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Effect of stepwise lignin removal on the enzymatic hydrolysis and cellulase adsorption

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ABSTRACT

Lignin can negatively impose on the enzymatic hydrolysis of pretreated lignocellulose through non-productive enzyme adsorption and/or steric hindrance. The effect of the residual lignin on enzymatic hydrolysis was evaluated with sugarcane bagasse (SCB) samples containing different lignin contents and their milled and enzymolytic lignins (MELs) retaining the intact protolignins. The results showed that the improvement of enzymatic hydrolysis of SCB would become insignificant when the lignin removal achieved above 64.0%, and the changes in chemical groups of lignin had slight impact on adsorbing enzymes. When the lignin retention in the pretreated SCB was less than 36.0%, the residual lignin would not significantly influence the enzymatic hydrolysis via enzyme adsorption and steric hindrance. The contributions of the residual lignins in pretreated SCB samples to the total enzyme adsorptions of pretreated SCB samples were only in the range of 9.6% to 15.5%, which were not predominant for the total enzyme adsorptions.

1. Introduction

Bioconversion of lignocellulosic biomass into biofuels and biochemical products which refers to pretreatment, enzymatic hydrolysis and fermentation has gained more and more attention due to its effectively sustainable recycling of lignocellulosic wastes, reduction of CO_2 emission, low energy input, weakly adverse environmental impact, and production of high value-added products (Limayem and Ricke, 2012; Mabee et al., 2011; Morais et al., 2015). Lignocellulosic biomass is mainly composed of cellulose, hemicellulose and lignin. Cellulose is a homopolysaccharide formed by glucose monomers with the linkage of β-1, 4-glycosidic bonds (Silveira et al., 2015). Hemicellulose is a heteropolysaccharide including hexose, pentose and sugar acid units (Limayem and Ricke, 2012). These two polysaccharides are the chief components of lignocellulose to be enzymatically depolymerized, and further fermented into biofuels or biochemicals in the bioconversion process.

Lignin which is an amorphous non-polysaccharide polymer consisting of phenylpropane units usually connects with polysaccharides especially hemicellulose at α-carbon and C-4 sites of the benzene ring through covalent bonds (Buranov and Mazza, 2008). Due to its non-degradation under the action of cellulase, lignin has been thought as the major recalcitrant component to inhibit the enzymatic hydrolysis of treated lignocellulose via the non-productive adsorption of hydrolytic enzymes and/or the steric hindrance to the access of enzymes to cellulose (da Costa Sousa et al., 2016; Li and Zheng, 2017; Min et al., 2014; Nakagame et al., 2011). However, the features of lignin remained in the treated lignocellulose like content, composition, structure, and molecular weight can take different effects on the enzymatic hydrolysis (Li and Zheng, 2017; Siqueira et al., 2017). It is necessary to preserve the original properties of lignin to accurately evaluate the impact of protolignin on the enzymatic hydrolysis when lignin is extracted from the untreated and treated lignocellulose. The milled wood lignin (MWL) and cellulolytic enzyme lignin (CEL) have been thought as the representatives of protolignin in lignocellulose (An et al., 2017; Obst and Kirk, 1988; Yu et al., 2014). Nevertheless, due to the isolation procedures, the MWL and CEL are only a partial of protolignin, and thus cannot really reflect the influence of intact protolignin on the enzymatic hydrolysis. In addition, the chemical characteristics of lignin like hydroxyl groups (Pan, 2008; Rahikainen et al., 2013; Yu et al., 2014), syringyl and guaiacyl units (Karkonen et al., 2014; Zhu et al., 2015) which have been reported to have relation to the enzyme adsorption would be changed in the pretreatment process (Jung et al., 2018; Ko et al., 2015). To obtain the original lignin from the untreated and

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treated lignocellulose would closely reveal the true action between protolignin and enzyme. This study isolated the milled and enzymolytic lignins (MELs) via the ball-milling and enzymatic hydrolysis of sugarcane bagasse (SCB) with different lignin contents. The MEL preparations contain nearly the whole protolignins of untreated and treated lignocellulose (Obst and Kirk, 1988), and would be useful to investigate the effect of chemical changes of lignin on enzymatic hydrolysis and truly reflect the action between protolignin and enzyme. Furthermore, the contributions of protolignins in the untreated and treated lignocelluloses to the total enzyme adsorption of lignocelluloses were seldom quantified. This study also calculated these contributions due to the intact extraction of protolignins.

2. Materials and methods

2.1. Materials

SCB was collected from Fenghao Alcohol Co., Ltd which located in Pingxiang city, Guangxi Zhuang Autonomous Region, China. The preliminary treatment of SCB including pulverization, screening, washing, drying, and storing was referred to the previous method (Wang et al., 2016). The cellulase containing 110.0 U xylanase/g powder was bought from Imperial Jade Biotechnology Co. Ltd. (China). The filter paper activity of cellulase was 113.8 FPU/g powder. The xylanase was purchased from Shanghai Macklin Biochemical Co., Ltd. (China), and its activity instructed by the product specification was 100,000 U/g powder.

2.2. Preparation of SCB samples and their MELs

SCB samples containing different lignin contents were prepared by 2% (W/V) NaOH solution at 80 °C for 10, 30, 60, 120 min with the solid-to-liquid ratio of 1:10. The solid residues of the treated SCB were collected and washed until their pH values were neutral, and then oven-dried at 60 °C to the constant mass.

The MELs of raw and treated SCB were extracted through ball milling followed with enzymatic hydrolysis in three repeated times. Except 48-h ball milling for the raw SCB at the first time, the ball milling at each time was conducted for 24 h in the planetary ball mill (PMQW, Nanjing Chishun Science & Technology Co., Ltd. China). The enzymatic hydrolysis was performed at 5% (W/V) solid loadings, 50 °C, pH 4.8, 150 rpm for 96 h under the action of cellulase and xylanase. The cellulase and xylanase were respectively loaded with 40 FPU/g cellulose and 30 U/g hemicellulose at the first time. For the following two times of enzymatic hydrolysis, the addition of xylanase to all SCB samples increased to 1000 U/g initial hemicellulose, and 40 FPU cellulase/g initial cellulose was added into the raw and 10-min NaOH-treated SCB samples, while 20 FPU cellulase/g initial cellulose was loaded into other NaOH-treated SCB samples. When the last time of enzymatic hydrolysis was finished, the hydrolyzed solid residues were collected, and the proteinase K with work concentration of 100 μg/mL was added to degrade cellulase and xylanase at 55 °C for 24 h. Then all the samples were placed in boiling water for 20 min to destruct proteinase K. After being centrifuged and washed for three times with deionized water, the MELs were obtained and dried naturally at the room temperature.

2.3. Enzyme adsorption

The enzyme solution of 17.6 mg/mL which was prepared with 0.05 M acetate buffer (pH 4.8) and cellulase was centrifuged for 5 min at 10000 rpm to get rid of the undissolved substances. The mixtures of 0.02 g SCB MELs and 2 mL enzyme solutions were respectively added into 25 mL conical flasks. After being sealed, the flasks were placed in a rotatory shaker at 50 °C, 150 rpm for 90 min. The supernatants of the slurries were obtained through centrifugation, and their protein contents were detected as the Bradford's method (Bradford, 1976). The absorbance was recorded by the microplate spectrophotometer (Eon, BioTek). The adsorption amount of enzyme was a ratio of the adsorbed (the subtraction of the enzyme amount in the supernatant after adsorption from the initial enzyme amount) to the initial enzyme amount (Wang et al., 2017). The adsorption of enzyme to SCB samples was also detected as the SCB MELs.

2.4. Enzymatic hydrolysis

The SCB samples with different lignin contents were added into 5 mL penicillin bottles. After being loaded with 2 mL 0.05 M acetate buffer (pH 4.8) and 20 FPU cellulase/g cellulose, the bottles were sealed to prevent moisture loss during the enzymatic hydrolysis which was carried out under the condition of 10% solid concentration, 50 °C, 180 rpm for 72 h.

2.5. Analytic methods

The compositional analysis was conducted as the NREL procedure (Sluiter et al., 2008). The surface morphologies were imaged by the cold field emission SEM (S 4800, Hitachi) at an accelerating voltage of 2.0 kV. The chemical structure of MELs were analyzed by ^{13}C CP/MAS solid-state NMR procedure (Avance III, Bruker) and FTIR test (TENSOR 27, Bruker Optics, Germany) with the potassium bromide (KBr) wafer technique (Wang et al., 2012). The sugar amounts of the hydrolysates were detected at 50 °C with a SH1011 column (Shodex) installed in the HPLC system (Waters 2698, USA). The flow rate of the mobile phase which was 5 mM H_2SO_4 was 0.5 mL/min. The enzymatic digestibility was calculated as the ratio of the practical quantities of cellobiose, glucose and xylose released from holocellulose to their theoretical amounts (Wang et al., 2017).

3. Results and discussion

3.1. Effect of lignin removal on enzymatic hydrolysis

With NaOH-treated time increasing from 10 min to 120 min, the glucan retention of SCB was slightly decreased from 93.9% to 88.7%, while the xylan retention of SCB was obviously reduced from 91.2% to 68.9%, and the lignin removal was distinctly increased from 48.3% to 74.3% (Fig. 1). It meant that as NaOH treatment intensifying, the glucan could be mostly retained in the treated SCB, but a part of xylan

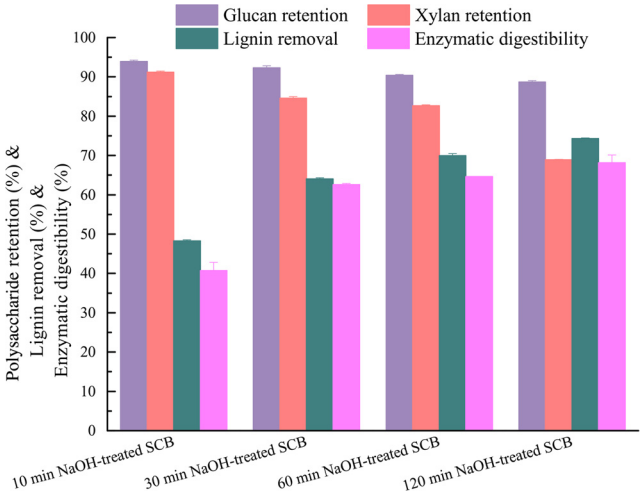

Fig. 1. Glucan and xylan retention, lignin removal of SCB treated by 2% NaOH at 80 °C for 10, 30, 60, and 120 min, and the enzymatic digestibility of treated SCB samples at 50 °C, 180 rpm for 72 h with cellulase loading of 20 FPU/g cellulose.