

# Pseudo-lignin Retarded Bioconversion of Sugarcane Bagasse Holocellulose after Liquid Hot Water and Acid Pretreatments

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Pseudo-lignin derived from the condensation of carbohydrate degradation products can retard the bioconversion of lignocellulose. In this work, liquid hot water (150 to 190 °C) and 1% H<sub>2</sub>SO<sub>4</sub> pretreatments (130 to 190 °C) were used on sugarcane bagasse holocellulose for 3 h to generate pseudo-lignin. The effects of pseudo-lignin generation on structural characteristics and bioconversion of substrates were evaluated. The results showed that the formation of pseudo-lignin increased the hydrophobicity of the substrates. After LHW pretreatments and acid pretreatments at low temperatures (<150 °C), most of the xylans were removed, yielding 2.1 to 5.4% pseudo-lignin. Increasing acid pretreatment temperature to 170 and 190 °C yielded 34.3% and 93.6% pseudo-lignin, respectively. After pretreatment, the accessibilities and bioconversions of substrates were enhanced by degradation of xylans, increasing glucose conversions and bioethanol productions of substrates from 53.2 to 85.3%, and 9.9 to 13.1 g/L, respectively. However, large amounts of pseudo-lignin were generated during acid pretreatments at 170 °C, reducing glucose conversion and bioethanol yield to 45.6% and 6.3 g/L, respectively.

*Keywords:* Pseudo-lignin; Bioethanol; Enzymatic hydrolysis; Structural characteristics

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## INTRODUCTION

The rise of global energy consumption necessitates the development of renewable energy sources to address vital strategic, economic, and environmental issues related to the depletion of fossil fuels (Himmel *et al.* 2007). Lignocellulose is an abundant and renewable feedstock for the production of fuels and chemicals. Sugarcane bagasse, a lignocellulosic residue from agriculture, can be utilized to produce bioethanol.

Sugarcane bagasse contains appreciable amounts of cellulose (35 to 45%), hemicelluloses (25 to 35%), lignin (21 to 25%), and minor amounts of extractives and inorganic compounds (Desouky *et al.* 2014). Carbohydrates in sugarcane bagasse can be depolymerized by chemical or enzymatic hydrolysis and further fermented by microorganisms to ethanol. However, the natural recalcitrance of cell walls makes polysaccharides difficult for microbes and enzymes to disintegrate. Thus, an essential pretreatment step is necessary to promote the accessibility of carbohydrates to attack by enzymes during hydrolysis. Among all the pretreatment technologies, liquid hot water (LHW), dilute acid (DA), and steam explosion (SE) pretreatments are commonly used to pretreat lignocellulose due to the effective removal of hemicelluloses and enhancement of

cellulose enzymatic conversion. In addition, LHW pretreatment has attracted a significant amount of attention due to the low cost of energy and alleviated equipment corrosion in comparison with pretreatments using acidic catalyst. However, several studies have found that the acid insoluble lignin content of residues from these pretreatments to be higher than the starting material (Li *et al.* 2005; Leschinsky *et al.* 2009; Jung *et al.* 2010; Xiao *et al.* 2011; Yang *et al.* 2012). Even taking holocellulose as substrate, acid-insoluble composition of the sample also increased after acid pretreatment (Sannigrahi *et al.* 2011; Hu and Ragauskas 2014). These acid-insoluble components are termed as pseudo-lignin and deposit on the surface of cellulose. Degradation products from carbohydrate fractions are the source of pseudo-lignin (Sannigrahi *et al.* 2011; Shinde *et al.* 2018). The structure of pseudo-lignin has been found to be lignin-like, containing carbonyl, carboxylic, methoxyl, aromatic, and aliphatic groups (Shinde *et al.* 2018). During pretreatment, the formed pseudo-lignin can reduce cellulose accessibility and unproductive bind cellulase (Kumar *et al.* 2013). Pseudo-lignin has been reported to be more detrimental to the enzymatic hydrolysis than dilute acid pretreatment lignin (Hu *et al.* 2013). However, pseudo-lignin has lower negative surface charge, hydrophobic nature, Langmuir constant, and binding strength than residual lignin in bamboo after dilute acid pretreatment, representing weaker negative impact on enzymes than residual lignin (He *et al.* 2018). Thus, the inhibition effect of pseudo-lignin depends on its physical chemical characteristics. In addition, the composition and amount of pseudo-lignin vary significantly depending on the type of biomass and pretreatment condition.

In this study, different conditions were used to pretreat holocellulose of sugarcane bagasse to generate pseudo-lignin. The effect of generated pseudo-lignin on structural characteristics of residues was measured by chemical composition, Fourier transform infrared (FT-IR) spectrometry, X-ray photoelectron spectroscopy (XPS), and dye adsorption. The effect of pseudo-lignin on bioconversion of holocellulose after pretreatments was studied by enzymatic hydrolysis and simultaneous saccharification and fermentation.

## EXPERIMENTAL

### Materials

Sugarcane bagasse was kindly supplied by Yingmao Sugar Co., Ltd. (Yunnan Province, China). After washing with distilled water, the material was oven-dried at 50 °C. In order to alleviate the effect of lignin on bioconversion of materials, the dried sugarcane bagasse was delignified using sodium chlorite under acidic condition to prepare holocellulose for pretreatments (Yang *et al.* 2015).

### Liquid Hot Water (LHW) and Dilute Acid Pretreatments (DAPs)

LHW pretreatments of holocellulose from sugarcane bagasse were performed in autoclaves at a solid to liquid ratio of 1:10. Then, the autoclaves were incubated in an oil bath at 150, 170, and 190 °C, respectively. DAPs were conducted in an autoclave at 130, 150, 170, and 190 °C with 1% H<sub>2</sub>SO<sub>4</sub> at a solid to liquid ratio of 1:10, respectively. After 3 h incubation, the hydrolyzates were collected by filtration and submitted to degradation products analysis. The solid fractions were thoroughly washed with distilled water until the filtrates were neutralized, oven-dried at 50 °C, and labeled according to pretreatment method and temperature. The solid samples were submitted to a list of characterizations and bioconversion.

## Enzymatic Hydrolysis and Simultaneous Saccharification and Fermentation (SSF)

The effect of pseudo-lignin on digestibilities of substrates obtained from pretreatments was investigated by enzymatic hydrolysis and SSF. The enzymatic hydrolysis experiments were carried out at substrate concentration of 5% (w/v) in 10 mL of 50 mM sodium acetate buffer (pH 4.8) with an enzyme loading of 15 FPU cellulase per gram of dry weight of substrate using an air bath shaking incubator at 150 rpm at 50 °C for 72 h. The enzyme cocktail used in hydrolysis experiments contained 100 FPU cellulase, 70 IU  $\beta$ -glucosidase, and 50 mg protein in 1 mL enzyme solution, which was kindly supplied by Shanghai Youtell Biochemical Co., Ltd., Shanghai, China. During enzymatic hydrolysis, 0.2 mL of hydrolyzates were sampled periodically and analyzed using a high-performance liquid chromatography (HPLC) system with a refractive index detector (RID) on an Aminex HPX-87 H column (Bio-Rad, Hercules, CA, USA) at 55 °C with 5 mM H<sub>2</sub>SO<sub>4</sub> at a flow of 0.6 mL/min for 55 min. All enzymatic hydrolysis experiments were performed in duplicate, and the average values and corresponding deviations were given.

SSF experiments were performed in 50 mL flasks containing 0.5 g cellulosic sample, 10 mL sodium acetate buffer (pH 4.8), and 10 g/L yeast extract. Prior to incubation, the mixture was sterilized in an autoclave at 121 °C for 20 min. The loading of cellulase and yeast (Angel Yeast Company Ltd., Yichang, China) in all SSF experiments were 20 FPU/g substrate and 3 g/L, respectively. After fermentation at 38 °C for 24 h, concentration of ethanol in broth was determined by HPLC system, as mentioned above. In addition, the number of yeast in each broth was accounted by hemocytometer. All SSF experiments were performed in duplicate, and average values and corresponding deviations were given.

## Analysis Procedures

The contents of glucan, xylan, and lignin in samples were determined based on the standard National Renewable Energy Laboratory (NREL) protocol (Sluiter *et al.* 2012). The quantitative analysis of degradation products was performed on the HPLC system as mentioned above. XPS measurements were performed on a K-Alpha spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with monochromatic Al K $\alpha$  X-ray source. High resolution spectra of carbon and oxygen were collected. The binding energy scale was calibrated by the C1s peak of 285 eV. X-ray powder diffraction patterns of the cellulosic samples were obtained using an XRD-6000 instrument (Shimadzu, Japan). The X-ray diffractograms were recorded from 5° to 35° diffraction angle  $2\theta$  using the reflection method at a scanning speed of 2°/min with Ni-filtered Cu K $\alpha$  radiation ( $\lambda = 1.54 \text{ \AA}$ ) at 40 kV and 30 mA.

The surface accessibilities of samples were determined as the amount of adsorbed direct orange (DO, which enters large pores or adsorbs on surface of substrate) and direct blue (DB, which populates small pores of substrate). In detail, 0.1 g of sample and 1 mL of sodium phosphate-sodium chloride buffered (pH 6.0) were put into six 50 mL polypropylene centrifuge tubes. DO (10 mg/mL) solution was added in a series of increasing volumes (0.2, 0.5, 0.75, 1.0, 1.5, and 2.0 mL) to the series of six tubes. DB solution (10 mg/mL) was added as the same volumes as that of DO into the tubes. Distilled water was added to make up the final volume in the centrifuge tubes to 10.0 mL. Then, the tubes were incubated at the 60 °C for 24 h with shaking at 200 rpm. After incubation, the tubes were centrifuged at 10,000 rpm for 5 min, and the supernatants were determined by a UV-Vis spectrophotometer at 624 and 455 nm. The amount of adsorbed dye was calculated as the difference between the concentration of the initial and final dye in the supernatant, as described previously (Chandra *et al.* 2008).

The hydrophobicity was determined by the adsorption of Rose Bengal with modification (Gessner *et al.* 2000). Briefly, 0.04, 0.08, 0.12, 0.16, and 0.2 g samples were added into five tubes, with 10 mL distilled water and 3 mL dye solution (40 mg/L) added to each tube. After incubating the tubes at 50 °C for 2 h under shaking, the dye concentrations left in the suspensions were determined with a UV-Vis spectrophotometer at 543 nm. The partitioning quotient (PQ) of each sample was calculated as the ratio of amount of Rose Bengal bound on surface to these released in dispersion medium. The PQ was plotted as a function of the total surface area resulting in straight lines.

## RESULTS AND DISCUSSION

### Yields and Chemical Composition

Under acidic and hydrothermal conditions, carbohydrates release monomeric sugars into the aqueous phase, and these can further degrade into furans, hydroxymethyl furfural, and acids. These compounds can be further condensed to form acid-insoluble enriched compounds termed as chars or pseudo-lignin (Kumar *et al.* 2013). After LHW pretreatments and DAPs, recoveries and chemical composition of residues are shown in Fig. 1. Sugarcane bagasse holocellulose contained 62.3% cellulose, 27.8% xylans, and 1.9% lignin (0.7% acid-insoluble lignin and 1.2% acid-soluble lignin). After LHW pretreatments, solid recoveries and xylan contents were reduced from 73.2 to 61.9% and 27.7 to 9.9% as pretreatment temperature increased from 150 °C to 190 °C, respectively. These results may reflect that  $H^+$  released from water under hydrothermal condition catalyzed the degradation of glycosidic bonds and the linkages between acetyl substitutes and backbone of xylans. The released acetyl group formed acetic acid further degraded xylans (Maurya *et al.* 2015). With degradation of xylans, the relative contents of glucose increased from 62.3 to 75.5%. The acid-insoluble lignin also increased from 0.7 to 2.9% with degradation of xylans. Compared with LHW pretreatments, more carbohydrates were degraded during DAPs, yielding 58.5% to 29.3% residues with minimal amount of xylans as pretreatment temperature increased from 130 °C to 170 °C. The acid insoluble lignin increased to 34.4% as carbohydrates decreased in residues. However, DAP at 190 °C led to carbonization of materials, making acid-insoluble lignin as the main compound of the residue (93.6%).

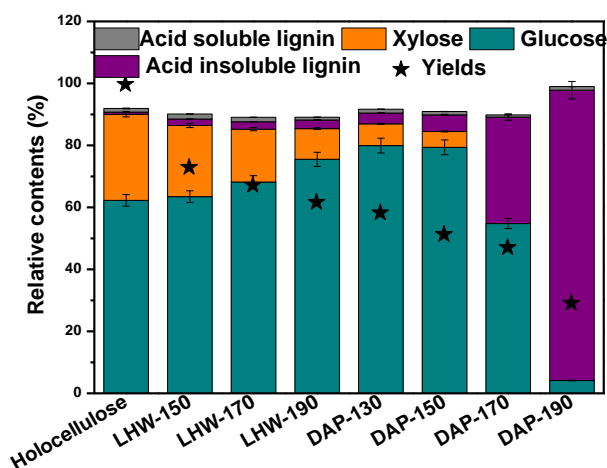


Fig. 1. Chemical composition of holocellulose and residues obtained from pretreatments

The compounds determined in hydrolyzates were consistent with the degradation of carbohydrates. The concentrations of glucose, xylose, FF, and HMF in hydrolyzates increased with pretreatment temperature (Table 1). However, these concentrations decreased as temperature increased to 170 and 190 °C under acidic conditions. These results suggested that degradation products might participate in reactions to form pseudo-lignin, introducing 34.4% and 93.6% acid insoluble lignin in solids, respectively. In acidic sugar degradation pathways, furfural (FF) and 5-hydroxymethylfurfural (HMF) are the main dehydration products of pentoses and hexoses, respectively, and they can rearrange to yield other aromatic compounds. For instance, FF can form 3,8-dihydroxy-2-methylchromone (DMC) (Popoff and Theander 1972), while the hydrolytic ring-open reaction of HMF generates 1,2,4-benzenetriol (BTO) (Luijckx *et al.* 1993; Chuntanapum and Matsumura 2009). Both DMC and BTO are the key intermediates for the formation of pseudo-lignin during DAP (Hu *et al.* 2012). Pseudo-lignin was also observed in the holocellulose from poplar after LHW and acid pretreatments (Hu *et al.* 2012; Sannigrahi *et al.* 2011; Zhuang *et al.* 2017). The pronounced amount of pseudo-lignin obtained after DAPs in this study might be ascribed to the long pretreatment time and high temperature.

**Table 1.** Chemical Composition of Degradation Compounds in the Hydrolyzates from LHW Pretreatments and DAPs (mg/L)

	Glucose	Xylose	Formic acid	Acetic acid	Levulinic acid	Hydroxy methylfurfural	Furfural
LHW-150	19.60	63.73	8.56	75.15	ND	ND	ND
LHW-170	21.37	69.64	10.64	71.20	ND	ND	ND
LHW-190	20.85	77.94	14.74	97.18	ND	ND	ND
DAP-130	53.35	98.65	115.30	87.07	ND	ND	ND
DAP-150	76.57	107.97	119.93	97.39	10.20	8.65	28.39
DAP-170	92.41	65.32	126.14	98.55	12.96	17.61	38.49
DAP-190	85.24	9.61	164.19	114.62	195.39	30.56	56.18

Note: ND, not determined.

### XPS Spectra

X-ray photoelectron spectroscopy is used to determine the elemental composition and the locations of atoms in samples (Kocaefe *et al.* 2013). The chemical shifts for carbon (C) and oxygen (O) in holocellulose, DAP-170, and DAP-190 are shown in Figs. 2a and b, respectively. The peaks at 285, 286, and 288 eV correlated to C-C/C-H, C-O, and C=O/O-C-O linkages, respectively. In holocellulose, C-O was the predominant linkage, followed by C-C/C-H and C=O/O-C-O linkages. After DAPs at 170 °C, the amount of C-C/C-H linkages increased alongside a decrease in C-O and C=O/O-C-O linkages. The absence of peaks for C-O and C=O/O-C-O in the spectrum of DAP 190 confirmed carbonization of holocellulose under this condition. In the spectra of oxygen, the intensity of the C-O peak also decreased after DAPs. These phenomena indicated that C-O of holocellulose was broken under acidic condition. The drastic decrease of intensities of peaks for C-O and O suggested that the oxygen-containing functional groups might participate in condensation reactions with the formation of pseudo-lignin during pretreatments (Ma *et al.* 2015). The

dissociated groups participated condensation reaction to form unconjugated ketones and carbon-carbon bonds in pseudo-lignin (He *et al.* 2008). The increased intensity of the peak for C-C bonds was evidence of condensation reactions. In a previous study, 1,2,4-trisubstituted benzene compound was isolated from acid pretreated hybrid poplar holocellulose (Hu *et al.* 2012).

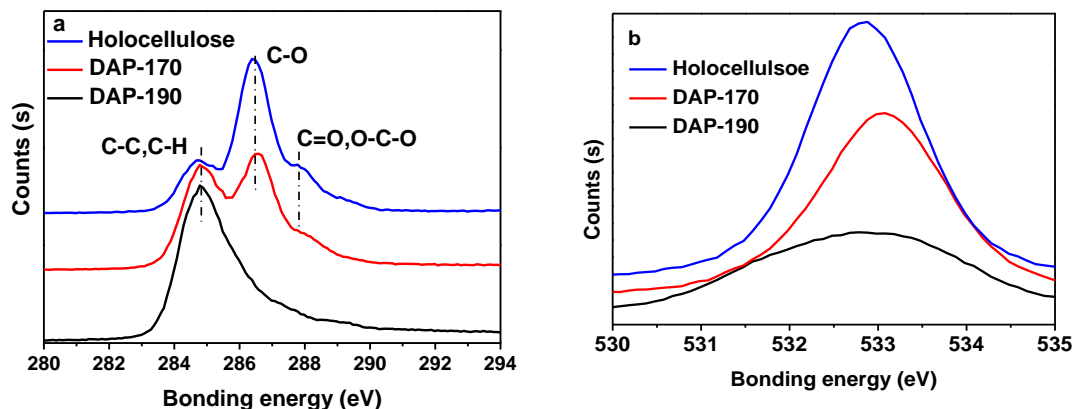


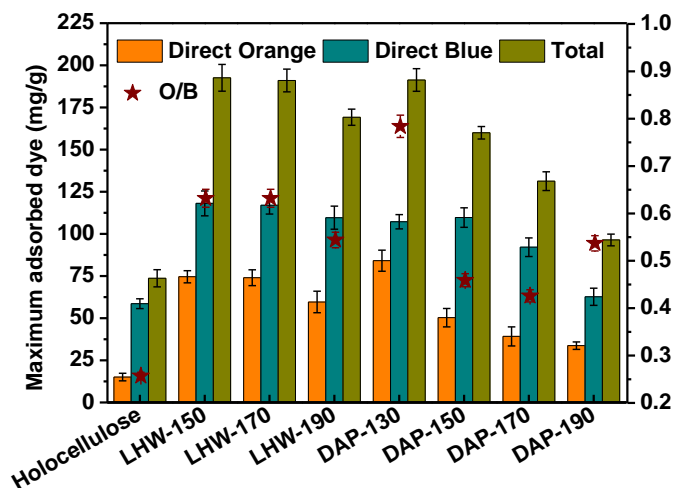
Fig. 2. XPS spectra of holocellulose and residues obtained from DAPs at 170 and 190 °C

### Accessibilities and Hydrophobicities

With regard to the digestibility of the lignocellulose, cellulose accessibility is a more important factor than the chemical composition, delignification, and cellulose crystallinity (Karimi and Taherzadeh 2016). Thus, increasing the accessibility for enzymes is one of the main objectives of pretreatment. Simons' staining method is used to estimate the accessibility of lignocellulose. In this method, DO molecules have a similar diameter as a typical enzyme and can be used to estimate the accessibility of substrate for cellulase. The ratio of DO and DB indicates the relative amount of large pores to small pores.

Figure 3 shows that LHW pretreatments and DAPs increased accessibility of holocellulose. After pretreatments, the absorbed DO and total dyes on samples increased from 15.1 to 74.6 mg/g (LHW-150) and from 73.7 to 192.6 mg/g (LHW-150), respectively. Residue from DAP at 130 °C adsorbed a similar level of total dyes as the LHW-150 but had a larger O/B ratio (0.784). This result might be due to the fact that acidic conditions cause degradation of a larger amount of xylans than the LHW pretreatments, releasing larger pores. This phenomenon was consistent with the composition analysis; the content of xylans in DAP-130 (7.0%) was less than that in LHW-150 (17.1%). However, further degradation of xylans caused the collapse of pores, decreasing cellulose accessibility to 160.0 mg/g (DAP-150 with 5.1% xylans).

In samples DAP-170 and DAP-190, the contents of xylans were reduced to undetectable and caused a decrease of substrate accessibilities to 131.3 and 96.4 mg/g, respectively. These results might be ascribed to the deposition of pseudo-lignin during its condensation onto the surface of materials, thus hindering the diffusion of the dye molecules into the substrates (Sannigrahi *et al.* 2011; Xiao *et al.* 2011). Compared with other studies, the accessibilities of the substrates from LHW pretreatments and DAPs of sugarcane bagasse holocellulose was much higher than that of DAPs of *Populus* for 0 to 60 min (Meng *et al.* 2013; 2015). The higher accessibility of untreated holocellulose was mainly due to the looser cell wall structure of sugarcane bagasse. The effect of delignification on cellulose accessibility is limited (Kumar and Wyman 2009; Meng and Raguakas 2014). Long pretreatment times increased the accessibility slightly above that from DAP of *Populus* at 160 °C for 1 h (Meng *et al.* 2015).



**Fig. 3.** Accessibilities of holocellulose and residues obtained from pretreatments

The removal of xylans increases cellulose accessibility and changes hydrophobicities of substrates (Kumar and Wyman 2009). The effects of LHW pretreatments and DAPs on hydrophobicities of samples are shown in Table 2. After LHW pretreatments and DAPs at 130 and 150 °C, the hydrophobicities of samples ranged between 18.7 and 32.3 L/g. The slight increase of hydrophobicities of substrates after pretreatments was attributed to the fact that ordered cellulose is more hydrophobic than amorphous xylans. The results from XRD indicated that pretreatment degraded amorphous xylans and cellulose, increasing the CrI of samples from 39.1 to 57.8. However, the residue after LHW pretreatment at 150 °C had lower hydrophobicity as than holocellulose. Thus, under this condition LHW pretreatment removed acetyl groups rather than the xylans. Kumar and Wyman (2009) suggested that acetyl groups may restrict cellulose accessibility by changing its hydrophobicity. The hydrophobicities of substrates DAP-170 and DAP-190 increased to 65.5 and 113.4 L/g as the pseudo-lignin contents increased to 34.4% and 93.6%, respectively. These results were ascribed to the fact that pseudo-lignin had more hydrophobic function groups than carbohydrates, which was consistent with the decreased O contents and increased intensity of signal for C-C bonds in XPS spectra.

**Table 2.** Crystalline Indexes (CrI) and Surface Hydrophobicities of Holocellulose and Residues from Pretreatments

	Holocellulose	LHW-150	LHW-170	LHW-190	Acid-130	Acid-150	Acid-170	Acid-190
CrI	39.1	46.0	47.4	51.6	48.6	53.2	57.8	23.4
Hydrophobicity (L/g)	26.5	18.7	27.1	30.1	28.6	32.3	65.5	113.4

### Enzymatic Hydrolysis and Fermentation

LHW pretreatments and DAPs introduced pseudo-lignin condensation, and changes of accessibility and hydrophobicity, which affected the combination between cellulase and substrate. Enzymatic hydrolysis conversions of samples after pretreatments are shown in Fig. 4a. The initial and final enzymatic conversion of holocellulose were observed as 10.4% and 53.2%, respectively. LHW pretreatments achieved an enhancement of cellulose conversion, increasing initial and final conversions to 13.9% and 82.4% (LHW-170), respectively. As pretreatment temperature increased to 190 °C, initial and

final glucan conversion decreased to 9.9% and 70.4%, respectively. The decrease might be ascribed the higher CrI of samples LHW-190 than other residues from LHW pretreatments (Table 2). DAP at 130 °C further improved final glucan conversion to 85.3% with initial conversion of 12.8%. However, further increases in temperature of DAPs to 150 to 190 °C caused an increase of CrI and decreases in both initial and final glucan conversions of residues. After pretreatments, the enhancement of initial glucan conversion was due to the degradation of xylans. Xylans can act as a barrier to enzymatic hydrolysis (Wiman *et al.* 2012). Removal of physical barriers enhances the availability of substrates and improves initial carbohydrate conversion (Pihlajaniemi *et al.* 2016).

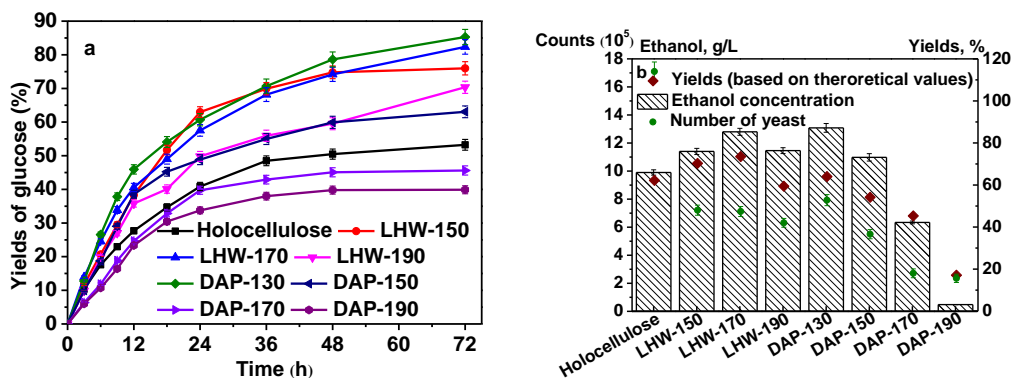
The final glucan conversion was negatively correlated with contents of pseudo-lignin in the residues. DAPs generated a larger amount of pseudo-lignin than LHW pretreatments at the same temperature. In this study, DAPs at 150, 170, and 190 °C formed 5.4%, 34.3%, and 93.6%, respectively, resulting in declines of glucose yields *via* enzymatic hydrolysis to 63.0%, 45.6%, and 39.9%, respectively. The initial glucose conversion of these samples also decreased to 9.9%, 6.4%, and 6.0%, respectively. These decreases reflect that pseudo-lignin forms physical barriers to prevent enzyme access to substrates, which decreases glucose yields. In addition, the pseudo-lignin contains hydrophobic groups that irreversibly bind to enzymes through hydrophobic interaction and inhibit enzyme activity (Hu *et al.* 2012).

The negative effect of pseudo-lignin on initial and final glucose conversion has also been confirmed by previous studies (Hu *et al.* 2012, 2013; Kumar *et al.* 2013; He *et al.* 2018). Xylans derived pseudo-lignin significantly retards cellulose hydrolysis, resulting in a 25% drop in cellulose conversion even with a negligible amount of pseudo-lignin after dilute acid pretreatment of xylan-Avicel mixture. The pseudo-lignin in the mixture also decreases the initial and final glucose conversion (Kumar *et al.* 2013). Pseudo-lignin from acid pretreatment of poplar holocellulose also inhibits the enzymatic hydrolysis of poplar holocellulose. When the pseudo-lignin increases from 10% to 40%, cellulose conversion decreases from 74% to 46% (Hu *et al.* 2012).

Holocellulosic residues with different amounts of pseudo-lignin were also submitted to SSF to production bioethanol; the yields of bioethanol are shown in Fig. 4b. LHW pretreatments and low temperature DAPs enhanced bioethanol production. The bioethanol yields were consistent with glucose conversion of enzymatic hydrolysis. After LHW pretreatments and DAPs, residues (LHW-170 and DAP-130) had higher ethanol yield as compared to other residues (12.8 g/L and 13.1g/L, respectively), which was consistent with the results of enzymatic hydrolysis. However, samples LHW-150, LHW-190, and DAP-150 had different enzymatic conversions, but a similar bioethanol production, as these residues contained similar amounts of cellulose. The bioethanol yields decreased to 6.3 and 0.5 g/L *via* fermentation of DAP-170 and DAP-190, respectively. The drop of bioethanol yield was because DAPs at 170 and 190 °C decreased the residual cellulose contents in substrates. In addition, large amounts of pseudo-lignin in these samples retarded bioethanol production.

Pseudo-lignin negatively affects the growth and metabolism of yeast. The number of yeast cells in the fermentation broth were counted. After fermentation for 24 h, there were  $9.5 \times 10^5$  yeast cells in the holocellulose fermentation broth. The fermentation broths of residues obtained from LHW pretreatments DAP-130 and DAP-150 contained a similar number of yeast cells ( $4.0 \times 10^5$ ). However, as the pseudo-lignin contents increased in substrates, the number of yeast cells decreased to  $1.5 \times 10^5$  and  $1.3 \times 10^5$  in the fermentation broths of DAP-170 and DAP-190, respectively. The inhibition mechanism of pseudo-lignin on yeast growth and metabolism will be considered in a future study.





**Fig. 4.** Enzymatic hydrolysis (a) and SSF (b) of holocellulose and residues obtained from pretreatments.

## CONCLUSIONS

1. Liquid hot water (LHW) pretreatments and dilute acid pretreatments (DAPs) degraded holocellulose and generated pseudo-lignin, accounting 2.1% to 93.6% of the residues.
2. Increasing pretreatment temperature caused degradation of a large amount of xylans and certain amount of cellulose, increasing accessibilities of residues.
3. Dilute acid pretreatment at 130 °C introduced the maximum glucose and bioethanol yields of substrate *via* enzymatic hydrolysis and SSF as 85.3% and 13.1 g/L, respectively.
4. Accumulation of pseudo-lignin after pretreatment with dilute acid at 170 °C led to an increase of hydrophobicities of residues, impeding bioconversion of residue and reducing glucose and ethanol yields to 45.6% and 6.3 g/L, respectively.

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