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A cost-effective integrated process to convert solid-state fermented sweet sorghum bagasse into cellulosic ethanol

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HIGHLIGHTS

- An integrated process was developed to produce cellulosic ethanol.
- Cellulosic ethanol can be cost-effectively produced from SS by using this integrated process.
- 69.49% theoretical yield of ethanol was achieved under the optimal condition.

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ABSTRACT

A cost competitive integrated technology to convert solid state fermented sweet sorghum bagasse (SS) into cellulosic ethanol which combined ethanol distillation, NaOH pretreatment and simultaneous saccharification and co-fermentation (SSCF) was presented in this study. After solid-state fermentation, the SS was distilled with 10% (w/w dry material, DM) NaOH to separate sugar-based ethanol and pretreat lignocelluose simultaneously in one step and one distillation stripper, then the NaOH pretreated SS was subsequently converted into cellulosic ethanol by SSCF. Results showed that 69.49% ethanol theoretical yield was achieved under the optimal condition based on this novel integrated process. This integrated technology can significantly reduce the energy consumption and capital cost for cellulosic ethanol production, and ensure cellulosic ethanol produced from SS cost-effectively.

1. Introduction

The past decades have witnessed a continuous depletion of fossil resources as world energy consumption continuously increased [1]. Biofuel development has bright prospects in addressing issues related to energy security [1–3]. Bioethanol is considered as the most promising alternative renewable energy to petroleum for the current stage. Sweet sorghum is regarded as a promising energy crops for bioethanol production as its stalk contains rich of both non-structural carbohydrates (sucrose, glucose and fructose) and structural carbohydrates (cellulose and hemicellulose) [4,5]. Fermentable sugars in sweet sorghum stems have been cost-effectively converted into sugar-based ethanol by new solid-state fermentation technology [6]. However, cost-competitive bioethanol production from sweet sorghum also requires the bioconversion of structural carbohydrates (cellulose and hemicellulose) into cellulosic ethanol, as there are approximately equal quantities of soluble carbohydrates and insoluble carbohydrates in sweet sorghum stalks [7].

As the first step in the biochemical conversion process to produce cellulosic ethanol, pretreatment plays a critical role in preparing biomass for enzymatic hydrolysis to fermentable sugars, and contributes to the most ethanol cost [8]. Hence, it is very important to improve pretreatment process to reduce capital investment and energy consumption [8,9]. NaOH pretreatment removes partial lignin and hemicellulose in the biomass by cleaving hydrolysable linkages in lignin and glycosidic bonds of polysaccharides [10]. Compared to other pretreatment technologies, lower temperatures, pressures and shorter processing time were utilized and fewer inhibitors were generated during NaOH pretreatment [11]. After NaOH pretreatment, the pretreated solid state fermented sweet sorghum bagasse (SS) can be efficiently converted into cellulosic ethanol by separate enzymatic hydrolysis and fermentation (SHF) or simultaneous saccharification and co-fermentation (SSCF). SSCF

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is more favorable for cellulosic ethanol production than SHF due to its lower capital cost, shorter processing time and higher ethanol yield [12]. However, the production cost of cellulosic ethanol is still high due to the complexity of the normal technology. Therefore, there is no commercial-scale cellulosic biofuels facilities are operating at present because cellulosic ethanol production needs integrated technologies for saving the capital cost of infrastructure, transport and storage and reducing the energy consumption.

In this study, a cost-effective integrated process to convert SS into cellulosic ethanol was presented. After solid-state fermentation, the SS containing sugar-based ethanol was solid state distilled and pretreated with NaOH in one step and one distillation stripper, which was signed as alkaline distillation process. The effects of alkali on the sugar-based ethanol recovery and pretreatment were investigated. After alkaline distillation process, the pretreated SS was converted into cellulosic ethanol by SSCF using an engineered strain of *Z. Mobilis* TSH-ZM-01 [13]. The SSCF process was optimized using a statistical method.

2. Materials and methods

2.1. Solid state fermented sweet sorghum bagasse (SS)

The SS, with 1–2 mm in diameter and 3–50 mm in length, was generated during solid-state fermentation. After solid-state fermentation, the collected SS mixing sugar-based ethanol was immediately stored in a sealed plastic bag at −20 °C to prevent any possible spoilage and release of ethanol. The dry weight and the composition of SS were determined using NREL's method [14,15].

2.2. Enzyme assay

The enzyme preparations, including Cellulase (CTec-3) and xylanase (HTec-3), were generously provided by Novozymes (Co., Ltd., China). The activity of Celluclase was 213.87 FPU/g, determined according to NREL's method [16]. The activity of xylanase was 17,658 U/g, measured according to the State Standard of the People's Republic of China (GB/T 23874-2009).

2.3. Microorganisms and media

An engineered strain *Z. Mobilis* TSH-ZM-01 [13] was used for SSCF. The microorganism was conserved in RM medium (1% yeast extract, 0.2% NaH_2PO_4, 2% glucose) at 4 °C. The microorganism was sub-cultured before each experiment in order to maintain its viability.

2.4. Alkaline distillation process

Around 4 kg SS was blended with 10% (w/w DM) NaOH concentrated solution completely and then loaded in a 50 L distillation stripper with 0.45 m in height, 0.4 m in diameter, which was designed and made by Tsinghua University. Around 0.04 MPa steam pressure was injected into the distillation stripper and the operation temperature was kept at about 100 °C during distillation stage. The operation time was 30 min starting at the first drop of the distillate was observed. After alkaline distillation, the distillate was collected and ethanol concentration was determined by a GC (SHIMADZU GC-14C) equipped with a flame ionization detector and column. The ethanol recovery was calculated as Eq. (1):

$$\text{Ethanol recovery yield } (\%) = \left(C_{Ethanol} \cdot V_{Distillate} / M_{Ethanol,\ initial}\right) \cdot 100 \quad (1)$$

$C_{Ethanol}$ represents ethanol concentration (g/mL) in the distillate; $V_{Distillate}$ represents the volume (mL) of distillate; $M_{Ethanol,\ initial}$ represents the initial content of ethanol remaining in SS. The whole slurry in the distillation stripper was solid–liquid separated by centrifuged and the separated solid residues were washed by tap water (until pH = 7.0) as required. The washed solids (usually called as substrate) were stored in a sealed plastic bag at −20 °C. The dry weight and the composition of substrate were determined using NREL's method [14,15]. Solid recovery yield was calculated as Eq. (2):

$$\text{Solid recovery yield } (\%) = M_{Substrate} / M_{SS} \cdot 100 \quad (2)$$

$M_{Substrate}$ is the mass of solids after alkaline distillation (g); M_{SS} is the mass of SS used in alkaline distillation (g). Xylan removal was calculated as Eq. (3):

$$\text{Xylan removal } (\%) = \left(M_{Xylan,\ SS} - M_{Xylan,\ remain}\right) / M_{Xylan,\ SS} \cdot 100 \quad (3)$$

$M_{Xylan,\ SS}$ represents the total mass of xylan in SS (g); $M_{Xylan,\ remain}$ represents the mass of xylan remained in alkaline distilled SS (g). Lignin removal was calculated as Eq. (4):

$$\text{Lignin removal } (\%) = \left(M_{Lignin,\ SS} - M_{Lignin,\ remain}\right) / M_{Lignin,\ SS} \cdot 100 \quad (4)$$

$M_{Lignin,\ SS}$ is the mass of lignin in SS (g); $M_{Lignin,\ remain}$ is the mass of lignin remained in alkaline distilled SS (g). The recovery of the compounds X (glucan and xylan) after alkaline distillation was calculated as Eq. (5):

$$\text{Recovery}_{\text{Compounds } X}\ (\%) = M_{\text{Compounds } X,\ remain} / M_{\text{Compounds } X,\ SS} \cdot 100 \quad (5)$$

$M_{\text{Compounds } X,\ remain}$ is the mass of compounds X in alkaline distilled SS (g); $M_{\text{Compounds } X,\ SS}$ is the mass of compounds X in SS (g).

2.5. SSCF

SSCF was performed according to Jin et al. [12] with minor modifications [12]. 10 g of autoclaved substrate was added to the 250 mL Erlenmeyer flask and diluted with sodium acetate buffer (50 mM) to reach a certain solid–liquid ratio. Prior to SSCF, substrate was firstly pre-hydrolyzed with a given charge of enzyme preparations at pH 4.8, 50 °C and 150 rpm for certain duration. After pre-hydrolysis, pH and temperature were adjusted to 6.0 and 32 °C, respectively. 10% (v/v) of concentrated YP (1% yeast extract, 10% peptone) was added to the Erlenmeyer flask containing enzymatic slurry as nutrients, and then hydrolyzed slurry was inoculated with 10% (v/v) of *Z. Mobilis* TSH-ZM-01 seed. Samples were taken at 0 h and 24 h, and centrifuged at 15,000 rpm, 4 °C for 10 min. The supernatant was stored in −20 °C freezer for the sugar and ethanol analysis.

2.6. Analytical methods

2.6.1. Monomeric sugars and ethanol

Concentration of monomeric sugars (glucose plus xylose) was measured by HPLC (Shimadzu LC-20AD, Tokyo, Japan) with a refractive index detector and a column (Bio-Rad HPX-87H, 250 mm × 4.6 mm). Ethanol concentration was determined with a GC (SHIMADZU GC-14C) equipped with a flame ionization detector and column. Ethanol theoretical yield was determined using Eq. (6):

$$\%Y_{\text{Ethanol theoretical yield}} = \frac{C_{Ethanol} \times V_{Final}}{M_{Xylan} \times 1.14 \times 0.511 + M_{Glucan} \times 1.11 \times 0.473} \times 100 \quad (6)$$