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Research paper

# Use of surfactant and enzymes in dry-grind corn ethanol fermentation improves yield of ethanol and distillers corn oil

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ABSTRACT

Distillers corn oil (DCO) is a valuable co-product of dry-grind corn ethanol process. It can be used for bio-fuel production and in animal feed. DCO can be present in different forms in the fermentation matrix, including oil adhering to solid surfaces such as cell wall and protein matrix, and oil contained in unbroken cells, which is difficult to partition to thin stillage by decanting. Effects of using surfactant (Tween® 80) and enzymes during fermentation on DCO partition to thin stillage and DCO recovery from the condensed corn distillers with solubles (CCDS) were investigated. There was more than 8 ~ 10% DCO adhered to wet cake solids in whole stillage produced by conventional procedure, and this part of DCO was moved to thin stillage when 500 ppm of Tween® 80 was used during fermentation. Enzymes reduced the particle size of wet cake solids and released more DCO from wet cake to thin stillage. However, the use of protease reduced oil recovery (4.0% versus 7.9% and 17.9%, protease versus control and non-starch polysaccharide hydrolyzing enzymes) by producing partially hydrolyzed protein, which may have acted as emulsifier. Moreover, a synergistic effect between the use of enzymes and Tween® 80 was found on DCO partition in thin stillage and its recovery from CCDS.

## 1. Introduction

Dry-grind corn ethanol industry has become the second largest corn user in the United States, producing 90% of the ethanol in the U.S. in 2015. This industry was very profitable in the past. However, due to the sharp decline in crude oil price, the price of dry-grind corn ethanol fell sharply in recent years (Irwin and Good, 2015; Wisner, 2015). The additional revenue streams from co-products of the dry-grind ethanol process are becoming more important. One such co-product is the distillers corn oil (DCO), which is the oil recovered from post-fermentation streams. The revenue from DCO has become more important, particularly in the low profit margin times (Jayasinghe, 2015).

The most widely used method for DCO recovery in dry-grind ethanol process is separating the oil from the condensed corn distillers solubles (CCDS) by centrifugation (Moreau et al., 2012). The oil recovery procedure was described in a U.S. Patent 7,601,858. In general, when the whole stillage is separated by decanting into thin stillage and wet cake, 40 ~ 60% of total oil in corn whole kernel is left in wet cake and the rest goes to thin stillage after decanting. The thin stillage is further evaporated to produce CCDS with 60 ~ 85% water content, and the DCO is separated from the CCDS by using a disk stack centrifuge (Cantrell and Winsness, 2009). Many efforts have been made to improve the DCO recovery from CCDS. Centrifugation coupled with oil recovery aid is easy to use and relatively effective in improving oil recovery from the CCDS. A number of commercial oil recovery aids have been designed for large-scale process, including FoodPro SA9843 corn oil yield improver (General Electric, Trevose, PA, USA), PTV M-5309 corn oil extraction aid (Ashland Chemical, Covington, KY, USA), Ashland DPI-428 (Ashland Hercules Water Technologies, Wilmington, DE, USA), and Hydri-Maize Demulsifier 300 (Hydrite Chemical Co., Waterloo, IA, USA). However, these products are designed for recovering oil from CCDS only, and there is limited information on how they affect the partitioning of oil during decanting from wet cake to thin stillage.

DCO is present in several different forms during the dry-grind corn ethanol process, including the oil adhering to surface of wet cake solids, like cell wall and protein matrix (Majoni et al., 2011). Based on our preliminary experiments, about 8 ~ 10% w/w of total corn oil that was only part of the total oil partitioned in wet cake was attached to solid wet cake particles, which did not partition to thin stillage fraction by decanting. The oil adhering to the wet cake surface is very similar to the oil stain on a fabric surface. Surfactants as cleaning agents work by reducing the surface tension and removing the oil as micelles. In our previous study on the distribution of different types of oil in CCDS, the use of surfactant mix resulted in a higher recovery of oil partially coming from surface adhering oil (Fang et al., 2015). This gives the

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basis for our hypothesis that the cleaning function of surfactants could be applied in dry-grind corn ethanol process to partition the oil from wet cake to the thin stillage to enable high recovery of oil in the later stage.

Surfactants have been applied in aqueous extraction processing to improve vegetable oil recovery. Sodium dodecyl sulfate was used to improve recovery of soybean oil (Campbell and Glatz, 2009) and canola oil (Tuntiwiwattanapun et al., 2013) by extracting the oil trapped in disrupted cellular matrices. The extended-surfactants, which are recently developed as a new class of surfactants that can significantly reduce the interfacial tension, extracted 93–95% of total oil from ground peanut and canola seeds (Do and Sabatini, 2010). However, due to the safety issue of the extended-surfactants, they are not allowed for human or animal consumption. To date, only a few reports on destabilization of oil-in-water emulsion by using food-grade-non-ionic surfactants are available. Fang et al. (2015) attempted to improve oil recovery from CCDS by using a Tween® 80-Span® 80-silica nanoparticle mixture. Zhang and Wang (2016) used Tween® 20 to improve peanut oil recovery. Both works showed an improved oil recovery as the result of emulsion destabilization by surfactant-protein competition on the emulsion interface. Thus, we believe that using surfactant at the beginning of dry-grind ethanol process could not only improve oil partition in thin stillage but also increase the oil recovery from CCDS by de-emulsification.

There is a large portion of oil (40 ~ 60% of total oil in corn) remaining in intact cells and protein/polysaccharide matrices of wet cake solids. Therefore, an enzyme hydrolysis of the solids might be an efficient way to release this part of the oil. Luangthongkam et al. (2015) reported that using a combination of cellulolytic enzymes, protease, and phytase during fermentation led to a higher oil partition in thin stillage. However, no research has been reported to confirm if hydrolyzing the non-fermentable components during fermentation step can improve oil recovery from CCDS. Therefore, the objectives of this research were 1) to determine the optimum level and best processing stage to add the surfactant (Tween® 80), and 2) to investigate the synergistic effects between surfactant and hydrolyzing enzymes (protease, cellulase, and pectinase) on ethanol production and oil recovery.

## 2. Materials and methods

### 2.1. Materials

Yellow dent corn produced in 2013 was procured from Honeyville (West Chester, OH). The corn was ground using a Fitz Mill (Model DAS 06, Fitzpatrick Co., Elmhurst, IL) at 3000 rpm to give coarsely material (average particle size of 0.44 mm). It contained 10.1% moisture, 5.1% (dwb) oil, 8.6% (dwb) protein, 1.1% (dwb) ash, and 74.6% (dwb) starch. α-Amylase (Novozymes, Franklinton, NC), glucoamylase (liquid, Spirizyme Excel XHS, Novozymes, Franklinton, NC), dry yeast (Saccharomyces cerevisiae; commercial grade currently being used in the ethanol industry) and antibacterial chlorine dioxide (commercial grade) were provided by Lincolnway Energy LLC, Ames, IA. Cellulase (75,000 CU/g) and pectinase (3500 ENDO-PG/g) were provided by Bio-Cat (Troy, VA). In this study, pectinase and cellulase were used as a mix (PC) with a ratio of 1:1 (w: w). Fermgen™ (acid protease in liquid form, activity of 1000 SAP units/g) was provided by DuPont Industrial Biosciences (Palo Alto, CA). The other chemicals, including Tween® 80 (polysorbate 80), hydrochloride acid, petroleum ether, and ethyl ether were purchased from Fisher Scientific (Fairlawn, NJ, USA).

### 2.2. Corn fermentation

The procedure of lab-scale fermentation is shown in Fig. 1. The liquefaction and simultaneous saccharification and fermentation of the corn slurry were performed in 250-mL round bottom flasks with Tornado IS6 Overhead Stirring System (Radleys Discovery Technologies, Shire Hill, Saffron Walden, UK) equipped with an anchored stirring shaft. Ground corn was mixed with cold DI water (or Tween® 80 aqueous solution) at a 1:2 ratio (w:w). The total amount of slurry was maintained at 225–230 g α-Amylase (0.15 mL) was added to the slurry and mixed at 81 °C for 3 h. Afterwards, the flasks were cooled to 30 °C in an ice bath, and the pH of the cooled slurry was adjusted to 4.0 with 3 M sulfuric acid. Chlorine dioxide (0.021 mL), ammonium sulfate (0.065 mL of 0.2 g/g water), *gluco*-amylase (0.15 mL) and dry yeast (0.15 g) were added. Fermentation was carried out at 30 °C for 64 h with continuous stirring (190 rpm). During fermentation, ethanol production was estimated by mass loss according to the following equation (Wang et al., 2009).

$$\text{Ethanolyield(g/100gdrycorn)} = 100 \times \frac{46 \times (gofmassloss)}{44 \times (gofdrycorn)}$$

Where 46 and 44 are molecular weights of ethanol and $CO_2$, respectively.

For the experiments of adding hydrolyzing enzymes during fermentation, 0.375 mL of Fermgen or 0.3 g of PC was added before starting the fermentation. For experiments of using Tween® 80 in fermentation, Tween® 80 water solutions of 200, 300, 400, 500, 600, 700, 800, 1000 ppm concentration were prepared and ground corn meal was mixed with Tween® 80 solutions instead of DI water.

### 2.3. Post-fermentation processing

A rotary evaporation (Rotavapor R-210 and Vacuum Pump V-700, Buchi, Switzerland) at 82 °C for 10 min was conducted to simulate the industrial distillation step. After this step, water was added to make up for the weight loss during rotary evaporation, giving the stillage a final solids content of 13% w/w. The whole stillage was then subjected to decanting following a procedure that simulates the industrial decanting process and performance (Wang et al., 2009) to obtain the thin stillage and wet cake fractions. Briefly, a self-designed laboratory decanting device was used, and the multiple-wash and centrifugal filtration (MWCF) procedure was applied to the whole stillage. The 4 sequential washings of the cake solids with the clear/top liquid generated from each step of centrifugal filtration produced a thin stillage with similar solids content, dry-matter yield, and wet yield compared to industrial thin stillage produced by decanting. The final wet yield, solid content and oil content of thin stillage and wet cake were measured. CCDS was made by condensing thin stillage with rotary evaporation at 75 °C for 30 min. The solid content of CCDS was adjusted to 28% with water. All CCDS samples were stored at 4 °C until use.

### 2.4. Oil recovery from CCDS

Oil recovery from CCDS was simulated by using the method of Fang et al. (2015). To compare the effect of surfactant use during fermentation, 2300 ppm Tween® 80 was added in CCDS before heating and shaking. In brief, 40 g of CCDS in a 250-mL centrifuge bottle was heated at 80–85 °C for 10 min at 100 rpm shaking in a shaker water bath (Model R-76, New Brunswick Scientific Co. Inc., NJ, USA). Immediately following heating and shaking, oil was separated using a Centra MP4 centrifuge (International Equipment Company, Needham Heights, MA, USA) at 3000 xg for 10 min. The oil layer was collected by washing the oil on the top with hexanes (5 washes with 20, 20, 10, 10, and 5 mL respectively). The solvent was removed by evaporation, then by vacuum drying. The weight of the oil which was freed oil and recovered by centrifugation was determined gravimetrically.

### 2.5. Surfactant recyclability with backset

Thin stillage sample was collected from corn fermentation with 500 ppm Tween® 80 as described in Section 2.3 and was used as backset