

Hydrolysis and Fermentation of Pericarp and Endosperm Fibers Recovered from Enzymatic Corn Dry-Grind Process

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ABSTRACT

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A modified dry-grind corn process has been developed that allows recovery of both pericarp and endosperm fibers as coproducts at the front end of the process before fermentation. The modified process is called enzymatic milling (E-Mill) dry-grind process. In a conventional dry-grind corn process, only the starch component of the corn kernel is converted into ethanol. Additional ethanol can be produced from corn if the fiber component can also be converted into ethanol. In this study, pericarp and endosperm fibers recovered in the E-Mill dry-grind process were eval-

uated as a potential ethanol feedstock. Both fractions were tested for fermentability and potential ethanol yield. Total ethanol yield recovered from corn by fermenting starch, pericarp, and endosperm fibers was also determined. Results show that endosperm fiber produced 20.5% more ethanol than pericarp fiber on a g/100 g of fiber basis. Total ethanol yield obtained by fermenting starch and both fiber fractions was 0.370 L/kg compared with ethanol yield of 0.334 L/kg obtained by fermenting starch alone.

Renewable sources of energy are needed to replace finite fossil fuels and meet energy needs in the future. Ethanol is fast becoming the renewable fuel of choice and its production is increasing exponentially in the United States and around the world. Most of the ethanol in the United States is produced from corn. The starch in corn kernel can be converted into sugars and fermented to produce ethanol. The U.S. ethanol production capacity is projected to increase to more than 6.0 billion gallons per year by the end of 2006 (MacDonald et al 2003). Most of this increase in the ethanol capacity, from a current capacity of 3.1 billion gallons, will come from new dry-grind corn plants. In a conventional dry-grind ethanol process, corn is ground and mixed with water to produce slurry. The slurry is cooked and the starch in the slurry is liquefied, saccharified, and fermented to produce ethanol. The remaining nonfermentables in the corn (germ, fiber, and protein) are recovered at the end of the dry-grind process as a feed coproduct called distiller dried grains with solubles (DDGS).

A process modification called enzymatic milling (E-Mill) has been developed for conventional corn dry-grind process for ethanol production (Singh et al 2004). The E-Mill process recovers germ, pericarp (coarse) fiber, and endosperm (fine) fiber separately as coproducts at the beginning of the dry-grind process before fermentation. The E-Mill process involves soaking corn kernels for a short period of time (6–12 hr) followed by coarse grinding and incubating with protease and starch-degrading enzymes for 2–4 hr. After incubation, germ and pericarp fiber are recovered by floatation, and the remaining slurry is screened on a 200-mesh (0.074 mm opening) sieve to recover the endosperm fiber (Singh et al 2004).

The coproducts (germ, pericarp fiber, and endosperm fiber) recovered in the E-Mill process can be used as feedstocks for the recovery of other valuable coproducts such as corn germ oil (from germ) and corn fiber oil (from pericarp and endosperm fiber). Corn fiber oil contains compounds that lower levels of serum LDL-cholesterol (Moreau et al 1998; Hicks and Moreau 2001). Pericarp

and endosperm fibers can also be hydrolyzed into C5 sugars, which are predominantly arabinose and xylose. Hydrolysis of corn fiber improves the extraction efficiency of corn fiber oil and does not affect its composition (Singh et al 2003; Dien et al 2004). Furthermore, these C5 sugars can be fermented by recombinant organisms and converted into ethanol, thus increasing the total ethanol production from corn. In this work, the recombinant *Escheria coli* strain FBR5 was used to ferment the C5 sugars to ethanol. While *E. coli* strains are capable of fermenting these sugars, they produce a mixture of ethanol and organic acids. The *E. coli* FBR5 has been mutated to eliminate production of these native products and transformed with a plasmid carrying the genes from *Zymomonas mobilis* (pyruvate decarboxylase and alcohol dehydrogenase) needed for redirecting the fermented sugars into ethanol production. A full description of this strain can be found in Dien et al (1998).

One of the unique features of the E-Mill process is that it recovers pericarp and endosperm fibers separately. Currently, the fiber recovered in a corn wet-milling process is a combination of pericarp and endosperm fibers and is called corn fiber (or white fiber). White fiber has been investigated extensively for hydrolysis and fermentation (Bothast et al 1996). However, no information is available on the hydrolysis and fermentation of pericarp and endosperm fibers individually. Previous work on fiber analysis shows that the structure and composition of pericarp and endosperm fibers were quite different in terms of arabinose and xylose content (Singh et al 2000). In this study, pericarp and endosperm fibers were evaluated as a feedstock for ethanol production.

MATERIALS AND METHODS

Experimental Material

A yellow dent corn hybrid grown during the 2002 crop season at the Agricultural Engineering Research Farm, University of Illinois at Urbana-Champaign, was field dried to $\approx 15.0\%$ moisture content and combine-harvested. Corn samples were hand-cleaned to remove broken corn and foreign material, packaged in plastic bags, and stored at 4°C until processing. Whole kernel moisture content was measured using a 103°C convection oven method (Approved Method 44-15A, AACC International 2000).

E-Mill Dry-Grind Laboratory Process

E-Mill dry-grind processing was done using 600-g corn samples. The corn samples were soaked in 1,200 mL of tap water for 12 hr. After soaking, samples were ground coarsely with remaining soak water plus an additional 500 mL of tap water and incubated with starch-degrading enzymes for 2 hr (55°C, pH 5.0)

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and protease enzymes (GC106, Genencor International, Palo Alto, CA) for 2 hr (45°C, pH 5.0) with intermittent gentle mixing of the sample every 30 min. After the enzyme incubation step, germ and pericarp fiber were recovered by floatation using the procedure developed by Singh and Eckhoff (1996) and Singh et al (1999), respectively. The remaining slurry was screened through a standard 200-mesh (0.074-mm opening) sieve and washed with 100 mL of distilled water to recover endosperm fiber. The slurry (after germ, pericarp fiber, and endosperm fiber removal) was liquefied, saccharified, and fermented using the conventional dry-grind ethanol procedure developed by Singh et al (2004). Four E-Mill runs were made in parallel and combined to obtain sufficient fiber for further experiments. Both pericarp and endosperm fiber fractions were dried, and the moisture content was determined using the two-stage convection oven method (Approved Method 44-18, AACC International 2000).

Composition of Pericarp and Endosperm Fiber Samples

The endosperm and pericarp fibers were analyzed for xylose and arabinose contents by hydrolyzing in 2M trifluoroacetic acid at 100°C for 1 hr. Sugars were measured using an HPLC equipped with an Aminex 87H column and an RI detector (Dien et al 1997). Crude protein, crude fat, ash, cellulose, lignin, starch content for pericarp, and endosperm fibers were determined by a commercial laboratory using standard analytical procedures.

Bacterial Strains, Growth Media, and Reagents

Saccharomyces cerevisiae (Y-2034, ARS Culture Collection, Peoria, IL) and *E. coli* FBR5 strains were stored frozen (-80°C) in 50% (v/v) glycerol stocks. Yeast cultures were 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 32°C. The *E. coli* FBR5 were routinely grown at 37°C on either LB plates with 10 mg/L of tetracycline added for plasmid maintenance or in anaerobic liquid LB cultures containing 4 g/L as previously described (Dien et al 1998). Enzymes were supplied by Novozyme (Franklinton, NC) and included cellulase (Celluclast 1.5 L; 48 international filter paper units/mL), β-glucosidase (Novozym 188; 66.8 × 10³ units/mL), and glucoamylase (Novozyme AMG300L). Sugars were purchased from Sigma (St. Louis, MO); chemical and media reagents were purchased from Fisher Scientific (Fairview, NJ).

Hydrolysis and Fermentation of Pericarp and Endosperm Fibers

Either 12.5 g, db, of the fine or coarse fibers were mixed with 125 mL of 1% (w/v) H₂SO₄ and treated at 121°C for 1 hr in an autoclave. The solids and syrup were separated using a glass fiber filter. The syrup was adjusted to pH 7.0 using Ca(OH)₂. The neutralized solution was centrifuged (10,000 × g, 15 min) to remove solids (including gypsum), filter sterilized (through a 0.22-μm

membrane filter), and stored at 4°C before fermentation. The schematic for the experimental protocol for *E. coli* FBR5 and *S. cerevisiae* fermentation is shown in Fig. 1.

Aliquots (20 mL) of syrup plus 5 mL of a 10× stock solution of LB and MOPS, pH 7 (100 g/L of tryptone, 50 g/L of yeast extract, 1M MOPS) were fermented using *E. coli* FBR5 in 50-mL Erlenmeyer flasks fitted with butyl rubber stoppers vented with 22-gauge needles. The inoculum was grown on anaerobic LB supplemented with 20 g/L of xylose and 0.1% acetate at 37°C for ≈18 hr. Fermentation cultures were inoculated with 2.5 mL of the culture grown overnight. To avoid carryover of residual xylose from the precultures, the inoculum was centrifuged and the collected cell pellet was transferred to the fermentation flasks. Fermentations were done at 35°C for 96 hr under 75 rpm agitation. The *E. coli* FBR5 fermentations were sampled daily.

The solids (≈2 g, db) were washed with distilled water. An aliquot was dried at 105°C, and weight loss was monitored to determine moisture content. The solids were mixed with 20 mL of water and sterilized by autoclaving. The sterile solids were mixed with 32 units of a filter-sterilized 1:1 mixture of cellulase and β-glucosidase (45 fpu/mL) and fermented using *S. cerevisiae* in basal YP (10 g/L of yeast extract and 20 g/L of Bacto peptone) medium containing 50 mM citric acid, pH 4.8. Fermentations were conducted in 50-mL Erlenmeyer flasks fitted with butyl rubber stoppers and vented with a 22-gauge needle. The *S. cerevisiae* inoculum was grown on YP supplemented with 5% glucose for 18 hr at

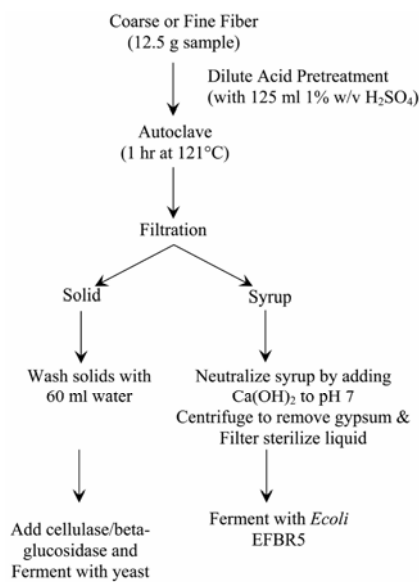


Fig. 1. Experimental protocol for hydrolysis and fermentation of fiber samples.

TABLE I
Compositional Analysis (dry basis) of Pericarp and Endosperm Fiber Fractions^a

Composition (%)	Pericarp Fiber	Endosperm Fiber	Corn Fiber ^b
Crude protein	11.87 ± 0.06	14.31 ± 0.08	10.98
Crude fat	5.83 ± 0.61	0.61 ± 0.05	2.53
Ash	0.97 ± 0.05	0.49 ± 0.08	0.60
Cellulose	13.17 ± 0.28	4.59 ± 0.38	17.51
Lignin	1.01 ± 0.06	0.52 ± 0.09	7.78
Starch	21.17 ± 1.51	57.51 ± 1.76	19.68
Xylose/galactose ^c	17.73 ± 0.41	4.72 ± 0.90	21.20
Arabinose	9.22 ± 0.17	3.32 ± 0.51	11.20
Acetate	1.98 ± 0.03	0.56 ± 0.09	1.71
Total	82.94 ± 0.02	86.62 ± 0.03	93.19

^a Average ± standard deviation.

^b Grohmann and Bothast (1997).

^c Galactose coelutes with xylose. Galactose was 21 and 70% of the total xylose/galactose value in pericarp and endosperm fibers, respectively.

TABLE II
Sugar Yields (% of maximum) in Sterile Syrup Recovered After Dilute Acid Treatment of Fiber

Fiber Fraction	Recovered Syrup (mL)	Glucose	Xylose	Arabinose	Total CHO	Acetate
Pericarp	87	84	75	72	79	74
Pericarp	85	83	76	73	79	72
Pericarp	84	78	71	71	74	74
Average	85	82	74	72	77	73
Endosperm	88	80	82	66	79	67
Endosperm	105	97	88	74	95	77
Endosperm	99	92	87	74	90	74
Average	97	90	86	71	88	73

TABLE III
Results from Fermentation of Syrup with *Escheria coli* FBR5^a

Fraction	Initial Sugar Conc. (% w/v)	EtOH Conc. (% w/v)	Residual CHO Conc. (% w/v)	Production Yield (% of maximum)	Metabolic Yield (% of maximum)	Process Yield (g of EtOH/100 g of fiber)
Pericarp	4.65	2.09	0.089	88.0	89.8	14.5
Pericarp	4.77	2.27	0.099	93.3	95.2	15.4
Pericarp	4.48	2.07	0.098	90.4	92.4	13.9
Average	4.63	2.14	0.100	90.6	92.5	14.6 ^a
Endosperm	6.21	2.77	0	87.4	87.4	19.5
Endosperm	5.93	2.70	0	89.3	89.3	22.7
Endosperm	5.98	2.72	0	89.3	89.3	21.6
Average	6.00	2.70	0	88.7	88.7	21.3

^a Average values are significantly different at $P < 0.05$.

30°C under 250 rpm agitation. The fermentation cultures were inoculated at an A_{600} of 0.5 nm for the yeast inoculum. The *S. cerevisiae* fermentation was done at 30°C for 96 hr under 100 rpm agitation. Samples were taken at the end of the fermentation to reduce ethanol evaporation.

Statistical Analysis

Both pericarp and endosperm fiber fermentations were conducted in triplicate. Fermentation samples from three replicates were analyzed using HPLC with at least two determinations. Analysis of variance (ANOVA) and Duncan's multiple range test (SAS Institute, Cary, NC) were used to compare means of ethanol yield for pericarp and endosperm fibers. The level selected to show statistical significance was 5% ($P < 0.05$).

RESULTS AND DISCUSSION

Composition of Pericarp and Endosperm Fibers

Pericarp fiber consisted of 13.2% cellulose, 17.7% xylose, 9.2% arabinose, and 21.2% starch (Table I). The total noncellulose carbohydrate composition was 48.1%. The components measured account for 82.9% of the dried material. The endosperm fiber, on average, consisted of 4.6% cellulose, 4.7% xylose, 3.3% arabinose, and 57.5% starch (Table I). The total noncellulose carbohydrate in endosperm fiber was 65.6%. The components measured account for 86.6% of the dried material in endosperm fiber. Other material (not determined) in pericarp and endosperm fibers include extractables and organic acids such as uronic, ferulic, and gluronic acid. Comparison between the composition of the pericarp and endosperm fibers and composition of corn fiber (which is a combination of pericarp and endosperm fibers) reported by Grohmann and Bothast (1997) shows comparable values, except for lignin. Grohmann and Bothast (1997) determined Klason lignin, which tends to give higher values.

As expected, residual starch content in the endosperm (fine) fiber was much higher than in the pericarp fraction. Most of the bound starch in the fiber fraction is within the endosperm fiber fraction (Dowd 1997). Previous reported starch contents from endosperm fiber had a range of 35–58% (Singh et al 1999, 2000). The compositional results are significant because they show fiber-

associated starch is primarily associated with the endosperm fractions. Therefore, the efficiency of the E-Mill dry-grind process can be significantly enhanced by recovering the starch from this fraction and converting it into ethanol.

Hydrolysis and Fermentation of Pericarp and Endosperm Fiber Using *E. coli* FBR5

The noncellulose carbohydrates present in pericarp and endosperm fibers were hydrolyzed to free sugar by dilute sulfuric acid treatment (Fig. 1). The syrup recovered after dilute acid pretreatment gave an average glucose yield of 82% for pericarp fiber and 90% for endosperm fiber fractions (Table II). The average yields of xylose and arabinose (in syrup) were 74 and 72%, respectively, for pericarp fiber. The average yields of xylose and arabinose in syrup were 86 and 71%, respectively, for endosperm fiber. The final average recovered sugars, based on noncellulose carbohydrates, were 77% for pericarp fiber and 88% for endosperm fiber fractions (Table II). Possible losses include sugars retained with the cake and unhydrolyzed carbohydrates. In this study, the cake was not washed for residual sugars to avoid diluting the syrup.

The hydrolysate was fermented with a recombinant *E. coli* strain that is capable of fermenting arabinose, glucose, and xylose into ethanol (Dien et al 1998). The initial sugars in the hydrolysate were 4.6% (w/v) for pericarp fiber and 6.0% for the endosperm fiber fraction (Table III). The higher sugar concentration for the endosperm fraction reflects its higher starch content. The sugars in hydrolysates were completely fermented by *E. coli* FBR5, which indicates a low level of inhibitors. The final average ethanol concentrations were 2.1% (w/v) for pericarp fiber and 2.7% (w/v) for endosperm fiber (Table III). Ideally, the *E. coli* FBR5 can yield a maximum of 0.51 g of ethanol/g of fermentable sugar. The yield efficiencies observed here were 90.5% of this value for the pericarp fiber and 88.7% for the endosperm fiber fraction. The expected ethanol yields based on processed fibers are 14.6 g/100 g of pericarp fiber and 21.3 g/100 g of endosperm fiber (Table II).

Hydrolysis and Fermentation of Pericarp and Endosperm Fiber Using *S. cerevisiae*

The dilute acid treatment, on average, solubilized 79% pericarp fiber and 90% endosperm fiber fraction, which reflects the high

TABLE IV
Results from Fermentation of Residual Solids with Yeast

Fraction	Solubilization (%)	EtOH Conc. (% w/v)	Ethanol Yield ^a	
			Residual Biomass (g/g of)	Starting Biomass (g/100 g of)
Pericarp	78.8	1.602	0.23	4.80
Pericarp	80.1	1.463	0.21	4.08
Pericarp	78.1	1.477	0.21	4.65
Average	79.0	1.514	0.22	4.51 ^b
Endosperm	92.0	0.708	0.20	1.64
Endosperm	92.1	0.863	0.25	1.99
Endosperm	86.7	0.809	0.14	1.87
Average	90.3	0.793	0.20	1.83

^a Average values are significantly different at $P < 0.05$.

^b Residual biomass is everything collected in the cake after hydrolysis. Starting biomass is the total amount of beginning biomass before hydrolysis.

TABLE V
Ethanol Yields from Pericarp Fiber, Endosperm Fiber, and Starch Fermentations^a

Fraction	Ethanol Yield (g EtOH/100 g fiber)		Total EtOH (g EtOH/100 g of fiber)	Fiber Yield ^b (%)	Ethanol Produced	
	FBR5 Ferm.	Yeast Ferm.			(g of EtOH/600 g of corn)	L/kg (gal/bu)
Pericarp	14.5	4.80	19.3	10.2	11.7	0.029 (0.21)
Pericarp	15.4	4.08	19.5			
Pericarp	13.9	4.65	18.6			
Average	21.3	1.80	23.1 ^a			
Endosperm	19.5	1.64	21.1	4.5	6.2	0.015 (0.11)
Endosperm	22.7	1.99	24.7			
Endosperm	21.6	1.87	23.4			
Average	14.6	4.50	19.1			
Starch ^b					180.0	0.334 (2.40)
Combined ^c					197.9	0.378 (2.72)

^a Average values are significantly different at $P < 0.05$.

^b After removal of germ, pericarp fiber, and endosperm fiber (Singh et al 2005).

^c Pericarp + endosperm + starch.

noncellulosic content of the fibers. The cellulose, which is not converted to sugars directly by acid hydrolysis, can be conveniently converted to glucose by treating with cellulases. End-product inhibition of cellulase enzymes by glucose can be avoided by treating the cellulose in the presence of *S. cerevisiae*. The yeast readily converts glucose formed by hydrolysis to ethanol, thereby preventing end-product inhibition (Fig. 1). When the residual fiber cakes were treated with cellulase, the final ethanol concentration was 1.51% (w/v) for pericarp fiber and 0.79% (w/v) for endosperm fiber (Table IV). The average ethanol yield was 0.22 g/g of residual biomass for pericarp fiber and 0.20 g/g of residual biomass for endosperm fiber. The average ethanol yield was 4.5 g for pericarp fiber and 1.8 g for endosperm on a starting biomass basis of g/100 g.

Combined Ethanol Yield from Pericarp Fiber, Endosperm Fiber, and Starch Fermentations

Combined average ethanol yield from fiber fermentations using *S. cerevisiae* and *E. coli* was 19.1 g/100 g of pericarp fiber and 23.1 g/100 g of endosperm fiber (Table V). Combined average ethanol yield was $\approx 20.5\%$ higher for endosperm fiber compared with pericarp fiber. Higher ethanol yield for endosperm fiber is due to a higher amount of starch in the endosperm fiber fraction compared with the pericarp fiber fraction. Total ethanol produced in L/kg (gal/bu) was 0.029 (0.21), 0.015 (0.11), and 0.334 (2.40) for pericarp fiber, endosperm fiber, and starch fermentations, respectively. Fermentation of both fiber fractions in addition to starch fermentation will increase ethanol yield in a dry-grind plant by 13.3%.

CONCLUSIONS

Both pericarp and endosperm fibers recovered from an E-Mill dry-grind process can be hydrolyzed into simple sugars by dilute

sulfuric acid treatment and enzymatic treatment with cellulases. The hydrolysates can be readily fermented by conventional yeast (*S. cerevisiae*) and recombinant organisms (*E. coli* FBR5) into ethanol, indicating a low degree of fermentation inhibitors. Average ethanol yield from fiber fermentations using *S. cerevisiae* and *E. coli* was 19.1 g/100 g of pericarp fiber and 23.1 g/100 g of endosperm fiber. Ethanol yield of endosperm fiber is slightly higher than that of pericarp fiber because of higher amounts of residual starch in the endosperm fiber fraction. By converting these fiber fractions into ethanol, the starch that is lost during the recovery of pericarp and endosperm fibers is converted into ethanol. Ethanol yield can be increased by 13.3% by converting these fiber fractions into ethanol along with the starch fraction. Therefore, there are two main advantages of converting corn fiber into ethanol in a dry-grind corn plant: 1) higher yield of ethanol can be achieved, and 2) the residual starch lost in fiber is converted into ethanol.

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