energy&fuels

Article

Cite This: *Energy Fuels* 2018, 32, 7636–7642

pubs.acs.org/EF

SPORL Pretreatment Spent Liquors Enhance the Enzymatic Hydrolysis of Cellulose and Ethanol Production from Glucose

Haifeng Zhou,*[,†,‡] Roland Gleisner,[‡] J.Y. Zhu,[‡] Yuanyu Tian,[†,§] and Yingyun Qiao[§]

[†]Key Laboratory of Low Carbon Energy and Chemical Engineering, College of Chemical and Environmental Engineering, Shandong University of Science and Technology, Qingdao 277590, China

[‡]USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin 53726, United States

[§]State Key Laboratory of Heavy Oil Processing, China University of Petroleum (East China), Qingdao 266580, China

Supporting Information

ABSTRACT: This study investigated the recycle utilization of SPORL pretreatment spent liquor. Three lignosulfonates (LSs) were purified from the spent liquor of SPORL pretreated Beetle-killed lodgepole pine (BKLP), Poplar NE222 (NE222), and Douglas-fir (FS10). The structural characterization showed that the apparent molecular mass and sulfur content of NE222-LS were lowest, but the phenolic group content was highest. FS10-LS, from a pH profiling SPORL pretreatment, had the highest apparent molecular mass but medium sulfur and phenolic group content. The spectral analyses exhibited that the guaiacyl unit was the main structure in BKLP and FS10 LSs, while NE222-LS mainly contained both guaiacyl and syringyl units. Both LSs and SPORL pretreatment spent liquors were used as additives to enzymatic hydrolysis of Whatman paper and ethanol production from glucose. LSs and liquors, from SPORL pretreated BKLP and NE222, could obviously enhance the enzymatic saccharification. Nevertheless, LS and liquor from SPORL pretreated FS10 presented a slight negative effect on enzymatic saccharification. All LSs and liquors with low concentration exhibited no inhibition on ethanol fermentation from glucose. When whole spent liquors without any detoxification were applied to prepare the fermentation medium with an initial glucose concentration of 100 g/L, the ethanol yield was almost the same as the control for BKLP and FS10 liquors. Nevertheless, the whole NE222 liquor without detoxification inhibited ethanol production thoroughly.

INTRODUCTION

Lignocellulose mainly consists of cellulose, hemicellulose, and lignin. The existence of lignin often inhibits enzymatic hydrolysis of cellulose because of the structural obstruction and non-productive binding of cellulase.[1−4] A strong correlation was confirmed between cellulose hydrolysis by cellulase and lignin extraction with ionic liquid pretreatment.[5] However, lignin originating from different biomass sources and methods (pulping or biorefinery pretreatment) have exhibited disparate influence on enzymatic hydrolysis of lignocellulose. The solvent extractable lignin from organosolv pretreated sweetgum showed positive influence, while the residual bulk lignin showed negative influence, on enzymatic hydrolysis of Avicel.[6] Ethanol organosolv lignin from hardwood could improve the enzymatic hydrolysis of Avicel considerably, which was suggested to be controlled by hydrophobicity and negative zeta potentials of lignin.[7−9] In addition, the modification of lignin, such as carboxylation,[10] alkylation,[11] sulfonation,[12,13] and non-covalent modification,[14] could enhance the enzymatic hydrolysis.

Our previous study showed that the effect of lignosulfonates (LSs), derived from pulping spent liquor, on enzymatic hydrolysis of cellulose was different for their complicated structure.[12,15] The large weight-average molecular mass (Mw) fraction with the lowest degree of sulfonation inhibited enzymatic hydrolysis of cellulose. However, a stimulation effect was observed when using the intermediate and smallest Mw fractions. SPORL pretreatment, a sulfite pretreatment to overcome recalcitrance of lignocellulose, also produces LS. The spent liquor from SPORL pretreated lodgepole pine, including LS, exhibited inhibition on enzymatic hydrolysis of cellulose at low concentration.[16] During lignocellulose pretreatment, the degradation of lignin generates cinnamic acid, vanillin, and other kinds of phenols. These soluble lignin degradation compounds in hydrolysates have been recently reported to inhibit the enzymatic hydrolysis and fermentation.[17−21] In addition, lignin coming from different biomass sources possesses a different effect on enzymatic hydrolysis.[4,7,22] Hardwood organosolv lignin improved enzymatic hydrolysis of organosolv pretreated sweetgum and loblolly pine, while softwood organosolv lignin inhibits it.[8] Compared with the poplar enzymatic lignin, the lodgepole pine lignin showed higher inhibition on enzymatic hydrolysis of Avicel; nevertheless, the corn stover enzymatic lignin showed negligible effect.[22] Therefore, we can hypothesize that the LSs, produced in different SPORL pretreatments, from different wood species, might possess a different effect on enzymatic hydrolysis of cellulose. Furthermore, we ask if it is possible that the SPORL pretreatment spent liquor can be directly used to enhance the enzymatic hydrolysis of pure cellulose. If so, it is not necessary to purify the LS from SPORL spent liquor.

However, the LSs or spent liquor applied in enzymatic hydrolysis remained in the enzymatic hydrolysate. In order to optimize the ethanol production from lignocellulose, not only

Received: March 14, 2018
Revised: June 18, 2018
Published: June 19, 2018

Downloaded via US DEPT AGRCLT NATL AGRCLTL LBRY on August 24, 2018 at 15:43:52 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

ACS Publications © 2018 American Chemical Society

DOI: 10.1021/acs.energyfuels.8b00864
Energy Fuels 2018, 32, 7636–7642

the effect of additives on enzymatic hydrolysis but also the effect on the subsequent fermentation should be determined. Generally, pretreatment processes produce inhibitors from the degradation of cellulose, hemicellulose, and lignin, many of which severely inhibit the ethanol yield and microbial growth.[21,23,24] The influence of residual surfactants on the subsequent fermentation process has been investigated.[25,26] Lee and co-workers studied the effect of non-ionic surfactant (Tween 20, Tween 80, and Triton X-100) on ethanol production of glucose, cellulose, and lignocellulose enzymatic hydrolysate.[26] The results showed that Tween 20 and Tween 80 could slightly enhance ethanol fermentation; however, Triton X-100, the best additive for enzymatic hydrolysis, showed a negative influence on ethanol fermentation. The addition of Tween 80, with an initial glucose concentration of 55 g/L, did not improve the ethanol yield.[25] Similarly, Tween 80 could enhance only the enzymatic hydrolysis but could not improve ethanol production during separate hydrolysis and fermentation (SHF).[27] When using simultaneous saccharification and fermentation (SSF) produced ethanol,[25,27−29] the application of non-ionic surfactants could enhance ethanol yield, in other words, which could achieve the same ethanol yield with reduced enzyme loading. However, the influence of LS or SPORL spent liquor on ethanol fermentation from glucose is still lacking.

SPORL pretreatment is an efficient pretreatment method for removing the recalcitrance of woody biomass, including softwood species. In our previous research we investigated the pilot-scale demonstration for lodgepole pine (50 kg) and Poplar (40 kg).[30,31] At a total solids loading of 20%, a terminal ethanol titer of 52.2 and 43.6 g/L with a yield of 288 and 247 L/tonne wood was achieved without any detoxification, respectively. In addition, a pH profiling method in SPORL was studied for Douglas-fir forest residues, which could reduce the production of inhibitors.[32] A terminal ethanol titer of 48.9 g/L at an ethanol yield of 297 L/tonne wood was achieved. Although this biorefinery method could be finished without washing and detoxification process, the recycle utilization of final spent liquor has significant implications. According to the results that lignin with different molecular structures showed diverse effects on enzymatic saccharification, the effect of different kinds of lignin on the fermentation process might also be different. SPORL spent liquor, containing different kinds of inhibitors, probably inhibits the ethanol fermentation process. Nevertheless, the question remains whether SPORL spent liquor could be applied safely for ethanol fermentation in moderate amounts or not. If SPORL spent liquor could be used directly for improving enzymatic hydrolysis and fermentation without any detoxification, the cost of SPORL pretreatment and its co-product application will be lower.

In this study, both SPORL pretreated hardwood and softwood spent liquor were chosen. In addition, the spent liquor from a specific SPORL pretreatment, a pH profiling method, was also investigated. LSs in SPORL pretreatment spent liquor were purified and characterized using functional group content measurements, GPC, UV, and IR. Moreover, the influence of the purified LSs and spent liquors on enzymatic saccharification of cellulose and ethanol fermentation from glucose was also studied.

MATERIALS AND METHODS

Materials. Beetle-killed lodgepole pine (BKLP), Poplar NE222 (NE222), and Douglas-fir (FS10) wood chips were provided by the USDA Forest Products Laboratory, Madison, WI, USA. Detailed information on these tree wood chips has been described previously.[30−32]

Commercial high purity sodium LS (D748) from sulfite pulping of softwood (typical sulfite loading 20−25% on wood, much higher than that used in SPORL of approximately 10% for softwoods and 3% for hardwoods) provided by LignoTech USA (Rothschild, WI) was used as received.

Commercial cellulase Cellic CTec3 (abbreviated CTec3) was provided by Novozymes North America (Franklinton, NC, USA). The filter paper activity of CTec3 was 217 FPU/mL as calibrated according to Wood and Bhat.[33] Whatman filter paper (grade 1, catalogue number 1001-150, Whatman International, U.K.) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals such as Folin Ciocalteau's phenol reagent (2 N), vanillin, glucose, sodium acetate, acetic acid, sulfuric acid, and sodium bisulfite were all ACS reagent grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

Saccharomyces cerevisiae YRH400[34] was obtained from the USDA Agricultural Research Service, National Center for Agriculture Utilization Research, Peoria, IL, USA. The strain was first grown on YPD agar plates and then cultured in a liquid YPD medium in a flask overnight on a shaking bed incubator (Thermo Fisher Scientific, model 4450, Waltham, MA, USA) as described previously.[35] The concentration of the cultured biomass was monitored by measuring the optical density at 600 nm ($OD_{600\ nm}$) using a UV−Vis spectrometer (model 8453, Agilent Technologies, Palo Alto, CA, USA).

SPORL Pretreatment. BKLP wood chips (40 kg in oven dry (OD) weight) were pretreated in a 390 L rotating wood pulping digester at 165 °C for 60 min. The sodium bisulfite and sulfuric acid loading on BKLP wood was 8 and 2.2 wt %, respectively. NE222 wood chips (40 kg OD weight) were pretreated in a 390 L rotating wood pulping digester at 160 °C for 40 min. The sodium bisulfite and sulfuric acid loading on NE222 wood was 3 and 1.1 wt %, respectively. FS10 wood chips (2 kg OD weight) were pretreated in a 23 L rotating reactor at 165 °C for 75 min with the pH profiling through a delayed acid application during pretreatment. The sodium bisulfite and sulfuric acid loading on FS10 wood was 12 and 2.2 wt %, respectively. The sulfuric acid was applied at 35 min during FS10 pretreatment by injection. The details of these tree SPORL pretreatments have been described previously.[30−32] The pretreated materials were recovered by wet solids and freely drainable liquor. The solid contents of the three spent liquors were 11.18%, 8.36%, and 14.2% for BKLP, NE222, and FS10, respectively. The LS concentration in three spent liquors was 31.05, 25.04, and 56.33 g/L for BKLP, NE222, and FS10, respectively. The concentrations of LS in the liquors was determined through UV absorption measurements through calibration using the purified LS of itself.

LS Separation. Three LSs abbreviated as BKLP-LS, NE222-LS, and FS10-LS were obtained from the three spent liquors mentioned above through filtration using filter paper followed by ultrafiltration according to a previously described procedure.[30] All of the LSs used are sodium LSs, as $NaHSO_3$ was used in all SPORL pretreatments.

Phenolic Group and Sulfur Content Measurements. The phenolic group (Ph−OH) contents of the LSs were determined as described previously.[36] The sulfur contents of the LSs were analyzed using inductively coupled plasma (ICP) mass spectrometry as described previously.[30]

Gel Permeation Chromatography (GPC). The purified LSs were analyzed by aqueous GPC using a Ultrahydrogel 250 column. The GPC system consists of an Agilent 1100 HPLC instrument equipped with a UV detector, an Optilab T-rEX RI detector (Wyatt Technology Corp.), and a DAWN HELEOS II (Wyatt Technology Corp.) multi-angle light scattering (MALS) detector. Six poly(styrenesulfonate) sodium salts with molecular masses ranging from 1100 to 976000 Da were used for calibration. The equation of the calibration curve was as follows: log Mw = 11.8 − 0.589 V (R^2 = 0.9960). Sodium nitrate solution of 0.1 mol/L was used as the eluent at 0.5 mL/min.

DOI: 10.1021/acs.energyfuels.8b00864
Energy Fuels 2018, 32, 7636−7642

Spectroscopy Analyses. Absorption characteristics of LS were analyzed by a UV−vis spectrometer (model 8453, Agilent Technologies, Palo Alto, CA, USA) and a Bruker Tensor 27 FTIR spectrometer in a range of 4000−400 cm^{-1}.

Enzymatic Hydrolysis. Enzymatic hydrolysis of Whatman filter paper was conducted at 2% (w/v) in 50 mM acetate buffer (25 mL) 50 °C and pH 5.5 in an incubator on a shaker (Thermo Fisher Scientific, model 4450, Waltham, MA, USA). The elevated pH of 5.5 was used to reduce non-productive cellulase binding to lignin as suggested previously.[13,16,37] The CTec3 loading was 5 FPU/g glucan. Glucose in the hydrolysate was measured using a commercial biochemistry analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH, USA). The application dosages of spent liquor to the Whatman paper suspension was based on the final LS concentration in the mixture. From the LS concentrations of the three spent liquors (specified in the Materials), the calculated volume amounts of BKLP, NE222, and FS10 spent liquors were 121 and 403, 150 and 499, and 67 and 222 μL, respectively, to achieve LS concentrations of 1.5 and 5.0 g/L in the Whatman paper-spent liquor mixtures. When 5 g/L spent liquor was added by solid content, the volume of BKLP, NE222, and FS10 was 112, 150, and 88 μL, respectively. Reported results were the averages of replicate hydrolysis runs with measurement errors of less than 2%.

Fermentation. Fermentation experiments were carried out in a flask on a shaker (Thermo Fisher Scientific, model 4450, Waltham, MA, USA) using pure glucose in 50 mM acetate buffer solution (50 mL) at pH 5.5. The glucose concentration was 100 g/L, and the shaker was operated at 150 rpm and 35 °C. The amount of cultured YRH400 added was based on optical density $OD_{600\ nm}$ = 3.5.

The fermentation broths were analyzed for glucose, inhibitors, and ethanol using an HPLC system (Ultimate 3000, Thermo Scientific, Sunnyvale, CA, USA) equipped with RI (RI-101) and UV (VWD-3400RS) detectors and BioRad Aminex HPX-87P and HPX-87H columns as described previously.[38]

RESULTS AND DISCUSSION

Apparent Molecular Mass Distribution and Functional Group Contents of LSs. The apparent molecular mass and functional group contents of the three SPORL LSs and the commercial sulfite pulping LS D748 are shown in Figure 1 and Table 1. The commercial LS, D748, had the greatest apparent molecular mass of Mw = 43300 Da among the four LSs, and NE222-LS had the smallest apparent molecular mass of Mw = 2800 Da. FS10-LS had the lowest polydispersity of 1.77.

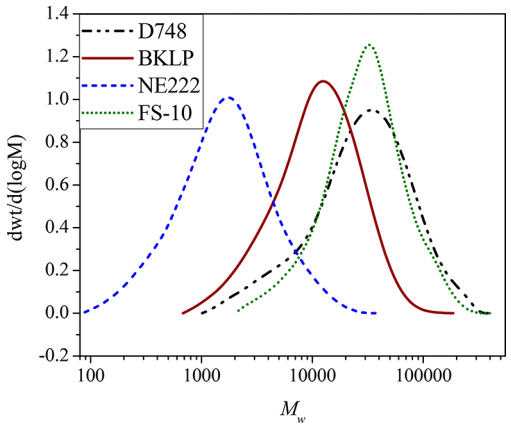

Figure 1. Apparent molecular mass distribution curve of LSs.

NE222-LS had the highest phenolic group content of 3.2 mg/g, with the lowest sulfur content of 42 mg/g (Table 1) due to the lowest sulfite loading (3 wt % $NaHSO_3$ on wood) among the SPORL runs. D748 had the highest sulfur content as expected due to the high sulfite loading in pulping softwoods (of approximately 20−25%). It should be noted that BKLP-LS had a sulfur content similar to D748 despite having a sodium sulfite loading (8 wt % $NaHSO_3$ on wood), perhaps due to the higher temperatures, lower than that of commercial wood pulping. The sulfur content of FS10-LS was lower than that of BKLP-LS despite a higher sulfite loading (12 wt % $NaHSO_3$ on wood) being applied in SPORL, most likely due to the higher pH in the first 35 min of the SPORL run using pH profiling.

Spectral Analyses of LS Chemical Structures. The structural characterizations of LSs from SPORL spent liquors were analyzed by their UV−vis and IR spectra (Figure 2). The major differences among the three LSs were due to the differences in the wood species.

The aromatic ring structure and chromophoric groups in LS resulted in strong absorption of UV light as shown by the peaks around 205 and 280 nm in Figure 2a.[39] The extinction coefficients and molar absorptivity (using Mw from GPC as the average molecular mass) of LSs were shown in Table 2. The extinction coefficient of BKLP-LS, NE222-LS and FS10-LS at around 205 nm was 87.6, 78.4, and 107.1 L/(g·cm), respectively. Generally speaking, the maximum absorption intensity of hardwood lignin is around 274−276 nm, while around 280−285 nm for softwoods.[40] This holds true for the LSs used in the present study. The maximum absorption intensity of NE222-LS was at 271 nm, while around 281 nm for BKLP and FS10, both are softwoods. The extinction coefficient of BKLP-LS, NE222-LS and FS10-LS at around 280 nm was 16.7, 15.9, and 17.1 L/(g·cm), respectively.

The IR absorption spectra show several chemical structural features of LSs according to peak assignments in the literature.[41−43] The strong broad band around 3427 cm^{-1} was due to the OH stretching in phenolic and aliphatic structures. The peaks at 2931 and 1461 cm^{-1} were contributed to the C—H stretching in methyl and methylene side chains. The peak at 1710 cm^{-1} originated from the unconjugated carboxyl, ketone stretching. The peaks at 1610, 1511, and 1423 cm^{-1} were associated with the aromatic skeletal vibrations. The spectral region from 1400 to 1000 cm^{-1} showed vibrations from different monolignol units with typical structural characteristics. For example, the breathing vibration of the syringyl (S) lignin unit at 1326 cm^{-1} for NE222-LS (but not softwood BKLP-LS and FS10-LS), as well the C—O stretch of G lignin around 1260 cm^{-1}. The peaks at 1165 and 1144 cm^{-1} and 1115 and 1091 cm^{-1} were from the C—H in-plate deformation in G and S lignin, respectively, as evidenced by (1) the strong intensity at 1115 cm^{-1} on the spectrum of NE222-LS but not on the spectra of BKLP-LS and FS10-LS anf (2) the spectra of BKLP-LS and FS10-LS both having a strong peak at 1144 cm^{-1}, but not the spectrum of NE222-LS, rather than a valley. These validated that NE222-LS contained G and S lignin units, typical of hardwood lignin, while BKLP and FS10 LSs primarily contained the G lignin unit. Representative structural features of LS were the peak at 1041 cm^{-1} from the S=O stretching, C—H deformation, and C—OH bending, and the peak at 617 cm^{-1} from C—S stretching, as well as the peak at 1217 cm^{-1} attributed to C—C, C—O, and C=O vibration.

Effect of LSs and SPORL Spent Liquors on Enzymatic Hydrolysis of Whatman Paper. SPORL spent liquor contains primarily LS in addition to residual sulfite, some

DOI: 10.1021/acs.energyfuels.8b00864
Energy Fuels 2018, 32, 7636−7642

Table 1. Functional Group Content and Apparent Molecular Mass Distribution of D748 and LSs Purified from Different SPORL Spent Liquors

LSs	production conditions	Ph−OH (mg/g)	sulfur (mg/g)	Mn (Da)	Mw (Da)	polydispersity
D748	commercial softwood sulfite pulping	1.628 ± 0.010	61.66 ± 1.27	19100	43300	2.27
BKLP	pilot-scale 40 kg wood chips; 60 min at 165 °C; 8 wt % $NaHSO_3$	1.707 ± 0.025	61.28 ± 1.78	6000	12300	2.06
NE222	pilot-scale 40 kg wood chips; 40 min at 160 °C; 3 wt % $NaHSO_3$	3.232 ± 0.006	42.46 ± 1.35	1000	2800	2.74
FS10	lab-scale 2 kg wood chips; 75 min at 165 °C; 12 wt % $NaHSO_3$; pH profiling	2.051 ± 0.014	53.16 ± 3.24	22400	39700	1.77

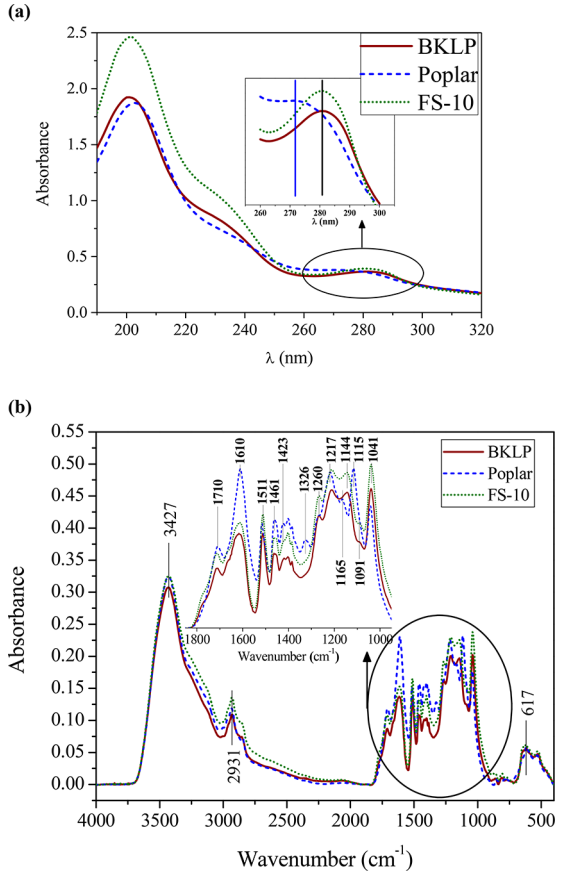

Figure 2. UV-vis (a) and FTIR (b) spectral characteristics of the three LSs separated from SPORL spent liquors.

Table 2. Extinction Coefficients and Molar Absorptivity of LSs from Different SPORL Spent Liquor Measured in Distilled Water

LSs	λ_{max} (nm)	a (L g^{-1} cm^{-1})	ε (10^{-4} L mol^{-1} cm^{-1})[a]
BKLP	281	16.7	20.5
	201	87.6	107.7
NE222	271	15.9	4.5
	203	78.4	21.9
FS10	281	17.1	67.8
	201	107.1	425.1

[a] ε was calculated using the Mw derived from GPC analyses.

dissolved sugars, such as xylose, mannose, and glucose, and sugar degradation products, such as furans (Table S1). It will be interesting to compare the effects of SPORL spent liquors with the LSs separated from these spent liquors on enzymatic hydrolysis of Whatman paper. The liquors were prepared to have an LS (Mw > 1000 Da) concentration of 1.5 g/L. The actual LS concentrations were probably greater than 1.5 g/L as the spent liquors also contained some molecules with low molecular mass (<1000 Da). As shown in Figure 3a, the

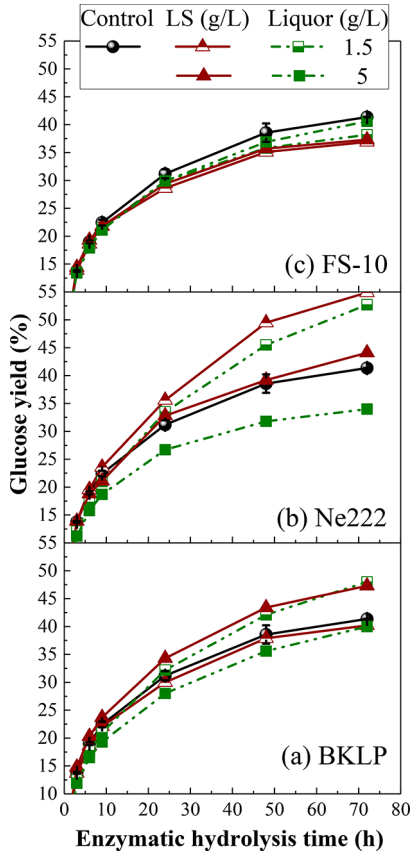

Figure 3. Effects of varying levels of different SPORL spent liquors and LSs separated from these liquors on enzymatic hydrolysis of Whatman paper: (a) BKLP, (b) NE222, and (c) FS10.

application of either the separated LS or the spent liquor from SPORL BKLP could have positive effects on glucose yields. . It appears that a high dosage of BKLP-LS at 5 g/L and a low dosage of BKLP spent liquor at 1.5 g/L improved glucose yields by approximately 15% at the end of 72 h of hydrolysis. Positive effects on glucose yields were more pronounced when using LS and liquor from NE222 than when using BKLP, as shown in Figure 3b. With the supplementary 1.5 g/L of NE222 liquor, the glucose yields increased by around 33%. Again, the high dosage of 5.0 g/L of spent liquor (NE222) reduced glucose yields; furthermore, the increment in glucose yields by NE222-LS at 5 g/L was lower than that at the lower dosage of 1.5 g/L. Very different effects on glucose yields were observed with the application of LSs from FS10 spent liquor. Application of separated LS or spent liquor from SPORL FS10 at both the lower 1.5 g/L and the higher 5.0 g/L dosages slightly reduced glucose yields (Figure 3c). When the

DOI: 10.1021/acs.energyfuels.8b00864
Energy Fuels 2018, 32, 7636−7642

application dosage of liquor was based on liquor solids, similar results as discussed above were observed (Figure S2).

Our previous studies indicated that LS molecular mass affects LS performance in enhancing cellulose enzymatic saccharification.[12,15] Lower molecular mass LSs performed better than higher molecular mass LSs. The LSs from NE222, despite their lower degree of sulfonation, performed better than those LSs from BKLP and FS10, which is mostly due to their molecular mass being lower than those from BKLP and FS10 (Table 1). However, NE222 with a higher concentration reduced glucose yields, which might be attributed to the higher content of phenolic groups. The syrinyl/guaiacyl phenolic OH groups boost the hydrogen bonding between lignin and cellulase, which can intensify undesirable non-productive binding.[44] As reviewed in our previous study, the effects of LS on cellulose enzymatic saccharification are complex.[15] The degree of LS sulfonation also plays a role, as does the hydrolysis pH. LSs from FS10 had an apparent molecular mass and lower degree of sulfonation similar to those of the commercial LS D748 and had similar effects in inhibition of glucose yields of Whatman paper (Figure S1). In this study, LS or spent liquor, originating from softwood or hardwood, could enhance the enzymatic hydrolysis of cellulose, except for LS or spent liquor from FS-10.

Effect of LSs and Liquors on Ethanol Fermentation. The above results indicate that NE222-LS and NE222 liquor at 1.5 g/L loading enhanced enzymatic saccharification of Whatman paper; thereby, the effects of NE222-LS and NE222 spent liquor at the same loading of 1.5 g/L on ethanol fermentation was investigated. The terminal ethanol concentration in the fermentation broth using a glucose solution of 100 g/L with the applications of different SPORL LS and spent liquor is shown in Figure 4. No inhibiting effects were

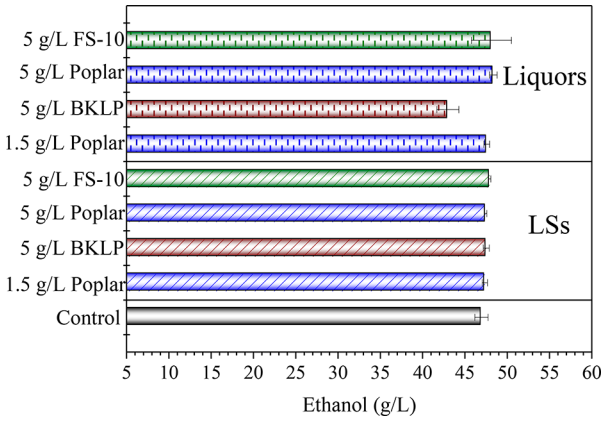

Figure 4. Influence of varying levels of different kinds of LSs and SPORL spent liquors on the ethanol yield of 100 g/L initial glucose.

observed with the addition of LSs or spent liquors on ethanol production except for the application of BKLP spent liquor at 5 g/L with a terminal ethanol concentration of 43.0 compared with 47.5 g/L for the control. It indicated that some kinds of low molecular mass molecules inhibited the ethanol fermentation. It is found that aldehydes and acids are key inhibitors in biomass pretreatment spent liquor.[24,45] However, the specific molecule in BKLP spent liquor which inhibits the ethanol fermentation from glucose needs to be further identified. Time-dependent glucose consumption and ethanol production were observed in Figures S3 and S4. LSs could slightly increase the glucose consumption rate, and the ethanol production rate also increased (Figure S3). For SPORL pretreatment spent liquor, only NE222-liquor exhibited this influence. Hence, there would not be inhibition influence of low concentration of LSs and liquors on ethanol fermentation from glucose except for 5 g/L BKLP liquor. Additionally, they might improve the fermentation of lignocellulose. Hence, generally speaking, both the purified LS and SPORL spent liquor could be used to improve the enzymatic saccharification and fermentation of lignocellulose.

For the purpose of evaluating the effect of SPORL spent liquor on ethanol production, we fermented the undiluted original SPORL spent liquor with the application of glucose (in solid) with concentration of 100 g/L in the spent liquor. The results of these experiments are shown in Figure 5. For the

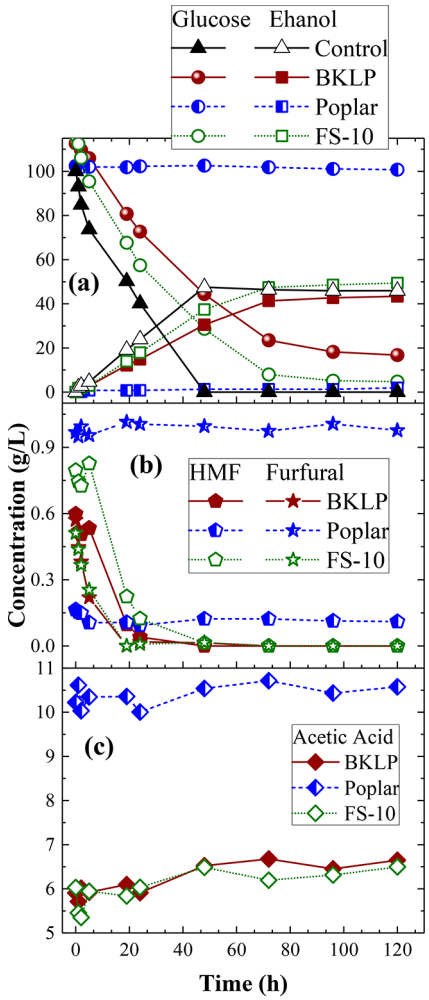

Figure 5. Comparisons of time-dependent (a) glucose and ethanol, (b) furans, and (c) acetic acid concentrations in the fermentation broth using the whole SPORL spent liquors at an initial glucose concentration of 100 g/L.

control test, a maximum ethanol concentration of 47.5 g/L was obtained after 48 h of fermentation using a pure glucose solution (concentration 100.0 g/L). For BKLP spent liquor, the initial glucose concentration was 112.7 g/L after glucose supplementation (Table S1). A terminal ethanol concentration of 43.4 g/L was obtained after 120 h of fermentation, with a conversion yield of 75.4%, which was lower than that of the

DOI: 10.1021/acs.energyfuels.8b00864
Energy Fuels 2018, 32, 7636−7642

control test (92.9%) and the 5 g/L BKLP spent liquor run (84.3%). For FS10 spent liquor, the initial glucose concentration was 116.3 g/L after glucose supplementation (Table S1). The terminal ethanol concentration of 49.4 g/L was obtained after 120 h of fermentation and resulted in a yield of 83.1%, which was higher than that of BKLP spent liquor but still lower than that of the control test. It indicated that the high ethanol concentration during FS10 spent liquor fermentation was due to the higher initial glucose concentration. Glucose prepared with whole NE222 spent liquor was unfermentable, which might be due to the high acetic acid concentration, high furfural concentration, high phenolic group contents, and the synergistic effects of these toxic products, especially. The maximum ethanol concentration was achieved at 120 h during spent liquor fermentation, while it only needed 48 h in the control test. Therefore, when the whole spent liquor was used to prepare fermentation medium, the effect of toxic products on the ethanol fermentation from glucose was still non-negligible just as the references reported. However, a low concentration of spent liquor could be used to improve the enzymatic saccharification and fermentation of lignocellulose.

CONCLUSIONS

LSs were purified from different SPORL pretreatment spent liquors, and the structural characterization of which was studied. The spectra analyses showed that BKLP-LS and FS10-LS had typical softwood characterizations. The guaiacyl unit was the main structure. NE222-LS mainly contained guaiacyl and syringyl units. The apparent molecular mass and functional group content of these three LSs were different from each other. The apparent molecular mass of NE222-LS was lowest, as was the sulfur content of NE222-LS. The sulfur content of BKLP-LS was highest, while the apparent molecular mass of BKLP-LS was medium. Three LSs and SPORL spent liquors were applied to Whatman paper. Both LSs and liquors from BKLP and NE222 could enhance the saccharification of Whatman paper. LS and liquor of FS10 slightly inhibited cellulose saccharification. In addition, the effect of LSs and liquors on ethanol fermentation from glucose were studied. A low concentration of LSs and liquors did not show an inhibition influence on ethanol fermentation from glucose. This indicated that purified LSs or SPORL spent liquors could enhance the enzymatic hydrolysis and fermentation of lignocellulose. Especially when the apparent molecular mass was moderate and the sulfur content was high, LS or spent liquor could enhance the enzymatic hydrolysis and fermentation of cellulose.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.energyfuels.8b00864.

Content of SPORL pretreatment spent liquors, influence of varying levels of different LSs including commercial LS D748 and SPORL spent liquors on enzymatic hydrolysis of Whatman paper, influence of 5 g/L LSs and SPORL spent liquor (solid content) on enzymatic hydrolysis of Whatman paper, influence of varying levels of different kinds of LSs (a) and SPORL spent liquors (b) on glucose consumption, and influence of varying levels of different kinds of LSs (a) and SPORL spent liquors (b) on the ethanol yield of 100 g/L initial glucose (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: zhouhaifeng-qd@163.com. Tel.: +86-532-86057766.

ORCID

Haifeng Zhou: 0000-0003-2126-3988
J.Y. Zhu: 0000-0002-5136-0845
Yuanyu Tian: 0000-0003-3326-7484

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the National Natural Science Foundation of China (21506117), the Key Research and Development Plan of Shandong (2018GGX104015), the Applied Basic Research Programs of Qingdao (17-1-1-23-jch), and the SDUST Research Fund (2018YQJH102, 2015RCJJ008).

REFERENCES

(1) Mooney, C. A.; Mansfield, S. D.; Touhy, M. G.; Saddler, J. N. The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. *Bioresour. Technol.* **1998**, *64* (2), 113−119.

(2) Kumar, L.; Arantes, V.; Chandra, R.; Saddler, J. The lignin present in steam pretreated softwood binds enzymes and limits cellulose accessibility. *Bioresour. Technol.* **2012**, *103* (1), 201−8.

(3) Mansfield, S. D.; Mooney, C.; Saddler, J. N. Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnol. Prog.* **1999**, *15* (5), 804−816.

(4) Berlin, A.; Balakshin, M.; Gilkes, N.; Kadla, J.; Maximenko, V.; Kubo, S.; Saddler, J. Inhibition of cellulase, xylanase and β-glucosidase activities by softwood lignin preparations. *J. Biotechnol.* **2006**, *125* (2), 198−209.

(5) Lee, S. H.; Doherty, T. V.; Linhardt, R. J.; Dordick, J. S. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. *Biotechnol. Bioeng.* **2009**, *102* (5), 1368.

(6) Lai, C.; Tu, M.; Yong, Q.; Yu, S. Disparate roles of solvent extractable lignin and residual bulk lignin in enzymatic hydrolysis of pretreated sweetgum. *RSC Adv.* **2015**, *5* (119), 97966−97974.

(7) Lai, C. H.; Tu, M. B.; Shi, Z. Q.; Zheng, K.; Olmos, L. G.; Yu, S. Y. Contrasting effects of hardwood and softwood organosolv lignins on enzymatic hydrolysis of lignocellulose. *Bioresour. Technol.* **2014**, *163* (7), 320−327.

(8) Lai, C.; Tu, M.; Li, M.; Yu, S. Remarkable solvent and extractable lignin effects on enzymatic digestibility of organosolv pretreated hardwood. *Bioresour. Technol.* **2014**, *156* (4), 92−99.

(9) Huang, Y.; Sun, S.; Huang, C.; Yong, Q.; Elder, T.; Tu, M. Stimulation and inhibition of enzymatic hydrolysis by organosolv lignins as determined by zeta potential and hydrophobicity. *Biotechnol. Biofuels* **2017**, *10* (1), 162.

(10) Nakagame, S.; Chandra, R. P.; Kadla, J. F.; Saddler, J. N. Enhancing the enzymatic hydrolysis of lignocellulosic biomass by increasing the carboxylic acid content of the associated lignin. *Biotechnol. Bioeng.* **2011**, *108* (3), 538.

(11) Lai, C.; Tu, M.; Xia, C.; Shi, Z.; Sun, S. L.; Yong, Q.; Yu, S. Lignin Alkylation Enhances Enzymatic Hydrolysis of Lignocellulosic Biomass. *Energy Fuels* **2017**, *31*, 12317.

(12) Zhou, H.; Lou, H.; Yang, D.; Zhu, J.; Qiu, X. Lignosulfonate to enhance enzymatic saccharification of lignocelluloses: role of molecular weight and substrate lignin. *Ind. Eng. Chem. Res.* **2013**, *52* (25), 8464−8470.

DOI: 10.1021/acs.energyfuels.8b00864
Energy Fuels 2018, 32, 7636−7642

(13) Lou, H.; Zhu, J. Y.; Lan, T. Q.; Lai, H.; Qiu, X. pH-induced lignin surface modification to reduce nonspecific cellulase binding and enhance enzymatic saccharification of lignocelluloses. *ChemSusChem* **2013**, *6* (5), 919−927.
(14) Lou, H.; Wang, M.; Lai, H.; Lin, X.; Zhou, M.; Yang, D.; Qiu, X. Reducing non-productive adsorption of cellulase and enhancing enzymatic hydrolysis of lignocelluloses by noncovalent modification of lignin with lignosulfonate. *Bioresour. Technol.* **2013**, *146* (10), 478−484.
(15) Lou, H.; Zhou, H.; Li, X.; Wang, M.; Zhu, J. Y.; Qiu, X. Understanding the effects of lignosulfonate on enzymatic saccharification of pure cellulose. *Cellulose* **2014**, *21* (3), 1351−1359.
(16) Wang, Z. J.; Lan, T. Q.; Zhu, J. Y. Lignosulfonate and elevated pH can enhance enzymatic saccharification of lignocelluloses. *Biotechnol. Biofuels* **2013**, *6*, 9.
(17) Tejirian, A.; Xu, F. Inhibition of enzymatic cellulolysis by phenolic compounds. *Enzyme Microb. Technol.* **2011**, *48* (3), 239−247.
(18) Michelin, M.; Ximenes, E.; Ladisch, M. R.; de Lourdes Teixeira de Moraes Polizeli, M. Effect of phenolic compounds from pretreated sugarcane bagasse on cellulolytic and hemicellulolytic activities. *Bioresour. Technol.* **2016**, *199*, 275−278.
(19) Kim, Y.; Ximenes, E.; Mosier, N. S.; Ladisch, M. R. Soluble inhibitors/deactivators of cellulase enzymes from lignocellulosic biomass. *Enzyme Microb. Technol.* **2011**, *48* (4−5), 408−415.
(20) Ximenes, E.; Kim, Y.; Mosier, N.; Dien, B.; Ladisch, M. Deactivation of cellulases by phenols. *Enzyme Microb. Technol.* **2011**, *48* (1), 54−60.
(21) Jönsson, L. J.; Alriksson, B.; Nilvebrant, N. O. Bioconversion of lignocellulose: inhibitors and detoxification. *Biotechnol. Biofuels* **2013**, *6* (1), 16.
(22) Nakagame, S.; Chandra, R. P.; Saddler, J. N. The effect of isolated lignins, obtained from a range of pretreated lignocellulosic substrates, on enzymatic hydrolysis. *Biotechnol. Bioeng.* **2010**, *105* (5), 871.
(23) Carter, B.; Squillace, P.; Gilcrease, P. C.; Menkhaus, T. J. Detoxification of a lignocellulosic biomass slurry by soluble polyelectrolyte adsorption for improved fermentation efficiency. *Biotechnol. Bioeng.* **2011**, *108* (9), 2053−2060.
(24) Cao, D.; Tu, M.; Xie, R.; Li, J.; Wu, Y.; Adhikari, S. Inhibitory Activity of Carbonyl Compounds on Alcoholic Fermentation by Saccharomyces cerevisiae. *J. Agric. Food Chem.* **2014**, *62* (4), 918−926.
(25) Ballesteros, I.; Oliva, J.; Carrasco, J.; Cabanas, A.; Navarro, A.; Ballesteros, M. Effect of surfactants and zeolites on simultaneous saccharification and fermentation of steam-exploded poplar biomass to ethanol. *Appl. Biochem. Biotechnol.* **1998**, *70−72*, 369−381.
(26) Lee, W.; Lee, J.; Lee, J.; Shin, C.; Kim, M.; Park, S. Effect of surfactants on ethanol fermentation using glucose and cellulosic hydrolyzates. *Biotechnol. Lett.* **1996**, *18* (3), 299−304.
(27) Tu, M.; Zhang, X.; Paice, M.; McFarlane, P.; Saddler, J. N. Effect of surfactants on separate hydrolysis fermentation and simultaneous saccharification fermentation of pretreated lodgepole pine. *Biotechnol. Prog.* **2009**, *25* (4), 1122−1129.
(28) Alkasrawi, M.; Eriksson, T.; Börjesson, J.; Wingren, A.; Galbe, M.; Tjerneld, F.; Zacchi, G. The effect of Tween-20 on simultaneous saccharification and fermentation of softwood to ethanol. *Enzyme Microb. Technol.* **2003**, *33* (1), 71−78.
(29) Jin, M.; Lau, M. W.; Balan, V.; Dale, B. E. Two-step SSCF to convert AFEX-treated switchgrass to ethanol using commercial enzymes and Saccharomyces cerevisiae 424A (LNH-ST). *Bioresour. Technol.* **2010**, *101* (21), 8171−8178.
(30) Zhou, H.; Zhu, J.; Gleisner, R.; Qiu, X.; Horn, E.; Negron, J. Pilot-scale demonstration of SPORL for bioconversion of lodgepole pine to bioethanol and lignosulfonate. *Holzforschung* **2015**, *70* (1), 21−30.
(31) Zhou, H.; Zhu, J. Y.; Gleisner, R.; Qiu, X.; Horn, E.; Sukumaran, R. K. High Titer Ethanol and Lignosulfonate Production from SPORL Pretreated Poplar at Pilot Scale. *Front. Energy Res.* **2015**, *3*, 16.
(32) Cheng, J.; Leu, S.-Y.; Zhu, J.; Gleisner, R. High titer and yield ethanol production from undetoxified whole slurry of Douglas-fir forest residue using pH profiling in SPORL. *Biotechnol. Biofuels* **2015**, *8* (1), 22.
(33) Wood, T. M.; Bhat, K. M. Methods for measuring cellulase activities. *Methods Enzymol.* **1988**, *160*, 87−112.
(34) Hector, R. E.; Dien, B. S.; Cotta, M. A.; Qureshi, N. Engineering industrial Saccharomyces cerevisiae strains for xylose fermentation and comparison for switchgrass conversion. *J. Ind. Microbiol. Biotechnol.* **2011**, *38* (9), 1193−1202.
(35) Zhou, H.; Lan, T.; Dien, B. S.; Hector, R. E.; Zhu, J. Y. Comparisons of Five *Saccharomyces cerevisiae* strains for Ethanol Production from SPORL Pretreated Lodgepole Pine. *Biotechnol. Prog.* **2014**, *30*, 1076−1083.
(36) Zhou, H.; Qiu, X.; Yang, D.; Xie, S. Laccase and Xylanase Incubation Enhanced the Sulfomethylation Reactivity of Alkali Lignin. *ACS Sustainable Chem. Eng.* **2016**, *4* (3), 1248.
(37) Lan, T. Q.; Lou, H.; Zhu, J. Y. Enzymatic saccharification of lignocelluloses should be conducted at elevated pH 5.2 - 6.2. *BioEnergy Res.* **2013**, *6* (2), 476−485.
(38) Zhou, H.; Zhu, J. Y.; Luo, X.; Leu, S.-Y.; Wu, X.; Gleisner, R.; Dien, B. S.; Hector, R. E.; Yang, D.; Qiu, X.; Horn, E.; Negron, J. Bioconversion of beetle-killed lodgepole pine using SPORL: Process scale-up design, lignin coproduct, and high solids fermentation without detoxification. *Ind. Eng. Chem. Res.* **2013**, *52* (45), 16057−16065.
(39) Brunow, G.; Lundquist, K.; Gellerstedt, G. Lignin. In *Analytical Methods in Wood Chemistry, Pulping, and Papermaking*; Sjöström, E., Alén, R., Eds.; Springer: Berlin, Germany, 1999; pp 77−124.
(40) Sakakibara, A.; Sano, Y. Chemistry of Lignin. In *Wood and Cellulosic Chemistry*; Hon, D. N.-S., Shiraishi, N., Eds.; Marcel Dekker, Inc.: 2001; pp 118−183.
(41) Hu, J.; Xiao, R.; Shen, D.; Zhang, H. Structural analysis of lignin residue from black liquor and its thermal performance in thermogravimetric-Fourier transform infrared spectroscopy. *Bioresour. Technol.* **2013**, *128*, 633−639.
(42) Boeriu, C. G.; Bravo, D.; Gosselink, R. J. A.; Van Dam, J. E. G. Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. *Ind. Crops Prod.* **2004**, *20* (2), 205−218.
(43) Shao, L.; Qiu, J. H.; Feng, H. X.; Liu, M. Z.; Zhang, G. H.; An, J. B.; Gao, C. M.; Liu, H. L. Structural investigation of lignosulfonate doped polyaniline. *Synth. Met.* **2009**, *159* (17−18), 1761−1766.
(44) Sun, S.; Huang, Y.; Sun, R.; Tu, M. The strong association of condensed phenolic moieties in isolated lignins with their inhibition of enzymatic hydrolysis. *Green Chem.* **2016**, *18* (15), 4276−4286.
(45) Xie, R.; Tu, M.; Elder, T. Substituent Effect of Phenolic Aldehyde Inhibition on Alcoholic Fermentation by Saccharomyces cerevisiae. *Energy Fuels* **2016**, *30* (4), 3078−3084.

DOI: 10.1021/acs.energyfuels.8b00864
Energy Fuels 2018, 32, 7636−7642