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Fermentation of "Quick Fiber" Produced from a Modified Corn-Milling Process into Ethanol and Recovery of Corn Fiber Oil

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Abstract

Approximately 9% of the 9.7 billion bushels of corn harvested in the United States was used for fuel ethanol production in 2002, half of which was prepared for fermentation by dry grinding. The University of Illinois has developed a modified dry grind process that allows recovery of the fiber fractions prior to fermentation. We report here on conversion of this fiber (Quick Fiber [QF]) to ethanol. QF was analyzed and found to contain 32%wt glucans and 65%wt total carbohydrates. QF was pretreated with dilute acid and converted into ethanol using either ethanologenic *Escherichia coli* strain FBR5 or *Saccharomyces cerevisiae*. For the bacterial fermentation the liquid fraction was fermented, and for the yeast fermentation both liquid and solids were fermented. For the bacterial fermentation, the final ethanol concentration was 30 g/L, a yield of 0.44 g ethanol/g of sugar(s) initially present in the hydrolysate, which is 85% of the theoretical yield. The ethanol yield with yeast was 0.096 gal/bu of processed corn assuming a QF yield of 3.04 lb/bu.

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The residuals from the fermentations were also evaluated as a source of corn fiber oil, which has value as a nutraceutical. Corn fiber oil yields were 8.28%wt for solids recovered following prtetreatment.

Index Entries: Bioethanol; corn fiber oil; *Escherichia coli*; pentose fermentations; *Saccharomyces cerevisiae*; β-glucosidase.

Introduction

In 2000, the United States produced more than 2 billion gallons of fuel ethanol, and >95% of this was from processed corn (Renewable Fuels Association, 2003). Ethanol is used as a fuel oxygenate, to meet goals required by the Clean Air Act 1990 amendment. Methyl *tert*-butyl ether (MTBE), which is made from petroleum-derived methanol, is the alternative oxygenate. However, MTBE has been identified as a major groundwater pollutant, and the federal government is moving to ban its use in gasoline. Fuel ethanol from corn is expected to double because it will be needed as a substitute for MTBE. Alternately, there is currently a proposal in the US Congress to relax the fuel oxygenate standard and require fuel ethanol usage as part of a National Renewable Fuel Standard.

Corn is processed for ethanol production by wet milling and dry grinding. Dry grinding accounts for 60% of the processed corn, and dry grind production capacity is growing more rapidly than wet milling. This growth in dry grinding capacity can be traced to the establishment of co-operatives owned by farmers, who favor this process because capital costs are much less than for wet mills. A major disadvantage of dry grinding compared to wet milling is the production of fewer coproducts.

Dry grind plants produce the following coproducts in addition to ethanol: carbon dioxide and a variety of high-fiber content animal feeds *(1)*. Carbon dioxide is produced during the fermentation, but because of its low selling price it is collected and sold by only a few of the dry grind ethanol processors. The animal feed products are manufactured from the fermentation residuals. The whole stillage is centrifuged or screened to yield distillers' wet grain (DWG) (more dense material) and thin stillage. The DWG is sometimes sold as is, but only locally because of its short shelf life. More often, it is combined with condensed thin stillage, dried, and sold as distillers' dried grains with solubles. By contrast, wet millers produce the following coproducts: corn oil, gluten meal, and corn gluten feed. Many wet millers also produce a variety of products from the starch in addition to ethanol. The final result is that the net cost of corn for ethanol production is lower for a wet mill compared to a dry grind operation.

The University of Illinois is developing a modified milling process, which would allow recoveries of the germ and hull fractions prior to fermentation *(2,3)*. The process involves soaking corn in water for a short period of time (12 h). The process has the following advantages over dry grinding: the potential for recovery of corn oil from recovered germ, an increased bioreactor capacity from prior separation of nonfermentables,

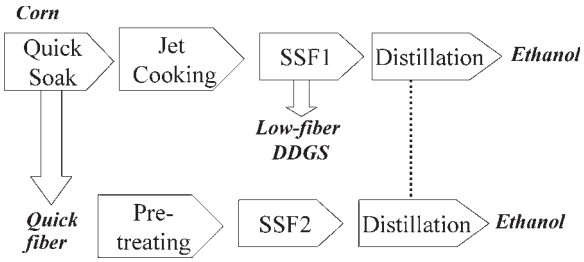

Fig. 1. Schematic of corn dry grind process with fiber conversion to ethanol. DDGS, distillers' dried grains with solubles.

and the ability to increase value and relative protein content for modified distillers' wet grains with solubles. It has been estimated that the modified process could generate an additional revenue of 5–7 ¢/gal *(4)*. The process is much less expensive to build than a traditional wet milling because of the shortened soaking time, fewer complex equipment needs, and avoidance of sulfuric acid steeping.

In the modified milling process, following steeping, the corn is milled and the germ and pericarp fiber are separated from the starch and gluten using hydroclones. The germ and pericarp fiber (quick fiber [QF]) are washed to recover additional starch, dried, and separated from each other by aspiration. The germ, which contains the corn oil, could be sold to an oil processor for extraction. The QF would be available for production of corn fiber oil, as well as an additional substrate for fermentation. Corn fiber oil has potential as a valuable coproduct because this oil contains relatively high levels of phytosterols *(5–7)*, which have been shown to lower cholesterol in several animal studies *(8,9)*. The phytosterol components include free phytosterols (St), phytosterol fatty acyl esters (St:E), and ferulate phytosterol esters (FPE). Corn fiber oil also contains stanols, which have additional value for lowering cholesterol.

QF has potential as a feedstock for ethanol fermentation because of its high carbohydrate content. A modified dry grind process that includes conversion of QF into ethanol is shown in Fig. 1. After fiber removal, the starch is liquefied, in part by jet cooking, and then undergoes simultaneous saccharification and fermentation (SSF1) to ethanol by the addition of yeast and glucoamylase. Converting QF to ethanol requires two additional processing steps: pretreatment and a SSF (SSF2). The ethanol streams for the starch and fiber fermentations can be mixed prior to distillation (Fig. 1, dotted line). Pretreatment prepares the cellulose for enzymatic saccharification and hydrolyzes the other carbohydrate components (i.e., residual starch and hemicellulose) into free sugars. The cellulose is saccharified enzymatically by cellulase. Fermentation of the fiber hydrolysate is more complicated than corn starch. The fiber contains a variety of carbohydrates including residual starch, hemicellulose, and cellulose. Hydrolyzing corn hemicellulose produces a mixture of sugars including arabinose, galactose, and xylose (ibid).

Saccharomyces cerevisiae ferments neither arabinose nor xylose. For the present study, these sugars along with glucose were converted to ethanol using recombinant ethanologenic *Escherichia coli* strain FBR5, which was developed by our laboratory *(10,11)*. This strain has been metabolically engineered to convert a wide spectrum of sugars to ethanol. Typical ethanol yields are 94% or greater of theoretical *(11)*. However, some mills may not wish to use recombinant organisms and, thus, fermentations were also carried out using *S. cerevisiae*. The yeast, however, is only capable of converting the sugars derived from cellulose and residual starch to ethanol.

Materials and Methods

Bacterial Strains, Growth Media, and Reagents

Media and protocols for routine maintenance of *E. coli* strain FBR5 have been previously described *(10)*. *S. cerevisiae* (Y-2034; ARS Culture Collection, Peoria, IL) was stored in 50% (v/v) glycerol stocks at –80ºC. The yeast culture was routinely maintained on YPD (10 g/L of yeast extract, 20 g/L of peptone, and 20 g/L of dextrose with 20 g/L of Difco agar added for solid medium) and incubated at 32ºC.

Enzymes were supplied by Novozyme (US Office: Franklinton, NC) and included cellulase (Celluclast® 1.5 L; 48 international filter paper units [IFPU]/mL), β-glucosidase (Novozym® 188; 66.8×10^3 IU/mL), and glucoamylase (Novozyme AMG300L). Sugars were purchased from Sigma (St. Louis, MO), and all other chemical and media reagents were from Fisher (Fairview, NJ).

Preparation of Quick Fiber

QF was prepared from no. 2 yellow dent corn as previously described *(2,12)*. Briefly, corn (1 kg) was soaked in water (2 L) for 12 h at 59°C. The corn was ground in a blender at 40% full power for 3 min followed by 46% full power for an additional 3 min, so as to separate out but not damage the germ. The germ, which is lighter than water, was isolated by flotation. The density of the remaining corn solution was adjusted by adding dried starch until the fiber fraction floated and could be removed by skimming off the surface. The coarse fiber was washed twice with water to remove added starch and stored at –20°C prior to hydrolysis.

Compositional Analysis of Biomass

Each sample was analyzed for moisture, carbohydrate, oil, and protein contents. Moisture was measured by drying the samples at 105°C until they reached a stable weight. Oil was measured using AOAC method 920.39 and protein by AOAC method 976.06, which is based on measuring total nitrogen. Starch was determined as previously reported *(13)*. Arabinose and xylose were determined by hydrolyzing the biomass with trifluoroacetic acid and analyzing for production of free sugars by high-

performance liquid chromatography (HPLC) as described previously *(14)*. Cellulose was determined using ASTM method E1758-95. Samples were analyzed for oil, protein, and starch by Analabs (Fulton, IL).

Optimizing Acid Loading for SSF Experiments

QF was pretreated with dilute acid for the *S. cerevisiae* SSF experiments. The amount of acid added per gram of biomass was optimized for complete hydrolysis of the hemicellulose and subsequent enzymatic hydrolysis of the cellulose. The QF (1.2 g) was mixed with 16 mL of various dilute H_2SO_4 solutions (0–12 g H_2SO_4/100 g of biomass [dry basis, db]) to give a solid loading of 7.0% (w of biomass, db/total w). The mixture was placed in stainless steel pipe reactors (40-mL working volume), which were placed in a fluidized sand bath (Model 01187-00 bath and 01190-72 temperature controller; Cole-Parmer, Vernon Hills, IL). The mixture was heated to and kept at 150ºC for 10 min before being quickly cooled in a water bath. The internal reactor temperature was monitored using a thermocouple probe inserted into one of the pipe reactors. The pretreated material was transferred to a test tube, neutralized with $Ca(OH)_2$ to pH 4.5, and citric acid buffer (pH 4.8, 50 m*M*) was added along with cellulase (0.3 mL) and β-glucosidase (0.3 mL); the total enzyme loading was 24 IFPU/g of QF. Thymol (0.025 mg/mL) was added to prevent microbial contamination. The biomass samples were incubated at 45°C with agitation for 48 h in a water bath (Dubnoff Metabolic Shaking Incubator; Precision Scientific, Chicago, IL). The hydrolysis reactions were sampled at 24 and 48 h for sugar concentrations. Each reaction was run in duplicate.

S. cerevisiae *SSF*

The biomass was pretreated as already described for the cellulase hydrolysis experiments with a 3.2% (w/w) H_2SO_4 loading. For SSF, 10.8 g (db) of pretreated material was placed in a 125-mL Erlenmeyer flask to which the following was added: cellulase (0.33% [v/v]), β-glucosidase (0.33% [v/v]), glucoamylase (0.046% [v/v]), and 10% (v/v) of a 10X YP stock (final concentration in medium: 10 g/L of Difco yeast extract, 20 g/L of Difco Proteose Peptone). The SSF was initiated by inoculation with *S. cerevisiae* to an OD_{600} of 0.5. The beginning solids for the SSF, including all additions, was 16.4% (w/w). The flasks were capped with rubber stoppers and pierced with a 22-gage needle to exhaust CO_2. The cultures were incubated at 32°C and agitated at 150 rpm (Refrigerated Innova® Shaker; New Brunswick Scientific, Edison, NJ) for 70 h. The fermentations were sampled each day for glucose and ethanol concentrations. All fermentations were run in duplicate.

E. coli *FBR5 Fermentations*

The QF was hydrolyzed using a different protocol than described for the *S. cerevisiae* fermentations, and, furthermore, the cellulose fraction was enzymatically hydrolyzed and fermented to ethanol. The QF was ground

with a coffee mill. The corn fiber was mixed with 1% (v/v) H_2SO_4 solution at a ratio of 1.2 g (db) biomass to 5.0 mL, placed in a shallow Pyrex® dish, covered with aluminum foil, and heated at 121°C for 1 h. After being allowed to cool, the liquid was separated from the solids by straining through cheesecloth. The recovered liquid portion was then treated as follows: first, the pH was adjusted to 10.0 by adding $Ca(OH)_2$. Second, 1 g/L of sodium bisulfite was added. Third, the liquid was warmed to 90°C and incubated at this temperature for 30 min. Finally, the liquid was neutralized with H_2SO_4 to pH 7.0. Following neutralization, the resulting precipitates, including gypsum, were removed by centrifugation (10,000*g*, 15 min). The recovered liquid was filter sterilized through a 0.22-µm membrane filter.

Bacterial fermentations were carried out in minibioreactors with automatic pH control that were constructed and operated as described previously *(14,15)*. Each 500-mL Fleaker® culture vessel contained 170 mL of hydrolysate supplemented with 20 mL of a 10X Luria-Bertani solution (10 g/L of tryptone and 5 g/L of yeast extract) and antifoam 289 (0.1 mL/L). Nitrogen was bubbled through the medium for 30 min prior to inoculation to remove oxygen. The fermentation vessels were each inoculated with a 5% (v/v) inoculum from an anaerobic culture of *E. coli* FBR5 grown overnight at 37°C. Fermentations were run at 35°C and stirred magnetically with 1 × 1 in. "X"-shaped stir bars at 300 rpm. The pH was set at 6.5 and maintained by the addition of a concentrated base solution (4 *N* KOH). Ethanol, sugars, organic acids, and optical densities (ODs) (550 nm) were determined periodically with 1.5-mL samples of cultures. Each experiment was run in duplicate.

Analytical Procedures

Activities for cellulase (FPU/mL) and β-glucosidase (IU/mL) were measured by the methods described previously *(16,17)*. ODs (1-cm light path) of cultures were monitored on a Beckman DU-640 Spectrophotometer (Fullerton, CA) at 550 (*E. coli*) or 660 nm (*S. cerevisiae*). Concentrations of sugars and ethanol were determined by HPLC using an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad, Richmond, CA) and refractive index detector. Samples were run at 65°C and eluted at 0.6 mL/min with 5 m*M* H_2SO_4.

Calculations

Ethanol yields and productivities for the fermentations were determined as previously described *(18)*. Ethanol yields for the QF are also reported on a per-bushel-of-corn-processed basis. The ethanol yield equation, which is similar to those derived in ref. *19*, is as follows.

$$\begin{aligned}\text{Ethanol}\left(\text{gal/bu}\right) = {} & \text{Dry Mass Yield}\left(\text{lb/bu corn}\right) \times \text{Carbohydrate Yield}\left(\text{lb/lb biomass}\right)\\ & \times 1.11\left(\text{lb free sugar/lb anhydrous sugar}\right)\\ & \times \text{Fermentation Yield}\left(\text{lb ethanol/lb fermented sugar}\right)\\ & \div 6.58\left(\text{lb ethanol/gal ethanol}\right)\end{aligned}$$

Table 1
Comparison of QF and Corn Fiber[a]

Component	Corn fiber (% w/w db)[a]	QF (% w/w db)
Starch	11–23	15
Cellulose	12–18	17
Xylan	18–28	22
Arabinan	11–19	11
Protein	11–12	11
Oil	2	1

[a]Data from ref. *25*.

The dry biomass yield for QF (db) was assumed to be 3.04 lb/bu. The data on carbohydrate composition for QF (db) are provided in Table 1. The fermentation yield for a theoretical ethanol yield is 0.51 lb of ethanol/lb of sugar(s).

Results and Discussion

Composition of QF

QF samples contained 15% (w/w) starch and 17% cellulose (Table 1). The total carbohydrate composition was 65%. Protein and oils accounted for 12%. The components measured account for 78% of the dried material, the residual material (not tested for) includes ash, extractables, lignin, and lipids. The composition of the QF was, as expected, similar to that found for corn fiber. Corn fiber and QF are both derived from the pericarp and tip portions of the kernel. Most notably, the QF contained approx the same amount of residual starch, which suggests that the modified milling process is as effective at separating starch from the pericarp as a full steeping protocol. Starch recovery is significantly improved compared to previous results for which the starch content of the QF was 42–46% w/w *(2)*. The current study used an improved process that included an additional starch washing step.

Pretreatment and SSF of QF Using S. cerevisiae

The noncellulose carbohydrates present in QF were converted directly to free sugars by hydrolyzing with dilute H_2SO_4. Cellulose was hydrolyzed enzymatically using industrial cellulase preparations. QF was pretreated at various sulfuric acid loadings (0–4.8% g of H_2SO_4/g biomass [db]) to determine the optimal amount required for complete hydrolysis. The temperature for the pretreatment was set at 150°C as suggested *(20)* for the similar substrate of corn fiber. The glucose yield was maximum (92% of starch and cellulose recovered as glucose) at acid loadings of 0.8–3.2% (w/w) (Table 2).

Table 2
Dilute Acid Hydrolysis and Saccharification of QF

Acid Loading (% w/w)[a]	Glucose (%)[b]	Xylose (%)	Arabinose (%)	pH after heating
0 7	5 ± 0	16 ± 0	39 ± 0	4.41 ± 0.07
0.8	92 ± 2	50 ± 6	77 ± 2	2.78 ± 0.00
1.6	90 ± 1	74 ± 9	86 ± 3	2.12 ± 0.07
3.2	92 ± 3	98 ± 6	98 ± 4	1.89 ± 0.07
4.8	87 ± 4	98 ± 8	87 ± 0	1.56 ± 0.09

[a] % g of H_2SO_4/g of biomass.
[b] % of theoretical yield.

Maximum yields for arabinose (98% of available arabinose recovered as free sugar) and xylose (98% of xylan recovered as xylose) occurred at 3.2% (w/w) (Table 2). These percentage yields correspond to recoveries of 0.11 g of arabinose, 0.38 g of glucose, and 0.22 g of xylose/g of QF (db). Heating, even without adding a mineral acid, was sufficient to recover 0.32 g of glucose/g of QF (db) or 75% of the available glucan.

For the yeast fermentations, QF was treated at a high solid loading (14.1% w biomass [db]/total w) and an acid loading of 3.2% (w/w). The highest possible solid loading was used, such that the material formed a mixable slurry. The pretreated QF was neutralized; mixed with cellulase, β-glucosidase, and glucoamylase (to ensure complete starch hydrolysis); and inoculated with *S. cerevisiae*. The cellulase loading was 15 FPU/g of cellulose. The native β-glucosidase activity of the cellulase mixture was supplemented because adding extra β-glucosidase activity has been reported to enhance the rate of SSF *(21)*. The combined hydrolysis and fermentation was completed within 72 h (Fig. 2), and the final ethanol concentration was 23.4 ± 0.1 g/L. The ethanol yield was 0.153 g of ethanol/g of QF (db) or 85 ± 1% of maximum ethanol possible based on the total amount of glucans added and the theoretical ethanol yield from glucose. Assuming that 3.04 lb (db) of QF is recovered per bushel of corn, the fermentation results suggest that an additional 0.096 gal of ethanol/bu can be gained by fermenting QF with *S. cerevisiae*. The theoretical yield, based on the total glucan composition of QF, is 0.113 gal/bushel of corn.

The oils present in the QF differ from those found in the germ (corn oil) and in particular are enriched for phytosterols, which are cholesterol-lowering agents *(5–9)*. A prior study with corn fiber demonstrated that the oils withstood dilute-acid) pretreatment and became enriched in the pretreated solids *(22)*. Therefore, it was of interest to determine whether dilute-acid-pretreated QF solids might also serve as a source for these valuable nutraceutical chemicals. Following SSF, the solids residue was recovered and analyzed for the presence of oils. It was determined that only 1.12% oils was present in the residual solids (post-SSF, Table 3), which is comparable with that found in untreated QF (1.24–3.49% oil as noted in ref. 2).

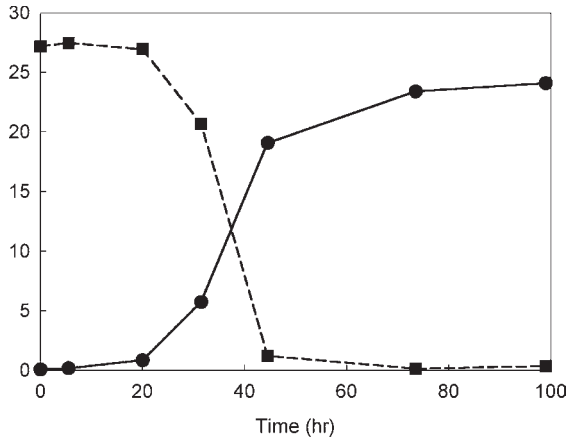

Fig. 2. H_2SO_4 loading was optimized for pretreating DWG for conversion to monomeric sugars. Each point is the average of duplicate runs. (■) Glucose; (●) ethanol.

Table 3
Recovery of Corn Fiber Oil from Process Fiber Residues

Fiber source	Total oil (% w/w)	Free sterol	FPE (wt% oil)	St:E (wt% oil)	Total sterols [a]
Pre-SSF [b]	8.15 ± 0.21	4.43 ± 0.19	3.27 ± 0.04	7.9 ± 0.1	15.6
Post-SSF [c]	1.12 ± 0.06	6.03 ± 3.74	5.82 ± 3.66	11.8 ± 5.7	23.6
Post-FBR5 ferm [d]	8.28 ± 0.14	5.80 ± 0.79	4.29 ± 0.69	12.2 ± 1.8	22.3

[a] Sum of prior three columns.
[b] Pretreated with dilute acid.
[c] Residual solids from SSF fermentation.
[d] Residual fiber from hydrolysate prepared for FBR5 fermentation.

One possible explanation for why the oils were not enriched is that they were diluted out by yeast and gypsum, from neutralizing with lime, mixed in with the recovered SSF residue. This possibility was partially tested by extracting the oils from the washed, pretreated QF prior to fermentation. The washed solids analyzed prior to neutralization and SSF contained 7 times more oil and 4.8 times more total phytosterols (pre-SSF, Table 3) than the recovered solid post-SSF. Therefore, pretreated QF is a valuable source of phytosterols, provided that they are recovered from the solids prior to SSF.

Pretreatment and Fermentation of QF Using E. coli *FBR5*

Sixty-five percent of the carbohydrates present in QF are in the form of pentoses, which *S. cerevisiae* does not ferment to ethanol. We have developed a recombinant *E. coli* strain that is capable of fermenting arabinose,

Table 4
Fermentation Results for Various Fibrous Feedstocks Using Ethanologenic Strain FBR5[a]

Feedstock	Initial sugar concentration			Maximum ethanol (% w/v)	Ethanol yield (g/g)	Ethanol productivity (g/[L·h])	Reference
	Arabinose (% w/v)	Glucose (% w/v)	Xylose (% w/v)				
QF	1.47	3.13	3.40	3.52 ± 0.03	0.44 ± 0.00	0.43 ± 0.04	This study
DWG[b]	0.79	1.96	1.23	2.12 ± 0.05	0.49 ± 0.01	0.71 ± 0.01	*11*
Corn fiber	2.00	2.80	3.70	3.74 ± 0.01	0.46 ± 0.00	0.77 ± 0.05	*11*

[a] Each result is based on duplicate fermentations.
[b] Broin Distiller wet grains.

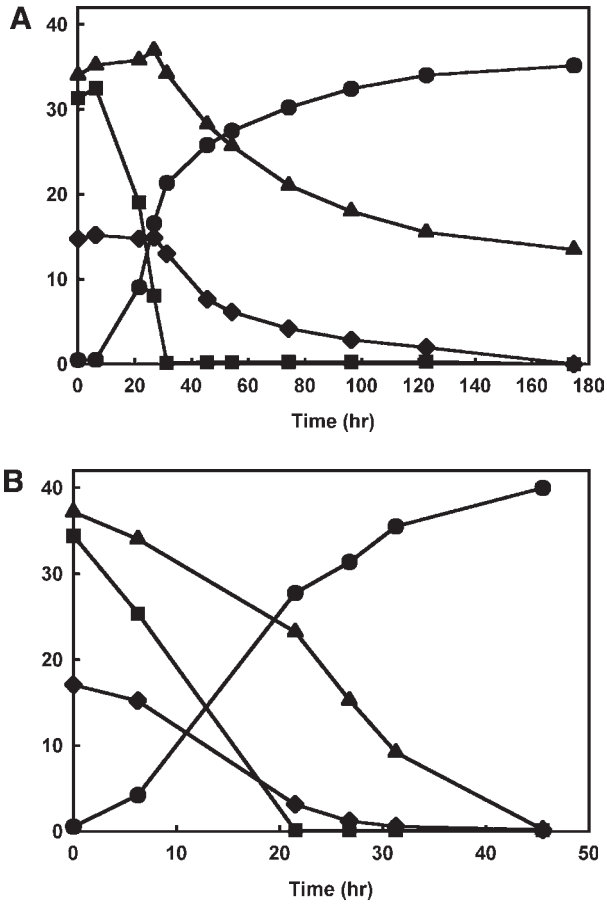

Fig. 3. **(A)** SSF of pretreated DWG with *S. cerevisiae.* Fermentations were performed in duplicate. **(B)** Fermentation of DWG liquid hydrolysate with *E. coli* FBR5. Fermentations were performed in duplicate. (▲) Xylose; (■) glucose; (◆) arabinose; (●) ethanol.

glucose, and xylose into ethanol *(10)*. The same pretreatment protocol was used for the present experiments as had been used previously for corn fiber produced by wet milling. Unlike the protocol described herein for the *S. cerevisiae* fermentations, only the liquid portion of the pretreated material was fermented to avoid a high solids content in the bioreactor. Cellulase was not added to the hydrolysate because cellulose partitions with the solids. The recovered liquid fraction from the hydrolysate contained 9.4% (w/v) total sugars (data not shown). *E. coli* FBR5 fermented all of the arabinose, glucose, and much of the xylose into ethanol (Fig. 3A). The final ethanol concentration was 3.51% (w/v), which is equal to 85% of the theoretical maximum ethanol yield based on the beginning sugar concentration of the medium (Table 4). The remaining 15% of the sugar(s) not converted to ethanol can be accounted for as residual xylose. The overall ethanol yield for FBR5 was 0.116 g of ethanol/g of QF (db).

A sugar mixture was prepared at a concentration similar to that of the QF hydrolysate using reagent-grade sugars. Fermentation of this sugar mixture served as a control. In contrast to the hydrolysate, all of the sugars were readily fermented (Fig. 3B), and the final production yield was 98% of theoretical. Furthermore, the ethanol productivity of the control fermentations was 57% faster than that of the hydrolysate. The difference in yield and rate can be attributed to microbial inhibitors that are formed during the hydrolysate preparation process *(23,24)*. Numerous inhibitors are formed during pretreatment and hydrolysis, and their effects are synergetic. One inhibitor that was detected in the QF was acetic acid (5 g/L), which arose from the acetyl side groups on the hemicellulose.

The additional yield per bushel of corn realized with FBR5 was 0.055 gal/bu. Ideally, 0.126 gal/bu would be realized by converting all of the pentosans and starch to ethanol at the theoretical ethanol yield (0.51 g of ethanol/g of fermented sugars). However, most of the loss in yield (73%) is associated with the pretreatment step. Fermentable sugars are lost during pretreatment by failing to recover all of the free sugars from the solid cake. The solids were not washed so as not to dilute the recovered sugars. Sugars can also be lost during pretreatment by degradation reactions; however, very little furfural and hydroxymethylfurfural were detected by HPLC in the hydrolysate (data not shown).

The residual solids from the QF pretreated for the FBR5 fermentation were analyzed for their oil content. The total oil content for the washed cake (Table 3) was 8.28% (w/dw) and the yield of total phytosterols was equal to 1.89% (w/dw). The yield compared favorably to that of an earlier study in which corn fiber was pretreated with dilute H_2SO_4 for which the yield of total phytosterols was 1.43% (w/dw) *(22)*. The pretreatment step concentrated the total phytosterols 26 times compared to untreated QF *(2)*.

As noted previously, QF is derived from the pericarp fraction of the corn. In dry grinding and wet milling, this fraction ends up in the DWG and corn fiber, respectively *(1)*. Both of these feedstocks have been converted to ethanol using *E. coli* FBR5 own this laboratory using the same protocol as described herein for QF (Table 4). Strain FBR5 produced the highest ethanol concentrations from corn fiber and QF, as expected because the prepared hydrolysates had approximately twice the concentration of sugars of the DWG. The ethanol production yields were also similar for corn fiber and QF, 86–90% of theoretical. However, FBR5 fermented the QF hydrolysate at a much slower rate (44% slower based upon average productivity) than the corn fiber hydrolysate. The slower rate suggests that the QF hydrolysate was more inhibitory to fermentation than the corn fiber. This result was unexpected and suggests that some difference in the collection of the two materials may be responsible. Possibly, the conventional steeping process "washes away" an inhibitor that is not removed during the much gentler steeping used to produce QF. One solution may be to include an additional step to further remove the inhibitors present following hydrolysis in addition to overliming.

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References

1 Dien, B. S., Bothast, R. J., Nichols, N. N., and Cotta, M. A. (2002), *Int. Sugar J.* **104(1241),** 204–207.
2 Singh, V., Moreau, R. A., Doner, L. W., Eckhoff, S. R., and Hicks, K. B. (1999), *Cereal Chem.* **76(6),** 868–872.
3 Wahjudi, J., Xu, L., Wang, P., Singh, V., Buriak, P., Rausch, K. D., Mcaloo, A. J., Tumbleson, M. E., and Eckhoff, S. R. (2000), *Cereal Chem.* **77(5),** 640–644.
4 Taylor, F., Mcaloon, A. J., Craig, J. C., Yang, P., Wahjudi, J., and Eckhoff, S. R. (2001), *Appl. Biochem. Biotechnol.* **94(1),** 41–49.
5 Moreau, R. A., Hicks, K. B., and Norton, R. A. (1998), US patent no. 5,843,499.
6 Moreau, R. A., Powell, M. J., and Hicks, K. B. (1999), *J. Agric. Food Sci.* **44,** 2149–2154.
7. Moreau, R. A., Singh, V., Nunez, A., and Hicks, K. B. (2000), *Biochem. Soc. Trans.* **28,** 803–806.
8 Hicks, K. B. and Moreau, R. A. (2001), *Food Technol.* **55(1),** 63–67.
9 Moreau, R. A., Whitaker, B. D., and Hicks, K. B. (2002), *Prog. Lipid Res.* **41(6),** 457–500.
10 Dien, B. S., Hespell, R. B., Wyckoff, H. A., and Bothast, R. J. (1998), *Enzyme Micro. Technol.* **23(6),** 366–371.
11 Dien, B. S., Nichols, N. N., O'Bryan, P. J., and Bothast, R. J. (2000), *Appl. Biochem. Biotechnol.* **84–86,** 181–196.
12 Singh, V. and Eckhoff, S. R. (1996), *Cereal Chem.* **73(6),** 716–720.
13 Dien, B. S., Bothast, R. J., Iten, L. B., Barrios, L., and Eckhoff, S. R. (2002), *Cereal Chem.* **79(4),** 582–585.
14 Dien, B. S., Hespell, R. B., Ingram, L. O., and Bothast, R. J. (1997), *World J. Microbiol. Biotechnol.* **13(6),** 619–625.
15 Beall, D. S., Ohta, K., and Ingram, L. O. (1991), *Biotechnol. Bioeng.* **38(3),** 296–303.
16 Ghose, T. K. (1987), *Pure Appl. Chem.* **59(2),** 257–268.
17 Hespell, R. B., Wolf, R., and Bothast, R. J. (1987), *Appl. Environ. Microbiol.* **53(12),** 2849–2853.
18 Nichols, N. N., Dien, B. S., and Bothast, R. J. (2001), *Appl. Microbiol. Biotechnol.* **56(1–2),** 120–125.
19 Gulati, M., Kohlmann, K., Ladisch, M. R., Hespell, R., and Bothast, R. J. (1996), *Bioresour. Technol.* **58(3),** 253–264.
20 Grohmann, K. and Bothast, R. J. (1997), *Process Biochem.* **32(5),** 405–415.
21. Grohmann, K. (1993), in *Bioconversion of Forest and Agricultural Plant Residues*, 1st Ed., Saddler, J. N., ed., C.A.B. International, Wallingford, UK, pp. 183–210.
22 Singh, V., Johnston, D. B., Moreau, R. A., Hicks, K. B., Dien, B. S., and Bothast, R. J. (2003), *Cereal Chem.* **80(2),** 126–129.
23 Palmqvist, E. and Hahn-Hagerdal, B. (2000), *Bioresour. Technol.* **74(1),** 17-24.
24 Palmqvist, E. and Hahn-Hagerdal, B. (2000), *Bioresour. Technol.* **74(1),** 25–33.
25 Leathers, T. D. (1998), *Soc. Ind. Microbiol. News* **48(5),** 210–217.