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Bioethanol from sugarcane bagasse: Focused on optimum of lignin content and reduction of enzyme addition

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ABSTRACT

To investigate the effect of delignification on enzymatic saccharification and ethanol fermentation of sugarcane bagasse (SCB), NaClO, NaOH, and Na_2CO_3 were used to prepare SCB with different lignin contents. We found that a lignin content of approximately 11% was sufficient for enzymatic saccharification and fermentation. Based on this result, an economical delignification pretreatment method using a combination of acid and alkali (CAA) was applied. Lignin content of 11.7% was obtained after CAA pretreatment with 0.5% w/v H_2SO_4 at 140 °C for 10 min and 1.0% w/v NaOH at 90 °C for 60 min. Presaccharification-simultaneous saccharification and fermentation (P-SSF) of the CAA-pretreated SCB resulted in an ethanol concentration of 43.8 g/L and an ethanol yield of 81.7%, with an enzyme loading of 15 FPU/g–CAA-pretreated SCB. Enzyme activities (filter paper, carboxymethyl cellulase, and β-glucosidase activities) were determined in liquid phase during P-SSF, indicating that the residual cellulase activity could be further used. Thus, fed-batch P-SSF was carried out, and an ethanol concentration of 43.1 g/L and an ethanol yield of 80.4% were obtained with an enzyme loading of 10 FPU/g–CAA-pretreated SCB. Fed-batch P-SSF was found to be effective to reduce enzyme loading.

1. Introduction

Concerns about environmental deterioration and a continuous depletion of fossil resources have been motivating the development of processes based on alternative energy sources. Bioethanol is an important alternative renewable energy because it has been widely blended with oil and consumed in internal combustion engines for the power of vehicles (Chen et al., 2012). It can decrease the use of petroleum since petroleum is a non-renewable resource (Brostow and Hagg Lobland, 2017). However, the majority of ethanol is produced from maize and sugarcane at present (Balat and Balat, 2009). When bioethanol is produced from the aforementioned feedstocks, the raw materials account for around 40–70% of the production cost (Quintero et al., 2008). Besides, production of ethanol from corn has the ethical debate of competition with food and animal feed. Therefore, production of ethanol from lignocellulosic biomass has been gained considerable attention worldwide, due to its abundance, inedibility, and renewability.

Lignocellulosic biomass is recalcitrant to microbial or enzymatic saccharification due to the presence of lignin seals and hemicellulose sheaths, and to the crystallinity of cellulose itself (Wang et al., 2015). Therefore, pretreatment is commonly required to increase its digestibility. Lignin is covalently linked to hemicellulose and fills the spaces between hemicellulose and cellulose (Yan et al., 2015), and it also non-productively adsorbs cellulases (Yu et al., 2011), hindering the enzymatic accessibility to cellulose. Therefore, delignification pretreatments are considered as valid approaches to facilitate enzymatic saccharification.

It was reported that enzymatic saccharification of delignified lignocellulosic biomass correlated with lignin content, and this correlation was biomass-dependent owing to the difference of lignin structure and distribution in various biomass (Yu et al., 2011). Rico et al. (2015) delignified *Eucalyptus* using laccase and methyl syringate as mediators; their results revealed that enzymatic saccharification efficiency was inversely correlated to lignin content in the range of 11.2–22.3%. Yu et al. (2011) reported that enzymatic

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saccharification of softwood (loblolly pine) and hardwood (mainly poplar and maple) delignified by sodium hypochlorite (NaClO) nearly leveled off with a lignin content <15%, while it sharply decreased with a lignin content more abundant than 15%. Similar results were obtained by Sookyeong et al. (2016); they showed that enzymatic saccharification efficiency was >84% with a lignin content <11%. Moreover, although a diluted sulfuric acid (H_2SO_4) pretreatment was used, a distinct relationship between lignin content of the biomass and enzymatic saccharification efficiency was found by Raud et al. (2016).

Sugarcane bagasse (SCB) is the most widely available material in South China and can become a promising feedstock for bioethanol production (Zhang and Wu, 2014); however, it is still unknown to what extent delignification affects the enzymatic saccharification and ethanol fermentation of SCB. Considering that pretreatment represents the second most expensive step in the conversion of lignocellulosic biomass into bioethanol, the great challenge of this technology is to find an appropriate strategy to remove the lignin to a certain extent, allowing effective enzymatic saccharification with low loads of enzymes, in a cost-effective and environmentally sustainable manner.

In this study, different delignification methods were used to investigate the effect of lignin content in delignified SCB on enzymatic saccharification and presaccharification-simultaneous saccharification and fermentation (P-SSF). Based on our results, an economical pretreatment combining acid and alkali (CAA) was optimized to decrease lignin content to approximately 11%. Subsequently, CAA-pretreated SCB was subjected to enzymatic saccharification and P-SSF. Furthermore, fed-batch P-SSF was carried out to reduce enzyme loading.

2. Materials and methods

2.1. Raw materials, enzymes, and yeast strains

Fresh SCB was kindly provided by Gui-gang Sugar Refinery (Guangxi, China); after air-drying, it was pulverized by a cutting mill (Pulverisette 15; Fritsch, Germany) with a 1-mm mesh sieve. SCB 1 comprised (% w/w, based on dry weight): glucan, 35.0 ± 0.7; xylan, 18.4 ± 0.4; lignin, 21.0 ± 0.3; ash, 0.8 ± 0.1, and SCB 2 comprised (% w/w, based on dry weight): glucan, 38.2 ± 0.2; xylan, 23.5 ± 0.6; lignin, 21.5 ± 0.4; ash, 3.3 ± 0.2.

The cellulase used in this study was Cellic CTec2 (hereafter called CTec2), which was kindly provided by Novozymes (Denmark). Filter paper activity (FPA), carboxymethyl cellulase (CMCase) activity, and β-glucosidase (BG) activity were: 134 ± 1 FPU/mL, 5001 ± 163 U/mL, and 7002 ± 120 U/mL, respectively.

Saccharomyces cerevisiae KF-7 was used to convert glucose to ethanol. It was constructed by protoplast fusion of the flocculating yeast strain IR-2 and the thermotolerant yeast strain EP-1 (Kida et al., 1992).

2.2. Preparation of sugarcane bagasse with different lignin contents

Delignification using NaClO, Na_2CO_3, and NaOH was carried out to prepare SCB with different lignin content.

2.2.1. Delignification using NaClO

SCB 1 (10 g, dry weight) and 400 mL of tap water were mixed in a 1000-mL Erlenmeyer flask and boiled for 4 h. The solid residue was recovered using a 0.04-mm mesh sieve and washed to reach constant pH. The residue was transferred to 400 mL of a solution with different concentrations of NaClO (0.6%, 0.9%, 1.2%, and 1.5% w/v), and each mixture was stirred at 200 rpm for 16 h using an RS-6D magnetic stirrer (As One, Osaka, Japan) at room temperature. The solid residue was recovered by filtration and washed with tap water to constant pH.

2.2.2. Delignification using Na_2CO_3 and NaOH

Na_2CO_3 delignification was carried out at 120 or 160 °C for 15 min in a pressure-resistant TEM-V glass reactor (Taiatsu Techno, Osaka, Japan). Raw SCB 2 (50 g, dry weight) was mixed with 50, 100, 200, or 400 mL of 5% w/v Na_2CO_3 solution, and tap water was added to reach a total liquid volume of 500 mL. The final solid loading and Na_2CO_3 concentration were 10% w/v and 0.5, 1.0, 2.0, or 4.0% w/v, respectively. NaOH delignification was similar to Na_2CO_3 treatment. SCB 2 was treated at 120 °C for 10 or 15 min with the NaOH concentration of 0.5 or 1.0% w/v. After delignification, the delignified SCB was recovered by filtration and washed with boiling tap water to natural pH.

The recovered delignified SCB was dried at 60 °C for compositions analysis, enzymatic saccharification, and ethanol fermentation.

2.3. Pretreatment of sugarcane bagasse

SCB was pretreated with a combination of diluted acid and alkali (CAA), using diluted H_2SO_4- and NaOH-solutions in a pressure-resistant TEM-V glass reactor. Raw SCB 1 (50 g, dry weight) was mixed with 50 or 100 mL of a 5% w/v H_2SO_4 solution; tap water was subsequently added to reach a total liquid volume of 500 mL, to obtain a solid loading of 10% w/v and a final H_2SO_4 concentration of 0.5% w/v or 1.0% w/v. Diluted H_2SO_4 treatment was carried out at 120, 130, 140, or 150 °C for 10 min. The solid residue was recovered by filtration and washed with tap water to obtain a constant pH value. The recovered solid residue was subsequently treated with a 1.0% w/v NaOH solution with a solid loading of 10% w/v at 80 or 90 °C for 30, 60, or 90 min. The solid residue was recovered by filtration and washed with boiling tap water to natural pH. Solid and liquid samples after diluted H_2SO_4 treatment and CAA pretreatment were taken for component analysis. The sugar recovery efficiency in the H_2SO_4-treated liquid ($R_{S\text{-}SL}$) was calculated using Eq. (1).

$$R_{S\text{-}SL} = \frac{C_{S\text{-}SL} \times V_{SL}/1000}{C_{S\text{-}M}/A \times m_M} \times 100\% \quad (1)$$

where $C_{S\text{-}SL}$ (g/L) is the glucose or xylose concentration in the H_2SO_4-treated liquid; V_{SL} (mL) is the volume of the H_2SO_4-treated liquid; 1/1000 is a conversion factor for milliliters to liters; $C_{S\text{-}M}$ is the glucan or xylan content of raw SCB; A is the coefficient converting polysaccharides to monosaccharides (1/0.9 for glucan to glucose and 1/0.88 for xylan to xylose); m_M (g) is the amount of raw SCB used for pretreatment.

2.4. Morphological characteristics and chemical structures analysis

Samples were dried to a constant mass at 60 °C prior to morphological characteristics and chemical structures analysis. Morphological characteristics were analyzed using a scanning electron microscope (SEM). Images were obtained using a JEOL-6700 F SEM (Jeol Ltd., Tokyo, Japan) with a magnification of 700 at 10 kV.

Chemical structures were characterized using a Fourier Transform Infrared (FT-IR) spectrometer (FT-IR-4200; Jasco, Japan). Before FT-IR analysis, approximately 500 μg of dried samples were mixed with 150 mg of KBr crystals, milled, and pressed into disks. IR spectra were obtained by averaging 16 scans from 2000 cm^{-1} to 400 cm^{-1} at a resolution of 4 cm^{-1}.

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