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Bioethanol production using the sodium hydroxide pretreated sweet sorghum bagasse without washing

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ABSTRACT

Sweet sorghum, with its multi-platform of resources of starch, sugar and cellulose, is considered a very promising energy crop to produce bioethanol. In this study, bioethanol production of sodium hydroxide (NaOH) pretreated sweet sorghum bagasse without solid substrate washing was investigated. The results showed that direct enzymatic saccharification and ethanol fermentation of NaOH pretreated solid substrate without washing significantly improved the fermentable sugars conversion and ethanol theoretical yield from 44.06 ± 0.93% and 44.85 ± 1.15% to 65.14 ± 0.19% and 61.81 ± 1.28%, respectively. Experiments where NaOH pretreated liquor (SL) was added into the thoroughly washed NaOH pretreated solid substrate showed the fermentable sugars conversion yield improved significantly. Furthermore, analysis of enzymes activities during enzymatic saccharification showed that SL maintained cellulase activity significantly, especially for that of xylanase activity. These results suggested that the NaOH pretreated lignocellulosic solid can be employed for direct enzymatic saccharification and subsequent ethanol fermentation without washing. This process would improve the sustainability and economic viability of bioethanol production.

1. Introduction

Bioethanol is considered an alternative liquid fuel and has been developed extensively for two decades [1]. Sweet sorghum is a promising herbaceous energy crops as it has high photosynthetic efficiency, high sugar yield and tolerance of harsh environments [2]. In recently years, many efforts have been made to commercialize the bioethanol production from the sweet sorghum [3–6]. During bioethanol production, pretreatment is necessary to eliminate the nature recalcitrance of lignocellulose to further bioprocess [7]. Among the leading pretreatment technologies, alkaline pretreatment is a relatively mild pretreatment method and considered more effective on herbaceous crops [3]. Mechanistically, alkali is believed to cleave the bonds of lignin and carbohydrate, which causes the dissolution of partial lignin and hemicellulose in the liquid phase [8]. During alkali pretreatment of lignocellulose, sugars and lignin-based degradation compounds were released in the pretreated slurry. These degradation compounds may influence the process of enzymatic saccharification and ethanol fermentation [9–11]. Excess enzyme loading or surfactant application could eliminate the negative effects caused by degradation compounds and achieve high enzymatic saccharification yields [12]. However, using excessive enzymes or additives is not economic for commercial bioethanol production. The thorough washing of the pretreated solid substrate is another way to remove sugar and lignin based degradation compounds [13,14]. However, the washing process consumed a significant amount of water, which caused either difficulties for commercialization or environmental concern in terms of water usage and waste treatment [12]. Therefore, development of efficient strategy for treatment of the pretreated slurry is the key to bioethanol production.

Recently, bioethanol production using the acidic pretreated biomass without washing has been explored by several researchers [14–16]. However, to the best of our knowledge, there is little information available using the alkali pretreated one. This study aimed to produce bioethanol using sodium hydroxide (NaOH) pretreated sweet sorghum bagasse without solid substrate washing.

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We also elucidated the influence of NaOH pretreated liquor (SL) on the enzymatic saccharification and subsequent ethanol fermentation by the strain *Zymomonasmobilis* TSH-ZM-01. We further investigated the impact of SL on enzymes activity during enzymatic saccharification process. The results that we obtained can be extended to other raw materials to develop a universal method for using alkali pretreated slurry for the bioethanol production.

2. Materials and methods

2.1. Solid-state fermented sweet sorghum bagasse (SS)

Advanced solid state fermentation (ASSF) technology was employed to produce SS by using a 50 L fermenter with 0.7 m in length, 0.3 m in diameter designed by Tsinghua University. ASSF was performed according to our previous study [3,17]. After ASSF processing, the collected SS was immediately stored in a sealed plastic bag at −20 °C to prevent any possible spoilage.

2.2. Enzyme assay

Enzymatic saccharification was carried out using commercial enzyme cellulase (CTec-3) and xylanase (HTec-3), both kindly provided by Novozymes investment Co. Ltd. (China). The activity of Celluclase was 158.37 FPU/g, determined based on Laboratory Analytical Procedures (LAPs) documented by NREL [18]. The activity of xylanase was 20,183 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 *Zymomonas mobilis* TSH-ZM-01 recombined by Tsinghua University was used as fermentation strain in the C5–C6 co-fermentation step. The ethanol producing bacterium, *Z. mobilis*, was metabolically engineered to tetracycline resistance and broaden its range of fermentable substrates to include the pentose sugar xylose [19]. The microbe was stored in rich medium agar slants containing glucose 20 g/L, yeast extract 10 g/L, NaH_2PO_4 2 g/L, and agar 15 g/L with a pH of 6.0 and in liquid medium of the same composition (without agar) by periodic transfers. For long term storage, stock cultures were maintained in 20% glycerol at −80 °C. The inoculum was prepared by thawing, transferring, and growing the frozen stock on a shaker incubator at 200 rpm and 32 °C for 16 h in 500 mL baffled flasks with rich medium. The inoculum was then centrifuged and resuspended in sterile deionized water twice for washing and prepared for inoculation at a 1.0 optical density (O.D.)

2.4. NaOH pretreatment and solid washing

NaOH pretreatment was performed as described in our another previous study [20]. At the completion of NaOH pretreatment, the entire pretreated slurry was collected and the weight and moisture content measured. The water was removed from the pretreated slurry by squeezing, using a 1 L hydraulic filter press designed by our lab, until the pretreated slurry (15% solid content, w/w) reached 35% (w/w) solid content, which simulating those conditions commonly used in commercial applications. The NaOH pretreated liquor (SL) was collected and stored at −20 °C. The product after the first squeezing of the substrate was considered to be unwashed substrate and the filtrate was measured. For substrate washing process, the unwashed substrate was rewetting by adding back deionized water equal to the filtrate volume. The substrate and deionized water were mixed thoroughly and then squeezed again to achieve 35% (w/w) solid content. This step was repeated as stated for each of the different multiplies of wishing. The relationship between washing frequency and the residual SL remained in washed substrate was described in Eq. (1):

$$\text{Residual SL in washed n times substrate (\%, v/w)} = 65\% \times 0.381^{n} \quad (1)$$

65% represents moisture content in washed substrate; 0.381 represents the SL concentration coefficient calculated by comparison of ratio of liquor volume after pressing/liquor volume before pressing. *n* represents washing times. In this study, n ranged from 0 to 5. The washing procedures and residual SL content in saccharification media were listed in Table 1. After washing 5 times, residual sugar and soluble lignin were not detected in the filtrate. Therefore, substrate washed 5 times was employed for composition analysis according to NREL's method [21]. Table 2 summarized the compositions of main component in solid and liquor fractions.

2.5. Enzymatic saccharification

Ten gram of washed sweet sorghum bagasse with different washing strategy was added into a flask. Samples were adjusted with distilled water to a solid loading of 10% (w/w). Tetracycline (40 mg/L) was added to inhibit microbial growth. Before enzyme loading, all the samples were neutralized to pH 5.0 with H_3PO_4. Then Cellulase enzyme (CTec-3, 8.0 FPU/g-glucan) and xylanase enzyme (HTec-3, 203 U/g-xylan) were added for hydrolysis at 50 °C on a rotary shaker incubator at 150 rpm for 72 h. Samples were taken periodically to determine sugar concentration in the hydrolyzed slurry. One control experiment without enzyme loading was performed as blank to determine the sugar released from washed substrate during enzymatic saccharification. These values were subtracted from the sugar concentration during enzymatic hydrolysis to calculate fermentable sugar conversion (Eq. (2)):

$$\text{Fermentable sugar conversion (\%)} = \frac{(C_{\text{glucose}} + C_{\text{xylose}}) \times V_{\text{hydrolysed slurry}}}{M_{\text{Glucan in substrate}} \times 1.11 + M_{\text{Xylan in substrate}} \times 1.14} \times 100 \quad (2)$$

C_{glucose} and C_{xylose} were the glucose and xylose concentration respectively in the hydrolysed slurry (g/L); $V_{\text{hydrolysed slurry}}$ was the hydrolysed slurry volume (L); M_{Glucan} and M_{Xylan} were the total mass of glucan and xylan respectively within 10 g substrate (g); 1.11 and 1.14 were the conversion factor of glucan and xylan to equivalent glucose and pentose, respectively. All the experiments were performed in triplicate.

2.6. C5–C6 co-fermentation of hydrolyzed slurry using Z. mobilis TSH-ZM-01

After enzymatic saccharification, 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 for nutrients. The pre-cultured *Z. mobilis* TSH-ZM-01 was inoculated at a 10% (v/v), and cultured on a rotary shaker incubator at 30 °C for 30 h without shake. Samples were taken periodically to obtain sugar and ethanol concentration. Sugar and ethanol concentration in control group were subtracted when calculating the sugar content and ethanol theoretical yield. The ethanol theoretical yield was calculated based on Eq. (3):

Table 1
Relationship between washing frequency and residual SL content in enzymatic saccharification media.

Washing frequency	0	1	2	3	4	5
Residual SL (%, v/w)	18.57	7.08	2.69	1.02	0.39	0.15