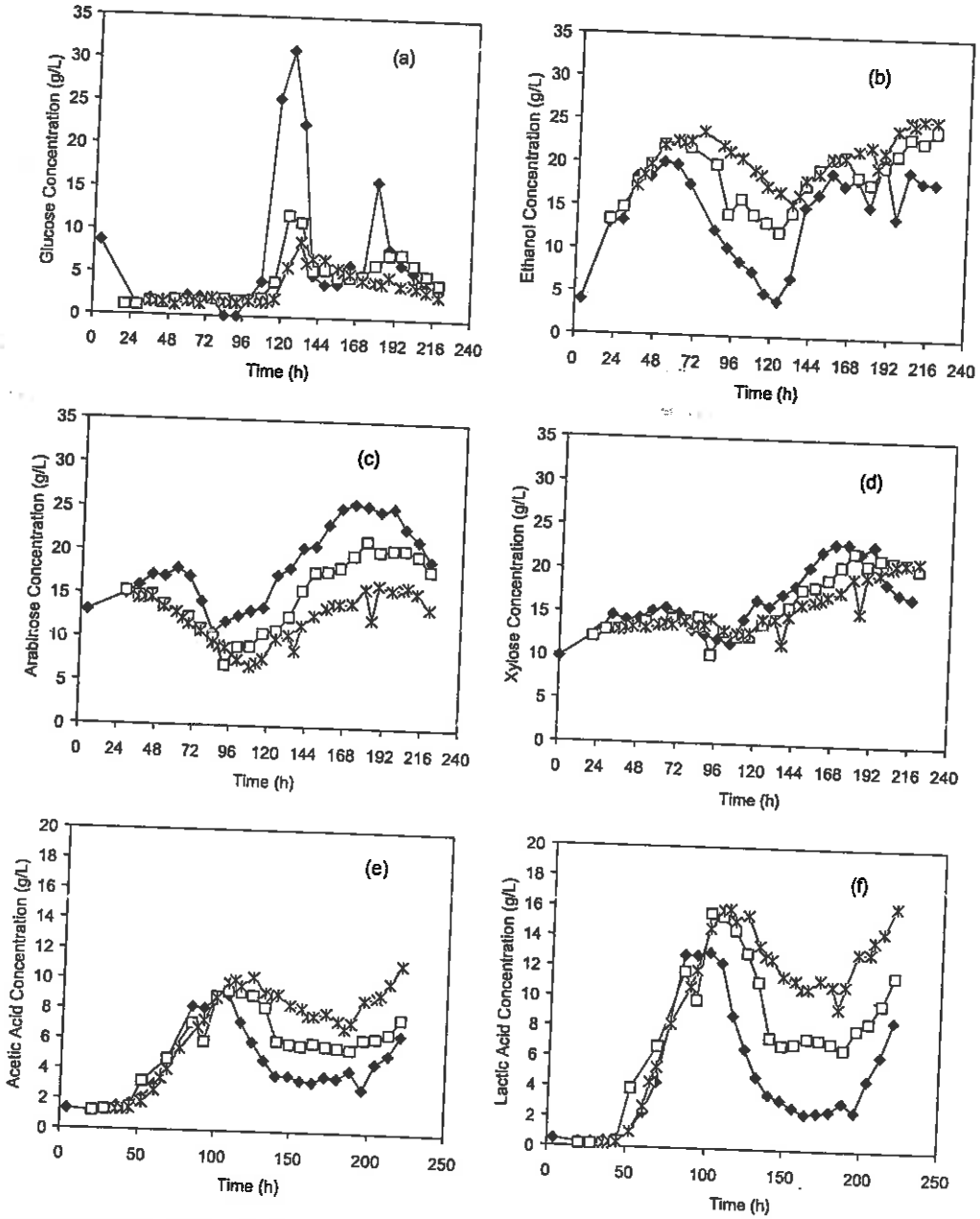

Fig. 3. Component concentration profiles in the first (◆), second (□), and third (×) 9000-l fermentors during Run 2. Quality control standard used to ensure measured values are accurate to ±3%.

problems that could occur in a commercial-scale facility. These were the first two runs performed in the PDU that integrated operations for feed handling, pretreatment, seed production, and fermentation and allowed for experience to be gained with a corn fiber feedstock and troubleshooting of equipment operation in preparation for more extensive runs. Problems with equipment operation and the control system were identified and corrected before longer six-week long runs were performed the following year (data not shown).

While resolving equipment problems was relatively easy, bacterial contamination was a more difficult problem. This issue is critical for the developing biomass-to-ethanol industry. Efforts were made in this work to maintain aseptic conditions, but contamination persisted and yeast and bacterial cell concentrations were similar (data not shown). The presence of a sugar (arabinose) not utilized by the yeast but consumed by the bacterial contaminant may have contributed to their rapid proliferation. High levels of lactic and acetic acid produced

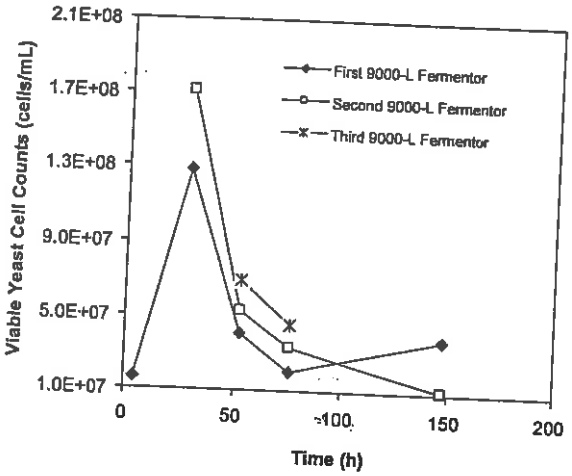

Fig. 4. Viable yeast counts in the first (◆), second (□), and third (✳) 9000-l fermentors during Run 2. Quality control standard used to ensure measured values are accurate to ±3%.

by the bacteria limited yeast performance. Genetic engineering has produced microorganisms with the ability to consume a wider variety of sugars (Ingram et al., 1998; Zhang et al., 1998), which may reduce the severity of contamination problems by limiting the availability of sugars for invading microorganisms.

Subsequent efforts focused on identifying the bacteria and the source of the contamination, and on ways to mitigate the impact of infection. Batch operation may be one method of reducing the impact, however, continuous operation is widely recognized for its ability to achieve higher overall plant productivities and in the absence of contamination is the more economical operating mode.

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