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Reducing non-productive adsorption of cellulase and enhancing enzymatic hydrolysis of lignocelluloses by noncovalent modification of lignin with lignosulfonate

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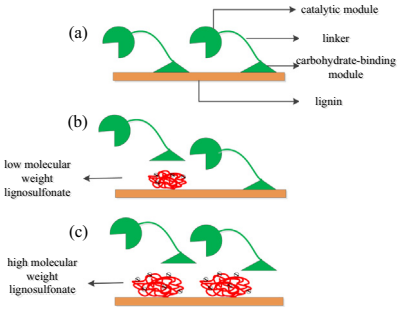
HIGHLIGHTS

- SL clearly reduced the non-productive adsorption of cellulase on lignin.
- SL with higher MW had stronger blocking effect on cellulase adsorption on lignin.
- Linear anionic aromatic polymers strongly blocked cellulase adsorption on lignin.
- Copolymer of lignin and PEG had stronger enhancement than PEG.

GRAPHICAL ABSTRACT



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ABSTRACT

Four fractions of one commercial sodium lignosulfonate (SXP) with different molecular weight (MW) and anionic polymers were studied to reduce non-productive adsorption of cellulase on bound lignin in a lignocellulosic substrate. SXP with higher MW had stronger blocking effect on non-productive adsorption of a commercial *Trichoderma reesi* cellulase cocktail (CTec2) on lignin measured by quartz crystal microgravimetry with dissipation monitoring. Linear anionic aromatic polymers have strong blocking effect, but they would also reduce CTec2 adsorption on cellulose to decrease the enzymatic activity. The copolymer of lignin and polyethylene glycol (AL-PEG1000) has strong enhancement in enzymatic hydrolysis of lignocelluloses, because it not only improves the cellulase activity to cellulose, but also blocks the non-productive cellulase adsorption on lignin. Apart from improving the cellulase activity to cellulose, the enhancements of enzymatic hydrolysis of lignocellulose by adding AL-PEG1000 and SXPs are the result of the decreased cellulase non-productive adsorption on lignin.

1. Introduction

Lignocellulosic biomass is a potentially renewable and abundant resource for biofuel and biomaterial production, which is significant to reduce human's reliance on the limited global fossil fuel consumption (Himmel et al., 2007). Despite of the extensive research efforts, large-scale utilization of lignocelluloses through converting cellulose and hemicellulose into fermentable sugars is

Abbreviations: SXP, the commercial sodium lignosulfonate; MW, molecular weight; LS, lignosulfonate; CTec2, commercial *Trichoderma reesi* cellulase cocktail; AL-PEG1000, copolymer of lignin and polyethylene glycol; QCM-D, quartz crystal microgravimetry with dissipation monitoring; ASP, sulfanilic acid–phenol–formaldehyde condensate; FDN, formaldehyde naphthalene sulfonate condensate; SAF, sulfonated acetone–formaldehyde condensate; SED, substrate enzymatic digestibility; AFM, atomic force microscope; CBM, carbohydrate-binding module; PEG, polyethylene glycol.

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Table 1
MW distribution and sulfur content of anionic polymers used in experiments.

Additives	MW distribution	Fractions	Sulfur content (%)
ASP	<10,000	ASP4	ND
	10,000–50,000	ASP3	7.10
	50,000–100,000	ASP2	6.75
	>100,000	ASP1	6.69
FDN	<10,000	FDN3	ND
	10,000–50,000	FDN2	10.88
	>50,000	FDN1	10.29
SAF	<2500	SAF4	8.25
	2500–10,000	SAF3	11.51
	10,000–50,000	SAF2	11.75
	>50,000	SAF1	10.83
SXP	<2500	SXP4	9.47
	2500–10,000	SXP3	7.65
	10,000–50,000	SXP2	6.30
	>50,000	SXP1	5.32

ND, not determined.

still limited by several factors (Jørgensen et al., 2007; Ding et al., 2012). The plant cell wall structure of lignocellulose is highly complex (Ding et al., 2012). Lignocellulose mainly contains of cellulose, hemicellulose, and lignin. Lignin can affect enzymatic hydrolysis by limiting access of the cellulase to the cellulose in cell wall (Ding et al., 2012; Leu and Zhu, 2013) as well as through the non-productive adsorption of cellulase (Palonen et al., 2004; Sewalt et al., 1997). Cellulase binds to lignin through hydrophobic (Eriksson et al., 2002), electrostatic (Berlin et al., 2006; Lou et al., 2013) and hydrogen bonding interactions (Sewalt et al., 1997). A pretreatment step is conducted to overcome recalcitrance of lignocelluloses (Ohgren et al., 2007). But lignin is usually partially removed and the cost is very high to achieve complete removal of lignin (Leu and Zhu, 2013). As a result, the non-productive adsorption of cellulase on lignin is unavoidable. Consequently, more cellulase dosages are required to achieve desired saccharification efficiency (Himmel et al., 2007; Zhu and Zhuang, 2012).

There are two routes to reduce cellulase non-productive adsorption on lignin, namely, covalent modification and noncovalent modification. Covalent modification of lignin, such as sulfonation (Lan et al., 2013; Lou et al., 2013) and carboxylation (Nakagame et al., 2011; Lim and Lee, 2013), would reduce cellulase non-productive adsorption on lignin to enhance the enzymatic hydrolysis. But it is usually achieved through pretreatment of lignocelluloses. By contrast, noncovalent modification of lignin can be more easily used. Surfactants, proteins and polymers have been reported to be effective to enhance enzymatic hydrolysis of lignocellulosic materials (Börjesson et al., 2007; Eckard et al., 2013; Kristensen et al., 2007). Further researches indicate that non-ionic surfactants are found more effective than cationic surfactants, whereas anionic surfactants lower cellulose hydrolysis (Eriksson et al., 2002; Kristensen et al., 2007). However, as an anionic polymer surfactant, lignosulfonate (LS) produced by sulfite pulping or sulfite pretreatment (Zhu et al., 2009) was reported to be effective to improve the enzymatic hydrolysis of lignocellulose (Wang et al., 2013; Zhou et al., 2013). The mechanism has yet to be fully elucidated.

Quartz crystal microgravimetry with dissipation monitoring (QCM-D) has been used to study cellulase adsorption on lignocellulosic and cellulosic films and monitor their enzymatic hydrolysis process (Hoeger et al., 2012; Sampedro et al., 2013). Well-defined and stable lignin model films have been utilized as substrates to investigate protein adsorption on lignin film (Salas et al., 2013) and competitive binding between proteins and blocking agents (Reimhult et al., 2008). Hence, it is of interest to study non-productive adsorption of cellulase on pure lignin films by QCM-D. In this work, a new rapid approach using QCM-D was developed to measure the cellulase adsorption on lignin. Then the impacts of SXP fractions with different MW on the cellulase non-productive adsorption on lignin film were investigated. Furthermore, three polymers, sulfanilic acid–phenol–formaldehyde condensate (ASP) and formaldehyde naphthalene sulfonate condensate (FDN) and sulfonated acetone–formaldehyde condensate (SAF), were used to elucidate the effect of the macromolecular structure feature on cellulase non-productive adsorption on lignin and enzymatic hydrolysis efficiency of pure cellulose. Furthermore, a copolymer of lignin and polyethylene glycol was prepared to reduce cellulase non-productive adsorption on lignin to improve enzymatic hydrolysis of lignocellulose.

2. Methods

2.1. Substrates

A pure cellulose substrate of Whatman filter paper (grade 1, catalogue number 1001 150, Whatman International, UK) was used in this study. Enzymatic hydrolysis lignin was prepared from the residue of simultaneous saccharification and fermentation of corn stover after steam explosion pretreatment (Henan Tianguan Group Corp., Ltd, China). It is processed successively by filtration, washing, filtration, washing, autoclaved sterilization, drying, grinding and sieving (40 mesh). Lignocellulosic substrate used in this experiment was a mixture of the cellulose (Whatman filter paper) and enzymatic hydrolysis lignin with the ratio of 7:3, which was used as a simplified lignocellulose to research the impact of lignin.

2.2. Enzymes

Commercial *Trichoderma reesi* cellulase cocktail Cellic® (CTec2) was generously provided by Novozymes North America (Franklinton, NC). The protein concentration of CTec2 is 73.6 mg/mL and its cellulase activity is 147 FPU/mL as calibrated by a literature method (Wood and Bhat, 1988).

2.3. Additives

SXP from poplar sulfite pulping was produced by Shixian papermaking Corp. Ltd. (Yanbian, Jilin province, China). SXP was classified into four fractions with polyether sulfone ultrafiltration membranes with the cut-off MW of 50,000 Da, 10,000 Da and 2500 Da by using an ultrafiltration apparatus (Wuxi Membrane Science and Technology Corp., China). The fractions with MW ranges of greater than 50,000 (SXP1), 10,000–50,000 (SXP2), 2500–10,000 (SXP3) and smaller than 2500 (SXP4) were used.

Anionic polymers including amino sulfanilic acid–phenol–formaldehyde condensate (ASP) and formaldehyde naphthalene sulfonate condensate (FDN) and sulfonated acetone–formaldehyde condensate (SAF) were synthesized in laboratory. Each of these three anionic polymers was separated into several fractions using ultrafiltration membranes. MW distribution and sulfonation degree of all anionic polymers were listed in Table 1.

Polyethylene glycol with the MW of 1000 Da (PEG1000, AR) was purchased from Guangdong Guanghua Sci. Techco. Ltd. (China).

AL-PEG1000 was synthesized by polyethylene glycol, epoxy chloropropane and alkali lignin with the mass ratio of 1:0.005:1. Alkali lignin was dissolved in sodium hydroxide with the concentration of 1.0 mol/L. After processed successively by extraction using butanone and centrifuge to remove insoluble lignin, AL-PEG1000 was precipitated out by adding diethyl ether, the MW was approximately 30,000 Da.