Comparison of Enzymatic (E-Mill) and Conventional Dry-Grind Corn Processes Using a Granular Starch Hydrolyzing Enzyme

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ABSTRACT

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A new low temperature liquefaction and saccharification enzyme STARGEN 001 (Genencor International, Palo Alto, CA) with high granular starch hydrolyzing activity was used in enzymatic dry-grind corn process to improve recovery of germ and pericarp fiber before fermentation. Enzymatic dry-grind corn process was compared with conventional dry-grind corn process using STARGEN 001 with same process parameters of dry solid content, pH, temperature, enzyme and yeast usage, and time. Sugar, ethanol, glycerol and organic acid profiles, fermentation rate, ethanol and coproducts yields were investigated. Final ethanol concentration of enzymatic dry-grind corn process was 15.5 ± 0.2% (v/v), which was 9.2% higher than conventional process. Fermentation rate was also higher for enzymatic dry-grind corn process. Ethanol yields of enzymatic and conventional dry-grind corn processes were 0.395 ± 0.006 and 0.417 ± 0.002 L/kg (2.65 ± 0.04 and 2.80 ± 0.01 gal/bu), respectively. Three additional coproducts, germ 8.0 ± 0.4% (db), pericarp fiber 7.7 ± 0.4% (db), and endosperm fiber 5.2 ± 0.6% (db) were produced in addition to DDGS with enzymatic dry-grind corn process. DDGS generated from enzymatic dry-grind corn process was 66% less than conventional process.

In the last five years there has been exponential growth in dry-grind ethanol production (RFA 2005). In the dry-grind process, corn is ground, mixed with water to form a slurry, cooked, liquefied, saccharified, and fermented to produce ethanol. Nonfermentable materials (germ, fiber, and protein) in corn are recovered at the end of the process as distillers dried grains with solubles (DDGS). The net corn cost (corn cost minus coproduct credits) is high in the dry-grind process due to low value of DDGS ($79/ton) (Baker and Allen 2004). New technologies are being developed to fractionate the corn kernel and recover nonfermentables before fermentation. One of the new technologies is the enzymatic dry-grind corn process (Singh et al 2005). In the enzymatic dry-grind corn process (Fig. 1), corn is soaked in water for a short period of time followed by coarse grinding with water (in addition to soak water). In two steps, the slurry is incubated with enzymes at different pH and temperatures. After incubation, germ and pericarp fiber are skimmed and endosperm fiber is screened. The remaining slurry is liquefied, saccharified, and fermented to produce ethanol. Protein content of DDGS from enzymatic dry-grind corn process was 58% and acid detergent fiber was 2% compared with 28% protein and 11% acid detergent fiber in conventional DDGS (Singh et al 2005).

Recently, a new granular starch hydrolyzing enzyme STARGEN 001 (Genencor International, Palo Alto, CA) for ethanol production has been developed. STARGEN 001 can convert starch into dextrins at low temperatures as well as hydrolyze dextrins into fermentable sugars. In this study, STARGEN 001 was used in the enzymatic dry-grind corn process to improve recovery of coproducts before fermentation. With STARGEN 001, only one step incubation was tested in enzymatic dry-grind corn process instead of two steps previously used (Singh et al 2005) and no liquefaction step was used (Fig. 2). The objective of this study was to compare the enzymatic dry-grind corn process with the conventional dry-grind corn process using STARGEN 001.

MATERIALS AND METHODS

A yellow dent corn 33A14 (Pioneer Hi-Bred International, Johnston, IA) grown in 2004 at the Agricultural and Biological Engineering Research Farm, University of Illinois at Urbana-Champaign, was used for this study. Corn samples were sieved over a 4.8 mm (12/64") round hole screen to remove broken corn and were hand-cleaned to discard foreign materials. Cleaned samples were packed in plastic bags and stored at 4°C. Before dry-grind processing, corn was equilibrated at room temperature. Whole corn moisture content was measured in triplicate using a 103°C convection oven method (Approved Method 44-15A, AACC International 2000).

Conventional Dry-Grind Corn Process

Triplicate cleaned corn samples were ground in a hammer mill (model MHM4, Glen Mills, Clifton, NJ) at 500 rpm using a 2-mm sieve with round holes (Fig. 3). The ground corn moisture content was measured (Approved Method 44-19, AACC International 2000). Corn (700 g) was mixed with 1,800 mL of water to obtain mash with 24.5% dry solids content before fermentation. The mash was adjusted to pH 4.2 using 10*N* sulfuric acid solution. Mash was incubated with 2 mL of STARGEN 001 and 1 mL of acid fungal protease GC106 (Genencor International, Palo Alto, CA) enzymes at 48°C for 3 hr with agitation at 20 rpm. After incubation, simultaneous saccharification and fermentation (SSF) was conducted to produce ethanol. Mash was cooled to 30°C and adjusted to pH 4.0 using 10*N* sulfuric acid solution. In SSF, 35 mL of yeast culture, 2 mL of STARGEN 001, 0.5 mL of GC106, and 0.5 g of $(NH_4)_2SO_4$ were added. Yeast culture was prepared by dispersing 11 g of active dry yeast (Fleischmann's Yeast, Fenton, MO) and 1 g of yeast malt broth in 89 mL of distilled water and agitated at 50 rpm at 30°C for 20 min (C24 Incubator Shaker, New Brunswick, NJ). It had a viable cell count of 1.8×10^8. The SSF process was performed in a 3L flask with an overhead drive (model DHOD-182, Bellco Glass, Vineland, NJ) for agitation. SSF was completed at 30°C for 72 hr at 50 rpm. Fermentation was monitored by taking 3-mL samples at 0, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, and 72 hr and analyzing them using HPLC. Each sample was centrifuged at 1,789 × *g* for 5 min (Centra CL3, Thermo IEC, Needham Heights, MA). HPLC analyses were done as performed by Singh et al (2005). After 72 hr of fermentation, ethanol was evaporated from the mash at 90°C for 3 hr. Remaining materials were dried in a convection oven at 49°C for 72 hr to produce DDGS. DDGS moisture content was determined (Approved Method 44-19, AACC International 2000).

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Enzymatic Dry-Grind Corn Process

Enzymatic dry-grind corn process as outlined by Singh et al (2005) (Fig. 1) was modified. The modified enzymatic dry-grind corn process using a new granular starch hydrolyzing enzyme STARGEN 001 is shown in Fig. 2. Corn (700 g) was soaked with 1,400 mL of water at 55°C for 12 hr. Soaked corn was ground coarsely with total of 1,000 mL of soak and tap water at 3,500 rpm for 8 min. Water used in the enzymatic dry-grind corn process was controlled to give ≈24.5% dry solids content before fermentation. One-step incubation was conducted with 2 mL of STARGEN 001 and 1 mL of GC106 enzymes at 48°C and pH 4.2 for 3 hr with agitation at 20 rpm. Germ and pericarp fiber were skimmed together. Skimmed germ and pericarp fiber were washed and dried in a convection oven at 49°C for 24 hr. Dried germ and pericarp fiber were separated using an aspirator (model 6DT4 Kice, Metal Product Co., Wichita, KS) at air pressure of 75 N/m^2 of water column. The remaining slurry, including wash water, was ground finely using a Quaker City plate mill (model 4-E, The Straub Co., Hatboro, PA). Dry solids content of the slurry was determined using a two-stage oven method (Approved Method 44-15A, AACC International 2000). SSF and ethanol evaporation were performed using the same procedure as used in conventional dry-grind corn process. Endosperm fiber was recovered by screening the remaining materials using a sieve shaker (RX-86, W. S. Tyler, Mentor, OH) with 100-mesh sieve (0.15-mm openings). DDGS recovery procedure for enzymatic dry-grind corn process was the same as the conventional process. Moisture content of germ, pericarp fiber, endosperm fiber, and DDGS were determined (Approved Method 44-19, AACC International 2000).

Data Analysis

Each treatment (enzymatic and conventional dry-grind corn processes) was replicated three times. Each sample was analyzed in duplicate using HPLC. Fermentation profiles (concentration vs. fermentation time) of ethanol, glucose, fructose, maltose, maltotriose, DP4+, glycerol, lactic acid, and acetic acid were plotted. Fermentation rates for both processes were calculated by ratio of ethanol concentration over final ethanol concentration. Theoretical ethanol yield (L/kg and gal/bu) was calculated based on corn test weight of 56 lb/bu (Kelsall and Lyons 2003) and total starch content of 73.2 ± 0.3% (db) scanned using a whole grain analyzer near-infrared transmission (NIT) (Omeg Analyzer G, Dickey-john, Springfield, IL). Actual ethanol yields (L/kg and gal/bu) were calculated based on final ethanol concentration. Ethanol conversion efficiencies of the two processes were calculated by ratio of actual ethanol yield over theoretical ethanol yield. Coproducts yields of germ, pericarp fiber, endosperm fiber, and DDGS were calculated based on total amount of dry matter of initial corn used.

RESULTS AND DISCUSSION

Ethanol Profile

For the first 18 hr of fermentation, ethanol profiles were comparable (Fig. 4). After 18 hr, enzymatic dry-grind corn process had a higher ethanol concentration compared with the conventional process. Final ethanol concentration of enzymatic dry-grind corn process was 15.5 ± 0.2% (v/v) compared with 14.2 ± 0.1% (v/v) for the conventional process. Approximately 9.2% higher final

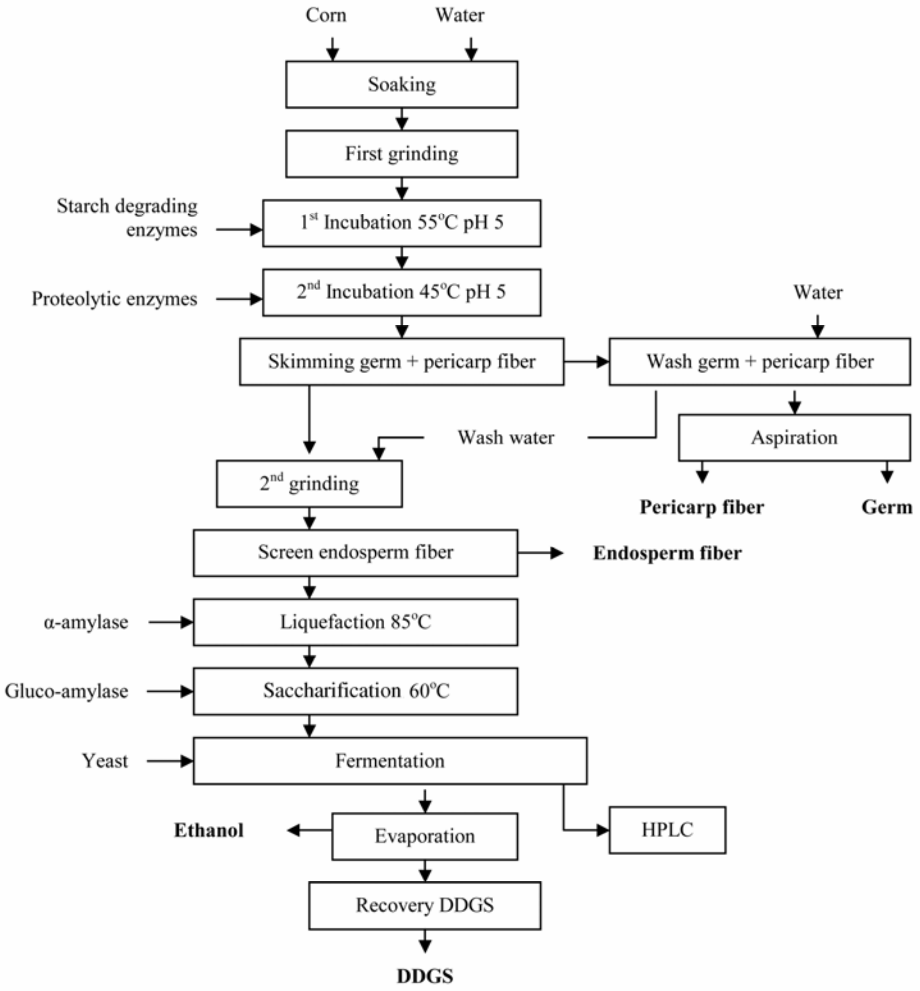

Fig. 1. Flowcharts of enzymatic dry-grind corn process described by Singh et al (2005).

ethanol concentration was achieved for enzymatic dry-grind corn processing because of higher fermentable substrate in the slurry. Higher fermentable substrate concentration in the enzymatic dry-grind corn process was due to removal of germ and pericarp fiber before fermentation. Producing higher ethanol concentration has potential benefits such as a lower utility requirement for concentrating and dehydrating ethanol. Higher concentration of ethanol also reduces the size of equipment in downstream processing (Wang et al 1999).

Sugar Profiles

Glucose. For the enzymatic dry-grind corn process, initial glucose concentration was 7.5% (w/v), which increased to 9.0% (w/v) at 4 hr, then exponentially dropped to negligible amount by 36 hr (Fig. 5). For the conventional process, initial glucose concentration was 6.0% (w/v), which increased to 7.0% (w/v) at 2 hr, then exponentially decreased to negligible amount by 12 hr.

Maltose. For the enzymatic dry-grind corn process, initial maltose concentration was 0.35% (w/v), which increased to 0.40% (w/v) at 2 hr and decreased to 0.18% (w/v) by 18 hr, then slowly decreased to 0.14% (w/v) by 72 hr (Fig. 6). For conventional process, initial maltose concentration was 0.30% (w/v), which increased to 0.37% (w/v) at 2 hr and decreased to less than 0.01% (w/v) by 18 hr. Differences in maltose profile for enzymatic and conventional dry-grind corn processes were very small and almost negligible.

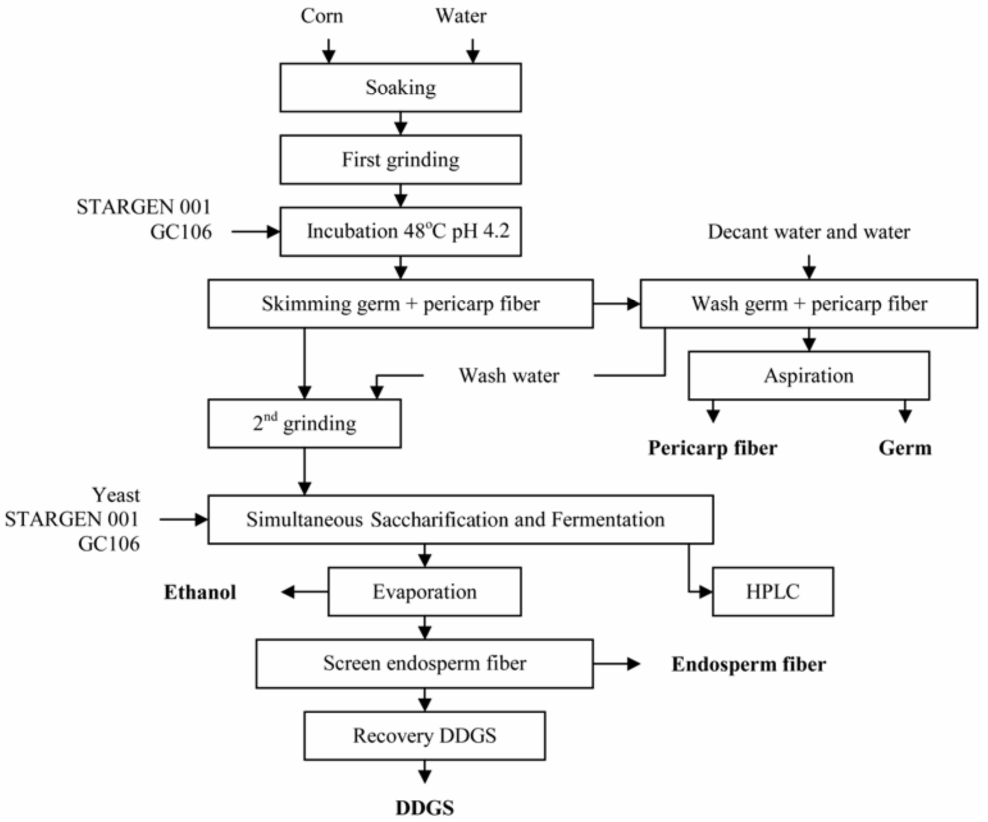

Fig. 2. Flowchart of enzymatic dry-grind corn process using a new granular starch hydrolyzing enzyme STARGEN 001.

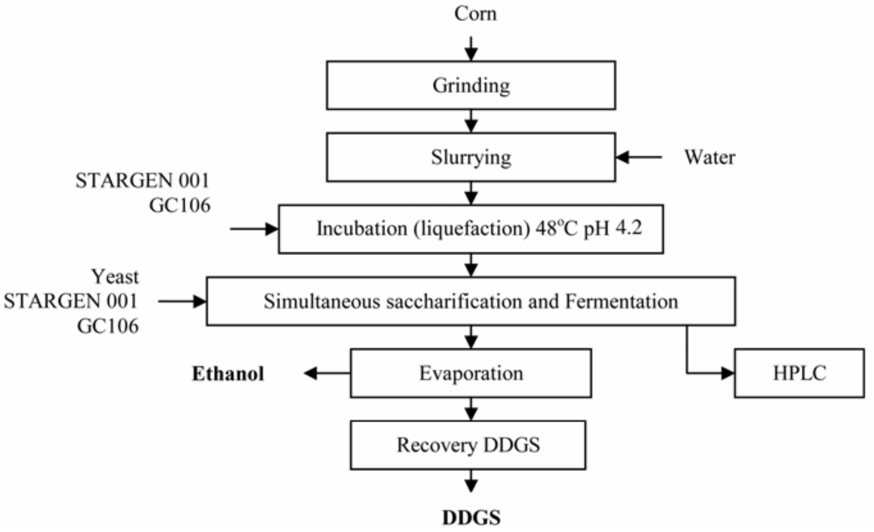

Fig. 3. Flowchart of conventional dry-grind corn process using a new granular starch hydrolyzing enzyme STARGEN 001.

Fructose, maltotriose, and DP4+. Sugar profiles of fructose, maltotriose, and DP4+ for both enzymatic and conventional dry-grind corn processes were similar (data not shown). Fructose profiles and maltotriose profiles were similar to glucose profile but with low initial concentrations of fructose 0.5% (w/v) and maltotriose 0.1% (w/v). For DP4+, initial concentrations of both processes were 0.38% (w/v), which decreased to 0.31% (w/v) for the remainder of the fermentation.

Glycerol Profile

Glycerol profiles for enzymatic and conventional dry-grind corn processes exhibited similar patterns as ethanol profiles. Glycerol concentrations for enzymatic and conventional dry-grind corn processes were comparable for the first 8 hr of fermentation. After 8 hr, glycerol concentrations of enzymatic dry-grind corn process were higher than conventional processing. Final glycerol concentration of enzymatic and conventional dry-grind corn processes were 0.5% (w/v) and 0.4% (w/v), respectively. After ethanol, glycerol is the second most important product of yeast alcoholic fermentation. It is an important compound, which helps to maintain the cell's redox balance. Typical glycerol concentrations are 1.2–1.5% in conventional corn dry-grind fermentation (Russel 2003).

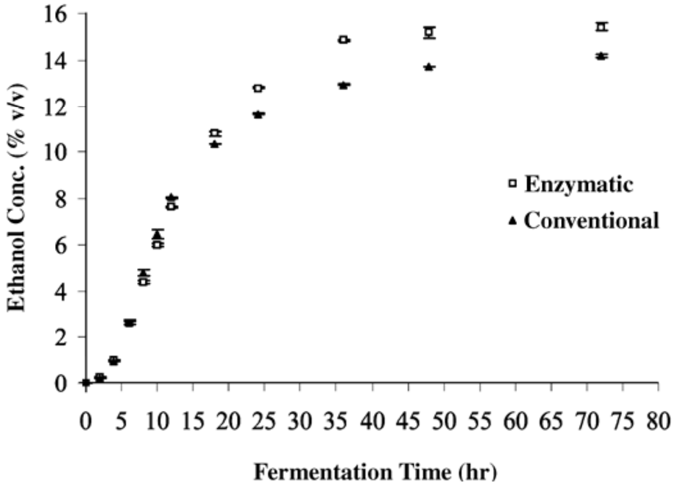

Fig. 4. Concentrations of ethanol during fermentation. Error bars ± standard deviations.

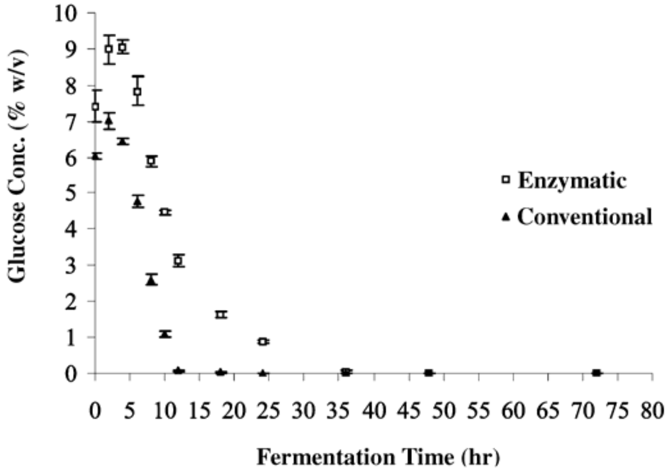

Fig. 5. Concentrations of glucose during fermentation. Error bars ± standard deviations

Organic Acid Profiles

High concentrations of organic acids, mainly lactic and acetic acids, indicate infection problems. Contaminating bacteria such as *Lactobacilli* convert glucose to lactic acid and acetic acid, resulting in lower ethanol yields and increasing yeast stress. Concentrations of lactic acid to >0.8% (w/v) and acetic acid to >0.05% (w/v) will cause stress on yeast (Russel 2003). Final lactic acid concentrations of enzymatic and conventional dry-grind corn processes were 0.02% (w/v) and 0.03% (w/v), respectively. Acetic acid in enzymatic and conventional dry-grind corn processes were not detected. Low lactic acid and acetic acid concentrations indicated there were no infection problems during fermentations in enzymatic and conventional dry-grind corn processes.

Fermentation Rate

At 36 and 48 hr of fermentation, ethanol concentrations of the enzymatic dry-grind corn process were 14.9 and 15.2% (v/v) and had reached 96.1 and 98.1% of maximum ethanol concentration, respectively (Table I). Ethanol concentrations of the conventional process were 12.9 and 13.7% (v/v) and had reached 90.8 and 96.5% (v/v) of maximum ethanol concentration at 36 and 48 hr, respectively. At 72 hr of fermentation, final ethanol concentration for the enzymatic dry-grind corn process was 15.5% (v/v) and for the conventional process was 14.2% (v/v). At 96% of maximum ethanol concentration, the enzymatic dry-grind corn process was 12 hr faster than the conventional process.

Ethanol Yields

Ethanol yields for enzymatic and conventional dry-grind corn processes were 0.395 ± 0.006 and 0.417 ± 0.002 L/kg (2.65 ± 0.04 and 2.80 ± 0.01 gal/bu), respectively (Table II). Theoretical ethanol yield was 0.457 L/kg (3.07 gal/bu) based on corn test weight 56 lb/bu and total starch content 73.2% (db). Ethanol conversion efficiencies for enzymatic and conventional dry-grind corn processes were 86.4 and 91.2%, respectively. Ethanol yield for enzymatic dry-grind corn process was 5.3% lower than conventional process because small amounts of starch and sugars were lost when germ and pericarp fiber samples were recovered before fermentation. Ethanol conversion efficiency for the enzymatic dry-grind corn process was lower than for the conventional process based on total starch content. Germ and pericarp fiber have starch associated with them. Removal of germ and pericarp fiber results in loss of starch. If extractable starch yield of 69.8% (db) obtained from the 1-kg wet-milling procedure by Eckhoff et al (1993) is considered, the theoretical ethanol yield is 0.437 L/kg (2.93 gal/bu) and ethanol conversion efficiency for enzymatic dry-grind corn process is 90.4%.

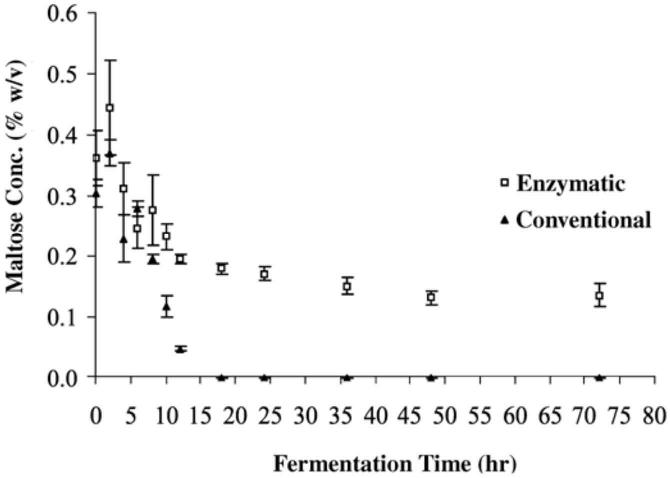

Fig. 6. Concentrations of maltose during fermentation. Error bars ± standard deviations.

TABLE I
Ethanol Concentrations and Fermentation Rates for Enzymatic and Conventional Dry-Grind Corn Processes[a]

	Enzymatic Process		Conventional Process	
Fermentation Time (hr)	Ethanol Conc. (% v/v)	% Maximum	Ethanol Conc. (% v/v)	% Maximum
0	0	0	0	0
2	0.3 ± 0.01	1.9	0.2 ± 0.01	1.4
4	1.0 ± 0.05	6.5	0.9 ± 0.02	6.3
6	2.6 ± 0.07	16.8	2.6 ± 0.09	18.3
8	4.3 ± 0.08	27.7	4.8 ± 0.13	33.8
10	6.0 ± 0.08	38.7	6.5 ± 0.19	45.8
12	7.6 ± 0.05	49.0	8.1 ± 0.04	57.0
18	10.8 ± 0.10	69.7	10.4 ± 0.01	73.2
24	12.8 ± 0.02	82.6	11.6 ± 0.03	81.7
36	14.9 ± 0.04	96.1	12.9 ± 0.03	90.8
48	15.2 ± 0.23	98.1	13.7 ± 0.01	96.5
72	15.5 ± 0.17	100.0	14.2 ± 0.05	100.0

[a] Mean ± standard deviation.

TABLE III
Coproduct Yield[a] (%, db) of Germ, Pericarp Fiber, Endosperm Fiber and Distillers Dried Grains with Solubles (DDGS) for Enzymatic and Conventional Dry-Grind Corn Processes

	Enzymatic Process	Conventional Process
Germ	8.0 ± 0.4	. . .
Pericarp fiber	7.7 ± 0.4	. . .
Endosperm fiber	5.2 ± 0.6	. . .
DDGS	9.8 ± 0.6	28.3 ± 0.1

[a] Mean ± standard deviation.

TABLE II
Ethanol Yield[a] for Enzymatic and Conventional Dry-Grind Corn Processes

	Enzymatic Process	Conventional Process
Dry solids in fermentation mash (%)	24.2 ± 0.7	24.5 ± 0.3
Ethanol concentration (% v/v)	15.5 ± 0.2	14.2 ± 0.1
Ethanol volume (mL)	292.2 ± 1.4	276.6 ± 4.3
Ethanol yield (gal/bushel)	2.65 ± 0.04	2.80 ± 0.01
Ethanol yield (L/kg)	0.395 ± 0.006	0.417 ± 0.002

[a] Mean ± standard deviation.

Coproducts

The specific gravity of the slurry was 1.085 after the enzyme incubation step and was higher than the specific gravity achieved by Singh et al (2005). Germ and pericarp fiber floated well on top of the slurry and made skimming of germ and pericarp fiber easier compared with previous enzymatic dry-grind corn process (Singh et al 2005). For the enzymatic dry-grind corn processing, 8.0 ± 0.4% (db) germ, 7.7 ± 0.4% (db) pericarp fiber, and 5.2 ± 0.6% (db) endosperm fiber were recovered (Table III). In this study, endosperm fiber was recovered after fermentation and ethanol evaporation. Singh et al (2005) recovered endosperm fiber before fermentation, which reduced ethanol yields because starch was lost with endosperm fiber.

For enzymatic and conventional dry-grind corn processes, DDGS yields (Table III) were 9.8 ± 0.6 and 28.3 ± 0.1% (db), respectively. DDGS yield for conventional processing was three times higher than enzymatic dry-grind corn processing. In other words, the enzymatic dry-grind corn process generated 66% less DDGS than the conventional process. Current growth in the ethanol industry could lead to overproduction of DDGS from conventional dry-grind corn process. Enzymatic dry-grind process can reduce the amount of DDGS as well as produce DDGS with higher protein and lower fiber content (Singh et al 2005).

CONCLUSIONS

A new granular starch hydrolyzing enzyme STARGEN 001 (Genencor International, Palo Alto, CA) was used in an enzymatic dry-grind corn process to improve recovery of germ and pericarp fiber prior to fermentation. The enzymatic dry-grind corn process with STARGEN 001 required no liquefaction and had one step of incubation instead of the two steps previously used. Enzymatic dry-grind corn process was compared with the conventional dry-grind corn process using the new enzyme with same process parameters of dry solid content, pH, temperature, enzyme and yeast usage, and time. Ethanol yields of enzymatic dry-grind ethanol and conventional processes were 0.395 ± 0.006 and 0.417 ± 0.002 L/kg (2.65 ± 0.04 and 2.80 ± 0.01 gal/bu), respectively. Compared with the conventional process, enzymatic dry-grind corn process had 9.2% higher ethanol concentration, higher fermentation rate, generated higher valuable coproducts (germ, pericarp fiber, and endosperm fiber), and produced 66% less DDGS.

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