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Enhancing Water Removal from Whole Stillage by Enzyme Addition During Fermentation

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ABSTRACT

The removal of water from coproducts in the fuel ethanol process requires a significant energy input. In this study, the addition of commercially available cell-wall-degrading enzymes was investigated to determine whether or not the enzymes could reduce the amount of water bound within the wet grains. This would have the effect of allowing more water to be removed during centrifugation, reducing the time and energy needed during the drying process. The experiment screened 15 cell-wall-degrading enzyme preparations. A significant reduction in water-binding capacity was found for a number of enzymes tested in the initial screening. The experiment was repeated and two enzymes were identified to have the highest whole stillage dewatering effect, 15 and 14% more water removed for enzyme preparations A and G, respectively. Adding different enzyme preparation amounts to the mash showed varying effects, with the potential to allow for an optimization of enzymes cost and energy savings. In some cases, an enzyme dosage of 0.5 mL worked as well, if not better, than a dosage of 1 mL. These results can translate into improvements in the overall energy efficiency of the process because the wet grains entering the drier would contain less moisture than in the conventional process thus requiring a shorter residence time in the drier.

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The U.S. ethanol industry produced almost 5 billion gallons of ethanol in 2006. This production capacity was expected to increase to at least 8 billion by 2012 but will likely reach this value much earlier. In October 2007, there were 132 operational ethanol plants with 79 under construction and 10 undergoing expansion (ACE 2007). Once operational, these plants will have a total production capacity of over 13 billion gallons per year.

The primary feedstock for ethanol production in the United States is corn. Using corn presents processing complexities that are not present when producing ethanol from sugar cane. This led to an ongoing debate over the production of ethanol that has primarily focused on whether or not the energy balance of the overall process is positive or negative. According to Farrell et al (2006), there are two major studies in the literature that report negative net energy values and four studies show positive net energy values. However, it is important to understand whether or not these studies have accounted for the economic value of the coproducts from ethanol production. These coproducts help to remove competing goods from the market that require energy to produce. The main coproduct that can directly compete in the marketplace is distillers dried grains with solubles (DDGS). According to the Association of American Feed Control Officials, DDGS is a byproduct from the grain distilling industry produced from drying the whole stillage down to at least 75% solids. Whole stillage a mixture of water and nonfermentables that remains after the ethanol is removed during distillation (AAFCO 2006).

Wet distiller grains (WDG) and DDGS are sold, for the most part, as feed for ruminants but they can be also consumed by swine, poultry, and other animals at lower dietary levels. Generally, DDGS contain ≈12% moisture by the time they are ready to be transported, whereas WDG contain 70% moisture. WDG have a higher nutritional value than dry grains because some components of the feed such as amino acids are sensitive to temperature and will degrade upon excessive heating (Tjardes et al 2002; Amezcua et al 2007). However, transportation and storage of wet feed present many problems, including a short shelf life and flowability problems (Ganesan et al 2006). Currently, the average cost of the wet feed is $32/ton, while the average cost of the dry feed is $120/ton (Paustian 2004; USDA 2007). Less drying would translate into smaller energy requirements for the process, helping to lower the cost of DDGS and make them more attractive in the marketplace (Miller 2000).

After centrifugation, the distillers grains (DG) still contain ≈65–70% moisture, and the drier is used to remove the excess moisture to achieve the desired 10–12% (Fig. 1). Cattle farmers prefer to feed livestock DDGS that have a color in the golden range. This can be difficult to achieve because DDGS are easily burned in the drier due to long residence times and high operating temperatures. The burned DDGS reduce the digestive abilities of ruminants (Shurson 2006). Furthermore, according to the economic model of fuel ethanol production previously published (Kwiatkowski et al 2006), decreasing the retention time of the DDGS in the dryer presents an important economic benefit that can save up to 6.2% in utility usage in the overall production process. Increasing the amount of water removed during centrifugation will make it possible to reach the necessary moisture content in the DDGS with a lower temperature and shorter residence time in the drier. The additional water removed is sent to the evaporator where it is removed 3 to 5 times more efficiently than in the drier. Even with the increase volume being sent to the evaporator, it will reduce the overall energy consumption of the process and ultimately reduce the production cost of ethanol. The challenge is to find a method to increase the amount of water removed from the stillage during centrifugation, and in doing so decrease the total amount of water removed and the residence time of the DDGS in the drier.

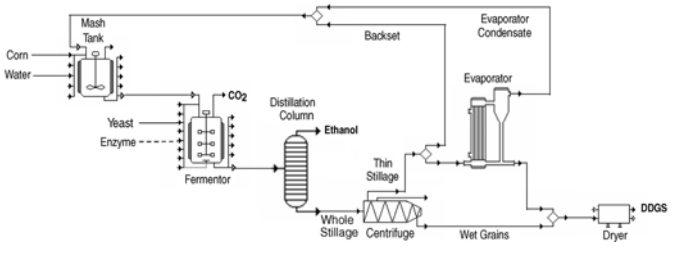

Fig. 1. Schematic diagram of dry grind process with emphasis on backend of process. Enzyme addition is represented by dashed line into fermentor. Process products are in bold.

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DDGS are composed of 30.7% crude protein, 10.9% crude fat, 33% neutral detergent fiber, and 16.2% acid detergent fiber, along with smaller amounts of water, amino acids, ash, calcium, and phosphorus (Shurson 2006). Acid detergent fiber is defined as the portion of the DDGS cell wall that is made up of cellulose and lignin. Neutral detergent fiber contains hemicelluloses as well as the cellulose and lignin components. The bonds formed between water molecules and polysaccharides can form in a number of different ways and with varying strengths. Maximum water-binding occurs when water molecules share one hydrogen bond with a polysaccharide. In this configuration, the molecule will be very hydrophilic due to an increase in water density. The presence of a polysaccharide carboxyl group can increase water density, as this specific interaction of bonds causes the polysaccharides to "fold on themselves to avoid loss of their rotational entropy" (Chaplin 2003). In polysaccharides from the corn kernel, the furanose sugars from the arabinoxylans present also create a volume of denser water surrounding them. If we are able to disrupt this hydrogen bonding structure, less water would be retained after centrifugation. The use of cell-wall-degrading enzymes such as cellulases, xylanases, and β-glucanases to hydrolyze and cleave cellulose and hemicellulose is one way to disturb this ordered environment of hydrogen bonds (de Vries et al 2001). The goal of the work presented here was to determine the viability of using cell-wall-degrading enzymes during fermentation to reduce the water-binding capacity of the distillers grains.

MATERIALS AND METHODS

The enzymes used in this research were donated by Genencor International (a Danisco Company, Palo Alto, CA) and Novozymes (Franklinton, NC). The corn used was a single hybrid variety (33A14) grown at the University of Illinois during the 2004 season. All chemicals used in this study are of analytical quality.

Mash Preparation

To prepare the mash, 1 kg of corn was removed from the cold room and equilibrated to room temperature. The corn was then ground in a Wiley laboratory mill equipped with a 20-mesh screen. Erlenmeyer flasks (250 mL) were labeled and their tare weights were recorded. Stir bars were also weighed together with stoppers and 21 gauge 1.5" needles. Microcentrifuge tubes used for collecting samples were labeled with fermentation date, ID, and sample number. A rubber stopper, a needle, and a stir bar were assigned to each fermentation flask. The needle was inserted into the rubber stopper. Each flask with a stir bar and rubber stopper with needle was weighed as an assembly.

Ground corn weighing 227 g (corn weight was adjusted using moisture content to give the desired final solids content of 25%) was added to 640 mL of water in 1-L flasks and adjusted to pH 5.8 ± 0.1 by adding 1*M* HCl solution. Spezyme Fred α-amylase (1 mL) was added to each flask which was placed on a preheated hot plate at 90°C with the stirring rate set to 120 rpm. The slurry was liquefied at 90°C for 1 hr. The slurry temperature was brought to 60°C using a water bath and 0.77 g of urea was added to each flask. The slurry was adjusted to pH 4.5 ± 0.1 by adding *1M* HCl solution and 0.4 mL of Optidex L-400 glucoamylase was added to the mash which was then saccharified at 60°C for 1 hr.

Mash Fermentation

The mash was cooled to 30°C, and 100 g was transferred to previously weighed Erlenmeyer flasks. Yeast suspension (Fleischmann's Active Dry Yeast, Fenton, MO) was prepared by mixing 3.3 g of yeast in 30 mL of distilled water and mixing for 10 min at room temperature. The suspension had a viable cell count of $\approx 1.8 \times 10^6$ cells/mL. Each flask was then inoculated with 1 mL of yeast suspension (0.11 g of dry yeast/100 mL of mash). The dewatering enzymes were added to each flask at the indicated amounts. A control flask was also prepared without enzyme addition. All flasks were then sealed with the stopper, the needle was inserted and flasks were moved to a temperature-controlled shaking incubator (30°C at 200 rpm) for 93 hr.

Analytical Techniques

Nine samples (1 mL) were taken from each of the flasks throughout the fermentation process. The samples were centrifuged (model 5415 D, Eppendorf, Westbury, NY) for 2 min at 16,110 × *g* and the supernatant (water removed after centrifugation) was filtered using a 0.2-μm syringe filter (model 4455T, Pall, Ann Harbor, MI) into labeled 1-mL microcentrifuge tubes and frozen until ready for HPLC analysis.

Samples were thawed and injected into a HPLC (model 2350, ISCO, Lincoln, NE) equipped with an Aminex HPX-87H Biorad (Hercules, CA) ion-exclusion column. Compounds were eluted from the column with an aqueous solution of 5 m*M* sulfuric acid, detected with a refractive index detector (model 1047A, Hewlett Packard, Palo Alto, CA) and quantified by HPLC software (Chrom Perfect Spirit v.4.17, Justice Laboratory Software, Fife, UK) using external standard calibrations.

At the end of fermentation, a 40-mL representative sample was taken from each of the 15 flasks and transferred to a 50-mL centrifuge tube (Corning, cat no. 430290, 29.1 mm o.d.). Each tube was then centrifuged in a bench-top centrifuge (model Z320, Hermle, Woodbridge, NJ) for 10 min at 1,400 × *g* to analyze the water-binding capacity of each enzyme treatment. The supernatant (water removed after centrifugation) from each flask was weighed, placed in a 55°C oven for 48 hr, moved to a 135°C oven for 2 hr, and then weighed again. The same was done with the solid pellets (wet grains) obtained after centrifugation.

Experimental Design

The procedure described above was used in three experiments. The first experiment had 16 fermenting flasks; one control flask and 15 flasks containing 15 different commercial enzyme preparations. The second experiment used six out of the 15 enzymes used in the first part and a control. The six preparations were chosen based on the highest dewatering capability (largest amount of water removed). Each of the six enzyme preparations was added in volumes of 0.1, 0.5, and 1 mL/100 g of mash. This experiment was done in duplicate. The last experiment used two out of the six enzymes used in the second experiment. Again, the two preparations chosen had the highest quantity of water removed after centrifugation. The mash volume was increased to 250 mL and the enzyme preparation amounts were scaled accordingly. This experiment was done in triplicate.

Statistical Analyses

A two-way analysis of variance (ANOVA) was used to compare means of the water amount removed after centrifugation for each enzyme treatment and the control as well as for the different enzyme volumes and the control. A one-way ANOVA was used to compare means of the water amount removed after centrifugation for the triplicate experiment. The *t*-test was used for each pair of enzyme treatments to compare means of the water amount removed (SPSS for Windows, Chicago, IL). The level selected to show statistical significance was 1% ($P < 0.01$).

RESULTS AND DISCUSSION

Initially, 15 commercial enzyme preparations were screened for the ability to dewater the whole stillage (Table I). These preparations were chosen based on marketed activities, as well as temperature and pH range. Single fermentations at high enzyme dosages (1 mL of enzyme/100 g of mash) were used in this run to identify enzyme preparations with the greatest dewatering poten-

tial. Significant improvements in dewatering were observed, with some enzyme preparations showing up to 14% more dewatering than the control (Fig. 2). The weight of the dry solid pellets obtained after centrifugation show some reduction in the enzyme-treated fractions when compared with the control sample (Fig. 3). This means that a portion of the solid phase was solubilized by the enzyme preparation during fermentation. The increase of solubles in the liquid phase was relatively small compared with the increase in water removal. Six enzyme preparations were chosen to be the most promising in terms of their ability to dewater the whole stillage. Enzyme preparations A, E, G, H, I, and M had the highest amounts of water removed after centrifugation as well as yielding the lowest wet pellet weights after centrifugation.

The same experiment was repeated for the enzyme preparations that were screened in the first part of this investigation. However, this time, the experiment was done using three different enzyme additions of 0.1, 0.5, and 1 mL for each enzyme treatment. All concentrations of enzyme preparation showed significant improvement over the control (Table II). Up to 15% more water was removed compared with the control for the mashes treated with A, H, and M. An increase in the amount of water removed during centrifugation was observed for both H and E with an increase in enzyme preparation addition. As the enzyme preparation E addition was increased from 0.1 to 1 mL, the amount of water removed increased from 4.9 to 14.2% compared with the control. In enzyme preparations G, A, M, and I, the difference observed was not significant when comparing 0.1 to 1 mL. The dry solid pellet weights of the enzyme-treated mashes all showed a significant reduction relative to the untreated control. When comparing enzyme additions, enzymes preparations E, H, I, and M all showed a small decrease in pellet weight from 0.1 to 1 mL of enzyme addition. Enzyme preparation A showed a small increase and G increased only when 1.0 mL of enzyme was added to the mash (Table II). In enzyme preparation A, the pellet weight remained almost constant even at different enzyme levels, indicating that only a small enzyme dosage (<0.1 mL tested) would be needed to produce the observed effects. In enzyme preparations that showed a downward trend, a higher enzyme dosage would be required to achieve the maximum effect. This may not be advantageous to the process due to the necessity of a higher enzyme-to-mash ratio and likely a higher enzyme cost.

Final ethanol yields were not significantly different for enzyme-treated mashes compared with the control (Table II). Also, there were no significant differences between the final ethanol yields for each enzyme-treated mash at the different enzyme levels. Thus, adding more enzyme preparation to the mash would not aid in increasing the final ethanol production.

The enzyme preparations that showed similar water removal ability at different enzyme additions were deemed favorable due to the need for smaller amounts of enzymes to achieve the best water-removal results. This would help improve the economics of the process by minimizing additional enzyme costs. Enzyme preparations A and G had this characteristic and were therefore chosen as the most efficient for whole stillage water removal. In enzyme preparations A and G, an enzyme addition of 0.5 mL showed the best efficiency in terms of highest water removal and lowest pellet weight. This addition was ideal for this investigation.

A third set of fermentation experiments was performed in which the above experiment was repeated in triplicate at a larger scale. The two most efficient enzyme preparations from the previous experiment (A and G) were used to treat the mash of 500 mL with an enzyme addition of 2.5 mL. Both preparations again showed a greater amount of water removed relative to the control. Enzyme preparation A had an average of 30.4 g of water removed, G had 30.0 g, and the control had only 26.4 g (Table III) from the 40-g subsamples. For A, this translated into an improvement of 15% more water removed after centrifugation than in the control. The wet solid pellet sizes were also smaller (30–35%) compared with the control. The dry pellet weights were reduced (10–20%) when compared with the control. The standard deviations calculated for each triplicate set showed that this experiment was reproducible and repeatable in terms of the amount of water that was removed after centrifugation and the solid pellet size that was left after the water was removed.

There were no significant differences in the final ethanol yields for the enzyme-treated mashes compared with the control for these runs (Table III). The final ethanol production from the triplicates also proved to be reproducible.

TABLE I
Key Indicating Enzyme Preparations Used in This Study

Enzyme Key	Enzyme Name
A	GC 220
B	AD9990209
C	AD990210
D	AD990208
E	AD990211
F	Multifect Xylanase
G	Multifect GC
H	GC 880
I	GC 440
J	GC 710
K	Protease 899
L	Pulpzyme
M	Multifect B
N	Multifect P3000
O	Viscozyme

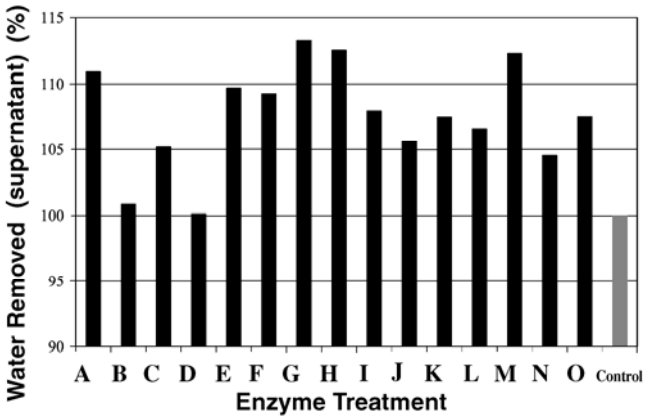

Fig. 2. Weight of water removed (supernatant) after centrifugation as a percentage of control for each enzyme-treated mash for a 40-g subsample of mash. Control with lowest amount of water removed taken as 100%.

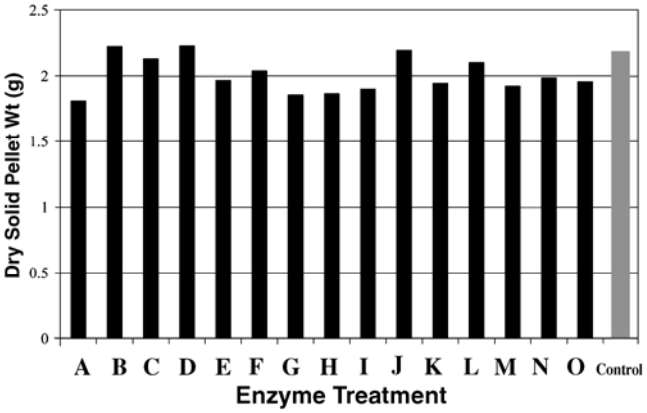

Fig. 3. Dry solid pellet weights/40 g of mash for each enzyme-treated mash and control. Weights measured from a 40-g subsample of 100 g of mash.

TABLE II
Amount of Water Removed After Centrifugation, Final Ethanol Yield, and Dry Pellet Weights Given for Enzyme-Treated Mashes and Control Sample[a]

Enzyme	Volume Added (mL)	H_2O Removed (%control)	Ethanol Yield (%v/v)	Dry Pellet Wt (g)
A	0.1	112 ± 0.188a	13.60 ± 0.11	1.80 ± 0.09
	0.5	115 ± 0.154a	13.50 ± 0.19	1.96 ± 0.19
	1	114 ± 0.101a	13.68 ± 0.35	1.77 ± 0.02
E	0.1	105 ± 0.055b	13.60 ± 0.33	2.17 ± 0.09
	0.5	112 ± 0.185a	13.34 ± 0.28	1.96 ± 0.09
	1	114 ± 0.242a	13.53 ± 0.18	1.92 ± 0.16
G	0.1	114 ± 0.372a	14.25 ± 0.02	2.08 ± 0.06
	0.5	112 ± 0.299a	14.32 ± 0.07	2.05 ± 0.06
	1	114 ± 0.120a	14.01 ± 0.28	1.87 ± 0.03
H	0.1	111 ± 0.27c	14.21 ± 0.31	2.08 ± 0.12
	0.5	115 ± 0.079a	13.87 ± 0.09	1.98 ± 0.04
	1	110 ± 0.143c	14.27 ± 0.21	1.84 ± 0.10
I	0.1	113 ± 0.204a	14.12 ± 0.11	2.38 ± 0.13
	0.5	112 ± 0.198a	14.10 ± 0.45	2.06 ± 0.10
	1	114 ± 0.173a	14.06 ± 0.16	1.88 ± 0.12
M	0.1	115 ± 0.264a	14.25 ± 0.13	2.07 ± 0.12
	0.5	112 ± 0.071a	14.42 ± 0.15	2.05 ± 0.11
	1	116 ± 0.308a	14.41 ± 0.16	2.00 ± 0.08
Control[b]	–	100 ± 0.28d	14.03 ± 0.28	2.67 ± 0.08

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.01$). Values are mean ± standard deviation of duplicate samples.
[b] Control had 26.66 ± 0.27 g of water/40 g of mash removed.

TABLE III
Average Wt of H_2O Removed, Solid Pellet, and Final Ethanol Yield for Mash Treated With Enzyme A and G[a]

Enzyme	H_2O Removed Avg (% control)	Wet Pellet Wt Avg (% control)	Dry Pellet Wt Avg (% control)	Ethanol Yield (% v/v)
A	115 ± 0.26a	65.39 ± 0.18	79.82 ± 0.04	14.17 ± 0.11
G	114 ± 0.68a	69.74 ± 0.11	90.30 ± 0.39	13.97 ± 0.29
Control[b]	100 ± 0.91b	100.00 ± 0.99	100.00 ± 0.07	14.19 ± 0.13

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.01$). Values are mean ± standard deviation of triplicate samples.
[b] Control had 26.41 ± 0.91 g of water/40 g of mash removed; wet pellet weight of 11.47 ± 0.96 g; dry pellet weight of 2.52 ± 0.07 g.

CONCLUSIONS

A significant reduction in water-binding capacity was found for a number of enzymes tested in the initial screening. Average dewatering improvements in whole stillage of 15 and 14% were observed for enzymes A and G, respectively, with 500-mL fermentations done in triplicate. The enzymes were able to disrupt the corn cell wall and release water bound within the grains. The addition of different enzyme amounts to the mash had varying effects, potentially allowing an optimization of enzyme cost with energy savings. In some cases, an enzyme dosage of 0.5 mL worked as well, if not better, than a 1-mL dosage. In enzyme A, there was a maximum effect shown with the lowest dose tested, indicating that significantly less of this enzyme could be used and still see a strong dewatering effect. Lower concentrations would be more economically optimal due to a lower enzyme cost.

The addition of these water-removing enzymes during fermentation of the dry grind corn-to-ethanol process will help in the dewatering of the whole stillage during centrifugation. Furthermore, there will be no capital cost associated with the added enzymes. By removing more water during centrifugation the energy cost of the DDGS drying process could be significantly reduced, which could translate directly into lower energy consumption, improved energy balance, and reduced ethanol production costs.

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