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Contrasting effects of hardwood and softwood organosolv lignins on enzymatic hydrolysis of lignocellulose

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HIGHLIGHTS

- Hardwood organosolv lignin enhanced enzyme hydrolysis.
- Softwood organosolv lignin inhibited enzyme hydrolysis.
- Lignin effects were associated with adsorption distribution coefficients.

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ABSTRACT

Identifying an appropriate parameter to elucidate effects of lignin on enzymatic hydrolysis is essential to understand the interactions between enzymes and lignin. Contrasting effects of hardwood organosolv lignin (EOL-SG) and softwood organosolv lignin (EOL-LP) on enzymatic hydrolysis were observed. The addition of EOL-SG (8 g/L) significantly improved the 72 h hydrolysis yields of organosolv pretreated sweetgum (OPSG) and loblolly pine (OPLP) from 49.3% to 68.6% and from 41.2% to 60.8%, respectively. In contrast, the addition of EOL-LP decreased the 72 h hydrolysis yields of OPSG and OPLP to 42.0% and 38.1%, respectively. A strong correlation between the distribution coefficients of cellulase enzymes on lignins and the changes of hydrolysis yields indicated that the inhibitory or stimulatory effects of organosolv lignins on enzymatic hydrolysis were governed by the distribution coefficients (R). The different R values probably were related to the electrostatic interactions, hydrophobic interactions and hydrogen bondings between enzymes and lignin.

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1. Introduction

The electrostatic interactions, hydrophobic interactions and hydrogen bondings between cellulases and lignin play essential roles in enzymatic hydrolysis of lignocellulosic substrates for biochemical conversion of renewable biomass to fuels and chemicals (Lan et al., 2013; Nakagame et al., 2011a; Palonen et al., 2004; Pan, 2008; Sewalt et al., 1997). Non-productive binding has been suggested as one of major factors in limiting the enzymatic hydrolysis by lignin. The negative and inhibitory effect of lignin has been studied extensively (Nakagame et al., 2011b; Rahikainen et al., 2011). Softwood hydrolytic lignin and residual lignin have been reported to show strong inhibition on endoglucanases, less inhibition on xylanases and beta-glucosidases (Berlin et al., 2006). The potential reason for softwood residual lignin inhibition was illustrated by strong binding ability and enzyme denaturation on lignin surface (Rahikainen et al., 2011). Compared with the enzymatic lignin from poplar (hardwood), the enzymatic lignin from lodgepole pine (softwood) showed higher inhibition on enzymatic hydrolysis of Avicel, but the enzymatic lignin from corn stover showed the negligible effect (Nakagame et al., 2010).

Although various types of lignins (Kraft lignin, cellulolytic enzyme lignin, residual lignin and organosolv lignin) have been investigated for their effects on enzymatic hydrolysis, the main emphasis has been put on the most potent inhibitory lignins than the less inhibitory lignins (Nakagame et al., 2011b; Pan, 2008). The studies on the strong inhibitory lignins have led to develop processes and technologies to reduce lignin inhibition such as adding surfactants (Eriksson et al., 2002), protein (Yang and Wyman, 2006) and polyelectrolytes (Ji and Lee, 2013) and modifying lignin (Palonen and Viikari, 2004). Non-ionic surfactant has been used to

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improve enzymatic hydrolysis of softwood substrates and cellulase recycling (Tu et al., 2007). Cationic polyelectrolytes have been used to enhance cellulase hydrolysis of cellulosic fiber by promoting productive binding between enzyme and cellulose (Ji and Lee, 2013). Previously, BSA has also been shown to adsorb on lignin binding sites irreversibly and improve the enzymatic hydrolysis of lignin-rich substrates (Yang and Wyman, 2006). Phenolic hydroxyl groups in lignin have been suggested to play an important role in affecting the enzymatic hydrolysis (Pan, 2008), subsequently they removed the negative effect by hydroxypropylation of free phenolic hydroxyl groups on lignin.

However, identifying the less inhibitory lignin and understanding their structural properties could be equally important, as this approach can help unearth the stimulatory effects of lignins and their corresponding feedstocks. Recently, it was reported that the extractable lignin from organosolv pretreatment of sweetgum enhanced the enzymatic hydrolysis of pretreated substrates significantly (Lai et al., 2014). This echoed another newly finding from Zhu's group on effects of lignosulfonate (Wang et al., 2013a,b; Zhou et al., 2013). They found the negative charged lignosulfonate within an appropriate molecular weight enhanced the enzymatic hydrolysis significantly (Zhou et al., 2013), in which the lignosulfonate has been suggested to act as a surfactant and reduce the non-productive binding. The highly sulfonated lignin (lignosulfonate) typically shows high hydrophilicity, which reduces the hydrophobic interaction between enzyme and lignin. In addition, the repulsive electrostatic interactions from negatively charged lignosulfonate could also reduce the non-productive binding between enzyme and lignin. However, still some commercial lignosulfonate with higher molecular weight and less surface charges showed negative effects on enzymatic hydrolysis (Zhou et al., 2013). Currently, new accurate characterization methods are lacking in distinguishing the positive and negative effects of lignins. Identifying an appropriate parameter to elucidate both inhibitory and stimulatory effects of lignins on enzymatic hydrolysis is essential to understand the mechanism of lignin inhibition and stimulation.

In this study, the stimulatory and inhibitory effects of organosolv lignins on enzymatic hydrolysis were compared to examine the potential difference between hardwood organosolv lignin and softwood organosolv lignin. Organosolv pretreated sweetgum (OPSG) and loblolly pine (OPLP) were used as the substrates along with Avicel. Cellulases enzyme distribution was determined during the enzymatic hydrolysis. Langmuir adsorption isotherms were used to characterize the adsorption affinity of enzyme on organosolv lignins. The correlation between adsorption distribution coefficients of enzyme on lignins and the changes of 72 h hydrolysis yields was established. The surface charges of two organosolv lignins were determined by potentiometric titration (Rahikainen et al., 2013). The hydrophobicity of two lignins was also estimated by Rose Bengal method (Gessner et al., 2000). The functional groups including phenolic hydroxyl, aliphatic hydroxyl and methoxyl groups were estimated by proton-NMR. The main objective of this work is to identify one potential parameter to distinguish the contrasting effects of lignin on enzymatic hydrolysis.

2. Methods

2.1. Organosolv pretreated biomass and organosolv lignin preparation

Sweetgum (*Liquidambar styraciflua*) and loblolly pine (*Pinus taeda*) wood chips (1.0 × 1.0 cm, L × W) were collected by Forest Products Laboratory at Auburn University. Organosolv pretreated sweetgum (OPSG) and organosolv pretreated loblolly pine (OPLP) were prepared in a 1.0 L Parr batch reactor (Parr Instrument Co., Moline, IL) as previously described (Lai et al., 2014). Briefly, sweetgum wood chips (80 g, dry weight) were soaked in 25% ethanol and 1% (w/w) sulfuric acid overnight, and then loaded into a reactor (7:1 liquor/solid ratio) and treated at 160 °C for 60 min. Loblolly pine wood chips were pretreated similarly with 75% ethanol at 170 °C for 60 min. After pretreatment, the pretreated slurry was separated into a solid fraction and a liquid fraction by filtration, and the solid substrates was washed with warm ethanol three times to remove the extractable lignin. The collected substrates were further washed with water for at least three times and then homogenized in blender for 30 s. After that, the wet pretreated substrates were collected by filtration and used for the following enzymatic hydrolysis (moisture content, ~75%).

To prepare ethanol organosolv lignin (EOL) from woody biomass, 3-fold volumes of water were added to organosolv spent liquor (the liquid fraction) after pretreatment. Organosolv lignin was precipitated and collected by filtration on Whatman No. 1 filter paper, and washed by warm water to remove the water-soluble compounds. Ethanol organosolv lignin from sweetgum (EOL-SG) was obtained from organosolv pretreatment of sweetgum with 75% ethanol at 160 °C, and ethanol organosolv lignin from loblolly pine (EOL-LP) obtained from organosolv pretreatment of loblolly pine with 75% ethanol at 170 °C. The chemical composition of pretreated substrates and ethanol organosolv lignins is summarized in Table 1.

2.2. Cellulase enzymes

Commercial cellulase preparation, Novozym 22C, obtained from Novozymes (Franklinton, NC) was used in enzymatic hydrolysis of Avicel (pure cellulose), OPSG and OPLP substrates. The filter paper enzyme activity of Novozym 22C was 100 FPU/mL, its β-glucosidase activity was 343 IU/mL. Cellulase C2730 from *Trichoderma reesei* ATCC 26921 was purchased from Sigma–Aldrich (St. Louis, MO) and used for cellulase adsorption isotherms determination.

2.3. Enzymatic hydrolysis and enzyme distribution

Enzymatic hydrolysis was performed in 50 mL of sodium citrate buffer (50 mM, pH 4.8) at 2% glucan (w/v) with commercial enzyme (Novozym 22C) at 50 °C and 150 rpm for 72 h. To achieve comparable hydrolysis yield, 5 FPU/g glucan was used in enzymatic hydrolysis of Avicel or OPSG, and 10 FPU/g glucan in enzymatic hydrolysis of OPLP. To investigate the effect of organosolv lignin on enzymatic hydrolysis, various amount of organosolv lignin (0, 2, 4, and 8 g/L) was added into the reaction solution with substrates respectively prior to the addition of cellulase enzymes. In addition, one control experiment was performed in the same condition with only organosolv lignin (8 g/L) and Novozym 22C to examine the potential sugar release from the hydrolysis of organosolv lignin. For the examination of pH effect, enzymatic hydrolysis of OPSG was carried out under pH 4.8 and 5.6 respectively with or without organosolv lignin (4 g/L). During the hydrolysis, the samples were taken from the hydrolysis solution at various time intervals to monitor the sugar release and free enzyme concentration in solution. The glucose and xylose content was quantitated by HPLC with Aminex HPX-87P column. The hydrolysis yield of the substrates was calculated from the released glucose content, as a percentage of the theoretical sugars available in the substrates. Initial hydrolysis rate was calculated based on the released sugars in the first 3 h of enzymatic hydrolysis. The free enzyme concentration in supernatant was determined by Bradford assay, and presented in the percentage of the total protein concentration. Enzymatic hydrolysis was carried out in duplicate.

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