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Improved Corn Ethanol Fermentation and Oil Distribution by Using Polysaccharide Hydrolyzing Enzymes

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Keywords
Corn Fermentation, Fiber, Fuel Ethanol, Oil Recovery, Polysaccharide Hydrolyzing Enzymes

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We determined the effects of a commercial proprietary formulation of polysaccharide hydrolyzing enzymes on ethanol fermentation performance, oil partitioning and recovery, and quality of dried distillers grains with solubles (DDGS) on a 1.5-L and 50-L fermentation scale. The enzyme was added at the start of fermentation. Whole beer was subjected to beer well incubation, distillation, and separation of thin stillage from the wet cake. The enzyme promoted faster ethanol production without affecting the final ethanol yield. The enzyme treatments resulted in 8–18% higher wet yield of thin stillage than the control, 13–21% of oil increase in thin stillage, and 11% fiber reduction in DDGS. Free oil recovery from thin stillage was improved by the enzyme treatments (13–53% increase). The present study shows that the use of the polysaccharide hydrolyzing enzymes can add benefits to ethanol plants by increasing corn oil yield and producing fermentation co-products with increased nutritional value and potentially broader applications in animal feeds.

KEYWORDS: Corn Fermentation, Fiber, Fuel Ethanol, Oil Recovery, Polysaccharide Hydrolyzing Enzymes.

1. INTRODUCTION

The U.S. fuel ethanol production capacity has steadily increased over the past decade. In 2012, U.S. fuel ethanol production was 13.8 billion gallons (903,000 barrels per day), which accounted for about 7.1% of total transport fuel consumption. Ethanol is at present the most significant biofuel in the United States, accounting for 94% of all biofuel production, according to U.S. Bioenergy Statistics (USDA, 2014). Most (>80%) ethanol is produced from corn via the dry-grind process (Haas, 2012). The major co-product of dry-grind process is distiller's dried grains with solubles (DDGS), which is the non-fermentable residue after ethanol fermentation, and it contains (on a dry matter basis) approximately 30% protein, 11% oil, 5% ash, and 54% fiber (Liu, 2011). About 34.7 million metric ton of DDGS was produced in 2013.

Ethanol plants have struggled to maintain profitability, which is highly variable depending upon corn price, demand and price of DDGS, tax credits, gasoline consumption, ethanol exports, and changes to the Renewable Fuels Standard (RFS) mandates (Irwin, 2014). New technologies for energy savings, higher yield of ethanol and higher value for co-products as well as improving oil separation will contribute to the profitability of producing ethanol.

Corn oil recovery has been recognized as one of the key operations in keeping many ethanol plants profitable in times of tight margins by improving incomes and diversifying plant revenue streams. During the past two years, the U.S. ethanol industry has widely implemented advanced corn oil extraction technology. It was estimated that at the end of 2013 about 80% of U.S. ethanol plants were recovering corn oil (Jessen, 2015). According to a report from the U.S. Energy Information Administration, although ethanol facilities with corn oil extraction have slightly higher production costs, their profit margins due to corn oil sales have remained positive and higher than the plants without oil recovery (U.S. EIA, 2014). Due to negative margins, many ethanol plants without oil recovery have chosen to shut down.

Corn oil recovered as a co-product of ethanol production, also referred to as distillers corn oil, is an economically attractive and renewable feedstock for biodiesel production. The current market price for inedible crude corn oil feedstock is about $0.30–0.35/lb ($0.13–0.15/kg), more than one-half the price of crude soybean oil, the major source for biodiesel (USDA, 2014). As biodiesel

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production has grown, the demand for the commonly available feedstocks (including vegetable oils, animal fats, and other recycled oils) has been increasing. In 2012, 76 million gallons of distillers corn oil was used for the production of biodiesel (Kotrba, 2014). The U.S. Environmental Protection Agency (EPA) estimated that corn oil will be "a significant contributor to the biodiesel volume required by the RFS2 rule." In order to reach the U.S. government's RFS2 mandate of 15 billion gal (56.8×10^6 m^3) of ethanol production from corn by 2022, about 5.2 billion lb (2.5×10^6 m^3) corn oil would be available and used to supply 40% of total annual U.S. output of biodiesel (EPA, 2010). With improved oil separation technology, this volume is expected to grow.

Oil removal from DDGS may also benefit handling and transportation of DDGS (less caking and improved flow properties) and expand the use of low-fat DDGS in non-ruminant livestock (Haas, 2012). Furthermore, the use of the distillers corn oil, which is inedible feedstock for biodiesel production, would not impact the cost and availability of oil for food. The distillers corn oil also contains high level of carotenoids that are valuable nutrients for feed applications (Moreau et al., 2010).

Reviews of current technologies for corn oil recovery from dry-grind ethanol plants were given in previous reports (Haas, 2012; Mueller and Kwik, 2014). Physical pretreatment on dry corn, front-end and back-end oil recovery in combination with heating, use of demulsifiers and polar solvents have significantly improved corn oil recovery compared to several years ago (Haas, 2012; Moreau et al., 2012; Majoni et al., 2011a; Wang et al., 2008, 2009; Allen and Rusnack, 2008; Randhava et al., 2008; Purtle and Zullo, 2013; Liu and Barrows, 2013; Winsness, 2012). In addition, efforts have been made to use enzymes to facilitate oil separation (Majoni et al., 2011b; Moreau et al., 2004). The proposed mechanism of enzyme actions on oil recovery was

(1) protease hydrolysis of the pseudo-membrane (olesin) surrounding corn oil bodies or the proteins that stabilize the oil-in-water emulsion, thereby releasing the oil (Majoni et al., 2011b; Tzen and Huang, 1992; Nobrega de Moura et al., 2008);

(2) fiber hydrolyzing enzymes further break the fragmented cell wall components, and then release the oil that is trapped in their intact network (Wang et al., 2008).

Cellulase, xylanase, and other fiber hydrolyzing enzymes in combination have been used to increase ethanol yield (Balcerek and Pielech-Przybylska, 2009; Sapińska et al., 2013; Abbas and Bao, 2013). Initially these fiber hydrolyzing enzymes were used to reduce the fiber content in DDGS (Hruby, 2012). We hypothesized that the non-starch polysaccharide hydrolyzing enzymes would facilitate the degradation of cell wall components and if added during fermentation, they would not only improve fermentation performance but also improve oil recovery, resulting in a DDGS with lower fiber and fat contents.

Therefore, the objectives of the present work were to determine the effects of polysaccharide hydrolyzing enzymes on ethanol fermentation performance, oil partitioning during post-fermentation processing, and DDGS quality. Polysaccharide hydrolyzing enzymes were added during simultaneous saccharification and fermentation. The finished beer was subjected to beer well incubation, distillation, and then decanting to separate thin stillage from the solids. Ethanol production, concentrations of residual sugars, acids, and glycerol, oil partitioning, and DDGS proximate composition were determined.

2. MATERIALS AND METHODS

2.1. Materials

Liquefied corn mash (containing 30–31% dry matters from 2012 harvested commodity corn), glucoamylase, antimicrobial chlorine dioxide, and dry yeast (commercial grade, currently being used in the ethanol plant) were acquired from a local dry-grind ethanol plant (Lincolnway Energy, Nevada, IA). A proprietary commercial blend of polysaccharide hydrolyzing enzymes, e.g., xylanases, cellulases, and glucanases, was provided by Direvo Biotechnology (Cologne, Germany). Other analytical-grade chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. 1.5-L Fermentation

Hot liquefied corn mash (1.5 L, 60–70 °C) obtained from a local ethanol plant (without storage) was added to a 2-L Erlenmeyer flask after a vigorous mixing. The temperature of mash was rapidly brought to 30 °C by using an ice water bath. The pH of the mash was adjusted to 4.0 by using 1 N sulfuric acid. Glucoamylase (0.04% w/w of corn), ammonium sulfate (150 ppm of mash), chlorine dioxide (110 ppm of mash), the polysaccharide hydrolyzing enzyme (4000 ppm of mash), and dry yeast (1 g), according to Wang et al. (2008), were added to the mash. The flask was capped with a foam stopper and incubated at 30 °C for 72 h in an orbital incubator shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at 150 rpm. The weight change of the whole flask was recorded periodically. The ethanol production during the fermentation was monitored based on the mass loss due to the production of CO_2 by using the equation (Wang et al., 2008): ethanol yield (g per 100 g dry corn) by mass loss = $100 \times$ ($46 \times CO_2$ production (g)/44)/dry corn mass (g); where 46 and 44 are the molecule weight of ethanol and CO_2. The dry corn mass was determined by the dry matter content of the initial corn mash. Three replicates with the addition of the enzyme, and three without (controls) were carried out at the same time.

2.3. 50-L Fermentation

Hot liquefied corn mash (44 L), obtained from a commercial ethanol plant 20–23 h earlier, was cooled to 30 °C and

held at ambient temperature overnight, and then added to a 50-L fermenter (ABEC Inc., Bethlehem, PA). The pH of the mash was adjusted to 4.0 by using 1 N sulfuric acid (appr. 2.5 L was used). Glucoamylase (0.04% w/w of corn), ammonium sulfate (150 ppm), chlorine dioxide (110 ppm), the polysaccharide hydrolyzing enzyme (4000 ppm), and dry yeast (29 g) were added. The fermentation was carried out at 30 °C. The mixing speed was 250 rpm in the first 6 h and then 200 rpm until finished. Samples were taken periodically for ethanol analysis by HPLC. After 72 h, 3 L of whole beer was collected in two 2-L Erlenmeyer flasks and incubation was done at 30 °C and 150 rpm for 23 h in an incubator shaker (the same one as used in 1.5-L fermentation runs) to simulate the industrial beer well holding step. The 72-h whole beer (before beer well) was subjected to the same analyses as the sample without further incubation. Three fermentation replicates were done with and without the addition of the enzyme.

2.4. Post-Fermentation Processing

As illustrated in Figure 1, whole beer with or without beer well incubation was split into two samples, with one sample rotary evaporated, and the other used as-is. The beer well treatment was not applied to samples from 1.5-L fermentation runs. The rotary evaporation (Rotavapor R-210 and Vacuum Pump V-700, Büchi, Switzerland) at 86 °C for 10 min was to simulate the industrial distillation step. The conditions of rotary evaporation were optimized in a preliminary study to maximize ethanol removal with a minimal loss of water. After the evaporation, water was added to compensate for the weight that was lost during heating, giving the distilled whole stillage a final solids content of 13.3%. Whole beer and distilled whole stillage were subjected to decanting following a procedure that simulates the industrial decanting process (Wang et al., 2009b). Thin stillage and wet cake were obtained after decanting. The wet yields and solid contents of thin stillage and wet cake, and oil contents of wet cake and whole beer were determined.

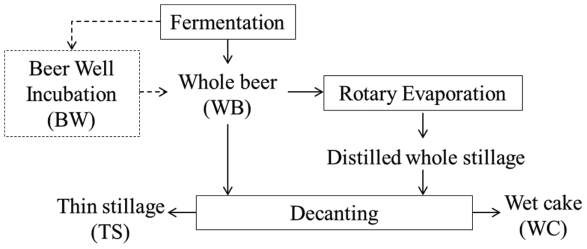

Fig. 1. Flow chart of post-fermentation processing and abbreviations. Beer well incubation (BW) was only carried out for 50-L fermentation to compare with the whole beer (WB) sample obtained after 72 h fermentation.

2.5. Free Oil Release in 50-L Fermentation

Free oil release in whole beer and thin stillage was determined by hexane (liquid–liquid) wash of the top floating oil after centrifugation following procedures of Wang et al. (2008). Briefly, 40 g of whole beer or thin stillage placed in 50-mL conical centrifuge tubes were centrifuged for 10 min at 3,000 g (IEC Centra CL3 centrifuge, ThermoFisher Scientific Inc., Waltham, MA). The oil floating on top of the supernatant was repeatedly washed with 2 mL hexane for 8 to 10 times until the hexane extract became colorless. Shaking/mixing was not applied during the hexane washing. The weight of free oil was determined gravimetrically after hexane was removed from the combined hexane extract. Free oil release was expressed as the percentage of free oil recovered from the total dry solids in whole beer or thin stillage. In the case of rotary evaporated samples, free oil was calculated based on the total dry solids in the distilled whole stillage and the corresponding thin stillage. Free oil release was determined for 50-L fermentations only, including the whole beer samples harvested at 72 h and those after beer well treatment.

2.6. Other Chemical Analyses

Solids (dry matter) content was determined by weight difference after oven-drying at 105 °C for 5 h. Oil content was determined by acid hydrolysis (AOAC Official Method 922.06), and protein content was determined by using the Dumas nitrogen combustion method with an ElementarVario MAXCN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) and a conversion factor of 6.25. Ash content was determined by heating the dry stillage at 550 °C for 5 h (AOAC Official Method 923.03). Fiber analysis was performed according to VDLUFA III, 6.5.1 (amylase treated neutral detergent fiber, aNDF) and VDLUFA III, 6.5.2 (acid detergent fiber, ADF) using an automated system Fibretherm FT12 (C. Gerhardt, Koenigswinter, Germany). Insoluble and soluble dietary fibers were determined using a commercial kit from Megazyme (Bray, Ireland) according to AOAC Method 991.43.

Ethanol production during 1.5-L fermentation was monitored by weight loss as described earlier. Final concentrations of ethanol, sugars, glycerol, and organic acids in the whole beer of 1.5-L and 50-L fermentations, and ethanol production during 50-L fermentation were measured by HPLC. The microfiltered sample was injected into a Shimadzu HPLC (Columbia, MD) equipped with a Rezex ROA-Organic Acid H+ (8%) LC column (300 × 7.8 mm, Phenomenex, Torrance, CA), degasser DGU-20A3R, isocratic pump LC-20AT, autosampler Sil-10AF, column oven CTO-20A, and a refractive index detector RID-10A at 40 °C. The column was eluted with 0.005 N sulfuric acid at a 0.6 mL/min flow rate at 60 °C. The system was standardized on a 3-point calibration standard (MSI HPLC2000.1-KT, Midland Scientific, Omaha, NE).

2.7. Calculations

The calculations of yield, mass distribution, and free oil release are described below using thin stillage as an example.

Wet yield of thin stillage (%)
$= 100 \times$ (g of thin stillage, as-is)
/(g of whole beer, as-is, before decanting)

Solid mass distribution in thin stillage (%)
$= 100 \times$ (g of dry matters in thin stillage)
/(g of dry matters in whole beer, before decanting)

Oil mass distribution in thin stillage (%)
$= 100 \times$ (1-oil mass distribution in wet cake)
$= 100 \times$ (1-(g of oil in wet cake, as-is)
/(g of oil in whole beer, as-is, before decanting))

Free oil in thin stillage (%)
$= 100 \times$ (g of free oil)
/(g of dry matters in thin stillage)

2.8. Statistical Analysis

All the chemical analyses were replicated three times. Triplicate fermentations were conducted on 1.5-L and 50-L scale. Two-way factorial analysis was performed with SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA) to test significant differences among all treatments and main effects at $\alpha = 0.05$. Statistics of mean comparison are labeled in the figures and tables, and the significance difference of the main effect of the polysaccharide hydrolyzing enzymes versus control, and heat versus no heat, is discussed below. Statistical significant interactions between enzyme and heat were not observed.

3. RESULTS AND DISCUSSION

3.1. Effects of Polysaccharide Hydrolyzing Enzyme on Fermentation Performance

Figure 2 shows the concentrations of residual sugars, glycerol, organic acids, and ethanol as determined by HPLC in the whole beer obtained from 1.5-L and 50-L scale fermentation as well as those from a dry milling ethanol plant where the same batches of corn mash was fermented without the use of the enzyme. In general, there were no significant difference in DP3, glucose, glycerol and acetic acid among the fermentations of laboratory runs and those from the commercial plant. The enzyme treatments on 1.5-L scale produced higher ethanol concentrations than those from 50-L scale, which may have been due to the difference in scale and fermenter mixing geometry. The ethanol concentration of the lab-scale runs ranged from 11.5 to 12.7% compared to 12.0% yield

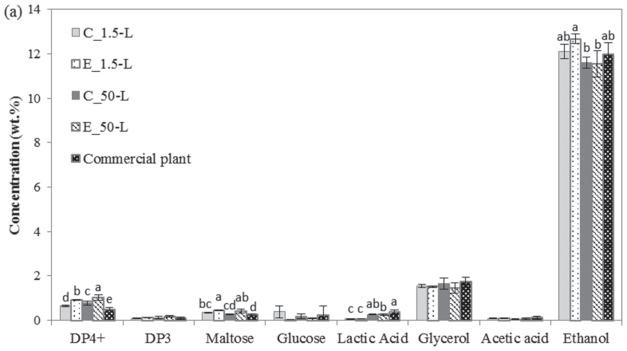

Fig. 2. Effect of enzyme on concentrations of residual sugars, organic acids, glycerol, and ethanol in the whole beers of 1.5-L and 50-L laboratory fermentations ($n = 3$) and that of a commercial ethanol plant ($n = 6$). Data of the commercial plant were from the samplings at drop points (54–56 h). C stands for control and E for enzyme. Different letters within each analyte denote significant difference with $p < 0.05$.

in the commercial plant. The enzyme was mainly composed of non-starch polysaccharides hydrolase, and was not expected to have significant impact on ethanol yield. The enzyme treatments of both 1.5-L and 50-L fermentations left slightly more maltose and less glucose than the

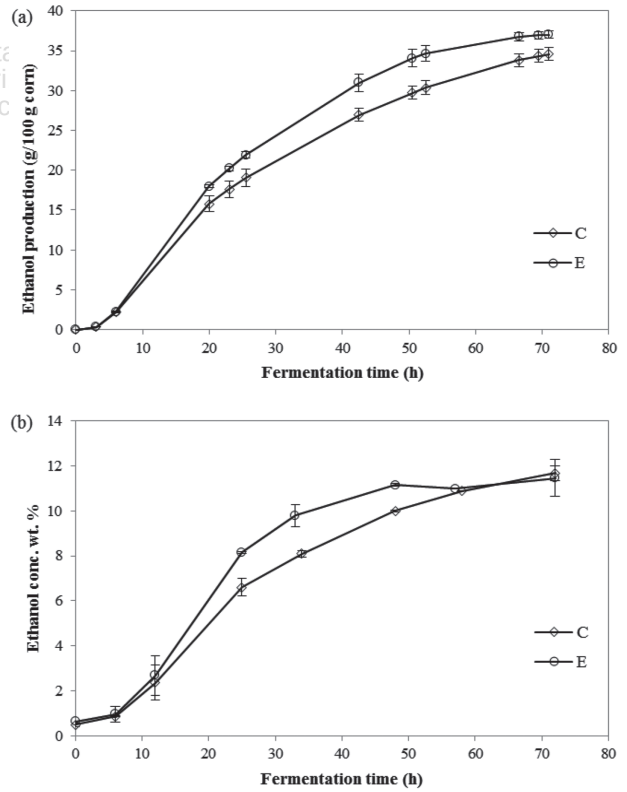

Fig. 3. Effect of enzyme on ethanol fermentation performance. (a) Ethanol production of 1.5-L fermentations ($n = 3$); (b) Ethanol production of 50-L fermentations ($n = 2$).
Notes: The third replicate of each treatment in (b) did not have a complete record and was not included. C stands for control and E for enzyme. Error bars represent one standard deviation.

Table I. Mass distribution (%) of dry matter and oil in thin stillage and wet cake (1.5-L)[a].

	Control		Enzyme	
	No heat	Heat	No heat	Heat
Thin stillage				
Dry matter	55.64 ± 1.12c	53.17 ± 0.51c	64.22 ± 0.74a	59.81 ± 1.72b
Oil	69.83 ± 10.27a	66.12 ± 3.87a	78.96 ± 3.09a	70.71 ± 8.12a
Wet cake				
Dry matter	44.36 ± 1.12a	46.83 ± 0.51a	35.78 ± 0.74c	40.19 ± 1.72b
Oil	30.17 ± 10.27a	33.88 ± 3.87a	21.04 ± 3.09a	29.29 ± 8.12a

Note: [a]Mean ± standard deviation followed by different letters within each row had significant difference with $p < 0.05$ ($n = 3$).

controls although the difference in glucose was not significant with $p > 0.05$. The laboratory runs had slightly higher amounts of DP4+. The difference in residual sugars may have been caused by difference in mixing and scale between our laboratory and the commercial ethanol plant operation. In addition, side activities of the enzyme might have affected the starch hydrolysis. Xylan degradation products might also contribute to the difference in residual sugars.

The ethanol yields are presented in Figures 3(a) and (b). Results from both 1.5-L and 50-L scale confirmed that the enzyme promoted faster ethanol production. Accelerated ethanol production occurred at 12–48 h. We attributed the faster ethanol production with the enzyme to the enzyme's effective hydrolysis of xylan, a cell wall component, thus reducing the viscosity of corn mash (Sapińska et al., 2013; Abbas and Bao, 2013) and giving α-amylase and glucoamylase better access to their substrates. We did batch-to-batch comparisons of ethanol production rate for 50-L fermentations with those that were completed in the commercial ethanol plant using the same batch of mash and found that the 50-L fermentations with the enzyme treatment had about the same or faster ethanol production rate than the commercial trials (data not shown). Laboratory control fermentations, however, took longer time than the commercial plant for ethanol production to reach stationary phase. It is worth noting that laboratory runs had the inoculation with dry yeast, whereas propagated yeast was used in the commercial plant. Our procedure was based on a previously published procedure as cited in the methods section. In summary, the addition of polysaccharide hydrolyzing enzyme did not negatively affect the ethanol yield or other fermentation-related parameters, but did increase the ethanol production rate.

3.2. Effects of Enzyme on Mass Distribution of Non-Fermentable Solids

Tables I to IV present the effects of enzyme and heat on the separation of thin stillage and wet cake (i.e., the wet yields of thin stillage and wet cake) and solids (dry matter) partitioning between the two fractions. Enzyme treatment led to significantly more solids (i.e., all dry matter including oil) partitioning into thin stillage than for the control, giving an increase of 8.6–9.7% without heating or 6.6–8.6% with heating (Tables I and II). Regardless of heating, wet yield of thin stillage from enzyme treatment were higher than for the control whereas solids content of the two treatment was similar (Tables III and IV), which explained why dry matter mass partitioning in the thin stillage was higher with enzyme treatment (Tables I and II). Likely, the fiber hydrolysis action of the enzyme reduced the water-holding ability of insolubles in the wet cake by interrupting the cell wall network. In addition, the enzyme might have reduced the particle size of the solids by degrading the fibers. Regardless, the dual function of the enzyme resulted in more liquid and finer solids partitioning in thin stillage. The wet yield of thin stillage ranged from 84.5–92.0%, with the highest yield of 92% for enzyme treatment without heating. These values were higher than the industrial thin stillage yield of 81% estimated by Wang et al. (2009b)

Table II. Mass distribution (%) of dry matter and oil in thin stillage and wet cake (50-L)[a].

	Control		Enzyme	
	No heat	Heat	No heat	Heat
Thin stillage				
Dry matter	53.27 ± 0.60bc	51.42 ± 2.02c	62.97 ± 0.86a	59.96 ± 4.89ab
Oil	65.21 ± 6.42bc	57.74 ± 3.93c	73.49 ± 2.46a	69.59 ± 8.74ab
Wet cake				
Dry matter	46.73 ± 0.60ab	48.58 ± 2.02a	37.03 ± 0.86c	40.04 ± 4.89bc
Oil	34.79 ± 6.42a	42.26 ± 3.93a	26.51 ± 2.46a	30.41 ± 8.74a

Note: [a]Mean ± standard deviation followed by different letters within each row had significant difference with $p < 0.05$ ($n = 3$).

Table III. Wet yield (%) and dry matter content (%) of thin stillage and wet cake (1.5-L)[a].

	Control		Enzyme	
	No heat	Heat	No heat	Heat
Thin stillage				
Wet yield	89.03 ± 0.16b	86.04 ± 0.26c	92.00 ± 0.20a	89.07 ± 0.68b
Dry matter content	6.49 ± 0.31b	7.61 ± 0.15a	6.89 ± 0.08b	7.80 ± 0.14a
Wet cake				
Wet yield	10.97 ± 0.16b	13.96 ± 0.26a	8.00 ± 0.20c	10.93 ± 0.68b
Dry matter content	41.95 ± 0.70b	41.29 ± 0.78b	44.13 ± 0.46a	42.76 ± 0.26ab

Note: [a]Mean ± standard deviation followed by different letters within each row had significant difference with $p < 0.05$ ($n = 3$).

and also higher than laboratory values (~83%) obtained in a previous study using the same decanting method (Wang et al., 2009b). The effect of fiber hydrolyzing enzymes on the wet yield of thin stillage is apparent. Higher yield of thin stillage means energy-saving in the subsequent DDGS drying steps, and thus is a favorable outcome. The thin stillage dry matter contents in the control and enzyme treatment were 6.5–8.1% and 6.9–8.7%, respectively, similar to typical industrial values 7.0–7.5% (Wang et al., 2009b). The dry matter mass partitioning in thin stillage of the control and enzyme treatment increased by 16–23% and 32–42%, respectively, over the industrial thin stillage (Wang et al., 2009b).

Heating affected the separation of thin stillage and wet cake (i.e., wet yield). Possibly, heating altered the interaction among various components in the stillage resulting in more compact network that held more water in the wet cake. Effects of heating on the wet yields of thin stillage and wet cake were not in agreement with the previous study (Wang et al., 2009b), which we attributed to the slightly different evaporation conditions. Heating, however, slightly increased the dry matter content of thin stillage. Heating removed most of the ethanol, which in turn, left a more viscous suspension, and enabled more of the fine particles to partition into in thin stillage. In general, enzyme facilitated the separation of thin stillage from wet cake. Heating increased the dry matter content in thin stillage, but decreased thin stillage wet yields, and thus did not affect the dry matter mass distribution in thin stillage and wet cake.

3.3. Effects of Enzyme on Oil Partitioning Between Thin Stillage and Wet Cake

Enzyme treatment increased oil partitioning in thin stillage of 50-L fermentations by 13% (with heating) and 21% (without heating) (Table II). The highest oil partitioning in thin stillage was 73% versus 27% in wet cake for the enzyme treatment without heating. Correspondingly, a 28% less oil was found in wet cake (with heating) and 24% less oil were found in wet cake by enzyme treatment (without heating). Although we observed the same trend in the 1.5-L trials, the differences of treatment means or main effects were not statistically significant due to large variations, which may have been resulted from the non-uniform distribution of oil in the wet cake. Oil mass distribution of 1.5-L and 50-L scale showed a pattern similar to those of dry matter mass distribution. We expected that more oil would be found in thin stillage due to the more total dry matter in thin stillage caused by the enzyme treatment. A previous study (Wang et al., 2008) showed that oil content in thin stillage strongly correlated with dry matter content.

Measurement of free oil release in whole beer and thin stillage was used to confirm the positive effect of the enzyme on oil partitioning. As seen in Table V, enzyme treatment led to significantly more free oil release from whole beer than the control did, giving a 96–150% increase. Thin stillage also seemed to have higher free oil yield by enzyme treatment than from the control although the difference was not statistically different. The oil in thin stillage emulsion is expected to be trapped by

Table IV. Wet yield (%) and dry matter content (%) of thin stillage and wet cake (50-L)[a].

	Control		Enzyme	
	No heat	Heat	No heat	Heat
Thin stillage				
Wet yield	87.97 ± 0.81ab	84.53 ± 1.19b	90.88 ± 0.61a	87.81 ± 2.41ab
Dry matter content	6.73 ± 0.52c	8.08 ± 0.47ab	7.35 ± 0.59bc	8.66 ± 0.11a
Wet cake				
Wet yield	11.86 ± 0.79ab	14.95 ± 1.41a	8.92 ± 0.57c	11.93 ± 2.33ab
Dry matter content	43.60 ± 0.65a	43.19 ± 0.47a	43.87 ± 0.96a	43.03 ± 1.39a

Note: [a]Mean ± standard deviation followed by different letters within each row had significant difference with $p < 0.05$ ($n = 3$).

Table V. Free oil recovered (% relative to total solids) from whole beer and thin stillage (50-L) with and without beer well incubation[a].

	Control		Enzyme	
	No heat	Heat	No heat	Heat
No beer well				
Whole beer	3.79 ± 0.92bc	2.44 ± 0.65c	7.44 ± 1.07a	6.10 ± 0.36ab
Thin stillage	8.15 ± 1.76a	2.82 ± 0.38b	9.95 ± 0.55a	3.19 ± 0.16b
Beer well				
Whole beer	3.75 ± 0.51b	2.77 ± 0.96b	7.82 ± 1.07a	6.58 ± 0.93a
Thin stillage	7.15 ± 1.27ab	2.55 ± 0.91c	10.97 ± 1.37a	3.48 ± 2.14bc

Note: [a]Mean ± standard deviation followed by different letters within each row had significant difference with $p < 0.05$ ($n = 3$).

polysaccharides, and present in the intact oil bodies (Wang et al., 2008; Majoni et al., 2011b). As expected, because polysaccharides were partially broken by the enzymes, more of the trapped oil would be released. However, the difference of free oil between the enzyme treatment and the control of non-beer well samples was not significant with $p > 0.05$.

Although heating did not affect the oil mass distribution in thin stillage and wet cake, it had detrimental effect on free oil recovery from thin stillage. A 3-fold reduction of free oil recovery from thin stillage was found in heated samples (for both non-beer well and beer well samples) (Table V). The impact of heating on free oil recovery in whole beer was also observed, but the reductions were not statistically significant ($p > 0.05$). Previous studies suggested that heating strengthened the hydrophobic interaction of oil and corn protein zein as well as the fiber, and therefore resulted in a poor oil recovery (Wang et al., 2009a; Majoni et al., 2011b; Wang et al., 2009b). The presence of ethanol may have helped solubilize the oil and break the emulsion that trapped the oil. It is quite evident that the removal of ethanol led to a decrease in free oil recovery. In fact, use of a polar solvent, such as ethanol, is an effective means to separate oil from thin stillage (Mueller and Kwik, 2014).

Results of oil partitioning in wet cake and thin stillage and free oil release of the present study suggest that the addition of enzyme has a great potential to increase oil recovery after fermentation. Previously, it was found that the use of protease and cellulase increased the yields of oil recovery from condensed corn distillers solubles from 60–65% to 70–83% (Majoni et al., 2011b). Cellulase alone gave oil yield of 80% from corn germ (Moreau et al., 2004). A Novozyme enzyme package (Olexa) is claimed to improve oil recovery by 15% by releasing the bound oil from oleosome (Novozyme, 2015). As summarized by Moreau et al. (2012), the yield of corn oil from dry grind ethanol production obtained after fermentation currently ranges from 25–80%, mainly via evaporation and centrifugation and additional processing aids. Unfortunately, enzyme treatments used in the ethanol industry for corn oil recovery are not often reported in the literature and mostly industrial information and technologies are proprietary. Therefore, a thorough discussion and comparison of our work with others is proven to be difficult.

The entire dry matter and oil analyses were also conducted for samples from beer well treatment. The beer well treatment gave a similar trend to those of non-beer well samples as discussed above; however, because of different mixing conditions used during beer well and fermentation and/or other unknown reasons, the results of beer well samples had large variations leading to statistically insignificant differences. The incubation time of beer well (23 h) was also much longer than the industrial typical time of 5 h. Thus, beer well data except for those of free oil release were not included in this report. Beer well treatment had no effect on the various parameters examined in the present study.

3.4. Effect of Polysaccharide Hydrolyzing Enzyme on DDGS Quality

Because the focus of the present study was not on oil separation, DDGS was obtained from whole beer without oil removal. The oil, protein, ash and total fiber (aNDF) contents of DDGS on dry weight basis were 9.5–12, 29.5–32.1, 4.9–5.6, and 30.1–34.0% (Table VI), which were within typical ranges of commercial DDGS (Liu, 2011). There was 11% reduction of total fiber in the DDGS from the enzyme treatment ($p = 0.058$). Consequently, the proportion of other components in DDGS of

Table VI. The composition of DDGS (% dry weight basis)[ab] produced under different conditions.

	1.5-L		50-L	
	Control	Enzyme	Control	Enzyme
Oil	11.4 ± 0.9	12.0 ± 1.6	9.5 ± 0.6	10.3 ± 1.1
Protein	30.0 ± 0.6*	32.1 ± 0.5*	29.5 ± 1.0*	32.1 ± 0.8*
Ash	5.3 ± 0.2*	5.6 ± 0.2*	4.9 ± 0.2*	5.3 ± 0.1*
aNDF[c]	–	–	34.0 ± 2.8	30.1 ± 1.2
ADF[c]	–	–	17.8 ± 2.8	13.3 ± 2.4

Notes: [a]DDGS without prior oil removal. [b]Asterisks in the same row of 1.5-L or 50-L denote significant difference with $p < 0.05$. [c]aNDF is amylase-treated neutral detergent fiber; ADF is acid detergent fiber; and –indicates not measured.

enzyme treatment (of 50-L runs) increased. The oil, protein and ash contents of enzyme treatment all increased by 8–9%. Results of 1.5-L runs gave a similar trend for DDGS composition of the control and enzyme treated materials.

4. CONCLUSIONS

The effects of polysaccharide hydrolyzing enzyme on ethanol fermentation performance as well as oil partition at 1.5-L and 50-L scale were in good agreement. The enzyme decreased ethanol fermentation time and the yield of thin stillage. More oil partitioned into thin stillage than into the wet cake, which may increase the profitability of ethanol plants if appropriate oil separation technology is applied. DDGS with low fat and reduced fiber contents can potentially be obtained after enzyme treatment, which may enable higher levels of feed addition for monogastric and aquatic animals. Additional benefit of using polysaccharide hydrolyzing enzymes during fermentation is that the introduction of this technology would not require further change in the design of an ethanol plant. However, the adverse effect of distillation on the oil partition needs to be considered in further scale-up. Moreover, research on the mechanism how polysaccharide hydrolyzing enzymes facilitate the corn oil separation in the dry-grind process is needed.

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