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Synergism of cellulase, xylanase, and pectinase on hydrolyzing sugarcane bagasse resulting from different pretreatment technologies

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HIGHLIGHTS

- NaOH pretreatment was the best among studied methods for sugar production.
- Synergism between cellulase and xylanase was obvious but substrate specific.
- Synergism between cellulase and pectinase was not observed for glucose production.
- Synergism between cellulase and xylanase correlated to xylan content.
- Replacement of 20% of the cellulase by xylanase enhanced the glucose yield.

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ABSTRACT

Sugarcane bagasse (SCB) resulting from different pretreatments was hydrolyzed by enzyme cocktails based on replacement of cellulase (Celluclast 1.5 L:Novozym 188 = 1 FPU:4 pNPGU) by xylanase or pectinase at different proportions. Lignin content of NaOH pretreated SCB and hemicellulose content of H_2SO_4 pretreated SCB were the lowest. NaOH pretreatment showed the best for monosaccharide production among the four pretreatments. Synergism was apparently observed between cellulase and xylanase for monosaccharide production from steam exploded SCB (SESB), NaOH, and H_2O_2 pretreated SCB. No synergism was observed between cellulase and pectinase for producing glucose. Additionally, no synergism was present when H_2SO_4 pretreated SCB was used. Replacement of 20% of the cellulase by xylanase enhanced the glucose yield by 6.6%, 8.8%, and 9.5% from SESB, NaOH, and H_2O_2 pretreated SCB, respectively. Degree of synergism between cellulase and xylanase had positive relationship with xylan content and was affected by hydrolysis time.

1. Introduction

Sugarcane bagasse (SCB) is a waste from sugar production (Li et al., 2013). It is estimated that approximately 100 million tons of dry SCB are produced globally every year (Cheng and Zhu, 2013). In comparison with other agricultural residues, SCB is considered as a rich solar energy reservoir (Pandey et al., 2000). SCB has high cellulose (approximately 50%) and hemicellulose content (approximately 25%) while low ash content. The high carbohydrate content which can be converted into fermentable sugars makes it a competitive candidate for biorefinery (Cheng and Zhu, 2013). Efficient enzymatic hydrolysis is one of the most important steps for biorefinery of SCB. For this purpose, many pretreatment methods have been developed to overcome the recalcitrance of the lignocellulose. Apart from the pretreatment, hydrolytic enzymes are considered as another essential factor. Therefore, optimization studies of enzyme complex to obtain high sugar yield have been done (Berlin et al., 2007; Billard et al., 2012; Boisset1 et al., 2001). Synergism between enzymes may play the major role to obtain a high sugar yield.

Synergism means that cooperation of different types of enzymes enhances the product yield (Kostylev and Wilson, 2012). There are different types of synergism interesting for biorefinery applications, the synergism between cellobiohydrolases, endoglucanases, and β-glucosidase for digesting cellulose (Fujii et al., 1991; Henrissat et al., 1985; Kostylev and Wilson, 2012) and the synergism between cellulase, xylanase, esterase, and mannanase for digesting pretreated lignocellulose (Hu et al., 2011; Kumar and Wyman, 2009a; Selig et al., 2008; Várnai et al., 2011). For studying the latter kind of synergism, the effectiveness of the supplementation of accessory enzymes was always explored (Kumar and Wyman, 2009a; Zhang et al., 2013). Under such case, enzyme protein loading was increased for the high sugar yield. For a supplementation system, synergistic effect often means the ratio of

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the product yield released by the enzyme cocktail to the sum of the product yield released by the individual enzymes when used separately in the same amounts as in the mixture.

Replacement part of the cellulase by accessory enzymes such as xylanase also improved the sugar yield (Hu et al., 2011). This is another good way to evaluate the synergism between cellulase and accessory enzymes without increasing the enzyme protein loading. Synergism could mean that the sugar yield was increased by substituting other kinds of enzymes for cellulase in the replacement system. Hu et al. (2011) reported that replacement of cellulase by xylanase enhanced the digestibility of steam pretreated corn stover. However, the effect of other pretreatments on such effectiveness was not explored.

Xylanase was selected as the accessory enzyme to enhance the digestibility of corn stover (Kumar and Wyman, 2009a) and polar (Kumar and Wyman, 2009b) resulted from different pretreatments. Additionally, supplementation of cellulase with the Multifect Pectinase increased the glucan conversion of the dilute acid-pretreated corn stover (Berlin et al., 2007). Multifect Pectinase also enhanced the glucan conversion of AFEX-treated rice straw (Zhong et al., 2009). Therefore, the xylanase and pectinase may also enhance the cellulose conversion for pretreated SCBs.

Steam pretreated corn stover, sweet sorghum bagasse, corn fiber, poplar, and lodgepole pine were used as substrate to study the synergistic action of accessory enzymes for sugar production. The results showed that such action was highly substrate specific (Hu et al., 2013). Furthermore, xylanase showed higher synergistic effect for hydrolyzing steam exploded hemp than fresh and ensiled hemp (Zhang et al., 2013). Synergism between cellulase and accessory enzymes seems to be substrate specific.

In the present study, steam exploded SCB (SESB), NaOH, H_2O_2, and H_2SO_4 pretreated SCB were employed to investigate the synergistic effect of accessory enzymes including xylanase and pectinase. The enzyme protein loading was fixed and replacement of different proportions of cellulase by accessory enzymes was used for hydrolyzing pretreated SCBs in order to obtain the best ratio of the enzymes. The relationship between synergism and chemical composition of the pretreated SCBs was also summarized.

2. Methods

2.1. Preparation of SCB

SCB was kindly provided by the Qianwu sugar refinery in Zhuhai, Guangdong, China. It was harvested and kept at room temperature after air-dried. Dried SCB was ground by using a hammer mill (FY130, Tianjin Taisitie Instrument Co., Ltd., China) and fractionated by using 100 and 200-mesh sieves. The fractions passed a 100-mesh sieve but retained by a 200-mesh sieve were used for alkali, acid, and H_2O_2 pretreatments.

2.2. Enzymes preparation

Celluclast 1.5 L from *Trichoderma reesei* and Novozym 188 from *Aspergillus niger* were provided by Novozymes (China). Endo-1, 4-β-Xylanase was purchased from Sigma–Aldrich (*Trichoderma longibrachiatum*). Pectinase was provided by Genencor (*A. niger*). The protein content of enzymes was determined by using Bovine Serum Albumin (Beyotime) as the protein standard (Sedmak and Grossberg, 1977).

2.3. Preparation of pretreated SCB

SESB was obtained as described previously (Li et al., 2014). The parameters of the steam explosion were: 220 °C, liquid/solid ratio of 1:1 (w/w), and residence time of 5 min. The obtained SESB was ground and fractionated. The fractions passed a 100-mesh sieve were collected to conduct the following experiments. H_2SO_4, NaOH, and H_2O_2 pretreated SCB were obtained as described previously (Silverstein et al., 2007). Briefly, the concentration of H_2SO_4, NaOH, and H_2O_2 was 2% (w/v) with the solid loading of 10% (w/v). Pretreatments were performed in an autoclave for 60 min at 121 °C. The pretreated solids were washed with hot deionized water (approximately 90 °C) until pH 6–7 for H_2SO_4 and NaOH pretreated solids. H_2O_2 pretreated solid was washed with 1000 mL of hot deionized water. Rinsed solids were oven-dried at 80 °C overnight for subsequent experiments.

2.4. Enzymatic hydrolysis

The enzymatic hydrolysis was carried out at a substrate loading of 2% (w/v) in 25 mL of 0.1 M citric acid/citric sodium buffer (pH 4.8), containing 80 μg/mL tetracycline and 60 μg/mL nystatin (dissolved in dimethylsulfoxide) to prevent microbial contamination. The reaction mixtures were placed in an orbital shaker incubator at 160 rpm, 45 °C. Cellulase including Celluclast 1.5 L and Novozym 188 with a ratio of 1 FPU:4 pNPGU was used in this work. For synergistic experiment, the protein loading was also a constant value of 6 mg/g substrate. The ratio of cellulase, xylanase, and pectinase was summarized and shown in Table 1. Samples (1 mL) were taken from the reaction mixture at specific times. Each sample from the hydrolysate was heated in boiling water for 10 min to deactivate the enzymes and then cooled to room temperature and subsequently centrifuged for 20 min at 8000 rpm. The supernatant was filtered through a 0.45 μm membrane filter (Sartorius, Gottingen, Germany) prior to analysis of glucose and xylose.

2.5. Analytical methods

The chemical composition of the lignocellulose was assayed by following the NREL method (Sluiter et al., 2008). Briefly, after a two-step analytical acid hydrolysis procedure, $Ba(OH)_2$ was added to 2.5 mL of acid hydrolysate to adjust the pH to 2 before analysis (Li and Xu, 2013). Acid soluble lignin was measured using a UV/Vis spectrophotometer (UNICO, UV-2000, Shanghai, China) at 240 nm. The pectin was extracted by using 1% (w/v) of ammonium oxalate solution for 1.5 h and then 0.5% (w/v) of ammonium oxalate solution for 1 h in a boiling water bath. Acidic ethanol (11 mL HCl dissolved in 1 L ethanol) was used to precipitate the pectin. The precipitated pectin was redissolved by alkali hot water (1.5 mL of $NH_3{\cdot}H_2O$ dissolved in 75 mL boiling water) before analysis by using the carbazole-H_2SO_4 method (Bitter and Muir, 1962). The pectin content was expressed as D-galacturonic acid (Aladdin, Shanghai) equivalent.

Cellulase activity was determined by following the NREL method (Adney and Baker, 1996).

Glucose and xylose were determined using a HPLC (Shimadzu, LC-15C) equipped with a refractive index detector (RID-10A). Aminex HPX-87H column with a Cation H+ Cartridge Micro-Guard column was used to separate these components. The column was operated at 55 °C with 5 mM H_2SO_4 as the mobile phase at the flow rate of 0.6 mL/min.

2.6. Calculations and statistical analysis

All experiments were conducted in duplicate and data are presented as mean values ± standard deviation (SD). Statistical analysis was carried out by the PASW statistics 18 using one-way ANOVA and Duncan's multiple range tests. Results were considered statistically significant at 95% confidence interval ($p < 0.05$):