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In-situ corn fiber conversion improves ethanol yield in corn dry-mill process

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ABSTRACT

Dry-mill process has been widely applied for ethanol production from corn. However, corn kernel fiber (mostly cellulose) is typically unconverted in the process. This work investigated the effect of corn cellulose conversion on the performance of dry-mill process. Solid loading and pH were optimized first for better design of cellulose conversion in the process. Cellulase was dosed for cellulose conversion during Simultaneous Saccharification and Fermentation (SSF). Addition of cellulase improved ethanol yield by 1.8% compared to traditional SSF without cellulase. Cellulose conversion reached 19.8%. Starch conversion was also enhanced from 98.3% to 99.0% with glycerol yield, which is a major side-product of ethanol fermentation, decreased by 7.3%. Residual starch and residual cellulose resulted from SSF were further studied for their degradability and it was found that 63.2% of residual starch and 14.3% of residual cellulose could be further degraded in the absence of ethanol. This work implies that cellulose conversion is of great benefit to corn ethanol process.

1. Introduction

With the development of industrialization and increase of population, global energy demand is increasing dramatically, and the most energy supplies are derived from non-renewable fossil fuel (Kumar and Singh, 2016). With consideration of environmental and social sustainability, renewable energy development has drawn much attention (Zhang et al., 2007). Bioethanol, which can be produced from starchy, sugary and cellulosic materials through fermentation, is considered as the most promising renewable fuel. Starchy crops (e.g. corn) are widely used for bioethanol production due to their availability, ease of conversion, storage capability for a long period and high ethanol yield (Balat, 2011). Currently, most fuel ethanol in United States is produced from corn by dry mill or wet mill process (Bothast and Schlicher, 2005). Although both processes are being employed for ethanol production, 67% of the commercial plants use dry-mill process.

Starch comprises 70–72% of the kernel weight on a dry basis, and is readily degraded to fermentable glucose, which can be further fermented to ethanol. However, the biodegradability of starch during enzymatic saccharification is largely determined by its structure that varies with regard to botanical sources and crop hybrids. This variation in structure affects overall starch conversion and thereby ethanol yield. In addition, the amylose and amylopectin ratio also affects physicochemical properties of starch and hence affects ethanol yield (Singh and Graeber, 2005; Yangcheng et al., 2013). Recent advances in improving ethanol yield and process profitability include the use of high yield corn varieties (Shi et al., 2013; Zabed et al., 2016), development of non-cooking hydrolysis technique with granular starch hydrolyzing enzyme (GSHE) (Li et al., 2014; Uthumporn et al., 2010; Wang et al., 2005), cell immobilization (Mishra et al., 2016; Najafpour et al., 2004), the use of superior yeast (Favaro et al., 2015; Nakamura et al., 1997; Shigechi et al., 2004), the enhancement of glucose release rate (Khanal et al., 2007) and recovery of high-value co-products (Somavat et al., 2016), etc.

Although technologies have been well developed to convert corn starch to ethanol, corn kernel fiber (mostly cellulose), which accounts for around 4 percent of total sugar in corn, is unutilized in the current process. Conversion of corn cellulose is of great interest in the field (Porter et al., 2007). However, most studies focused on conversion of isolated corn fiber (Gáspár et al., 2007; Van Eylen et al., 2011) or DDGS (dried distillers' grains with solubles) resulted from dry-mill process (Kim et al., 2010; Lau et al., 2008). In-situ conversion of cellulose during dry-mill process is not well studied. Cellulase is a mixture of different cellulytic enzymes that act synergistically on the cellulose molecules to degrade cellulose to glucose (Awafo et al., 2000). The addition of cellulase in the corn ethanol process to in-situ convert

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cellulose could potentially improve ethanol yield as it not only degrades cellulose but may also synergize with glucoamylase to further hydrolyze recalcitrant starch. Cellulase can help disrupt cell wall structure of grain and promote the release of contents including starch and protein, which plays the auxiliary role in improving ethanol yield of starch. Meanwhile, the addition of cellulase may decrease viscosity of fermentation broth and improve its rheological property (Knutsen and Liberatore, 2010), which facilitates saccharification and fermentation.

There are two stages in corn ethanol process that cellulase can potentially be dosed, namely liquefaction stage and Simultaneous Saccharification and Fermentation (SSF) stage (performs enzymatic saccharification and fermentation in the same bioreactor). Liquefaction stage uses alpha-amylase and carries out at above 85 °C. This temperature is much higher than most cellulases can tolerate. The other stage cellulases can be dosed is the SSF stage. However, there are several factors need to be investigated. SSF performs at 30 °C, which is lower than the optimal temperature of cellulase (50 °C). Fermentation causes pH decrease rapidly in the first 24 h and results in high ethanol concentration, which both affect cellulase activity. Therefore, it remains unknown how well cellulase can perform during SSF.

The main objective of this work was to in-situ convert corn cellulose during dry-mill process and investigate the effect of cellulase addition on SSF performances, including starch conversion, cellulose conversion and ethanol yield. Residual starch and residual cellulose resulted from SSF were further studied for their degradability.

2. Materials and methods

2.1. Materials

Corn flour was generously given by China Oil & Foodstuffs Corporation (COFCO), Anhui, China. The glucan and xylan contents of corn were 81.96% and 2.50%, respectively. The starch and cellulose contents of corn were 77.86% and 4.10%, respectively. The alpha-amylase, glucoamylase and cellulase used in this study were supplied by Genencor Bio-Products Co. Ltd., Shandong Longda Bio-products Co. Ltd. and Vland Biothech Co. Ltd., respectively. *Microorganism and seed culture preparation*

Saccharomyces cerevisiae was obtained from Angel Yeast Co., Ltd. Seed culture of *S. cerevisiae* was prepared in YPD medium (10 g/L yeast extract and 20 g/L tryptone) with 100 g/L glucose in a 250 mL Erlenmeyer flask with a working volume of 120 mL. The culture was incubated at 30 °C and 150 rpm under micro-aerophilic conditions with sterile and breathable filtering membrane for 24 h.

2.2. Simultaneous saccharification and fermentation (SSF)

The corn flour was mixed with deionized water to make slurry at different solid loadings (27%, 30% and 33% w/w). For liquefaction, the pH of the slurry was adjusted to 5.7 using 40% sodium hydroxide solution and 0.048 g of α-amylase was used per 100 g dry corn. The liquefaction was performed in a shake flask at 85 °C for 4 h using water bath with intermittent mixing. The pH of the cooling liquefied mash was adjusted to 4.6 using sulfuric acid for SSF process. SSF was carried out in a 250 mL Erlenmeyer flask with a total mixture weight of 150 g in triplicate at 30 °C, pH 4.6, and 150 rpm. Yeast seed culture was centrifuged at 4000 rpm for 10 min to obtain yeast cell pellet, which was used for inoculation. The initial OD_{600} for SSF was 1.0, which means 0.44 g yeast cells were used per 1 L fermentation broth. Urea (1.28 g/kg mash) and glucoamylase (0.1 g/100 g dry corn) were added. A rubber stopper with a needle piercing through it was used to cap the flask to maintain largely anaerobic conditions. To monitor the fermentation performance, about 2 mL of samples were drawn at 0, 4, 16, 24, 36, 48, 72, 96 and 120 h and centrifuged at 10,000 rpm for 10 min. The supernatants were immediately sterile filtered through a 0.22 μm filter for HPLC analysis.

2.3. Analytical methods

The concentration of maltose, glucose, xylose, organic acids, glycerol and ethanol was analyzed by HPLC with a Biorad Aminex HPX-87H column. The analysis conditions were as follows: column temperature (65 °C), mobile phase (5 mM H_2SO_4, 0.6 mL/min).

Total sugar and oligomeric sugar were analyzed according to procedures of NREL-TP-510-42618 and NREL-TP-510-4262, respectively. The starch content was analyzed using NREL analytical procedure NREL-TP-510-42624. Cellulose content was obtained by subtracting starch from total sugar. Ethanol yield was determined based on the theoretical ethanol yield from consumed glucose, which is 0.51 g ethanol/g sugar.

Moisture content of biomass was measured by a moisture analyzer (METTLER TOLEDO). The number of yeast cells in the fermentation broth was counted using a hemocytometer (Shanghai Qiujing biochemical reagents Co. Ltd) and microscope (CX31RTSF, OLYMPUS). The cells were stained with methylene blue dye (0.1%) which stains dead cells blue while living cells remain uncolored. The OD value of yeast was determined using ultraviolet and visible spectrophotometer (TU-1810, Beijing Purkinje General Instrument CO. Ltd) at 600 nm.

2.4. Effect of pH and ethanol on cellulase activity

Cellulase activity was assayed at different pH (3–5) and different concentrations of ethanol (0–20%) with the modified method of NREL-TP-510-42628 using filter paper as substrate. Citric acid-sodium citrate buffer (0.025 M) at different pH and ethanol solution were prepared and sterilized prior to use. Different amount of ethanol was added to the citrate buffer to form an ethanol-buffer mixture with ethanol concentrations of 0, 5, 10, 15, 17.5 and 20% (v/v). With the modified NREL-TP-510-42628 method, 0.5 mL of diluted enzyme was added into 1 mL of citrate-buffer-ethanol mixtures with different pH and ethanol concentration. After 60 min of hydrolysis reaction at 50 °C, 3 mL DNS reagent was added to measure the cellulase activity.

2.5. Effect of ethanol concentration on yeast activity

The ability of yeast to consume glucose under different concentrations of ethanol was examined in the medium containing 5 g/L glucose and various concentrations of ethanol. The glucose solutions were prepared with citric acid-sodium citrate buffer (0.025 M, pH 4.5) and sterilized prior to use. Ethanol concentration tested ranged from 0 to 20% (v/v). The yeast cells were inoculated at an OD_{600} of 1.0 (0.44 g/L broth), 3.0 (1.32 g/L broth) and 6.0 (2.64 g/L broth) and cultured at 30 °C, 150 rpm for 48 h. The consumption of glucose was analyzed after incubation.

2.6. Degradability of residual starch and residual cellulose

After SSF at 30% solid loading, the whole broth without any treatments was used as control group to study the degradability of residual starch and residual cellulose. At the same time, to remove ethanol effect, ethanol was evaporated from SSF broth (60 g) with rotary evaporator (RE100-Pro, Scilogex) at 65 °C, 10 min and 0.07 MPa. The broth without ethanol was performed as experimental group. The degradability of residual starch was carried out in a 100 mL Erlenmeyer flask with a total mixture weight of 60 g in triplicates. Alpha-amylase (0.048 g/100 dry corn) was added to make another round liquefaction at 85 °C for 4 h. Glucoamylase (0.1 g/100 g dry corn) was then added to hydrolyze at 30 °C or 60 °C and 150 rpm for 24 h. Residual starch content and released glucose were then analyzed again. The degradability of residual cellulose was tested by adding cellulase (0.6 g/100 dry corn) to the broth for hydrolyze residual cellulose at 50 °C or 30 °C and 250 rpm for 48 h.