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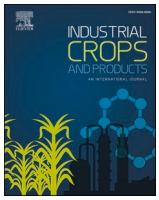

High-efficient fractionation of poplar chips by ternary deep eutectic solvents system for elevating enzymatic hydrolysis

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ABSTRACT

The traditional acid-based binary deep eutectic solvents (DESs) have the disadvantages of relatively low separation efficiency and relatively serious damage to lignin structure. In this study, salicylic acid, as the third component, was introduced into DESs fractionation system, and the effect of ternary DESs (t-DESs) on biomass separation and subsequent enzymatic hydrolysis was studied. After t-DESs fractionation, the separation efficiency for the components of lignocellulosic biomass was obviously improved, which the cellulose purity of residual substrate reaching 84.17 %, and the removal of hemicelluloses and lignin reaching 91.2 % and 75.3 % respectively. Compared to the DES fractionation without salicylic acid, the t-DES fractionation reserved more linkage bonds in lignin structure, including β—O—4, β—5, and β—β. Especially, 28.3 % of the β—O—4 substructure was remained in t-DES lignin, while that of the DES fractionation without salicylic acid only reached 22.5 %. The enzymatic conversion of the substrate by t-DESs at 110 ℃ reached 82.7 %.

1. Introduction

The utilization of fossil fuels, such as coal and petroleum, has caused many environmental concerns, pushing human society forward technologically and scientifically (Sekoai et al., 2020). It is an urgent and immediate demand to explore alternative resources to replace these fossil fuels, thus addressing global environmental challenges (Jin and Wei, 2023). Lignocellulosic biomass has been considered one of the most promising resources due to its sustainable and renewable features (Sindhu et al., 2016), which mainly consists of three components, cellulose, hemicelluloses, and lignin (Kellock et al., 2019). All of these components could be used as raw materials to produce biofuels, bio-materials, and chemicals. For example, the carbohydrates, including cellulose and hemicelluloses can be enzymatically fermented to produce bioethanol (Talebi Amiri et al., 2019). However, the stubborn cell wall structure of native lignocellulosic biomass makes it difficult to be directly transformed (Mankar et al., 2021), thus an efficient fractionation process of carbohydrates and lignin is the critical step for the further valorization (Yang et al., 2022).

To overcome the recalcitrance and accomplish the high-value utilization of lignocellulosic biomass, various fractionation strategies have been developed, including physical, such as physical or chemical methods (Paudel et al., 2017; Zhao et al., 2022). Among of these chemical fractionation processes, a novel fractionation system composed of deep eutectic solvents (DESs) has captured widespread attention due to the applied solvents are less toxic, biodegradable, and environmentally friendly (Abbott et al., 2003). Moreover, the DESs can be produced by a simple synthetic procedure without the need for complex purification steps (Mankar et al., 2021). DESs have presented a strong ability to fractionate lignocellulosic materials into their main components (Xu et al., 2021). It was found that the vast majority of DESs possessed well biocompatible and had little effect on enzyme activity, which makes them of great value in biorefinery (Lehmann et al., 2014). It has been proven that the ether bonds in lignin macromolecules could be easily cleaved during DESs treatment, showing the potential to depolymerize and dissolve lignin under mild conditions (George et al., 2015). However, to achieve a higher delignification rate, the DESs fractionation systems usually require a relatively severe cooking

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conditions, such as high temperature or long reaction time (Wang et al., 2020; Xu et al., 2016).

Although severe treatment conditions will lead to a high delignification rate, it will also lead to intense condensation of lignin, forming new C—C linkage bonds (Liu et al., 2021). The formed C—C bonds could increase the hydrophobicity of lignin and further reduce the enzymatic conversion of cellulose substrate (Sheng et al., 2021; Wu et al., 2023). The lignin condensation can also lead to lignin deposition on the fiber surface, thus hindering the efficient contact between enzymes and cellulose through steric hindrance (Huang et al., 2022; Wu et al., 2022). At present, binary acid-based DESs are widely applied in the separation of lignocellulosic biomass components and lignin extraction (Wang and Lee, 2021; Guo et al., 2020). Most current acid-based binary DESs treatments generally suffer from low separation efficiency of lignocellulosic biomass and low enzymatic hydrolysis efficiency (Liu et al., 2021). To control the condensation reaction of lignin structure, developed a ternary DESs, that is, adding the third component into binary DESs to achieve cleavage/stabilization or derivatization/protection of lignin. The addition of the third component can protect the lignin side chain α-OH, thus inhibiting the conformation of lignin α-carbocation, further protecting *β*—O—4 linkage and limiting the lignin condensation. At present, it has been reported that aldehydes (formaldehyde, acetaldehyde) and alcohols (ethylene glycol and other diols) can be used as protective agents for lignin side chain α-OH (Lan et al., 2018, 2019; Shuai et al., 2016).

In this work, another strategy is put forward, a small molecular aromatic monomer salicylic acid was introduced into DESs system, which is expected to capture the formed α-carbocation in lignin structure and further protect *β*—O—4 bonds, thus producing relatively complete lignin. Firstly, a new type of choline chloride-lactic acid-salicylic acid ternary DESs (t-DESs) was synthesized and applied to the separation of lignocellulosic biomass. The potential role of salicylic acid in the t-DESs fractionation system removal of lignin was studied by the structure characterization of the obtained lignin. Furthermore, the effect of the t-DESs fractionation system on the subsequent enzymatic conversion of cellulose residue was further assessed. We expect that this study could broad the utilization of DESs in the separation of biomass components and build a platform for efficient utilization of biomass components.

2. Materials and methods

2.1. Materials

The poplar chips were purchased from a paper mill in Yantai, Shandong Province. The chips were milled, and the wood powder between 40 and 60 mesh was collected and kept in the cold room (4 ℃) for further use. Choline chloride (98 %), lactic acid (92 %) and salicylic acid (99.5 %) were provided by Macklin Biochemical Co., Ltd. (Shanghai, China). Cellic CTec3 (abbreviated CTec3, 150 FPU/g) was provided by Novozymes Biotechnology Ltd Co., Ltd. (China).

2.2. Synthesis of t-DESs and biomass fractionation

The synthesis of t-DESs was carried out in a round-bottom flask. The choline chloride (HBA), lactic acid, and salicylic acid (HBD) were mixed in the round-bottom flask with different molar ratios, heated, and stirred at an oil bath (80 ℃) until the DESs was clear. The molar ratio of HBD to HBA gradually increased from 1:4–1:5.5 according to the different ratios. After that, the DESs was transferred to a sealed container and stored in a desiccator for later use.

The biomass fractionation by DESs was performed in a thick-walled pressure bottle. Wood powder (5 g) and t-DESs (100 g) were mixed and reacted in an oil bath (90–120 °C) for different times (1–12 h). When the reaction was complete, ethanol (3 times) was added immediately to terminate the reaction and then the mixture was filtered. The filtered residue was washed with fresh ethanol. The residue and liquid were collected respectively. The liquid was concentrated, and the concentrated extraction was dropped into HCl solutions (pH 2, 10 times) to precipitate lignin, which was further purified by tetrahydrofuran (THF) and ether. Finally, the reaction temperature was 110 °C, the reaction time was 6 h, and the ratio was 1:4:1 (chloride/lactic acid/salicylic acid at 1:4:1). The lignin extracted by DESs with salicylic acid and without salicylic acid was named CLS and CL, respectively.

2.3. Milled wood lignin (MWL) extraction

The milled wood lignin of poplar was extracted according to the method of Zhang (Zhang et al., 2017). Briefly, the ball-milled poplar (20 g) was extracted twice with dioxane/water (200 mL; 96:4, v/v) for 24 h. After the suspension was removed by centrifugation, the obtained solution was concentrated under reduced pressure. After freeze-drying, the crude milled wood lignin obtained was dissolved in acetic acid/-water (20 mL; 9:1, v/v) and precipitated into water (400 mL). The precipitated lignin was filtered and then freeze-dried, dissolved in 1,2-dichloroethane/ethanol (10 mL; 2:1, v/v), and precipitated into diethyl ether (200 mL). The lignin sample obtained was freeze-dried, referred as MWL.

2.4. Cellulose accessibility determination

Cellulose accessibility in biomass samples was determined by Congo red dye (DR28) adsorption, as previously described. Specifically, a certain gradient concentration of DR28 solution was first prepared, and the biomass samples was fully mixed with the solution at a substrate load of 1 % (w/v). The solution was soaked at 50 °C and 150 rpm for 24 h. After the soaking, the supernatant was taken and the absorbance was measured at 498 nm using an ultraviolet spectrophotometer. The maximum adsorption capacity of the biomass samples was determined according to the previously drawn curve to characterize the accessibility of cellulose.

2.5. Determination of surface lignin coverage

X-ray photoelectron spectroscopy (XPS) analysis was applied to determine surface lignin coverage of biomass samples as described previously. The ratios of oxygen to carbon on the biomass surfaces were obtained from the XPS spectra, and used to assess the surface lignin coverage according to the following equation.

$$\text{Surface lignin coverage} = \frac{O/C_{sample} - O\Big/C_{carbohydrates}}{O/C_{lignin} - O\Big/C_{carbohydrates}} \quad (1)$$

Where O/C_{lignin} is 0.33 and $O/C_{carbohydrates}$ is 0.83.

2.6. Enzymatic hydrolysis

The enzymatic hydrolysis of substrate (wood powder or residuals after fractionation) was performed in an oscillating table at a constant temperature (50 °C) and a constant rotation speed (80 rpm). The substrate was mixed with acetic acid-sodium acetate solution (pH 4.8) in a sterilization flask at a consistency of 5 %, and then adding the cellulase (20 FPU/g substrate). The hydrolysate was pipetted at different times and added buffer after each sample to maintain a substrate concentration of 5 %. The hydrolysate was determined by ion exchange chromatography (Thermo ICS-5000 +) and the conversion of cellulose was calculated following the publication (Wu et al., 2022).

2.7. Analytical procedure

The chemical compositions of wood powder and residuals after fractionation were determined according to NERL standard (Kou et al.,

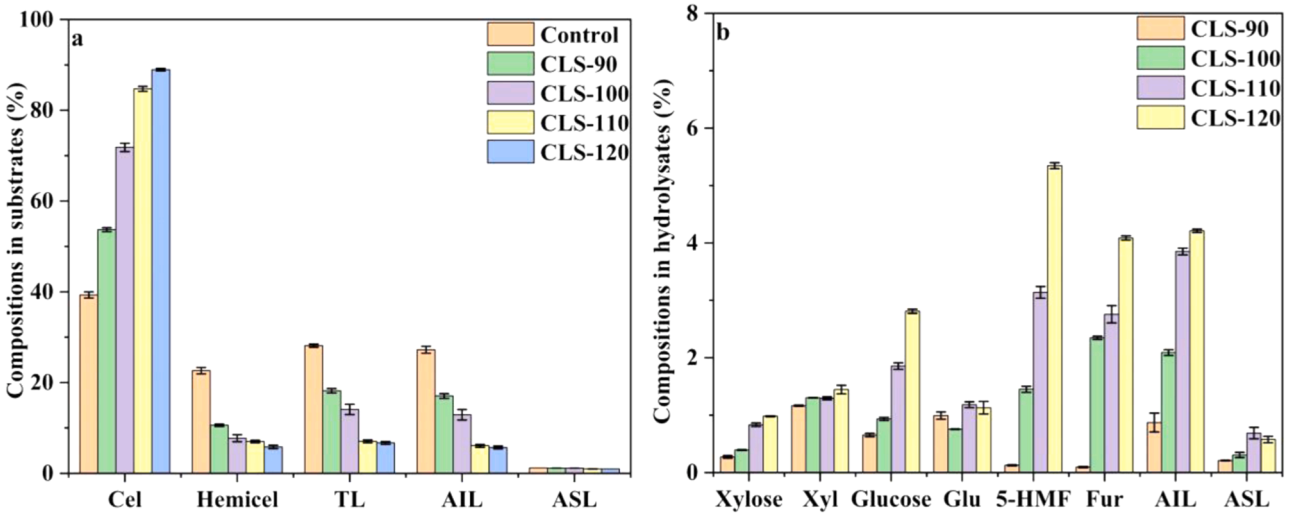

Fig. 1. (a) Effect of temperature on main components in substrates and (b) hydrolysates.Note: Cellulose (Cel); Hemicellulose (Hemicel); Total lignins (TL); Acid insoluble lignin (AIL); Acid soluble lignin (ASL); Xylan (Xyl); Glucan (Glu); Furfural (Fur); 5-hydroxymethyl furaldehyde (5-HMF).

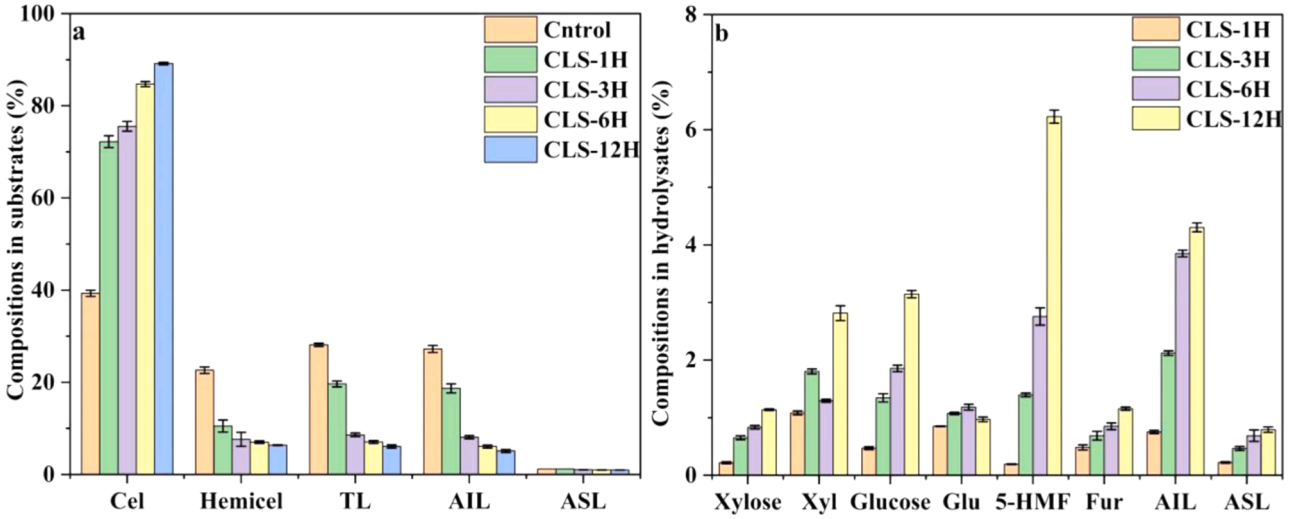

Fig. 2. (a) Effect of time on main components in substrates and (b) hydrolysates. Note: Cellulose (Cel); Hemicellulose (Hemicel); Total lignins (TL); Acid insoluble lignin (AIL); Acid soluble lignin (ASL); Xylan (Xyl); Glucan (Glu); Furfural (Fur); 5-hydroxymethyl furaldehyde (5-HMF).

2017). Soluble sugars in the wood powder, residuals, and hydrolysates were determined by ion chromatography (IC) equipped with Dionex Carbo Pac PA 20 (Thermo Fisher Scientific, USA). The isocratic mobile phase consisted of solvent A (NaAC 1 mM), solvent B (H_2O), solvent C (250 mM NaOH), and solvent D (50 mM NaOH). The molecular weight of lignin was determined by gel permeation chromatography (GPC) (Tolbert2et al., 2014). The 2D HSQC NMR and 31 ^{P} NMR spectra were recorded on a 400 MHz Bruker AVANCE III NMR spectrometer according to previous researches (Holtman et al., 2006).

3. Results and discussion

3.1. Effect of t-DESs treatment on the fractionation of biomass components

Plant cell walls were highly recalcitrant due to the cross-linked structure between carbohydrates and lignin. Hemicelluloses, an amorphous biopolymer in lignocellulosic biomass, is composed of straight chains (hexose and pentose) and branched chains (uronic acid) of heteropolysaccharides. It connects cellulose fibers through weak hydrogen bonds and van der Waals forces (Lu et al., 2021). In the DESs pretreatment process, hemicelluloses can be removed by destroying the intermolecular hydrogen bonds between carbohydrates and lignin, and forming intermolecular hydrogen bonds between DESs and hemicelluloses. Efficient fractionation treatment was a prerequisite for separating biomass components, increasing the surface area of cellulose fibers for subsequent high-value utilization, and removing lignin (Basak et al., 2023). The treatment conditions, such as temperature, time, and the composition of DESs are the key factors to affect the fractionation of biomass. Above all, the effect of the temperature on the fractionation of biomass was discussed by controlling the reaction time (6 h) and compositions of t-DESs (chloride/lactic acid/salicylic at 1:4:1), and the results were shown in Fig. 1. The chemical compositions of the raw material (poplar wood powder) were: 39.81 % cellulose, 20.73 % hemicelluloses and 25.57 % total lignin (AIL 27.24 %, and ASL 1.24 %). The cellulose content in the solid residual after treatment was increased with the temperature rise. The accumulation of cellulose in the solid residual was benefit for the enzymatic conversion. The maximum cellulose content in the solid residual was 84.71 %, compared with raw material, which increased by 45.4 %. The content of lignin and

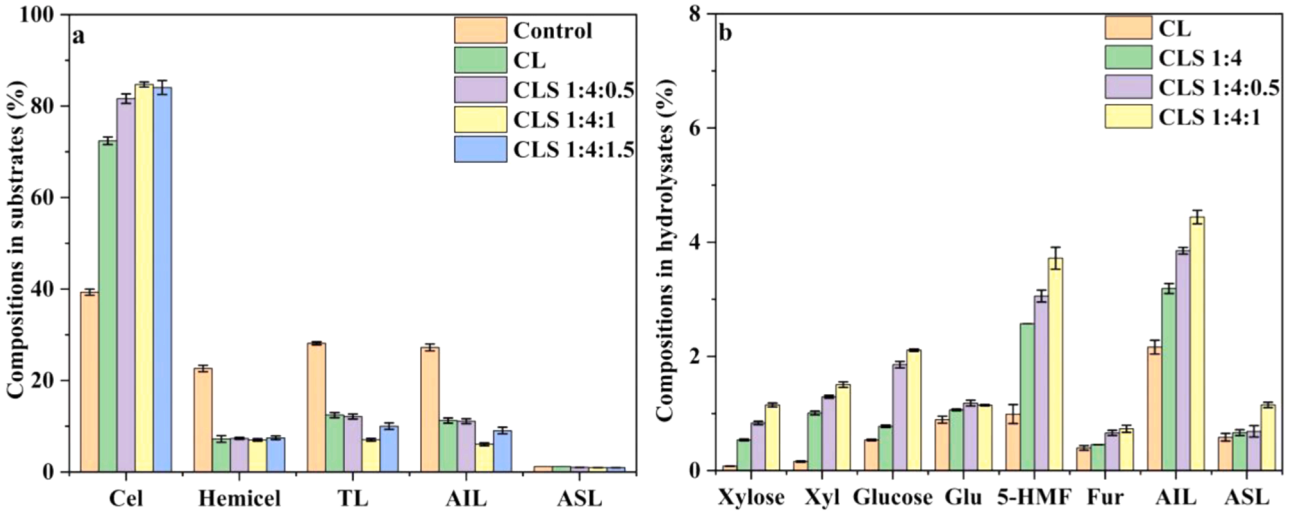

Fig. 3. (a) Effect of DES ratios on main components in substrates and (b) hydrolysates. Note: Cellulose (Cel); Hemicellulose (Hemicel); Total lignins (TL); Acid insoluble lignin (AIL); Acid soluble lignin (ASL); Xylan (Xyl); Glucan (Glu); Furfural (Fur); 5-hydroxymethyl furaldehyde (5-HMF).

Table 1
Cellulose accessibility and surface O/C ratio.

Sample[a]	Accessibility[b] (mg/g)	O/C[c]	Surface lignin coverage[d] (%)
Control	120.6	0.45	76.3
CLS-DES	384.3	0.70	26.7
CL-DES	331.7	0.62	42.4

Note:
[a] biomass raw materials obtained by different treatment methods.
[b] surface accessibility of cellulose.
[c] the ratio of C to O on cellulose surface.
[d] lignin surface coverage on cellulose surface

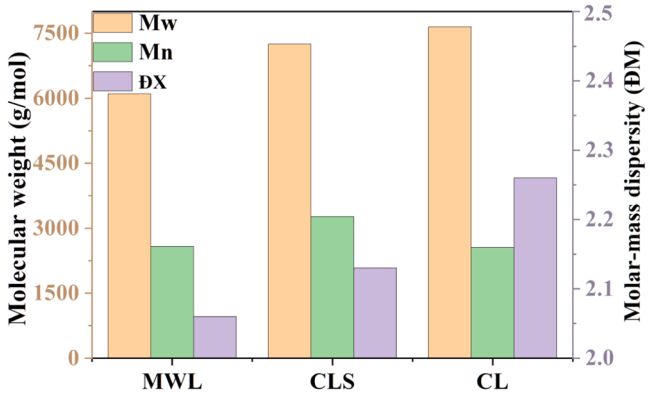

Fig. 4. Effect of DES on molecular weight of lignin. Note: ĐM = Mw/Mn, use of the term "polydispersity index" for Mw/Mn or other terms involving the word "polydispersity" is strongly discouraged (Stepto, 2009).

hemicelluloses in the substrates presented an opposite trend to that of cellulose. This phenomenon could be attributed to the cleavage of their connecting bonds in the molecular structure of hemicelluloses and lignin, which leads to the degradation and dissolution of lignin and hemicelluloses. The removal of lignin and hemicelluloses increased cellulose content in the substrates which could expose more bond sites of enzymes on the surface of cellulose. The maximum removal ratios of hemicelluloses and lignin reached 91.2 % and 75.3 %, respectively, when the treatment temperature was 120 °C. The chemical compositions contents in the extraction were increased with the increasing of the temperature, which further verified the results of solid residues. The lignin (AIL and ASL) content increased rapidly with the temperature increased from 90 to 110 °C. When the temperature was 110 °C, the lignin content in extraction reached 5.32 %, which is more than 4 times that extracted at 90 °C. The sugars in the extraction can be divided into monosaccharides (xylose and glucose) and polysaccharides (xylan and glucan). The sugar content was increased with the increase in temperature, which was related to the accelerated degradation of hemicelluloses under a higher temperature. The hemicellulose degraded products, such as furfural and HMF, exhibited a similar trend with that of the sugars. Remarkably, the content of HMF and furfural presented a significant rise when the temperature increased from 110 °C to 120 °C, which may be due to the higher treatment temperature led to the extensive degradation of the extracted sugars. Compared with that of 110 °C, was 5.47 and 4.03 %, increased by 80.7 and 45.2 %, respectively. The higher concentration of furfural and HMF would adversely affect the subsequent enzymatic hydrolysis (Toquero and Bolado, 2014). As mentioned above, aiming to improve the subsequent biological transformation of biomass, 110 °C reaction temperature is more suitable for this DESs fractionation.

The reaction time is also an important factor during the biomass fractionation process. The effect of reaction time on biomass fractionation was investigated, and the results were shown in Fig. 2. The cellulose content in the substrates was increased with the increasing of treatment time. The cellulose content was 84.3 % after 6 h of DESs treatment, which was 45.4 % higher than that of raw material. This is mainly due to the enrichment of cellulose during the treatment. When the treatment time increased to 12 h, the cellulose content showed only a slight increase (89.41 %). Meanwhile, both lignin and hemicelluloses content in the substrate were decreased and these contents in the extraction were increased with the increasing of the treatment time. The results revealed that the extension of the treatment time was beneficial for the removal of both lignin and hemicelluloses during t-DESs fractionation. Importantly, the content of furfural and HMF were significantly increased with the treatment time increased from 6 to 12 h, which were increased by 23.43 and 56.45 %, respectively. Therefore, extending the treatment time could in turn limit the enzymatic conversion of substrates due to the continuous generation of carbohydrate degradation products (furfural and 5-HMF).

As shown in Fig. 3, the effects of different molar ratios of DESs on the biomass fractionation were further analyzed. Compared to the DESs fractionation process without salicylic acid added, the t-DESs fractionation significantly extracted more lignin and hemicellulose, thus

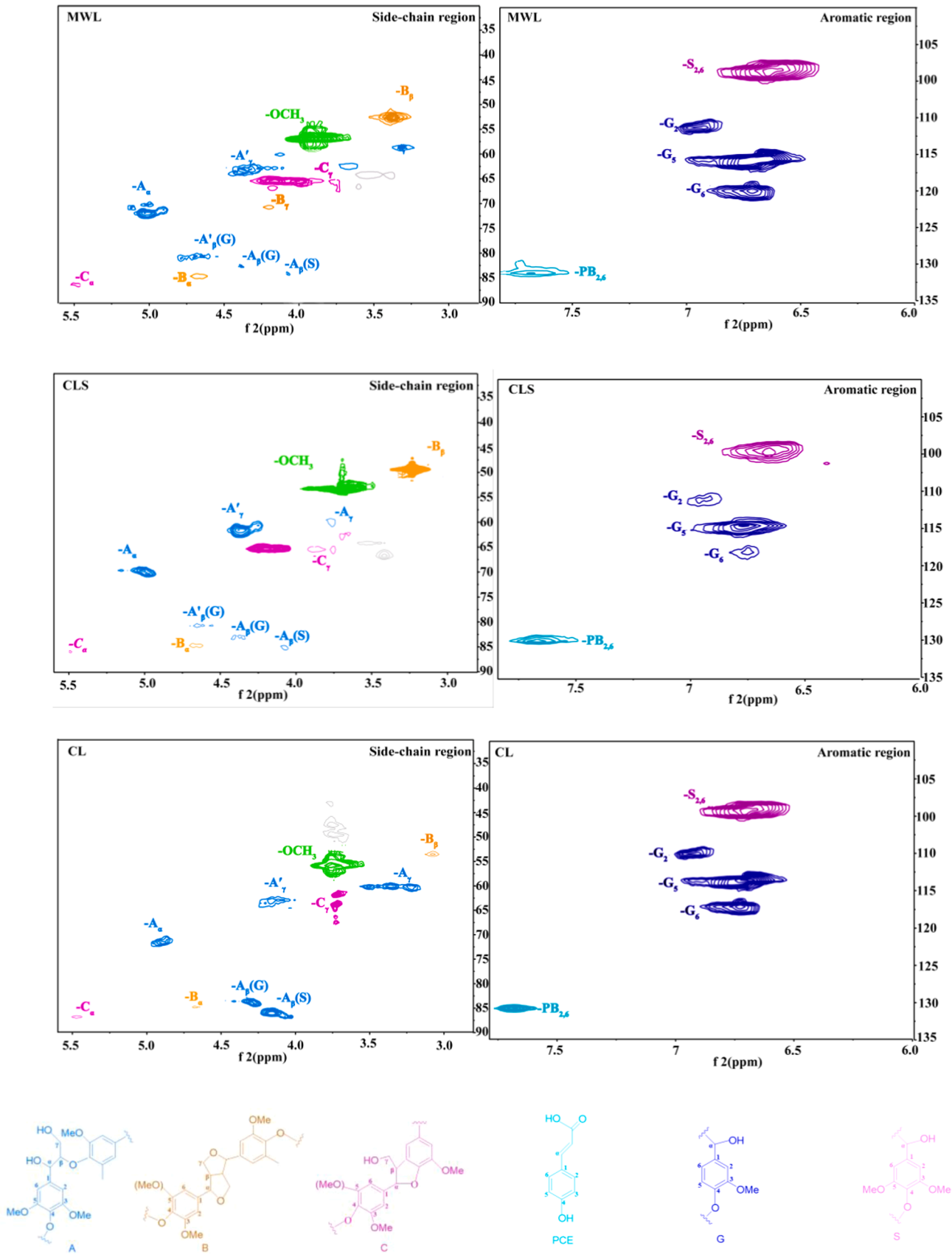

Fig. 5. Aromatic and side-chain regions of the MWL, CL and CLS in the 2D HSQC NMR spectra.

Table 2
Quantification of the lignin fractions by 2D-HSQC NMR method.

	MWL	CLS	CL
β—O—4[a]	52.6	28.3	22.5
β-β[a]	7.1	7.7	7.3
β—5[a]	2.1	2.5	2.2
S/G[b]	0.8	1.4	1.7

Note:
[a] Results expressed per 100 Ar based on quantitative 2D-HSQC spectra (Overend et al., 1997).
[b] S/G ratio obtained by the equation S/G ratio = $0.5I(S_{2,6})/I(G_2)$

obtaining a relatively pure cellulosic substrate. This result revealed that the participation of salicylic acid greatly improved the separation efficiency in t-DESs fractionation process. With the increase of salicylic acid content, the cellulose in the substrate increased first and then decreased. The highest cellulose content (84.71 %) in the substrate was obtained with an optimal t-DESs ratio 1:4:1 (chloride/lactic acid/salicylic acid at1:4:1), which was 12.37 % higher than that of DESs fractionation process without salicylic acid. The further increase in salicylic acid concentration led to a decrease in cellulose content, which may be related to the solvent bias towards acidity (Tan et al., 2019). Correspondingly, the content of hemicelluloses and lignin in the substrate was first decreased and then increased with increasing salicylic acid ratio. The maximum removal rate of hemicelluloses and lignin at the optimal ratio of t-DESs were 82.44 % and 74.13 %, respectively. In contrast, the DESs fractionation process without salicylic acid removed 68.53 % of hemicelluloses and 63.76 % of lignin under the same reaction time and temperature. Besides, with the increase of the salicylic acid, the content of hemicellulose degradation productions (furfural and 5-HMF) in the extraction was increased.

In summary, the optimal reaction conditions for the ternary system have been established as a temperature of 110°C, a duration of 6 hours, and a t-DESs ratio of 1:4:1 (chloride/lactic acid/salicylic acid at a 1:4:1 ratio). Under these conditions, the cellulose purity in the substrates increased to 84.17 %, and the removal rates for hemicelluloses and lignin improved to 91.2 % and 75.3 %, respectively. In previous studies, the ratio of AIL to ASL can be used to evaluate the condensation of lignin (Li et al., 2024a, 2024b). The AIL/ASL ratio of the substrate treated with salicylic acid (12.4) was significantly lower than that of the substrate without salicylic acid (17.2), indicating that the degree of repolymerization of residual lignin in the t-DESs pretreated substrate was lower. Additionally, the recovery rates from the hydrolysates are 3.27 % for glucose, 0.86 % for glucan, 2.77 % for xylose, and 1.18 % for xylan. These results indicated a successful optimization of the reaction conditions for enhanced substrate purity and recovery of valuable components.

At the same time, the separation efficiency was compared with other DES systems (see Table S1). Wang et al. (2020)) used a new hardwood lignin-based DES prepared by p-hydroxybenzoic acid (PB) and choline chloride (ChCl) to effectively pretreat lignocellulosic biomass at 160 °C for 3 h. The removal rate of hemicellulose reached 87.3 %, but the removal of lignin was only 69 %. Guo et al. (2019) using silicotungstic acid accompanied with ChCl/glycerol treatment could remove 89.5 % of lignin, but the removal rate of hemicellulose was low (58.3 %). Li et al. (2021)) prepared lignin-containing cellulose nanofibrils using DES composed of acetic acid and choline chloride at 90 °C for 9 hours with the cellulose purity of 77.5 %. In our work, the separation efficiency for the components of lignocellulosic biomass was obviously improved, which the cellulose purity of residual substrate reaching 84.17 %, and the removal of hemicelluloses and lignin reaching 91.2 % and 75.3 %, respectively.

3.2. Effect of cellulose accessibility and surface lignin distribution

The accessibility of cellulose was characterized by DR28. The results were shown in Table 1. The accessibility of cellulose after DESs treatment was significantly improved, with the accessibility of cellulose treated with salicylic acid increased from 331.7 to 384.3 (mg/g), compared with raw materials.

The surface coverage of lignin had an adverse effect on the subsequent enzymatic hydrolysis (Li et al., 2024b). The specific performance was that the residual lignin on the surface of the cellulose would bind to the enzyme non-productively, affecting the efficiency of enzymatic hydrolysis. As shown in Table 1, the surface lignin coverage of the raw material (76.3 %) reduced to 42.4 % after DESs pretreatment, especially the surface lignin coverage after adding salicylic acid treatment reduced to 26.7 %.

3.3. Characterization of lignin structure

The molecular weight of the extracted lignin was characterized, and the results were shown in Fig. 4. It can be clearly seen that the lignin

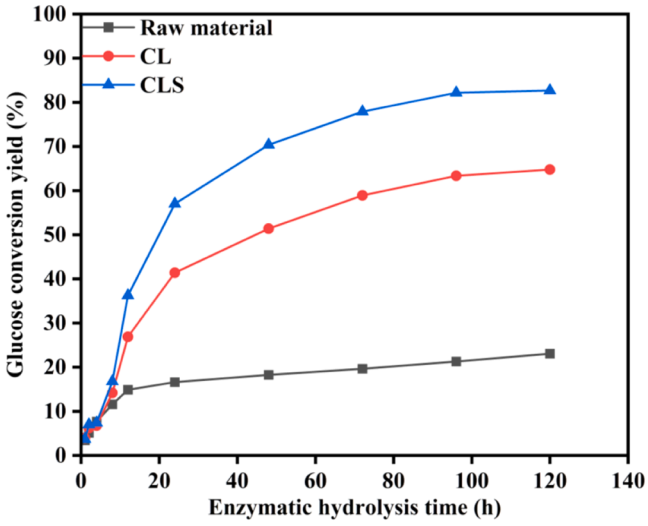

Fig. 6. Effect of DES in substrates on enzymatic hydrolysis.

Table 3
Quantification of functional groups (mmol/g) in DES lignin and MWL processing conditions by a quantitative ^{31}P NMR method.

Lignin samples	Aliphatic OH (mmol/g)	Phenolic OH (mmol/g)					Carboxylic acid (mmol/g)
		Syringyl		Guaiacyl		p-Hydroxyl	
		C[a]	NC[b]	C	NC		
MWL	6.63	0.04	0.29	0.11	0.65	0.74	0.16
CLS	4.29	0.14	0.88	0.22	0.81	0.89	0.71
CL	4.57	0.18	1.02	0.26	0.72	0.86	0.69

Note:
[a] C, condensed.
[b] NC, non-condensed

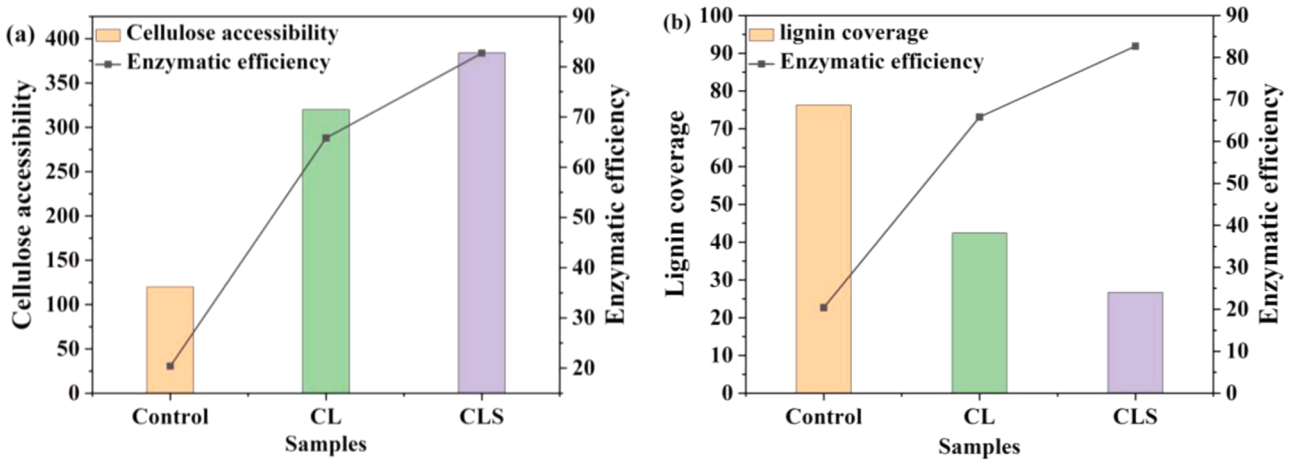

Fig. 7. (a) Enzymatic hydrolysis efficiency and cellulose accessibility, (b) enzymatic hydrolysis efficiency and lignin surface coverage.

extracted by DESs has a higher molecular weight than MWL (Mw=6102), which is an inevitable condensation reaction in the process of lignin removal. However, it can also be found that the lignin obtained by adding salicylic acid treatment has a relatively low Mw (Mw=7246) and a lower molar-mass dispersity (ĐM) (ĐM =2.16) than that without salicylic acid treatment (Mw=7681 and ĐM =2.26).

To fully understand the delignification mechanism during the t-DESs fractionation process, we investigated the structural characterization of the extracted lignin. The chemical structure of the precipitated lignin was analyzed using 2D HSQC NMR, as shown in Fig. 5.

Compared with MWL, lignin retained its basic structural units after DESs treating. it indicated that the structural integrity of lignin did not change significantly during the delignification process. The reduction in signal intensity from the DESs lignin spectra corresponded to A_{α}, A_{γ}, and A'_{γ} structures suggested the breakage of β—O—4 linkages. The detected signals for B_{α} (β—5) and B_{β} (β—β) showed only slight changes, indicating the high stability of C—C bonds during the DESs fractionation process. The main mechanism of lignin removal by DESs fractionation is through the breakage of β—O—4 linkages under acidic conditions. The lignin structure obtained under these conditions typically undergoes significant changes, making it difficult to be utilized. Especially under high temperature and acidic conditions, the depolymerization of lignin is more serious (Zhang et al., 2017).

Compared to lignin macromolecules, salicylic acid, with its simple structure and small steric hindrance, can be used as a cation scavenger to combine with carbocations, retaining more β—O—4 bonds without affecting the lignin removal. As Table 2 shows that the content of β—O—4 bonds in MWL was 52.6 %, whereas it was only 28.3 % and 22.5 % in t-DESs lignin and DESs lignin, respectively. Notably, t-DESs retained a relatively more complete lignin structure while simultaneously achieving a higher lignin removal rate. The increase in the S/G ratio after DESs treatment indicated that G-type units were more susceptible to be removed, aligning with previous results (Wen et al., 2013). The functional group was characterized by quantitative ^{31}P NMR (Pu et al., 2011), and the results were presented in Table 3. Compared to MWL, the aliphatic -OH of both t-DESs and DESs lignin were significantly reduced, indicating that the aliphatic -OH was more easily oxidized under harsher conditions during the treatment processes. In addition, compared with MWL, the S-OH and G-OH in t-DESs and DESs lignin increased significantly. The increase in phenolic -OH content is mainly due to the cleavage of β—O—4 bond in the DESs treatment (Jiang et al., 2019). Compared with CL, CLS contains relatively more aliphatic -OH and fewer S-type phenolic -OH, indicating that the addition of salicylic acid had a certain protective effect on β—O—4 bond, further corroborating previous conclusions in 2D NMR (Su et al., 2021).

3.4. Enzymatic hydrolysis of substrates

The bio-transformation is an effective way to achieve high-value utilization of pretreated cellulose-rich substrate, therefore the enzymatic hydrolysis efficiency was further evaluated, and the results were shown in Fig. 6. The glucose conversion rate of the untreated poplar powder was only 20.4 % after 120 h of enzymatic hydrolysis, while the pretreated substrates using t-DESs and DESs fractionation showed significant growth for the glucose conversion rate. The enzymatic hydrolysis of the substrate from t-DESs fractionation reached 82.7 %, which was 16.9 % higher than that of the substrate treated (65.8 %) by DESs fractionation without salicylic acid. This result revealed that the fractionation processes broke the highly interconnected structure of cell wall via removing lignin and hemicelluloses, improving cellulose accessibility. This is attributed to the fact that t-DESs treatment removes most of the lignin and cellulose, exposing more cellulose to improve the accessibility of cellulose (Huang et al., 2019). In Fig. 7, it can be seen that with the increase of cellulose accessibility and the decrease of lignin surface coverage, the enzymatic hydrolysis efficiency was also significantly improved. The analysis of lignin and hemicellulose removal has been discussed in the Section 3.1. It was observed that the efficacy of enzymatic hydrolysis is positively correlated with the extent of hemicellulose and lignin removal. Thus, the decreased residual lignin content was beneficial for the enzymatic hydrolysis. In addition, the residual cellulose after DES treatment was characterized by X-ray photoelectron spectroscopy (XPS, see Fig.S1). It can be clearly seen that the carboxyl group content on the surface of residual cellulose increased significantly (0.71 mmol/g) after the DESs treatment. Especially, the carboxyl groups increased to 14.1 % (Ternary DES) from 7.1 % (Binary DES) (see Table S2). It indicated that DES-treated lignin had a higher better hydrophobicity (Nakagame et al., 2011), which will significantly reduce the non-productive combination of lignin and enzyme and improve the efficiency of enzymatic hydrolysis.

4. Conclusion

Salicylic acid, as a lignin-like small molecule monomer, was introduced into DESs fractionation system, a new type of choline chloride-lactic acid-salicylic acid ternary DESs (t DESs) was synthesized and applied to the separation of main biopolymers of biomass to promote the subsequent enzymatic conversion of cellulose. Compared with the traditional binary DESs treatment, t-DESs fractionation system could obtain a higher removal rate of lignin and hemicellulose, as well as a higher purity cellulose residue. Importantly, a relatively complete lignin structure was remained and 28.3 % of the β—O—4 substructure was remained in t-DESs lignin, which could reduce the non-productive

adsorption with enzyme and further enhance the saccharification rate of cellulose. Compared with the substrate without salicylic acid treatment, the enzymatic saccharification rate of substrates increased from 65.8 % to 82.7 %.

CRediT authorship contribution statement

Menghua Qin: Writing – review & editing, Supervision. **Yongchao Zhang:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Xiaoqian Chen:** Supervision, Software, Conceptualization. **Shuzhen Ni:** Supervision, Resources, Investigation, Formal analysis. **Chunlin Xu:** Writing – review & editing, Resources, Funding acquisition, Data curation. **Yingjuan Fu:** Writing – review & editing, Resources. **Hongyu Zhang:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Ruijie Wu:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.120489.

Data availability

Data will be made available on request.

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