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Enhanced enzymatic saccharification of corn stover by in situ modification of lignin with poly (ethylene glycol) ether during low temperature alkali pretreatment

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HIGHLIGHTS

- In situ lignin modification was performed during alkali pretreatment with PEGDE addition.
- In situ lignin modification enhanced enzymatic hydrolysis of corn stover.
- Lignin modification reduced the non-specific binding of *exo*-glucanase and β-glucosidase.
- Lignin-based surfactant showed no positive effect on enzymatic hydrolysis of PAPCS.

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ABSTRACT

A novel pretreatment process of corn stover was established in this study by in situ modification of lignin with poly (ethylene glycol) diglycidyl ether (PEGDE) during low temperature alkali pretreatment. The addition of PEGDE obviously improved the enzymatic hydrolysis by covalently modifying the residual lignins in substrates. Under the optimized conditions (pretreated with 10% (w/w) NaOH and 10% (w/w) PEGDE at 70 °C for 2.5 h), the total fermentable sugar yield was increased by 46.4%, from 23.7 g to 34.7 g per 100 g raw materials. Additionally, the remaining activities of *exo*-glucanase and β-glucosidase in supernatant were increased by 58.6% and 40.6% respectively, demonstrating that the enhancement of enzymatic hydrolysis was mainly due to the alleviation of enzyme non-productive binding. Although the isolated lignin modified with PEGDE enhanced the enzymatic hydrolysis of substrates as well, this in situ lignin modification provided an efficient but simple way to improve enzymatic saccharification.

1. Introduction

Corn stover is one of the most abundant agricultural residues in China. The availability of corn stover in China was 0.84 billion tons, whose combustion can generate a huge energy, nearly equal to 11.2% of China's total energy consumption in 2013 (Zhang et al., 2016). However, 17–26% of the total corn stover residues were burned in open fields causing serious environmental problems (Li et al., 2007). Concerns of these environmental pollution issues, along with the potential fossil fuels depletion, have stimulated researches on biorefinery of lignocellulosic resources. Bioconversion of lignocelluloses to biofuels and biochemicals is considered as a promising approach to the large-scale utilization of corn stover (Menon and Rao, 2012). However, one of the greatest impediments for the biorefinery industry is enzymatic saccharification of recalcitrant lignocellulosic biomass (Arantes and Saddler, 2010). It is acknowledged that both enzyme- and substrate-related factors suppress the efficient enzymatic hydrolysis. Enzyme-related factors include enzyme deactivation, the loss of enzyme synergism, as well as end-products inhibition (Gunjikar et al., 2001; Holtzapple et al., 1990; Mansfield et al., 1999). In addition, substrate-related factors have been observed to play more important roles in enzymatic hydrolysis, including the impeditive effects of lignin and hemicellulose, cellulose crystallinity, degree of polymerization (DP) of cellulose and the accessible surface area

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(Leu and Zhu, 2013; Öhgren et al., 2007; Wiman et al., 2012; Yoshida et al., 2008).

Aiming to weaken the adverse influence of the substrate-related factors, pretreatment is conducted prior to enzymatic hydrolysis, by partially removing hemicellulose or lignin, decreasing the crystallinity or DP of cellulose, or increasing the surface area (Alvira et al., 2010; Chandra et al., 2007). Various pretreatment methods have been applied to facilitate the enzymatic saccharification of corn stover, such as dilute acid pretreatment, lime pretreatment, ammonia recycle pretreatment, and sodium hydroxide pretreatment (Esteghlalian et al., 1997; He et al., 2010; Kim and Holtzapple, 2005; Kim and Lee, 2005). As compared to the widely used acid pretreatment, alkali pretreatment has been reported to remove lignin more efficiently and reserve the majority of hemicellulose (Sun and Cheng, 2002). Additionally, it could also efficiently enhance the enzymatic digestibility of corn stover, as compared to other pretreatments including dilute acid, lime and aqueous ammonia steeping followed by dilute acid hydrolysis (Ming et al., 2009). Currently, due to the development of chemical recovery technology, alkali pretreatment exhibits the great potential in lowering the pretreatment cost and minimizing environmental pollution (Wu et al., 2011a). To further cut down the capital expenditure, dilute alkali pretreatment performed at low temperature was proposed for the herbaceous biomass (Wu et al., 2011a; Wu et al., 2011b). However, low temperature inevitably weakened the promotion of pretreatment on enzymatic hydrolysis efficiency, and therefore increasing the enzyme usage to achieve the high sugar yields. This might lead to cost concerns for the large-scale bioconversion of lignocelluloses. Moreover, the certain amount of residual lignins remained in the alkali pretreated materials still hindered enzymatic hydrolysis.

Lignin is well known to detrimentally impact enzymatic saccharification by physical blocking and enzyme non-productive binding (Rahikainen et al., 2011). It has been proposed that lignin adsorbed enzyme protein via hydrophobic, electrostatic, and hydrogen bonding interactions (Nakagame et al., 2011). Therefore, surfactants, such as poly (ethylene glycol) (PEG), and tween, were supplemented during enzymatic hydrolysis process to relieve the nonproductive adsorption of cellulases, which produced the higher enzymatic hydrolysis yields and the more efficient enzyme recovery (Eriksson et al., 2002; Li et al., 2016). Moreover, the surfactant-assisted pretreatment has been investigated as well (Qing et al., 2010; Pandey and Negi, 2015). The addition of surfactants during pretreatment enhanced enzymatic hydrolysis probably by increasing lignin removal and reducing non-productive binding of cellulases (Qing et al., 2010).

Recently, water soluble lignin-based surfactant has been prepared by the reaction between epoxidized PEG derivatives and lignins under the alkali conditions. The phenolic hydroxyl groups in lignins could be deprotonated, and converted into the reactive phenolates. Then the phenolates behaved as nucleophiles attacking the terminal epoxide moieties in PEG derivatives, thus producing oxyethylated lignin (Passauer et al., 2016). It has been reported that the resulting modified lignin might act as surfactant, and improved enzymatic hydrolysis by physically modifying the residual lignins in pretreated substrates, thus reducing the non-productive binding of enzymes (Aso et al., 2013; Winarni et al., 2014; Lin et al., 2015). However, the original lignins had to be isolated from the lignocelluloses prior to the graft modification. Then the modified lignins needed to be recovered, purified, and added during the enzymatic hydrolysis. To simplify this process, a one-pot process was established to pretreat the lignocelluloses and modify the lignins with poly (ethylene glycol) diglycidyl ether (PEGDE) simultaneously. We hypothesized that the addition of PEGDE during the alkali pretreatment could covalently modify not only the alkali lignins in the pretreatment liquor but also the residual lignins in the substrates. Thus, the PEG chains that were introduced on the lignin surfaces could reduce the adsorption of enzymes on lignins. Importantly, this in situ modification of lignins could facilitate the enzymatic saccharification of low temperature pretreated corn stover with dilute alkali. The effects of in situ lignin modification on enzymatic hydrolysis, and enzyme adsorption were studied to estimate its potential application prospect.

2. Materials and methods

2.1. Materials and enzymes

Corn stover, harvested from Xuzhou, Jiangsu province, China, was air-dried and ground by a laboratory mill to pass 20 mesh screen. This ground corn stover was used in the following alkali pretreatment experiments. PEGDE with an average molecular weight of 500 Da was purchased from Sigma-Aldrich, and applied as a graft modifying agent.

Cellulase enzymes (L-100) with 227 FPIU/mL of filter paper activity were kindly provided by Youtell Biochemical limited company, Hunan province, China, and used in the enzymatic hydrolysis experiments.

2.2. Alkali pretreatment with or without poly (ethylene glycol) diglycidyl ether

Alkali pretreatment of corn stover was carried out in a solid to liquid ratio of 1:10 in three-necked round-bottom flasks with mechanical stirring, using a water bath at 70 °C for 3 h. The ground corn stover was treated with varied sodium hydroxide loadings ranging between 10% and 40% (w/w) based on the dry weight of biomass. To achieve the in situ modification of lignin, alkali pretreatment of corn stover was performed with various PEGDE loadings ranging between 5% and 30% (w/w) based on the dry weight of biomass. The pretreatment time and the time point of PEGDE addition were optimized as well, to obtain the optimal conditions of PEGDE-assisted pretreatment. After pretreatment, the solid residues were washed with tap water to remove the soluble products and chemicals, and then collected on the filtration cloth. The resultant alkali pretreated corn stover without PEGDE was assigned as APCS; while the PEGDE-assisted alkali pretreated corn stover was assigned as PAPCS.

To figure out whether PEGDE could react with cellulose, Avicel (PH-101, Sigma-Aldrich) was pretreated with 10% (w/w) NaOH and 30% (w/w) PEGDE at 70 °C for 3 h.

2.3. Enzymatic hydrolysis of pretreated substrates

Enzymatic hydrolysis was performed at 2% (w/v) glucan concentration with a commercial enzyme loading of 20 FPIU/g-glucan, at 50 °C, pH 4.8 and 150 rpm for 72 h. To investigate the effects of in situ lignin modification on enzymatic hydrolysis, the enzymatic hydrolysis of APCS and PAPCS was conducted. The samples were taken from the hydrolysis solution at various time intervals. The concentrations of glucose and xylose were quantitated by HPLC with Aminex HPX-87H column. The glucose or xylose yields were calculated from the contents of released glucose or xylose, as percentages of the theoretical glucose or xylose available in the substrates. Each enzymatic hydrolysis experiment was carried out in duplicate, and each data point was presented as the average of two replicates.