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Protease increases fermentation rate and ethanol yield in dry-grind ethanol production ☆

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HIGHLIGHTS

- The benefits of protease addition during corn ethanol fermentations were evaluated.
- Increase in rates and ethanol yields were demonstrated with protease addition.
- The effects of using an inorganic nitrogen supplement were compared.
- Process modeling was used to evaluate the economic effects.
- The decrease in overall processing costs was found to be as high as 4¢/gallon.

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ABSTRACT

The effects of acid protease and urea addition during the fermentation step were evaluated. The fermentations were also tested with and without the addition of urea to determine if protease altered the nitrogen requirements of the yeast. Results show that the addition of the protease had a statistically significant effect on the fermentation rate and yield. Fermentation rates and yields were improved with the addition of the protease over the corresponding controls without protease. Protease addition either with or with added urea resulted in a higher final ethanol yield than without the protease addition. Urea addition levels >1200 ppm of supplemental nitrogen inhibited ethanol production. The economic effects of the protease addition were evaluated by using process engineering and economic models developed at the Eastern Regional Research Center. The decrease in overall processing costs from protease addition was as high as $0.01/L (4¢/gal) of denatured ethanol produced.

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1. Introduction

The production of fuel ethanol from corn has significantly increased during the last 15 years. In 1997, the annual US production of fuel ethanol was <5.7 billion L/year (1.5 billion gal/year). In 2013, production is expected to reach almost 53 billion L (14 billion gal) (RFA, 2013). During this period, processing modifications have also increased the overall plant efficiency (Hettinga et al., 2009). Reductions in energy and water needed to produce ethanol have decreased while the yield of ethanol from corn has increased.

Lantero and Fish (1993) showed that the addition of a protease during fermentations with corn could increase the rate of fermentation and the final ethanol yield. Additional work has demonstrated the importance of free amino nitrogen content on fermentation rate and yield for high gravity wheat fermentations and showed the effects of added proteases (Jones and Ingledew, 1994a,b). It has also been shown that not all nitrogen sources are equally effective as yeast supplemental nitrogen sources and some (such as glycine) may be detrimental (Thomas et al., 1993). Other effects on fermentation products by proteases have also been shown. Perez-Carrillo et al. (2012) showed how a neutral pH protease treatment affects free amino nitrogen (FAN) and fusel alcohol production in a separate protease treatment done between liquefaction and saccharification for both corn and sorghum. They showed increasing FAN levels for corn and sorghum by 60% and 30%, respectively. Klosowski et al. (2010) showed in fermentations with corn that an alkaline protease and other enzyme preparations could increase fermentation rates and ethanol yields.

It is clear from the above mentioned works, as well as others, that proper yeast nutrition and nitrogen supplementation is critical for efficient fuel ethanol production. The objectives of the work presented here were to examine the existing practices in the production of fuel ethanol from corn in relation to supplemental nitrogen and how the use of a commercial protease could impact the overall economics of the production process.

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2. Methods

2.1. Materials and enzymes

Enzymes used in the present study were gifts of DuPont Industrial Biosciences (Palo Alto, CA). SPEZYME FRED® (thermostable α-amylase) and OPTIDEX® L-400 (glucoamylase) were used for the corn mash preparations as described below. FERMGEN® (FG) is an acid protease from *Trichoderma reesei* and was used in most of the comparative studies. An additional protease (GC 106) was also used. The activities for both FG and GC 106 were 1000 SAP units/g. One SAP unit is defined as the amount of enzyme that liberates one micromole of tyrosine per minute from a purified casein substrate incubated at pH 3.0 and 37 °C.

Thin stillage (TS) was obtained from a commercial corn ethanol facility that does not add protease. The TS was stored frozen until used. It was thoroughly mixed after thawing and prior to being used with mashing in order to mix the suspended solids.

2.2. Corn grinding and analysis

Commercial yellow dent corn was ground in a Bunn (Springfield, Ill) (model G2) burr mill coffee grinder. The gap was set so that the ground corn produced would pass through a 2 mm screen. Moisture content of the ground corn was determined by using AOAC Official Method 930.15 (AOAC International, 2005).

2.3. Mash preparation

The appropriate amounts of ground corn (adjusted for moisture content) and water were added to a beaker to make either a 30% or 35% total solids slurry and the total mass was measured to later compensate for water loss due to evaporation. A mechanical mixer was used to continually mix the corn and water suspension. The pH was adjusted to 5.8 with 1 *N* HCl and alpha-amylase (SPEZYME FRED®, DuPont Industrial Biosciences) was added at a dosage of 0.5 mL/kg of mash (2 kg/MT dry corn). The slurry was heated to 90 °C on a hot plate and held for 60 min. The mash was then cooled to 30 °C and supplemental N (400 ppm) was added as urea if the experiment required. The pH was then reduced to 4.5 with 1 *N* HCl and glucoamylase (OPTIDEX® L-400, DuPont Industrial Biosciences) was added at a dosage of 0.4 mL/kg of mash (1.6 kg/MT dry corn). The total weight was readjusted by adding water to compensate for evaporation losses. Active yeast was added (1.1 g/kg of mash) to start the fermentation (Red Star Ethanol Red, Lesaffre, Milwaukee, WI).

To prepare mash for experiments where the solids content was varied, a 35% total solids slurry was initially prepared. The mash was then divided by weight and diluted with water to obtain the lower solids contents. In experiments where backset (thin stillage) was used, a portion of the water was replaced by the appropriate amount of TS in order to reach 12% w/w. Supplemental N (400 ppm) was added as urea if the experiment required.

To prepare the mash for experiments for determining if additional nitrogen could improve ethanol yields, a corn mash was prepared without urea at 30.3% corn solids. The prepared mash was partitioned into flasks and 1 mL of urea solution added to give the final mash supplemental nitrogen (from urea) ranging from 0 to 2000 ppm. Additional flasks with Fermgen were prepared without added urea for direct comparison. The final mash concentration after urea addition was 30% w/v solids. Flasks were incubated and analyzed as with previous studies.

2.4. Fermentation

Corn mash was distributed (100 g) into each pre-weighed 250 mL Erlenmeyer flasks equipped with rubber stoppers and 21-gauge needles to vent CO_2 produced during fermentation. The appropriate dose of protease (if required) was added to each flask and a final flask weight was taken. Flasks were incubated with shaking at 200 rpm for 72-h at 30 °C and periodically weighed to determine loss due to CO_2 production. After fermentation, the final flask mass was measured and a small (1 mL) sub-sample was removed for HPLC analysis.

2.5. Fermentation rate analysis

The total starch content of the corn used for mash preparation was determined by using AACC Method 76–13.01 (AACC International, 2000). Using the starch content, the mash solids content and the mass loss of the flasks during the fermentation period, the percent theoretical weight loss was calculated for each time point measured. This method allowed comparison between different mash preparations having varying solids contents and to determine how close the fermentation was to theoretical completion.

2.6. HPLC analysis

The sub-sample taken after fermentation was centrifuged at 16,000*g* and the supernatant was filtered through a 0.2 μm filter (Acrodisc, PALL Life Sciences, Ann Arbor, MI). The sample was then analyzed using an Agilent 1200 HPLC (Santa Clara, CA). The HPLC was equipped with a refractive index detector and an ion exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA). The column temperature was maintained at 65 °C and 5 mM sulfuric acid was used for elution at 0.6 mL/min. The column was calibrated by using analytical standards of maltodextrins (DP4+), maltotriose (DP3), maltose, glucose, fructose, succinic acid, lactic acid, acetic acid, glycerol, methanol and ethanol. Samples and standards were injected (5 μL) with a displaced volume loop. The results were analyzed using the Agilent ChemStation software. All analyses were done by using averaged values for duplicate injections.

2.7. Amino acid analysis

Amino acids were analyzed by an HPLC method using an Agilent 1100 HPLC system and method for amino acid analysis (Agilent Technologies, 2000). The method uses a dual derivatization reaction done in the injector loop immediately before injection. This allowed for analysis of soluble amino acids without additional sample preparation by using the same filtered samples taken for fermentation product analysis described above. Three enzyme levels were tested and samples were taken at 24 h intervals for soluble amino acid analysis.

2.8. Economic evaluation

To evaluate the overall economics of protease addition, a modification to an existing technical model for a 151 million L/yr (40 million gal/yr) (MGPY) corn to ethanol dry grind facility was made. The simulation was developed at the United States Department of Agriculture (USDA) Agricultural Research Service, Eastern Regional Research Center in Wyndmoor, PA using SuperPro Designer®, version 8.5 (SuperPro Designer®, Intelligen, Inc., Scotch Plains, NJ) (Kwiatkowski et al., 2006). The economic portion of SuperPro Designer® uses cost engineering methods which are generally accepted and used by industry (AACE, 1990; Ramírez et al., 2009).