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Addition of cellulolytic enzymes and phytase for improving ethanol fermentation performance and oil recovery in corn dry grind process

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ABSTRACT

Application of hydrolytic and other enzymes for improving fermentation performance and oil recovery in corn dry-grind process was optimized. Non-starch polysaccharide enzymes (BluZy-P XL; predominantly xylanase activity) were added at stages prior to fermentation at optimum conditions of 50 °C and pH 5.2 and compared with conventional fermentation (30 °C, pH 4.0). Enzyme applications resulted in faster ethanol production rates with a slight increase in yield compared to control. The thin stillage yield increased by 0.7–5% w/w wet basis with corresponding increase in solids content with enzyme treatment after liquefaction. The oil partitioned in thin stillage was at 67.7% dry basis after treatment with hydrolytic enzymes during fermentation. Further addition of protease and phytase during simultaneous saccharification and fermentation increased thin stillage oil partitioning to 77.8%. It also influenced other fermentation parameters, e.g., ethanol production rate increased to 1.16 g/g dry corn per hour, and thin stillage wet solids increased by 2% w/w. This study indicated that treatments with non-starch hydrolytic enzymes have potential to improve the performance of corn dry-grind process including oil partitioning into thin stillage. The novelty of this research is the addition of protease and phytase enzymes during simultaneous saccharification and fermentation of corn dry-grind process, which further improved ethanol yields and oil partitioning into thin stillage.

1. Introduction

The US corn production in 2014 was approximately 14.2 billion bushels, with roughly 30% utilized for ethanol production (NCGA, 2015). Ethanol has been the most significant source of total biofuel usage in the US (94%), of which about 82% is produced using corn dry-grind process (Wang et al., 2009a). In this process, ground corn is liquefied, saccharified, and fermented to convert monomeric glucose to ethanol. Non-fermentable residues result in a coproduct called distiller's dried grains with solubles (DDGS) after separation and drying with condensed solubles of thin stillage. On dry basis, DDGS usually contains 27.4, 11.7, 4.4, and 56.5% w/w of protein, oil, ash, and total carbohydrate, respectively (Liu, 2008). Approximately 40 million tons of DDGS were produced in 2012 and projected to reach 43 million tons in 2014 (Wisner, 2014). DDGS are also utilized as animal feed, with various incorporation levels for cattle and non-ruminant animals, higher fiber percentages limiting usage in the latter. Ethanol producers need to improve desirable characteristics in DDGS as animal feed to enhance its incorporation levels. Application of hydrolytic and other enzymes during processing could modify non-starch polysaccharides (NSP) more favorably for feed application and recover more oil upstream to make the process more profitable.

Corn oil is a higher-value coproduct of corn dry-grind process and is concentrated from 4% in corn kernel to about 14% in DDGS (Wang et al., 2008a; Wang, 2008b). Higher levels of oil in DDGS are sometimes undesirable and affect feed quality negatively; for example, higher amounts of oil could interfere with milk production in cattle and bacon texture in DDGS-fed swine (Wang et al., 2009b). Recovery of corn oil from the stillage will create a higher-value product stream than DDGS. Technologies for corn oil recovery from dry grind process are reported in the literature. Effect of physical treatments like grinding and flaking (Lamsal and Johnson, 2012), heating and solvent introduction before and after the corn dry-grind process (Majoni et al., 2011a; Wang 2008a; Wang et al., 2009a) were reported to enhance process performance. Use of hydrolytic enzymes is an environment-friendly and affordable method that can benefit corn dry-grind process (Johnston and McAloon, 2014), including recovery of corn oil (Majoni et al., 2011b).

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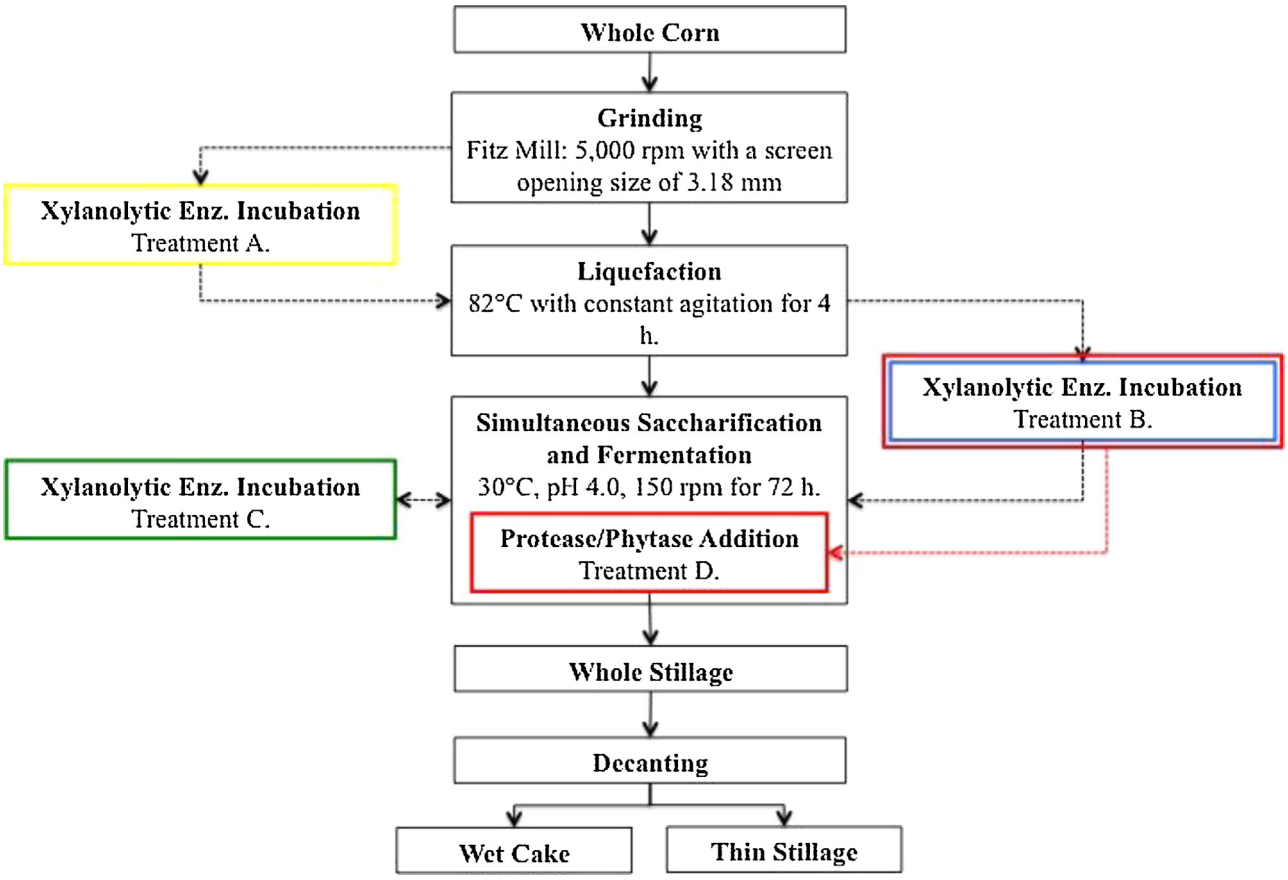

Fig. 1. Flow chart for BluZy-P XL enzymatic treatment at different processing stages for corn dry-grind ethanol process. Treatments A–C stand for xylolytic enzymes incubation at pre-liquefaction, post-liquefaction, and during-fermentation, respectively. Treatment D was a combination of Treatment B with protease and phytase supplementation during simultaneous saccharification and fermentation.

Corn oil is mostly stored in germ cells as oil bodies or oleosomes and is secluded by phospholipids and layer of oleosin, an alkaline protein (Huang, 1996; Danso-boateng, 2011). During corn dry-grind process, oil bodies can be trapped between non-starch polysaccharide and protein matrix. Addition of protease and NSP hydrolyzing enzymes during the corn dry-grinding process can degrade such barriers and enhance oil recovery. Proteases are also suggested for free amino nitrogen production and utilization by yeast during fermentation (Vidal, 2010) that could result in higher ethanol production rates and yields.

This study compared the performance of corn dry-grind process upon addition of NSP hydrolytic enzyme cocktail (BluZy-P XL) and other enzymes. The application of BluZy-P XL cocktail, provided by Direvo Industrial Biotechnology GmbH (Cologne, Germany), during the simultaneous saccharification and fermentation (SSF) at 30 °C for 60 h was compared with treatments at optimal enzyme conditions (pH, temperature, and process stages). Combination of the said enzyme cocktail with protease and phytase in corn dry-grinding process was also compared for enhanced performance indicators.

2. Materials and methods

Yellow dent #2 corn used in the study was obtained from Iowa State University's research farm and stored at 15% moisture content in airtight bags placed inside an airtight plastic bin at 4 °C. Corn contained 67% starch, 7% protein, and 3% lipid w/w on wet basis, the remainder being fiber, ash, and moisture. Corn was ground by using a hammer mill (Fitz Mill model DAS 06; Fitzpatrick Co., Elmhurst, IL) at 5,000 rpm with 3.18 mm screen opening (screen # 1531-0125). The ground corn meal had a particle size distribution of 4, 22, and 74 w/w% retained on mesh numbers 20, 12, and pan, respectively.

Liquid α-amylase Spezyme Xtra (13,642 R-amylase units/g) and protease GC 212 (2000 SAP units/g; SAP, spectrophotometer acid protease) were provided by Genecor international (Palo Alto, CA). Glucoamylase Spirizyme Excel XHS (Novozymes, Franklinton, NC), phytase Phytaflow (20,000 FYT/g, Novozymes, Bagsvaerd, Denmark), and dry yeast (*Saccharomyces Cerevisiae*) were provided by Lincoln way Energy (Neveda, IA). Lactrol (462 g virginiamycin/lb) was purchased from PhibroChem (Ridgefield Park, NJ). The BluZy-P XL enzymes cocktail, with mostly xylanase activity, was acquired from Direvo Industrial Biotechnology GmbH (Cologne, Germany). Other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

2.1. Optimal temperature and pH conditions for addition of enzymes cocktail

The enzyme cocktail BluZy-P XL obtained from the company was experimental mix with a broad range of temperature and pH conditions, which needed narrowing down for best performance in corn dry-grind process being followed. Ground corn was mixed with distilled water at the ratio of 1:2 in 250-mL Erlenmeyer flaks for a total slurry weight of 200 g. For pH and temperature optimization experiments, the pH of slurry was adjusted to 3.8, 4.5, and 5.2 with 6.0 N sulfuric acid and incubated in incubator shaker at 150 rpm at 35, 42, and 50 °C for 1 h. This range of temperature and pH was chosen following enzyme data sheet that showed a broader activity range. BluZy-P XL cocktail (400 ppm) was added and shaken steadily in incubator shaker at 150 rpm for 1.5 h (Innova