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Lignosulfonate To Enhance Enzymatic Saccharification of Lignocelluloses: Role of Molecular Weight and Substrate Lignin

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ABSTRACT: This study conducted an investigation of the effect of lignosulfonate (LS) on enzymatic saccharification of lignocelluloses. Two commercial LSs and one laboratory sulfonated kraft lignin were applied to Whatman paper, dilute acid and SPORL (sulfite pretreatment to overcome recalcitrance of lignocelluloses) pretreated aspen, and kraft alkaline and SPORL pretreated lodgepole pine. All three lignin samples inhibited cellulose saccharification of Whatman paper, but enhanced the saccharification of the four lignocellulosic substrates. The level of enhancement was related to the molecular weight and degree of sulfonation of the lignin as well as the substrate lignin structure. When different molecular weight (MW) fractions of one commercial LS (SXP), generated from sulfite pulping of hardwood, were applied to the Whatman paper, the large MW fraction (SXP1) with the lowest degree of sulfonation inhibited cellulose saccharification while the intermediate (SXP2) and smallest (SXP3) MW fractions enhanced saccharification. All MW fractions enhanced saccharification of the four lignocellulosic substrates with maximal enhancement by the smallest MW fraction, SXP3. The enhancement was most significant for the kraft lodgepole pine substrate and least significant for the SPORL pretreated lodgepole pine using all three LS and SXP fractions. The results suggest that LS acts as a surfactant to enhance pure cellullose saccharification. When LS is applied to lignocelluloses, it acts as a surfactant to block bound lignin from binding cellulase nonproductively leading to enhanced saccharification.

INTRODUCTION

Enzymatic hydrolysis of cellulose has been identified as one of the key steps for biochemical conversion of lignocellulosic biomass to biofuel and bioproducts using the sugar platform.[1] It is well-known that lignin can inhibit enzymatic hydrolysis of cellulose through physical blockage to limit cellulose accessibility[2] and nonproductive binding of cellulase to lignin.[3−7] Removal of nonproductive cellulase binding is critical to reducing enzyme dosage while achieving high sugar yield from lignocelluloses,[8,9] because acidic pretreatment often enriches lignin content in the pretreated lignocellulosic solid substrates due to simultaneous removal of other components such as hemicelluloses.[10] Near complete removal of lignin through further delignification is not only expensive but also unnecessary in terms of improving cellulose saccharification.[11] As a result, nonproductive cellulase binding to lignin is unavoidable in enzymatic hydrolysis of lignocelluloses.

Previously, we demonstrated that the application of a SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses[12]) hydrolysate containing lignosulfonate (LS) can result in net enhancement of enzymatic saccharification of lignocelluloses.[8] The enhancement is proportional to the amount of LS applied. We hypothesized that LS can act as a surfactant to block bound lignin (referring to lignin remaining in solid substrate after pretreatment) that has a high affinity to cellulase from binding cellulase nonproductively. Confirmation experiments using a purified commercial sodium LS of softwood also achieved enhanced saccharification efficiency though with less effect. The enhancement effect of nonionic surfactants on enzymatic saccharification of lignocelluloses is well-known and has been extensively studied.[7,13−16] Ooshima et al.[15] reported that nonionic and amphoteric surfactants enhanced saccharification, especially the nonionic surfactant Tween 20, while anionic surfactants did not. They also found that the higher the crystallinity of the substrate cellulose, the larger the enhancement effect of surfactants. However, using LS to enhance enzymatic saccharification of lignocellulose was not recognized because of the conventional belief that lignin inhibits enzymatic saccharification through nonproductive binding of cellulase. This belief was supported by the observed inhibitive effect of commercial LS on enzymatic saccharification of pure cellulose substrates.[16,17] Furthermore, the enzymatic saccharification efficiency of an unwashed aspen substrate (containing LS) from SPORL pretreatment with a low sulfite dosage of 3% on wood was lower than that of its corresponding washed aspen substrate due to LS inhibition.[16] LS, an ionic surfactant, contains both hydrophobic phenylpropanoid units and hydrophilic groups (sulfonic, phenolic hydroxyl, and carboxylic acid groups).[18] The existence of hydrophilic groups provides LSs with good surface activity in a variety of applications, such as dispersants for coal−water slurry and cement−water suspensions,[19,20] and oil well drilling additives.[21] The hydrophilic surface of lignosulfonate can have less affinity to cellulase because hydrophobic interaction is the primary driving force for protein adsorption.[22,23]

Commercial LSs are coproducts from sulfite pulping. LS can also be produced through sulfonation of kraft lignin or

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reactions between sulfite and lignocelluloses, such as SPORL. LSs from different origins have different properties, such as degree of sulfonation and surface hydrophilicity, molecular weight (MW) and its distribution, etc. These properties affect its effectiveness for an intended purpose.[19,20] The purpose of this study is to evaluate the effects of the origins and MW of LS on enzymatic saccharification in order to explain the varied enhancements observed in our previous studies using LS from SPORL and a commercial source.[8,16] The effectiveness of LS on enzymatic saccharification is also dependent on substrate lignin because the enhancement was achieved through the interactions between LS and bound lignin on the lignocellulosic solid substrate. Therefore, two hardwood (aspen) substrates pretreated by dilute acid (DA, the most widely studied process) and SPORL, and two softwood (lodgepole pine) substrates pretreated by alkaline kraft pulping (as DA is ineffective in removing softwood recalcitrance) and SPORL, were investigated in this study. The effect of LS on enzymatic xylan saccharification was also evaluated. Since LS is derived from lignocelluloses, the application of LS to enzymatic saccharification of lignocelluloses may have less unintended negative impacts than other additives for enhancing cellulose saccharification at reduced enzyme dosages. Therefore, this study has significance for increasing enzymatic hydrolysis yields and reducing the associated costs.

MATERIALS AND METHODS

Lignocellulosic Substrates. Three types of solid substrates were used in this study: a pure cellulose substrate of Whatman filter paper (grade 1, catalog number 1001 150, Whatman International, U.K.), pretreated aspen using SPORL[24] and dilute acid (DA), and pretreated lodgepole pine using SPORL[25] and alkaline pulping. The aspen wood chips were from the same batch used in previous studies.[10,24,26] The SPORL and DA pretreatments were conducted on 2 kg oven-dry (od) batches using a 23 L lab wood pulping digester as described previously.[24] The lodgepole pine wood chips were from beetle-killed logs collected from the Arapaho-Roosevelt National Forest. Procedures similar to those described previously were used to pretreat wood chips in the laboratory digester.[25] Both the SPORL and DA pretreated wood chips were disk refined to produce solid lignocellulosic substrates for the present study. Alkaline pulping of the same lodgepole pine wood chips was also conducted. The pretreatment and pulping conditions are listed in Table 1. The chemical compositions of the resultant solid aspen and lodgepole pine substrates were analyzed (Table 2). Whatman filter paper was torn into small pieces of approximately 1 cm × 1 cm and then wetted by deionized (DI) water at 5% solids concentration (w/v). The wetted paper was gently disintegrated into pulp using a disintegrator (Model 73-06-01, TMI, Ronkonkoma, NY, USA) for 5000 revolutions at 312 rpm at room temperature. After 10 min, the pulp was filtered by a nylon membrane with a pore size of 0.45 μm. Then the pulp cake was processed again in the disintegrator.

Table 1. List of Pretreated Lignocellulosic Substrates Studied along with Pretreatment Conditions

sample label	method	chem charges on wood (wt %)	T (°C)	time (min)	sep wash.
aspen					
AS-DA	DA	H_2SO_4: 1.1; $NaHSO_3$: 0.0	170	25	yes
AS-SP	SPORL	H_2SO_4: 1.1; $NaHSO_3$: 3.0	170	25	yes
lodgepole pine					
LP-KP	KP	NaOH: 18.2; Na_2S: 12.6	170	100	yes
LP-SP	SPORL	H_2SO_4: 2.2; $NaHSO_3$: 8.0	165	70	yes

Table 2. Chemical Compositions of Pretreated Lignocellulosic Substrates Listed in Table 1

sample label	Klason lignin (%)	glucan (%)	xylan (%)	mannan (%)
AS-DA	27.2	62.6	3.02	0.72
AS-SP	26.3	65.7	3.24	0.74
LP-KP	1.5	78.1	7.85	6.26
LP-SP	34.3	56.5	2.17	1.63

Enzymes. Commercial cellulase enzymes Cellic CTec2 (abbreviated CTec2) and Multifect xylanase were generously provided by Novozymes North America (Franklinton, NC, USA) and Genencor (Palo Alto, CA, USA), respectively. A Bio-Rad (Bradford) protein assay kit and bovine serum albumin (BSA) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). BSA was used as a standard to calibrate the protein content of CTec2 by the Bradford method.[27] The protein concentration of CTec2 was 73.6 mg/mL and its cellulase activity was 147 filter paper units (FPU)/mL as calibrated by a literature method.[28] Sodium acetate, sulfuric acid, and sodium bisulfite were used as received from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were ACS reagent grade.

Lignosulfonates. High purity sodium LS (D748) from sulfite pulping of softwood was donated by LignoTech USA (Rothschild, WI, USA). A second commercial sodium LS (SXP) was a byproduct of sulfite pulping of poplar wood from Shixian Papermaking Co. Ltd. (Jilin, China). It consists of approximately 70% LS, 11% reductive substances (mainly sugar acids), low molecular weight organic compounds, inorganic salts, and other impurities. The LS used in the present study was centrifuged to remove water insolubles. It was then separated into three fractions by ultrafiltration (Wuxi Membrane Science and Technology Co., China) using polyethersulfone (PES) membranes with cutoff molecular weights of 2500, 30 000, and 50 000 Da. The fractions with molecular weight ranges of greater than 50 000 (SXP1), 30 000−50 000 (SXP2), 2 500−30 000 (SXP3) were used. The actual molecular weights of these fractions were measured separately (Table 3). The fraction of molecular weight less than 2500 was discarded because of its high content of salts and impurities. Sulfonated kraft lignin (SKL) was produced by laboratory sulfonation of a commercial kraft poplar wood lignin from Tongdao Papermaking Co. Ltd. (Hunan, China). The sulfur content and molecular weights of all lignin samples

Table 3. Molecular Weight Distribution and Elemental Sulfur Content of the Lignin Samples

lignosulfonate	M_n (Da)	M_w (Da)	M_w/M_n	sulfur (wt %)
SXP	2400	9100	3.79	6.75
SKL	1000	2500	2.50	5.27
D748	4800	14000	2.92	5.98
SXP1	8100	21000	2.59	5.33
SXP2	3800	6400	1.68	6.35
SXP3	800	1700	2.13	7.81

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studied are listed in Table 3. We assumed that sulfur in LS was almost exclusively from sulfonic acid groups; therefore it is a good measure of LS hydrophilicity.

Enzymatic Hydrolysis. Enzymatic hydrolysis of lignocellulosic substrate was conducted at 2% (w/v) in 25 mL of 50 mM acetate buffer with pH 5.5 on a shaker/incubator (Thermo Fisher Scientific, Model 4450, Waltham, MA, USA) at 50 °C and 200 rpm. Elevated pH 5.5, higher than the commonly used pH 4.8−5.0, can significantly reduce nonproductive cellulase binding to lignin to enhance lignocellulose saccharification as demonstrated in our recent studies.[8,9,29] Acetic acid or 5% (w/w) NaOH was used to adjust the pH of the substrate suspension to pH 5.5 after the addition of LS. The CTec2 loading was 5 FPU/g glucan. A low CTec2 dosage was used to better reflect the enhancement effect by LS. For xylanase supplementation experiments, 10 mg of protein Multifect xylanase/g of glucan was used. Aliquots of 500 μL were taken periodically (3, 6, 9, 24, 48, and 72 h) for glucose analysis after centrifuging at 13000*g* for 5 min. Control experiments without the addition of LS were also carried out for comparison. Each data point is the average of two analyses. The data from duplicate runs were used to calculate the mean values and standard deviations used as error bars in plots.

Analytical Methods. The chemical compositions of the untreated and pretreated lignocelluloses were analyzed as described previously.[30] All lignocellulosic samples were Wiley milled (Model 2, Arthur Thomas Co., Philadelphia, PA, USA) to 20 mesh (~1 mm) and hydrolyzed in two stages using sulfuric acid of 72% (v/v) at 30 °C for 1 h and 3.6% (v/v) at 120 °C for 1 h. Carbohydrates of the hydrolysates were analyzed by high performance anion exchange chromatography with pulsed amperometric detection (ICS-5000, Dionex). The same method was used to determine xylose for xylanase supplementation studies. Klason lignin (acid insoluble) was quantified gravimetrically.[31] For fast analysis, glucose in the enzymatic hydrolysates was measured using a commercial glucose analyzer (YSI 2700S, YSI Inc., Yellow Springs, OH, USA).

Sulfur Content Analysis. The sulfur contents of the substrates were analyzed using inductive coupled plasma (ICP) mass spectrometry. The solid substrate suspensions were shaken well before sampling. Aliquots of samples were digested at 145 °C for 15 min in a microwave oven (MDS-2000, CEM Corp., Matthews, NC, USA) with approximately 5 mL of HNO_3 and 3 mL of 30% H_2O_2 before ICP optical emission spectrometry analysis.

Determination of Lignin Molecular Weight. The lignin molecular weight distribution was determined by gel permeation chromatography (GPC). Aqueous GPC was applied using Ultrahydrogel120, Ultrahydrogel250, and Ultrahydrogel 500 columns (Waters Corp., Milford, MA, USA) to separate lignin fractions and monitored using a UV detector at 280 nm (Model 2487, Waters Corp., Milford, MA, USA). Polystyrenesulfonates in the range from 1000 to 100 000 Da were used as standards for calibration. A 0.10 M $NaNO_3$ solution (pH 8) was used as eluent at 0.50 mL/min. All samples were prepared in double DI water and filtered using a 0.22 μm syringe filter.

RESULTS AND DISCUSSION

Effects of Different Sources of LS on Enzymatic Hydrolysis. The application of 5 g/L LS SXP from sulfite pulping of hardwood had a negligible effect on substrate cellulose enzymatic digestibility (SCED) of Whatman paper (pure cellulose) (Figure 1). SCED is defined as the percentage

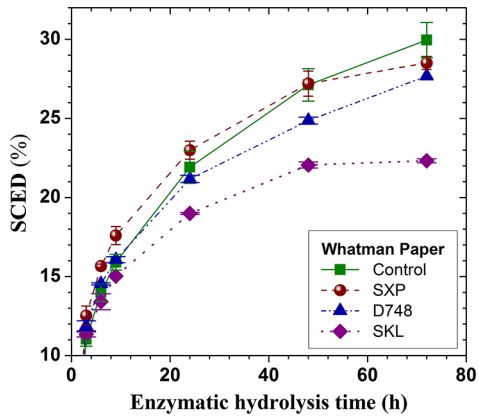

Figure 1. Effects of different lignosulfonates on time-dependent substrate cellulose enzymatic digestibility (SCED) of Whatman paper (pure cellulose). Lignosulfonate concentration = 5 g/L.

of substrate glucan enzymatically saccharified to glucose. The application of purified LS D748 and sulfonated kraft lignin (SKL) reduced the SCED of Whatman paper (Figure 1). The reduction was very small for D748 from sulfite pulping of softwood, agreeing with our previous studies that showed a slight reduction in enzymatic cellulose saccharification.[8,17] The reduction, however, is significant using SKL, suggesting the difference in lignin structure and surface properties between LS and sulfonated lignin SKL can affect lignin and cellulase interactions. The observed net effects of LS or sulfonated lignin on the enzymatic hydrolysis of pure cellulose are a result of the inhibition by nonproductive binding of cellulase to LS or sulfonated lignin and enhancement by the LS or sulfonated lignin as a surfactant.[7,8,15] It is possible that some kraft lignin may not be sulfonated through post sulfonation treatment. The sulfur content of SKL is also the lowest among the three lignin samples (Table 3), suggesting SKL is less hydrophilic and therefore has a higher affinity to cellulase than either SXP or D748, leading to increased nonspecific binding to cellulase.

When the same LS samples were applied to enzymatic hydrolysis of lignocellulosic substrates, enhancement of cellulose saccharification was observed (Figure 2). The four substrates were separately washed to eliminate nonspecific binding of cellulase by the dissolved lignin from the respective pretreatments. This agrees with our initial study using LS from SPORL pretreatment of lodgepole pine applied to pretreated lodgepole pine and aspen substrates.[8] It is believed that LS acts as a surfactant to prevent bound lignin (unseparated by pretreatment and remaining in the solid substrate) binding cellulase nonproductively. However, the degrees of enhancement of saccharification by SXP, SKL, and D748 were all different for each of the four substrates. SKL produced less increase in SCED than either D748 or SXP, probably due to the same reasons discussed previously. The enhancement of SCED by SKL for the dilute acid pretreated aspen (AS-DA) and SPORL pretreated lodgepole pine (LP-SP) were negligible. Less enhancement was observed for the SPORL pretreated aspen (AS-SP) compared to dilute acid pretreated aspen (AS-DA) and likewise for the SPORL pretreated lodgepole pine(LP-SP) compared to kraft treated lodgepole pine (LP-KP). The bound lignin on SPORL pretreated solid substrates,

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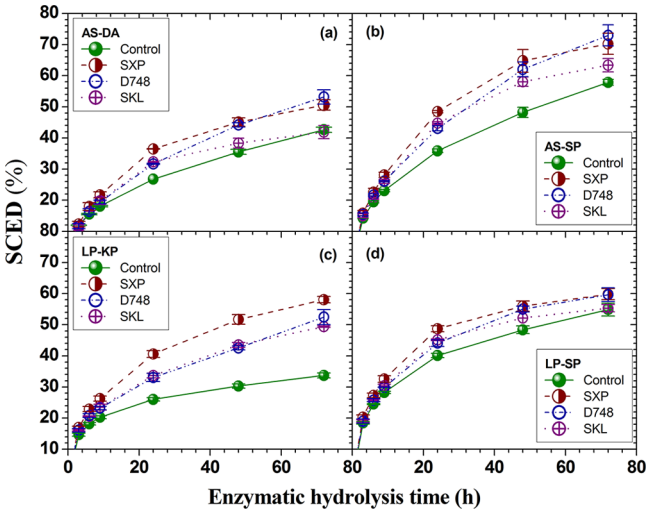

Figure 2. Effects of different lignosulfonates on time-dependent substrate cellulose enzymatic digestibility (SCED) of four lignocelluloses. Lignosulfonate concentration = 5 g/L. (a) Dilute acid pretreated aspen (AS-DA); (b) SPORL pretreated aspen (AS-SP); (c) kraft pulping produced lodgepole pine (LP-KP); (d) SPORL pretreated lodgepole pine (LP-SP).

AS-SP and LP-SP, are also sulfonated and maybe had weaker nonproductive hydrophobic interactions with cellulase than AS-DA and LP-KP. Maximal enhancement of cellulose saccharification was observed from the LP-KP despite its lowest lignin content (Figure 4d). SCED was increased from 35 to 58%, or by 65%, when 5 g/L SXP was applied. This suggests that kraft lignin has a higher affinity to cellulase than dilute acid and SPORL pretreated lignin. SXP contains a small amount of sugar. The residue sugar in a SXP solution at SXP concentration equal to the SXP application dosage of 5 g/L was found to be 0.12 g/L, which is equivalent to less than 1% in SCED.

LS Molecular Weight on Enzymatic Hydrolysis. Different MW fractions of SXP (Table 3) affect enzymatic saccharification of Whatman paper differently. The highest MW fraction SXP1 reduced SCED, while the SXP2 and SXP3 increased SCED (Figure 3). The increase in SCED was very small using the intermediate MW fraction SXP2 but very significant for the smallest MW fraction SXP3, increased from

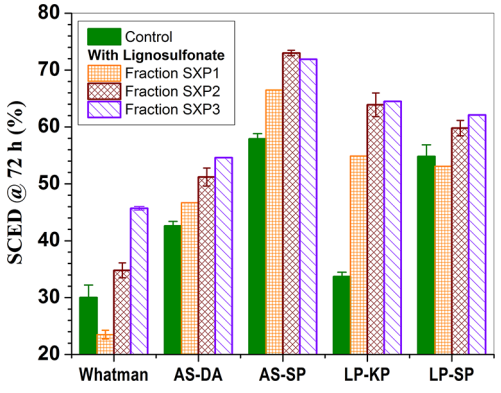

Figure 3. Effects of different MW fractions of a commercial lignosulfonate (SXP) on substrate cellulose enzymatic digestibility (SCED) of Whatman paper and four lignocelluloses. Lignosulfonate concentration = 5 g/L.

30 to 46%, or by over 50%. This suggests that the MW of LS can affect its effectiveness in enhancement of SCED of pure cellulose. The smallest MW fraction also has the highest sulfur content (Table 3), suggesting that SXP3 is the most sulfonated or hydrolphilic and having the lowest affinity to cellulase and thus minimizing nonproductive binding. Therefore, it is an excellent surfactant to enhance cellulose saccharification as conventional nonionic surfactants do. Mechanistic understanding of the MW effect is not possible due to the lack of a plausible explanation of the enhancement of pure cellulose saccharification by nonionic surfactants, although attempts were made.[7] Further study is needed.

The effects of MW on cellulose saccharification of lignocellulosic substrates vary with the substrate itself. All fractions increased SCED (Figure 3). This is because the affinities of all SXP fractions to cellulase are lower than the bound lignin in substrates to result in reduced nonproductive cellulase binding when applying LS. However, the differences in enhancement between applying SXP2 and SXP3 are smaller than that observed applying the two fractions to Whatman paper (Figure 3). Similar to the results presented in the section Effects of Different Sources of LS on Enzymatic Hydrolysis, the applications of SXP fractions to LP-KP have the maximal improvement in SCED. While SXP1 had a negative effect on enzymatic saccharification of Whatman paper, it had a negligible effect on LP-SP and some small positive effects on AS-DA and AS-SP, but greatly increased SCED of LP-KP from 33 to 55%, or by approximately 65%. These results suggest that the effectiveness of different SXP fractions on lignocellulose saccharification is controlled by the relative affinities to cellulase between the SXP fractions and the bound lignin on the lignocellulosic substrate. The bound lignin on SPORL pretreated substrate, especially LP-SP with 8% sulfite charge, is more hydrolphilic than the bound lignins of AS-SP (with 3% sulfite charge) and LP-KP (no sulfite). As a result, applications of SXP (Figure 2) or SXP fractions (Figure 3) on LP-KP were more effective by blocking a more hydrophobic lignin in LP-KP than that on AS-SP or LP-SP that contains a relatively hydrophilic lignin with less affinity to cellulase due to the presence of sulfonic acid groups. It is clear that the level of reducing nonproductive cellulase binding determines the effectiveness of LS on enhancing enzymatic saccharification of lignocelluloses. This is despite an incomplete picture of the interactions among LS, cellulase, and bound lignin in solid substrates through hydrophobic,[22] electrostatic,[9,32] and other forces as well as the potential to form chemical complexes[17] or produce cellulase structure modifications.[22,33]

The results in Figure 3 also indicate that the SCED of Whatman paper was lower than the corresponding values of the lignocellulosic substrates even for the corresponding control runs. This is because that enzymatic hydrolysis experiments were conducted using a buffer solution of pH 5.5, which is optimal for enzymatic saccharification of lignocellulosic substrates but not optimal for saccharification of pure cellulosic substrate.[9,29] Furthermore, fiber hornification due to drying in making Whatman paper caused pore collapse and significantly reduced enzymatic hydrolysis efficiency as we demonstrated previously.[34] In contrast, all lignocellulosic substrates studied were never dried.

Effects of Dosages of SXP Fractions on Enzymatic Hydrolysis. Understanding of the effect of LS application dosage on lignocellulose saccharification is very important. A nondiminishing effect as reported previously[8] is a good feature

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for enzymatic saccharification of the whole slurry of sulfite (such as SPORL) pretreated lignocelluloses to make full use of the LS produced in the pretreatment. On the other hand, a significant effect at low LS dosage is favorable for using LS as an additive to reduce LS dosage. The results indicate that significant effects were observed at low dosages of SXP1, SXP2, and SXP3 when applied to AS-DA (Figure 4a), AS-SP

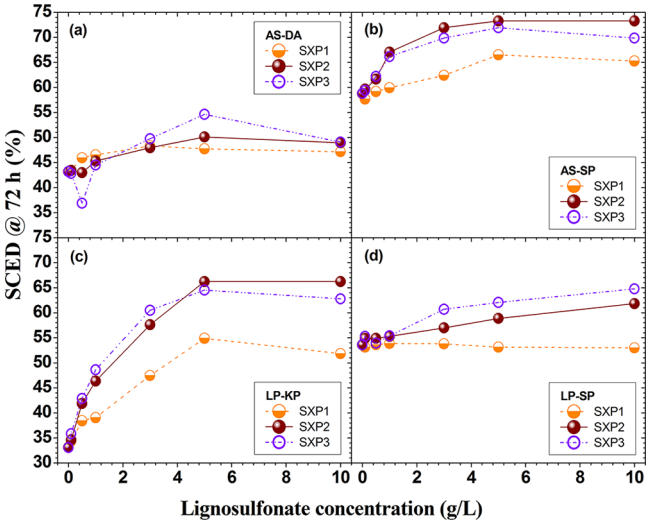

Figure 4. Effects of the loading of three MW fractions of SXP on substrate cellulose enzymatic digestibility (SCED) of four lignocelluloses. (a) Dilute acid pretreated aspen (AS-DA); (b) SPORL pretreated aspen (AS-SP); (c) kraft pulping produced lodgepole pine (LP-KP); (d) SPORL pretreated lodgepole pine (LP-SP).

(Figure 4b), and especially to LP-KP (Figure 4c). Minimal effects were observed at dosages beyond 5 g/L for these three substrates. A nondiminishing effect, though a smaller overall effect than those shown in Figure 4a–c, was observed when SXP2 and SXP3 were separately applied to SPORL pretreated lodgepole pine LP-SP (Figure 4d). This indicates an advantage of SPORL pretreatment due to lignin sulfonation which can facilitate enzymatic saccharification using the whole slurry (solids and liquor), eliminating the separation of pretreated solids from liquid (containing LS) and subsequent washing of the solids while still achieving high yield at low cellulase dosages. This has been demonstrated in our previous studies.[8,25] The results in Figure 4c suggest that LS is an excellent additive to enhance enzymatic saccharification of alkaline pretreated substrate LP-KP. SCED can be increased by approximately 100% at SXP2 and SXP3 concentration of 5 g/L.

Effects of LS on Enzymatic Xylan Saccharification. Application of nonionic surfactant was found to increase enzymatic xylan saccharification though not as pronounced as in cellulose hydrolysis.[13] All three fractions of SXP were used to evaluate the application of LS on xylan saccharification. LP-KP and AS-SP were chosen for the study because of their high xylan content among the four lignocellulosic substrates (Table 2). The application of the commercial cellulase enzyme mixture CTec2 (control), which has xylanase activity, produced significant xylan hydrolysis (Figure 5a), as represented by substrate xylan enzymatic digestibility (SXED). SXED is defined as the percentage of substrate xylan enzymatically saccharified to xylose. An increase in SXED was observed when SXP2 was applied in addition to CTec2. The increase in SXED is more pronounced for LP-KP, which has a higher xylan

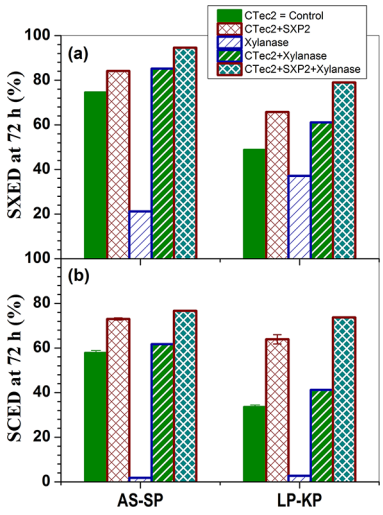

Figure 5. Effects of the intermediate MW fraction SXP2 at 5 g/L on enzymatic saccharification of a SPORL pretreated aspen (AS-SP) and a kraft pulping produced lodgepole pine (LP-KP) with and without xylanase supplementation. (a) Substrate xylan enzymatic digestibility (SXED) at 72 h; (b) substrate cellulose enzymatic digestibility (SCED) at 72 h.

content than AS-SP. SXED was increased from 49 to 66%, or by 35%, for LP-KP. The supplementation of xylanase to CTec2 achieved a level of xylan saccharification similar to those achieved by supplementing SXP2 to CTec2 for both LP-KP and AS-SP. This clearly indicates that SXP2 can increase enzyme activity (from xylanase or from both xylanase and cellulase assuming synergistic effect) equivalent to the amount of xylanase supplemented (10 mg of protein/g of glucan). Further enhancement of xylan saccharification by xylanase supplementation was achieved when SXP2 was additionally applied for both substrates (Figure 5a). SXED showed significant increases from 49 to 79% for LP-KP and from 75 to 95% for AS-SP.

Supplementation of xylanase had a limited effect on cellulose saccharification (Figure 5b) because CTec2 was optimized for maximal cellulose saccharification by the enzyme manufacturer. Xylanase supplementation had some effect on LP-KP compared to AS-SP because of the higher xylan content of LP-KP. The gains in SCED for both substrates with xylanase supplementation are slightly higher than that achieved using xylanase alone without CTec2. This suggests some synergistic effects between cellulase and xylanase; i.e., xylan saccharification increased the cell wall pore area and therefore cellulose accessibility to enhance cellulose hydrolysis and in turn improved xylan accessibility to xylanase. This synergistic effect is more pronounced for LP-KP, which has a higher xylan content. It also suggests that further enzyme cocktail optimization is possible for a given lignocellulosic substrate because CTec2 was optimized using a diluted acid pretreated corn stover, different from the two substrates used in the present study. The synergistic effect is also observed with the application of SXP2. The SCED can be increased from 33 to 74% for LP-KP with xylanase supplementation and SXP2 at a CTec2 dosage of only 5 FPU/g glucan.

■ CONCLUSIONS

This study demonstrated that LS can enhance enzymatic saccharification of lignocelluloses. The enhancement varies with

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LS molecular weight as well as the lignin of the lignocellulose. LS fractions with low MW were more effective than LS fractions with higher MW. Furthermore, LS with small MW can also enhance enzymatic saccharification of pure cellulose. Application of LS to lignocelluloses with hydrophobic lignin produced more improvement in saccharification than those with relatively hydrophilic lignin. It is postulated that LS with low affinity to cellulase due to its hydrophilic surface acts as a surfactant to block bound lignin on lignocellulose that is hydrophobic and has a high affinity to cellulase, which resulted in reduced nonproductive binding of cellulase and enhanced enzymatic saccharification of lignocelluloses. Further study is needed to understand the mechanism of enhancement of pure cellulose saccharification by nonionic surfactants and low MW LSs.

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Notes

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