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Using polyvinylpyrrolidone to enhance the enzymatic hydrolysis of lignocelluloses by reducing the cellulase non-productive adsorption on lignin

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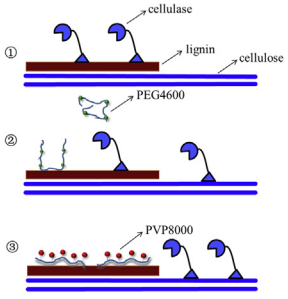
HIGHLIGHTS

- PVP could greatly enhance the enzymatic hydrolysis of lignocelluloses.
- The adsorption layer of PVP8000 on lignin was more stable than that of PEG4600.
- Lignin adsorbed PVP8000 was more hydrophilic than that adsorbed PEG4600.
- PVP8000 reduced 73.1% of the cellulase non-productive adsorption on lignin.

GRAPHICAL ABSTRACT



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ABSTRACT

Polyvinylpyrrolidone (PVP) is an antifouling polymer to resist the adsorption of protein on solid surface. Effects of PVP on the enzymatic hydrolysis of pretreated lignocelluloses and its mechanism were studied. Adding 1 g/L of PVP8000, the enzymatic digestibility of eucalyptus pretreated by dilute acid (Eu-DA) was increased from 28.9% to 73.4%, which is stronger than the classic additives, such as PEG, Tween and bovine serum albumin. Compared with PEG4600, the adsorption of PVP8000 on lignin was larger, and the adsorption layer was more stable and hydrophilic. Therefore, PVP8000 reduced 73.1% of the cellulase non-productive adsorption on lignin and enhanced the enzymatic hydrolysis of lignocelluloses greatly.

1. Introduction

Lignocelluloses are the most abundant biomass resources, which mainly contain cellulose, hemicellulose and lignin (Balat, 2011). With the increased greenhouse effect and the depletion of nonrenewable resources, bioconversion of lignocelluloses to biofuel and value-added products is of great significance in substituting traditional fossil fuel. Ethanol production from lignocellulosic biomass comprised the following main steps: pretreatment of lignocelluloses, hydrolysis of cellulose and hemicellulose, sugar fermentation, distillation of ethanol (Sanchez and Cardona, 2008). Low enzymatic hydrolysis efficiency and high dosage of cellulase were the main factors that restrict the industrialization of the second generation bioethanol (Saini et al., 2015).

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One of the most critical issues for the low enzymatic hydrolysis efficiency was the non-productive adsorption of cellulase on the lignin in substrates (Lin et al., 2016; Rahikainen et al., 2013), which decreased the effective concentration of cellulase in enzymatic hydrolysates. The adsorption of cellulase on lignin was almost irreversible (Gao et al., 2014; Yang and Wyman, 2006) and the adsorption capacity of cellulase on lignin is greater than that on cellulose (Tu et al., 2009). The main interactions between cellulase and lignin were hydrophobic interaction, electrostatic interaction and hydrogen bonding interaction (Nakagame et al., 2011; Rahikainen et al., 2013). The interactions between softwood kraft lignin and cellulase form *T. reesei*, ATCC 26921 were studied by atomic force microscopy, and found the interactions following the order: hydrophobic interaction > electrostatic interaction > hydrogen bonding interaction (Qin et al., 2014). However, the interactions between cellulase and lignin were varying for different substrates. For example, the maximal adsorbed cellulase of steam-exploded Lodgepole pine and ethanol-pretreated Lodgepole pine were 101.05 mg/g and 87.69 mg/g respectively (Tu et al., 2009). Strategy to reduce the non-productive absorption of cellulase on lignin was an effective way to enhance the enzymatic hydrolysis of lignocelluloses, reduce the cellulase dosage and improve the recovery of cellulase.

Many additives could effectively enhance the enzymatic hydrolysis of lignocelluloses by reducing the non-productive adsorption of cellulase on lignin (Cai et al., 2016; Lou et al., 2013a). In order to resist cellulase adsorption on lignin, additives needed to be adsorbed on lignin firstly, and then the non-productive adsorption of cellulase on lignin was reduced by the resistance of additives to cellulase. So during enzymatic hydrolysis, additives played a role of resisting protein adsorption. Reducing the non-productive adsorption of cellulase on lignin by pre-adsorption of additives had two crucial factors: (1) whether additives can form stable adsorption layer on lignin; (2) the ability of additives to resist cellulase.

Polyethylene glycol (PEG) was recognized as one of the best protein-resistant chemicals (Zong et al., 2015), and often used to enhance the enzymatic hydrolysis of lignocelluloses (Li et al., 2012). During enzymatic hydrolysis, PEG adsorbed on lignin mainly through hydrophobic interaction, while the ether linkage (–O–) trended into water and formed a hydrated layer on the surface of lignin to resist cellulase adsorption on lignin (Zhang et al., 2011). Many studies suggested that nonionic surfactants and polymers which contain ($-CH_2CH_2O-$) unit could effectively enhance the enzymatic hydrolysis of lignocelluloses by resisting cellulase adsorption on lignin (Monschein et al., 2014; Ouyang et al., 2010). Recent research suggested that polyvinylpyrrolidone (PVP) could be used in resisting protein as PEG (Choi et al., 2013; Kanagaraj et al., 2015). While PVP with good water solubility had not been reported as an additive for lignocellulosic enzymatic hydrolysis. PVP not only contains a long carbon chain which has hydrophobic interaction with lignin, but also contains polar amidic groups (–(CO)–N–) which could form the hydrated film to resist proteins. The polyurethane membrane which modified by PVP could effectively resist the adsorption of bovine serum albumin and lysozyme (Yuan et al., 2015). So PVP was used as additive in enzymatic hydrolysis in this work.

PVP had excellent physiological inertia and good biocompatibility, it wasn't involved in human metabolism (Cong et al., 2014). PVP had been widely used in the field of medicine, food and cosmetics (Liu et al., 2016; Leone et al., 2011). Firstly, effect of PVP on the enzymatic hydrolysis of lignocelluloses under different conditions was investigated. Then effect of PVP on the enzymatic hydrolysis of lignocelluloses was compared with classic additives, such as tween, PEG, and bovine serum albumin (BSA). The interaction between lignin and PVP, and effect of PVP on the hydrophobicity of lignin film was studied. The enhancing mechanism of the enzymatic hydrolysis of lignocelluloses by PVP was proposed. The results of this study could be applied to synthesize more efficient additives for lignocellulosic enzymatic hydrolysis.

2. Materials and methods

2.1. Materials

PVP8000, PVP10000, PVP24000, PVP58000 and PVP1300000 were bought from Shanghai Aladdin Biochemical Technology Co., Ltd; PVP160000 and PVP360000 were purchased form Sigma–Aldrich Shanghai trading Co., Ltd.

PEG1000, PEG2000, PEG4000, PEG6000, PEG8000 and PEG10000 were get form Shanghai Macklin Biochemical Co., Ltd; PEG4600 was purchased form Sigma–Aldrich Shanghai trading Co., Ltd.

Tween20 and Tween80 were purchased form Kermel chemical reagent Co., Ltd, Tianjing. Bovine albumin (BSA) was got form CapitalBio Co., Ltd, Shanghai.

Commercial cellulase enzyme Cellic CTec2 (abbreviated CTec2) derived from the fungus *Trichoderma reesei* (*Hypocrea jecorina*) was provided by Novozyme China (Shanghai, China). The protein concentration of cellulase is 73.6 mg/mL and its cellulase activity is 147 FPU/mL according to the literature method (Wood and Bhat, 1988).

Eucalyptus wood (Eu) was cut from Qinbei district of Qinzhou city in Guangxi province. Masson pine (Ma) was also obtained from Qian Xiang town of Dongyang city in Zhejiang province. Specific trees were selected based on size, tree age, and overall health. All logs were debarked and then cut wood into chips by machine. The wood chips which less than 50 mm and greater than 20 mm in length were selected by screening. The thickness of the accepted chips ranged from 1 to 5 mm. The chips were kept frozen at −4 °C until used.

Lignin used for lignin film was isolated from eucalyptus using organosolv ethanol process. Briefly, 100 g of od eucalyptus chips were loaded into a horizontal rotary digester (KRK 2611, Japan) with 300 g of 65 wt% ethanol aqueous solution. The extraction was conducted at 165 °C for 1 h. Then the extract solution was precipitated into 1 L cold water. The resultant lignin was filtered out, thoroughly washed with water, dried under vacuum and sieved by 40 mesh.

All chemicals were analytical grade and used as received. Milli-Q water was used for the preparation of all solutions.

2.2. Pretreatments of lignocelluloses

Pretreatments were carried out in a laboratory horizontal rotary digester (KRK 2611, Japan), which were described in detail in the previous study (Cai et al., 2016). About 500 g of od wood chips were loaded in the reactor. Wood chips were subjected to pretreat using bisulfite with sulfuric acid prior to size reduction. The pretreatment liquor to od wood chip solid ratio was 3:1. The sodium bisulfite dosages on od untreated wood (w/w) were 0, 4%, 8% respectively, and sulfuric acid dosages on od untreated wood (w/w) were 1.1%, 2.2% respectively, which resulted in pH of the pretreatment solutions ranging from 1.4 to 2.1. At the end of the pretreatment, the collected solids from pretreatment were fed directly into a laboratory High Consistency Disc Mill (2500-II, Japan) to produce substrates for enzymatic hydrolysis. The mechanical disk milling was carried out in ambient conditions with the disk gap of 0.2 mm. The biomass collected from size reduction was washed and dewatered for three times by a drying machine, then stored in the refrigerator at 4 °C. Solid content of substrates was about 20–30%.