

Impacts of hydrogen bond donor structure in phenolic aldehyde deep eutectic solvent on pretreatment efficiency

Jiae Ryu¹, Mairui Zhang¹, Yunxuan Wang², Ruoqian Li¹, Kwang Ho Kim³, Arthur J.

Ragauskas^{2,4,5}, Gyu Leem^{6,7}, Min Bum Park^{1,8}, Chang Geun Yoo^{1,6*}

¹ Department of Chemical Engineering, State University of New York Environmental Science and Forestry, Syracuse, NY 13210, United States

² Department of Chemical & Biomolecular Engineering, University of Tennessee-Knoxville, Knoxville, TN 37996, United States

³ Department of Wood Science, University of British Columbia, Vancouver BC V6T 1Z4, Canada

⁴ Joint Institute for Biological Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States

⁵ Center of Renewable Carbon, Department of Forestry, Wildlife, and Fisheries, University of Tennessee Institute of Agriculture, Knoxville, TN 37996, United States

⁶ Institute for Sustainable Materials and Manufacturing, Syracuse, NY 13210, United States

⁷ Department of Chemistry, State University of New York Environmental Science and Forestry, Syracuse, NY 13210, United States

⁸ Department of Energy and Chemical Engineering, Incheon National University, Incheon 22012, Republic of Korea

*Corresponding author: cyoo05@esf.edu

KEYWORDS: phenolic aldehyde, deep eutectic solvent (DES), biorefinery

ABSTRACT

As a rising green solvent for biomass processing, deep eutectic solvents (DESs) have shown their effectiveness in biomass processing. In this study, phenolic aldehydes with different numbers of methoxy groups, including 4-hydroxybenzaldehyde (HBA, no methoxy), vanillin (VA, monomethoxy), and syringaldehyde (SA, dimethoxy), that can be derived from the lignin and were employed to synthesize DES with choline chloride (ChCl). The presence of methoxy groups in the hydrogen bond donor structure affected DES properties as well as biomass pretreatment performance. The high thermal stability of phenolic aldehyde DESs was shown with over 225 °C onset temperature. The hydrogen bond donor with one aldehyde and one hydroxyl group at the para position without methoxy group (ChCl-HBA) showed the highest xylan removal and delignification, reaching 59.3% and 88.0%, respectively, leading to the highest enzymatic hydrolysis yield. Sonication after pretreatment further enhanced the hydrolysis yields, achieving 83.3% of glucan conversion and 50.1% of xylan conversion. In the lignin-rich fraction, the recovered lignin showed low weight-average molecular weight under 2000 g/mol with relatively uniform molecular weight dispersity below 1.5. This study provides insights into how the chemical structure of hydrogen bond donors in DESs affects biomass processing and paves the way for designing an effective lignin-derived DES in future biorefinery processes.

1. Introduction

Due to the shortage of resources and environmental pollution caused by petroleum usage, the utilization of lignocellulosic biomass has been introduced as a potential solution for those global challenges.^{1,2} The application of lignocellulosic biomass to replace fossil resources enables carbon neutrality and reduces the emission of greenhouse gases.³⁻⁵ However, the intrinsic recalcitrance

factors of biomass have to be overcome for industrial utilization.⁶ Various biomass pretreatment methods have been designed to reduce the recalcitrance factors, including physical, chemical, biological, and their combinations.⁷ Depending on the target products and biomass species, different pretreatment approaches have been selected and optimized. Moreover, recent studies not only aim to maximize biomass conversion efficiency but also emphasize cradle-to-grave life cycle assessment; therefore, the greenness of processing solvents and operating conditions have to be considered. For these reasons, various biomass-derived solvents have been investigated for biorefinery processes.^{8,9}

Deep eutectic solvents (DESs) emerged as a new type of biomass processing solvent, composed of hydrogen bond acceptor and hydrogen bond donor via sharing bonding interactions between molecules.¹⁰ Various types of hydrogen bonding, such as ionic, doubly ionic, and neutral in diverse constituents, offer the solvent with different physicochemical and thermodynamic properties.¹¹ The formation of DES is achieved by a significant depression of the melting point or glass transition temperature via an enthalpy change of mixture.^{12,13} The DESs have been considered as alternative conventional ionic liquids (ILs), which have already proven to be effective biomass processing solvents, with cheaper components and simpler synthesis methods.¹⁴ The combinations of DESs are countless and lead to achieving a wide range of uses. Especially natural deep eutectic solvents (NADESs) or renewable DESs, formed with nature-derived resources for both hydrogen bond acceptor and donor, have been highlighted and applied to biomass pretreatment.^{9,15,16} A wide variety of chemicals, including lactic acid, glycolic acid, oxalic acid, malic acid, guaiacol, *p*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, vanillin, catechol, 4-hydroxybenzyl alcohol, and *p*-coumaric acid have also been investigated in DES pretreatment studies as a hydrogen bond donor.¹⁷⁻²³ As for hydrogen bond acceptors, ChCl, a naturally available quaternary ammonium salt

with a cation and chloride anion has been widely used, and other natural components have been introduced to investigate an ideal DES combination for biomass pretreatment using betaine, L-carnitine, and allyltrimethylammonium chloride with various types of hydrogen bond donors.²³⁻²⁵ Among them, lignin-derived DESs have been introduced as renewable DESs, which could be achieved from the biorefinery process, leading to a sustainable closed-loop biorefinery using a generated aromatic compound during processing.²¹

The strategy of green chemical media from lignin for biomass processing has been studied to improve sustainability and accomplish the circular economy of biorefinery processes.^{21, 26} Lignin can be obtained from plant cell walls by biomass pretreatments and then processed further to form several phenolic compounds.²⁷⁻²⁹ These phenolic compounds-based DESs have been pretreated with several biomass feedstocks in their optimum processing conditions.^{19-21, 30, 31} Also, the different molar ratios and combinations of lignin-derived DESs were compared to evaluate the pretreatment efficiency. Kim et al. tested several phenolic compounds including vanillin, catechol, 4-hydroxybenzyl alcohol, and *p*-coumaric acid synthesized with *ChCl* for DES formation and compared their biomass pretreatment performance.²¹ Wang et al. prepared 4-hydroxybenzylaldehyde DES and compared the performance of biomass pretreatment with *p*-hydroxybenzoic acid and *p*-coumaric acid.³² Also, Zhou et al. reviewed and summarized the efficiency of DESs on biomass pretreatment with many combinations of hydrogen bond acceptors and donors.³³ However, these were applied to different biomass feedstock and conducted under different processing conditions; therefore, it is difficult to elucidate the impact of HBD's chemical structure on DES performance directly.

In this study, phenolic aldehydes having different functional groups were tested for DES formation as well as biomass pretreatment. In specific, the effects of methoxy groups in lignin-

derived phenolic aldehydes on biomass pretreatment were investigated, to represent the main aromatic units derived from lignin resources such as syringyl, guaiacyl, and *p*-hydroxyphenyl units. The phenolic aldehydes can easily be produced by oxidative depolymerization of lignin.³⁴ Also, a closed-loop process was identified using phenolic aldehyde DESs, part of which could be derived from aldehyde-rich engineered biomass through hydrothermal depolymerization.^{15, 29} Therefore, 4-hydroxybenzaldehyde (HBA, no methoxy), vanillin (VA, monomethoxy), and syringaldehyde (SA, dimethoxy), differing in the number of methoxy groups, were selected as hydrogen bond donors for DES formation with ChCl as a hydrogen bond acceptor. The thermophysical properties of DESs have been rarely discussed, influencing the performance of biomass pretreatment as a new processing chemical. While understanding the formation, and thermal characteristics of DESs will be crucial for biomass processing.³⁵ The thermal properties of the DESs were studied, and the biomass pretreatment efficiencies were characterized based on factors like chemical composition and enzymatic hydrolysis yields. This study aims to establish a fundamental understanding of DES formation and pretreatment performance based on the chemical structure changes of hydrogen bond donors with different methoxy groups. This information can be applied to advanced computational tools like machine learning for designing promising lignin-derived DESs for future biorefinery processes.

2. Materials and Methods

2.1. Materials

Sugar maple was obtained from forest properties of the State University of New York College of Environmental Science and Forestry (SUNY ESF). The biomass was Wiley-milled and screened to 20 mesh size for characterization and pretreatment. The chemical compositions of sugar maple including glucan (45.2%), xylan (23.2%), Klason lignin (24.1%), ash (1.6%), and extractives

(0.9%) were characterized based on the NREL procedure.³⁶ ChCl (98%) and VA (99%) were purchased from Sigma-Aldrich and Acros, respectively. HBA (98%) and SA (98%) were obtained from Alfa Aesar. Avicel®, enzyme (Cellic CTec2), sodium acetate trihydrate (99%), and sodium azide (99%) were purchased from Sigma-Aldrich. All other chemicals were purchased from VWR and used without further purification.

2.2. DES preparation

The phenolic aldehyde DESs were synthesized using ChCl as a hydrogen bond acceptor with three different hydrogen bond donors, HBA, VA, and SA, in a 1:2 molar ratio, respectively. The choline chloride and phenolic aldehyde were loaded in the 150 mL Pyrex bottle and blended with a vortex mixer until they were mixed homogeneously. ChCl-HBA and ChCl-VA DES were formed at 100 °C for 30 minutes, and ChCl-SA DES was prepared at 160 °C for 4 hours in an oil bath with magnetic stirring. The formed DESs were stored in a vacuum chamber with silica gel at room temperature to prevent moisture absorption.

2.3. Biomass pretreatment

Sugar maple was soaked in the prepared DES at 1:10 solid-to-liquid ratio (w/w) and pretreated at 160 °C for 3 hours with 500 rpm stirring. The reaction condition was determined from our previous study.³² Once the pretreatment was finished, the reaction was quenched with ethanol and water solution (1:1, v/v) and cooled down at room temperature. The pretreated biomass was vacuum filtered with qualitative P2 grade filter paper. The solid residue was further washed with ethanol and water solution until the filtrate turned colorless and transparent. The post-processing of the pretreated biomass was conducted by overnight soaking followed by 5 times of individual sonication (3 minutes per time) in the ethanol and water solution (1:1, v/v) to avoid heating the solution.

The liquid-liquid extraction was applied to the liquid fractions after DES pretreatment to recover the lignin through membrane filtration. The liquid fraction after pretreatment was blended with water and ethyl acetate in a Pyrex bottle. The recovered lignin was obtained from ethyl acetate phase through membrane filtration using an Amicon stirred ultrafiltration cell (UFSC20001, Amicon Corporation) with a 1 kDa regenerated cellulose membrane disc. The liquid was stirred at 100 rpm to prevent the filter cake formation, and pressure inside the stirrer was controlled to 20 psi with nitrogen gas. The remained lignin on the membrane filter was air-dried and stored for further analysis.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of untreated and pretreated biomass was performed at 1 wt% of solid loadings under the condition of pH 4.95, with sodium acetate buffer (50 mM) and sodium azide (0.02%). The enzyme loading was 15 FPU/g of biomass. The enzymatic hydrolysis was carried out in an incubator with a shaking speed of 150 rpm at 50 °C for 72 hours. A 1 mL of liquid aliquot was taken from flasks at 6, 12, 24, 48, and 72 hours and heated at 95 °C for 5 minutes to quench the enzymatic reactions. After quenching, the hydrolysates were filtered through a 0.22 µm nylon filter for the HPLC analysis.

The glucan and xylan conversion were calculated as follows:

$$\text{Glucan conversion} = \frac{\text{Total release glucose (g)} \times 0.9}{\text{Theoretical amount of glucan in biomass (g)}} \times 100$$

$$\text{Xylan conversion} = \frac{\text{Total release xylose (g)} \times 0.88}{\text{Theoretical amount of xylan in biomass (g)}} \times 100$$

Here, the conversion factors of glucose to glucan and xylose to xylan were 0.9 and 0.88, respectively.

The inhibition effect of each DES component on enzymatic hydrolysis was tested with Avicel using different concentrations of phenolic aldehydes and ChCl. Avicel was loaded at 1 wt% in 50 mL reaction volume. ChCl, HBA, VA, and SA were added to the solution at different concentrations (0.0025, 0.005, 0.01, and 0.02%). Other factors, including sodium acetate buffer concentration (pH 5.0), sodium azide concentration, and enzyme loading were the same as the hydrolysis reaction for pretreated biomass. The hydrolysates of untreated and pretreated biomass were tested by the HPLC as mentioned above.

2.5. Characterizations of phenolic aldehyde DESs

2.5.1. Thermal properties of the phenolic aldehyde DESs

The formation of eutectics was confirmed by characterizing the thermal behavior of each DES by a differential scanning calorimeter (DSC, TA Instrument Q200). The DES samples (3–5 mg, completely dried) were loaded into a T-zero hermetic pan and sealed with lid for DSC testing. The analysis was conducted from -80 to 60 °C with a ramping rate of 3 °C/minute in two sequential heating–cooling cycles, purging with 50 mL/minute of nitrogen purging. The isothermal was applied for 2 minutes to each stage except at the end of cycle 1, maintaining at -80 °C for 60 minutes.

The thermogravimetric analysis (TGA, TA Instrument Q500) was also conducted to measure the thermostability of the synthesized DESs. The prepared DESs were stored in a vacuum container with silica gels before measurement. Approximately 5 to 10 mg of samples were placed on the T-zero hermetic pan with a pin-hole lid to prevent unexpected weight changes due to the vaporization of liquid while the heat increased. The samples were heated to 600 °C at 10 °C/minute under the nitrogen atmosphere with a nitrogen flow rate of 60 mL/minute.

2.5.2. Fourier transform infrared (FT-IR) spectroscopy

The pure chemicals of DES constituents and prepared DESs were scanned by Fourier transform infrared/near-infrared spectrometry (FT-IR/NIR Spectrometer, PerkinElmer) with 32 scans and recorded between a wavelength range between 4000 cm^{-1} and 600 cm^{-1} . The baseline was corrected by employing the software (PerkinElmer Spectrum Version 10.4.3).

2.6. Analytical methods

2.6.1. Chemical compositional analysis

The compositional measurement of pretreated biomass was performed according to the National Renewable Energy Laboratory standard protocol.³⁶ To measure the chemical composition of untreated and pretreated biomass, in brief, 0.3 g of the oven-dried weight of the sample was placed in 3 mL of 72% sulfuric acid. The mixture was placed in a 30 °C water bath for 1 hour and stirred with a glass rod. Then, the acid concentration was diluted to 4% diluted with additional deionized water into a 125 mL Pyrex bottle and the mixture was heated at 121 °C for 1 hour in an autoclave. The hydrolysate was filtered through a glass filtering crucible, and the weight of Klason lignin was measured by gravimetrically considering the weight of the remained ash in the residues by heating at 575 °C in a muffle furnace. The measurement of sugar composition in the filtrate was carried out with a high-performance liquid chromatography (HPLC, Agilent Technologies 1260 Infinity) equipped with a Biorad Aminx HPX-87H column and a RefractoMax 520 refractive index (RI) detector. The liquid was filtered with a 0.2 μm nylon syringe filter before analysis. All tests were duplicated.

2.6.2. Scanning electron microscopy (SEM) analysis

To explore the morphology of untreated and pretreated biomass, a scanning electron microscopy (SEM) analysis was conducted. The samples were vacuum-dried prior to SEM analysis. All samples were coated with gold and palladium on metal stubs with carbon conductive

tape using a Denton Vacuum sputter coater. The SEM images were taken using a JEOL-JSM-IT100LA Scanning electron microscope. The electron gun parameters were set as follows: 10 kV and 70 pA.

2.6.3. Cellulose degree of polymerization

Cellulose tricarbanilate was prepared from untreated and DES-pretreated biomass to evaluate the cellulose degree of polymerization.^{32, 37} The biomass sample (0.3 g) was treated with 6 mL of 25 wt% peracetic acid aqueous solution at 25 °C for 24 hours. Then deionized water was used to wash the peracetic acid-treated holocellulose until the pH of the filtrate reached neutral. The air-dried holocellulose was treated with 17.5 wt% of sodium hydroxide aqueous solution at 25 °C for 2 hours. The concentration of alkaline was subsequently dropped to 8.75% by adding the deionized water into the reaction bottle and the mixture was stirred at 25 °C for another 2 hours. The α -cellulose was obtained by centrifugation and washed with 1% peracetic acid and deionized water, followed by freeze-drying. The cellulose tricarbanilation was conducted by mixing 15 mg of α -cellulose and 0.5 mL of phenyl isocyanate in 4 mL of pyridine. The mixture was stirred at 70 °C for 24 hours and a further 24 hours with an additional 0.5 mL of phenyl isocyanate. The cellulose derivate was regenerated by dropping the mixture slowly into a 70% methanol aqueous solution. The cellulose tricarbanilate was washed with a methanol-water mixture and deionized water and dried under vacuum at 40 °C for 6 hours. Before testing, the cellulose derivate was dissolved in tetrahydrofuran (THF) and filtered into a 2 mL HPLC vial through a 0.45 μ m syringe PTFE filter. The molecular weight was analyzed with the Agilent gel permeation chromatography (GPC) SECurity 1200 system with RI and a UV detector. The molecular weights of tested cellulose were measured to calculate DP_w and DP_n . The repeating unit of tricarbanilated cellulose is 519 g/mol.

2.7. Characterization of recovered lignin

The structural characterization of recovered lignin was performed using two-dimensional heteronuclear single quantum coherence nuclear magnetic resonance (2D HSQC NMR) analysis using a Bruker AVANCE III HD 800 MHz NMR equipped with a TCI cryoprobe. The recovered lignin (~30 mg) was dissolved in 0.6 mL of deuterated dimethylsulfoxide (DMSO- d_6) and loaded in an NMR tube for analysis. The processing of the hsqcetgpsp pulse program was controlled with acquisition parameters with 32 scans and 1 s of relaxation delay, with time domains of 1024 data points in the F1 (^{13}C) and 1024 data points in the F2 (^1H) dimensions. The spectral widths were 219 and 12 ppm in the F1 (^{13}C) and F2 (^1H), respectively.

Recovered lignin was acetylated to measure the weight-average (M_w) and number-average (M_n) molecular weights, and dispersity ($\mathcal{D}$). The lignin was acetylated in pyridine/acetic anhydride (1:1, v/v) with continuous stirring for 24 hours followed by the previous study.³² The acetylated lignin was recovered by rotary evaporation, dissolved in THF, and placed into the 2mL HPLC vial after being filtered through 0.45 μm syringe PTFE filters. The molecular weight was determined by an Agilent GPC SECurity 1200 system with four Waters Styragel columns (HR 1, HR 3, and HR 4E) and a Waters 2489 UV/Vis detector. The data processing was conducted with Breeze software. The cellulolytic enzyme lignin (CEL) was isolated as a native lignin from untreated sugar maple by ball milling and enzymatic hydrolysis followed by dioxane extraction, according to the previous study.³⁸

3. Results and Discussion

3.1. Effects of hydrogen bond donor structures on phenolic aldehyde DESs formation

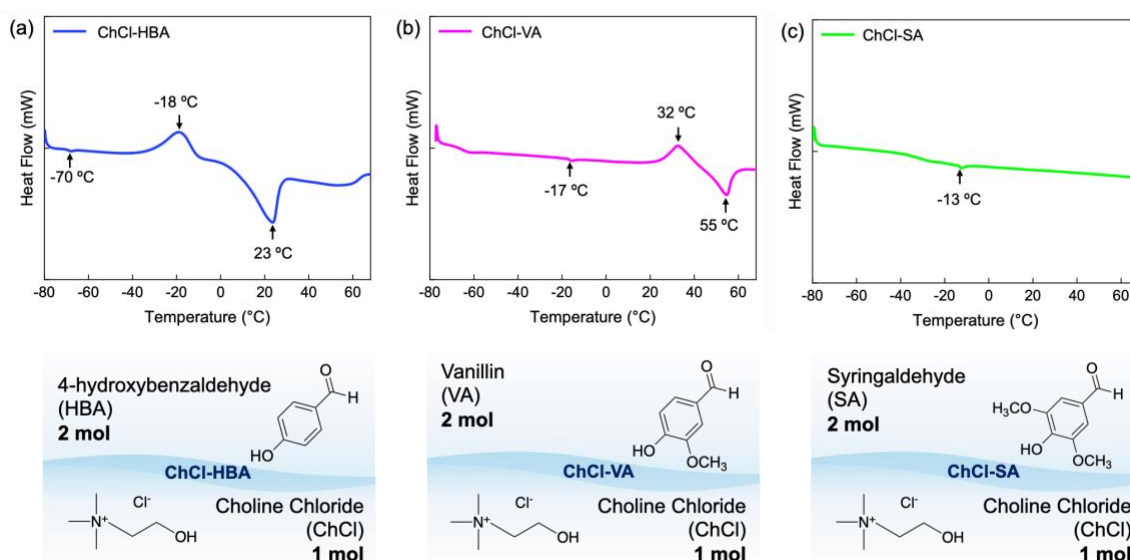


Figure 1. Phase transition temperatures of (a) ChCl-HBA, (b) ChCl-VA, and (c) ChCl-SA.

The DES can be formed via melting temperature depression in a eutectic mixture, with significantly lower thermal behavior of pure ingredients.¹² As lignin-based DESs, previous studies reported that HBA and VA could form clear and homogeneous liquids with ChCl.^{21, 32} The formation of phenolic aldehyde DESs was tested with the molar ratios of ChCl to phenolic aldehydes from 1:1 to 1:4. Homogeneous liquids were observed only at a 1:2 molar ratio for ChCl-HBA DES, 1:2 and 1:3 for ChCl-VA DES, and 1:2 to 1:4 for ChCl-SA DES (**Figure S1**). Since all three DESs formed at a 1:2 molar ratio, the phenolic aldehyde DESs at this ratio were used as biomass processing solvents to compare the performance of pretreatment in this study.

The formation of deep eutectic solvents can also be verified with their thermodynamic behavior. The phase transition temperatures, including glass transition temperature (T_g), cold crystallization temperature (T_{cc}), and melting temperature (T_m), of each DES at a 1:2 molar ratio are presented in **Figure 1** and **Table S1**. The results were reported after heating-cooling-heating cycles to remove the thermal history³⁹ of three types of DESs shown in **Figure S2**. The second heating cycle provided a better thermal transition diagram than the first heating. The formation of

DES was confirmed by comparing the T_m of the eutectic mixture with its pure chemical components. According to the literature, T_m of ChCl, HBA, VA, and SA are 302 °C,⁴⁰ 119 °C,⁴¹ 82 °C,⁴² and 110 to 112 °C,⁴³ respectively. ChCl-HBA showed the lowest T_m at 23 °C, which was a significantly lower temperature than the pure ChCl and HBA. ChCl-VA showed a higher phase transition temperature than ChCl-HBA but still had a lower T_m (55 °C) than the pure chemical components of the DES. It was difficult to observe the T_m of ChCl-SA and this DES only exhibited a T_g in the temperature range of DSC operation between -80 and 70 °C. The T_g of ChCl-HBA, ChCl-VA, and ChCl-SA were -70, -17, and -13 °C, respectively.

To support the interaction in phenolic aldehyde DES, the FT-IR analysis of prepared DES and its pure chemicals was conducted (**Figure S3**). All three types of DESs had strong broad bands ranging from 3200–3600 cm^{-1} , implying the presence of hydrogen bonds between ChCl and hydrogen bond donors.⁴⁴ The O—H stretching vibration of ChCl showed at 3221 cm^{-1} , and the peaks of phenolic aldehydes at 3160, 3162, and 3257 cm^{-1} were HBA, VA, and SA, respectively. Wang et al. also reported the O—H stretching vibration of DESs depends on its intermolecular interaction between the acceptor and donor. The interaction (O—H $\cdots$ Cl) increases bond length, resulting in the red shifts when tested with DESs composed of ChCl and diols (1,2-butanediol, 1,3-butanediol, 1,4-butanediol, and 2,3-butanediol) as hydrogen bond donor.⁴⁵ In phenolic aldehyde DESs, the evident peak between the O—H stretching vibration region was observed at 3158 cm^{-1} in ChCl-HBA DES, 3168 cm^{-1} in ChCl-VA, and 3238 cm^{-1} in ChCl-SA DES. Compared to the O—H stretching vibration frequency of ChCl, the higher red shifts were observed in ChCl-HBA followed by ChCl-VA, and ChCl-SA. It implies the hydrogen bond interaction between the DES constituents can be decreased with additional numbers of methoxy groups. In brief, these results indicate that phenolic aldehyde DESs were successfully prepared, and the different

thermodynamic behaviors and hydrogen bond interaction of the phenolic aldehyde eutectic mixtures were affected by the number of methoxy groups in their chemical structure. This difference in thermodynamic behavior plays an important role in explaining the difference in each DES performance in the following sections.

3.2. Thermal stability of phenolic aldehyde DESs as a biomass processing solvent

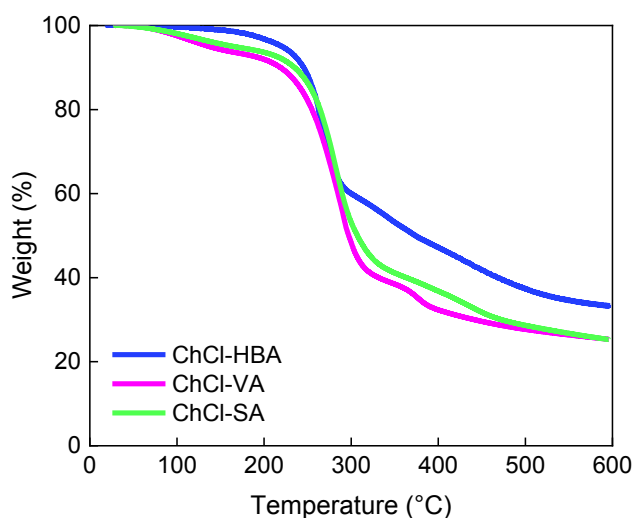


Figure 2. Thermal degradation behaviors of phenolic aldehyde DESs.

Table 1. Onset temperature of phenolic aldehyde DESs and their pure chemicals.

DES	T _{onset} (°C)	Hydrogen bond acceptor	T _{onset} (°C)	Hydrogen bond donor	T _{onset} (°C)
ChCl-HBA	240	ChCl	293	HBA	145
ChCl-VA	225			VA	135
ChCl-SA	242			SA	177

The thermal degradation behavior of the processing solvent is critical to determine the biomass pretreatment conditions. It is related to the feasible reaction temperature range as well as the thermal stability of the solubilized biomass components during the processing, influencing the quality of the fractionated components such as lignin. The thermal stability of DES is known to be

comparable with ionic liquids, enabling high processing temperatures with low vapor pressure.⁴⁶ The interactions between the hydrogen bond acceptor and donor are crucial for the thermal stability of DESs.⁴⁴ Herein, the onset decomposition temperatures of the phenolic aldehyde DESs were measured by TGA to investigate the thermal stability of phenolic aldehyde DESs (**Figures 2** and **S4** and **Table 1**). All three DESs showed onset decomposition temperatures over 225 °C, indicating their high thermal stability. In previous studies, the onset decomposition temperature of ChCl-urea DES (1:2 mol/mol) was reported to be 173 °C, and ChCl-phenylpropionic acid (1:2 mol/mol) was 156 °C.⁴⁷ Compared to those other DESs, the phenolic aldehyde DESs applied in this study have a broader processing temperature range, up to around 225 °C.

The onset decomposition temperatures of phenolic aldehyde DESs were notably different depending on the chemical structure of their hydrogen bond donors. ChCl-VA showed the lowest onset decomposition temperature among the three types of DESs at 225 °C, followed by ChCl-HBA (240 °C) and ChCl-SA (242 °C). Compared with the thermal degradation behavior of the pure chemicals, the studied phenolic aldehyde DESs showed enhanced thermal stability (**Table 1** and **Figure S4**). The lowest onset temperature of the hydrogen bond donor was 135 °C (VA), while the highest temperature was 177 °C (SA), leading to the highest thermal stability of ChCl-SA among the three DESs. Still, the onset temperatures of ChCl-HBA, ChCl-VA, and ChCl-SA were lower than the temperature of ChCl measured at 293 °C in this work. However, unlike pure ChCl which fully decomposed at 311 °C, the prepared DESs maintained over 40% of their weight over 300 °C. In particular, ChCl-HBA showed higher thermal stability during elevated temperatures up to 600 °C. One of the strong interactions, hydrogen bonding in DES can explain this high thermal stability.^{48, 49} ChCl-HBA preserved 60% of its weight at 300 °C and 33% was retained at 600 °C,

which was higher than the other two DESs. This result indicates that ChCl-HBA has the strongest hydrogen bonding among the three DESs.

3.3. Effects of phenolic aldehyde DES pretreatments on chemical composition of sugar

maple

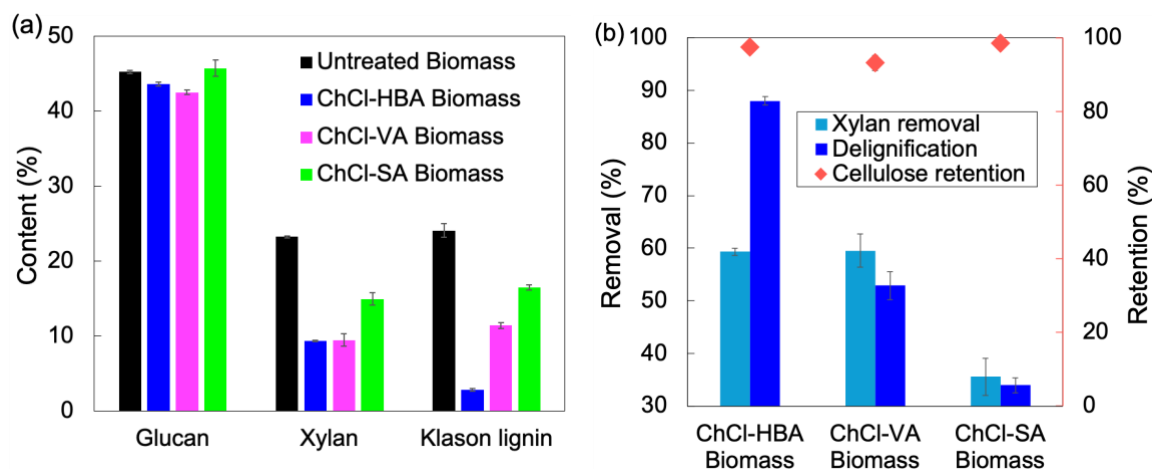


Figure 3. (a) Chemical composition of untreated and pretreated sugar maple and (b) cellulose retention, removals of lignin and xylan using ChCl-HBA, ChCl-VA, and ChCl-SA. *Note.* Chemical composition was calculated based on the dried weight of untreated sugar maple.

Herein, phenolic aldehyde DESs were used as a renewable processing solvent to improve fermentable sugar production from sugar maple. The pretreatments were conducted using the three DESs, ChCl-HBA, ChCl-VA, and ChCl-SA, at 160 °C for 3h. Each pretreatment performance was compared to elucidate the impacts of the chemical structure of phenolic aldehydes as a hydrogen bond donor. The chemical composition of untreated sugar maple was 45.2% glucan, 23.2% xylan, 24.1% Klason lignin, 1.6% ash, and 0.9% extractives. **Figure 3a** and **Table S2** show the changes in the chemical composition of sugar maple by each DES pretreatment. Interestingly, the methoxy groups in the phenolic aldehyde hydrogen bond donors resulted in different lignin content of pretreated biomass. ChCl-HBA, which has no methoxy groups on the hydrogen bond donor,

resulted in the lowest lignin contents of 2.9%, followed by ChCl-VA, and ChCl-SA DES pretreated biomass containing 11.4% and 16.5%, respectively. Regarding the carbohydrate contents, ChCl-HBA and ChCl-VA pretreated biomass showed similar xylan contents of 9.4% and 9.5%, while ChCl-SA pretreated biomass had 15% xylan. However, ChCl-VA pretreated biomass had the lowest glucan content of 42.5%, followed by ChCl-HBA pretreated (43.6%) and ChCl-SA pretreated biomass (44.6%), implying slight degradation of cellulose structure during the DES pretreatments.

The cellulose retention, delignification, and xylan removal by phenolic aldehyde DES pretreatments are shown in **Figure 3b** and **Table S3**. Overall, 90% of cellulose was preserved after all three DES pretreatments. ChCl-VA pretreated biomass showed the lowest cellulose retention at 93.2%, while pretreatment with ChCl-HBA, and ChCl-SA retained 97.5% and 98.5% cellulose, respectively. The xylan removal and delignification of ChCl-HBA from sugar maple were 59.3% and 88.8%, respectively. A similar amount of xylan was removed from sugar maple using ChCl-VA, which has one methoxy group in the hydrogen bond donor structure but it showed lower delignification than ChCl-HBA did. ChCl-SA, which has two methoxy groups in the hydrogen bond acceptor, resulted in the lowest pretreatment performance based on xylan and lignin removal, being 35.6% and 33.9%, respectively. Based on the FT-IR results in **Figure 3S**, the highest hydrogen bond interaction was estimated in ChCl-HBA DES, which has a hydroxyl group on phenolic aldehyde (no methoxy) as a hydrogen bond donor. Also, it showed the most effective pretreatment performance, removing lignin from the biomass with minimal cellulose loss. The additional methoxy groups to phenolic aldehyde hydrogen bond donors reduced the efficiencies of xylan removal and delignification during the pretreatment, with the decrease of hydrogen bond interactions compared to ChCl-HBA DES.

3.4. Effects of phenolic aldehyde DES pretreatments on morphological properties of sugar maple

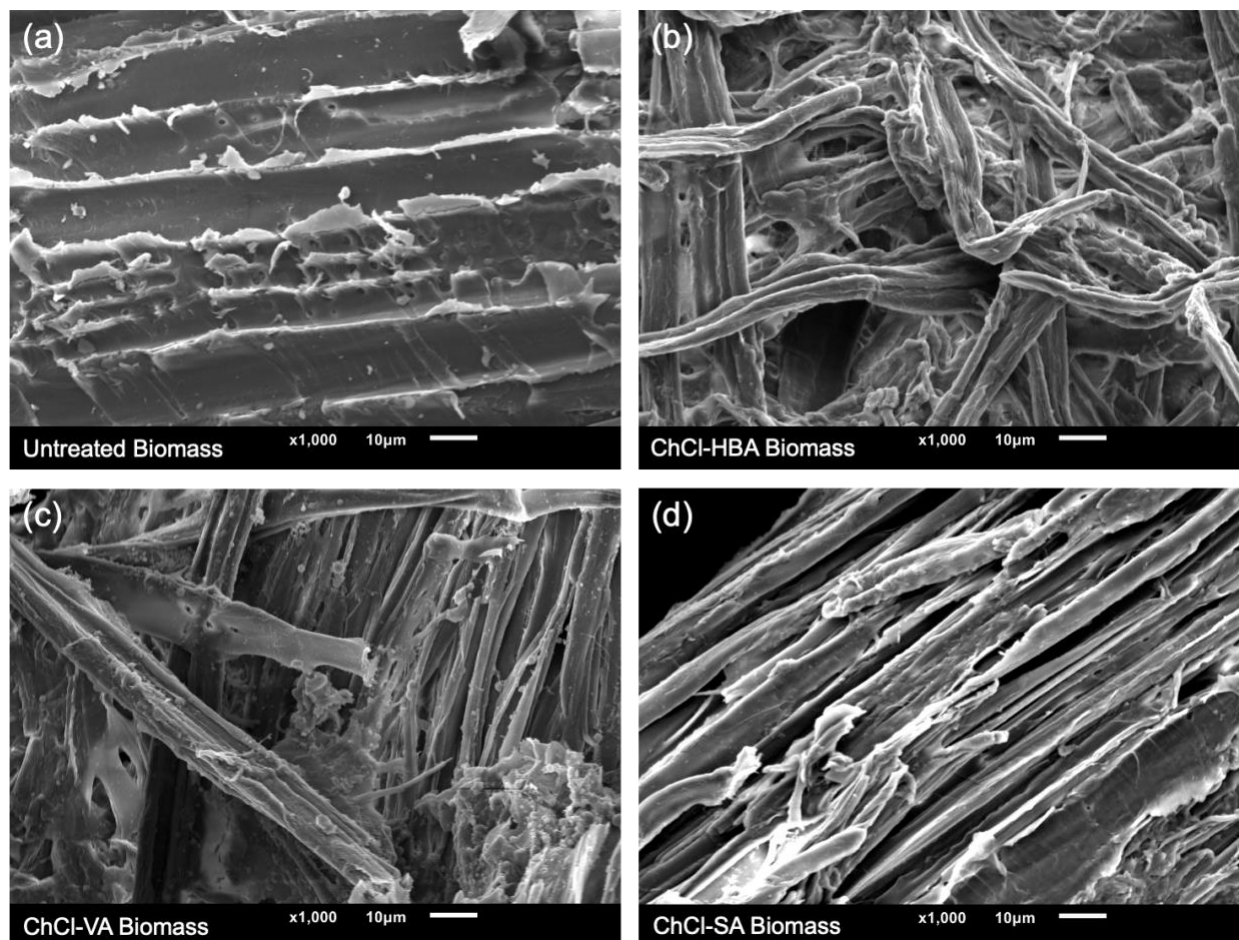


Figure 4. Morphological observation for (a) untreated biomass, pretreated sugar maple from (b) ChCl-HBA, (c) ChCl-VA, and (d) ChCl-SA.

Once the recalcitrance component such as lignin was removed from phenolic aldehyde DES pretreatment, the morphological changes were observed depending on the number of methoxy groups of phenolic aldehydes (**Figure 4**). The untreated biomass showed a packed arrangement. However, the pretreated biomass from ChCl-HBA DES disordered as individual fibers among the three types of hydrogen bond donors. During biomass processing, the removal of lignin and xylan reveals more cellulose structure and increases the surface area of biomass, increasing enzymatic accessibility and digestibility.⁵⁰ Surface morphologies of biomass showed differences depending

on the number the methoxy groups of hydrogen bond donors. ChCl-VA DES pretreated biomass showed both fiber bundles and some of isolated fibers. ChCl-SA pretreated biomass mostly showed the fibers bundles. The results suggested that the surface area is varying depending on the number of methoxy groups in phenolic aldehyde DESs, due to the performance of xylan and lignin extraction during the pretreatment.

3.5. The effects of phenolic aldehyde DESs on enzymatic hydrolysis of sugar maple

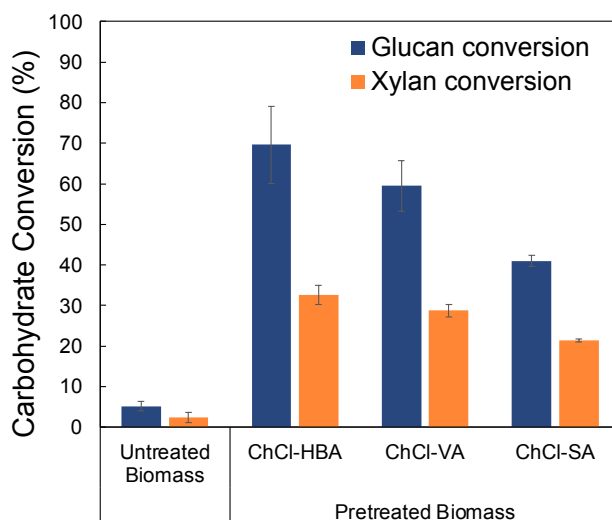


Figure 5. Glucan and xylan conversions of phenolic aldehyde DES pretreated sugar maple after 72 hours of enzymatic hydrolysis.

The enzymatic hydrolysis yield directly influences biomass utilization, such as biofuel production. The conversion efficiency of phenolic aldehyde DES pretreated sugar maple was evaluated by glucan and xylan conversions by enzymatic hydrolysis (**Figure 5** and **Table S4**). Glucan and xylan conversions of all three DESs pretreated biomass were higher than those of untreated sugar maple (5.2% and 2.4%, respectively). The carbohydrate conversion yield of ChCl-HBA pretreated biomass was the highest, followed by ChCl-VA, and ChCl-SA. ChCl-HBA pretreatment resulted in 69.6% glucan conversion and 32.6% xylan conversion after 72 hours of enzymatic hydrolysis. However, when the number of methoxy groups in the hydrogen bond donor

increased, the carbohydrate conversions of the DES pretreated biomass were decreased. Glucan conversion was 59.5% with ChCl-VA pretreated biomass and 41.0% of ChCl-SA one. Similarly, the xylan conversion of ChCl-HBA pretreated biomass was 32.6% after 72 hours of enzymatic hydrolysis, followed by ChCl-VA pretreated (28.8%) and ChCl-SA pretreated (21.4%). These results implied that the conversion of carbohydrates was influenced by the pretreatment solvents. However, as shown in **Figure S5**, the glucan conversion of ChCl-HBA pretreated biomass at 72 hours of enzymatic hydrolysis was only ~70%, and less than 30% of glucan was converted at 12 hours of enzymatic hydrolysis. To improve the digestibility of enzymatic hydrolysis, further processing for the pretreated biomass can be considered.

3.6. Effect of post-processing assisted by sonication with aldehyde DESs pretreated biomass

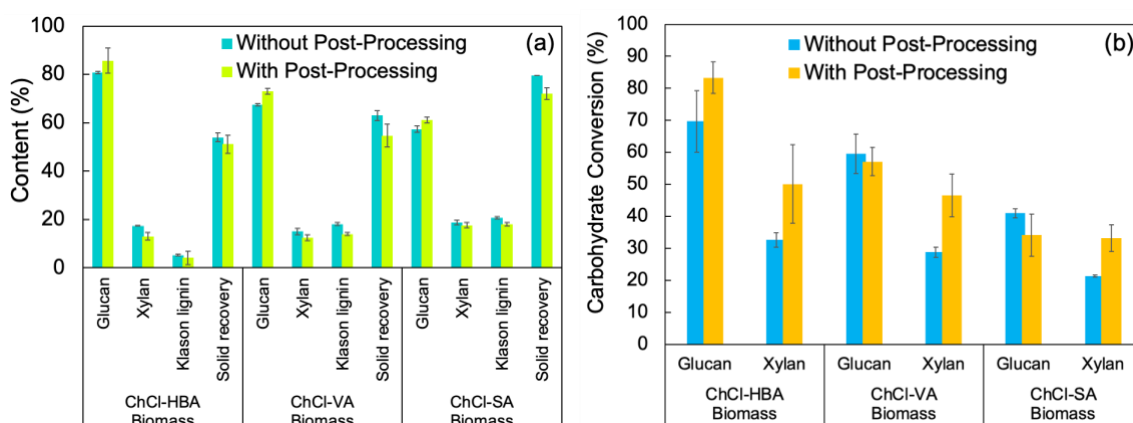


Figure 6. Changes of (a) chemical compositions of pretreated biomass and post-processed biomass and (b) their glucan and xylan conversions after 72 hours of enzymatic hydrolysis.

Although the applied DES pretreatments notably changed the chemical composition of sugar maple, as shown in **Figure 3** and **Tables S2** and **S3**, their enzymatic hydrolysis yields were not effectively improved (**Figures 5** and **S5** and **Table S4**). It was reported that the remaining DES in the biomass caused an inhibition effect on the enzymatic hydrolysis of biomass.⁵¹ Therefore, a post-processing step by sonication was carried out on the pretreated biomass to further reduce the recalcitrance. Overall, the glucan content increased, while xylan and lignin contents decreased

after the post-processing (**Figures 6a and S6 and Tables S5–S6**). The post-processed ChCl-HBA biomass showed 5.0% higher glucan content and 4.3% lower xylan contents than the corresponding pretreated biomass, without significant changes in lignin content. The post-processed ChCl-HBA also showed 13.7% higher glucan conversion and 17.5% improved xylan conversion after 72 hours of enzymatic hydrolysis (**Figure 6b and Tables S7**).

While the post-processed ChCl-VA biomass had 17.7% higher xylan conversion than the pretreated biomass, the glucan conversion was not significantly enhanced. In chemical composition, the post-processed ChCl-VA biomass had 5.6% higher glucan contents, 2.6% lower xylan content, and 4.1% lower lignin content than the pretreated biomass. ChCl-SA pretreated biomass also showed small changes in chemical composition after post-processing, with 3.7% higher glucan contents, while the 1.2% xylan and 2.6% lignin content decreased. The inhibition effect on the enzymatic hydrolysis of biomass can be caused by various factors, such as remaining DES and its components.⁵¹ Therefore, glucan digestibility of cellulose (i.e., Avicel) was tested with different concentrations of hydrogen bond acceptor and donor, to understand the inhibitory effects of the DES constituents (**Figure S8**). Results showed that even a small amount of VA, and SA reduced the glucan conversion, while ChCl and HBA resulted in similar glucan conversion at 72 hours of enzymatic hydrolysis. Since the inhibitory effects of free HBA and ChCl on glucan digestibility were not significant, the enhanced glucan conversion of ChCl-HBA pretreated biomass with post-processing can be caused by other parameters.

3.7. Impacts of phenolic aldehyde DES pretreatments on cellulose degree of polymerization in sugar maple

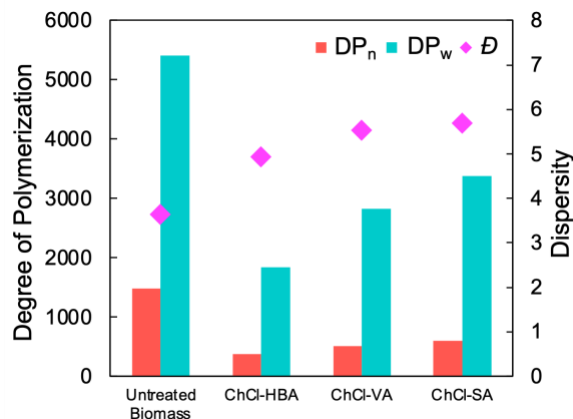


Figure 7. Number-average degree of polymerization (DP_n), weight-average degree of polymerization (DP_w), and dispersity (D) of cellulose in untreated and phenolic aldehyde DESs pretreated sugar maple.

While DES pretreatment primarily changed the chemical composition of sugar maple, it also affected the characteristics of cellulose in the biomass, potentially influencing the glucan conversion yield in enzymatic hydrolysis. Cellulose degree of polymerization has been reported as an indicator of the recalcitrance of biomass.^{32, 52} This information provides how much reducing ends are available in cellulose and shows a good correlation to glucose production.^{37, 52} **Figure 7** and **Table S8** show the DP_n , DP_w , and D of cellulose in the untreated and DES pretreated biomass to understand the effects of phenolic aldehydes on cellulose in sugar maple. The DP_w of untreated sugar maple cellulose was 5414 and its D was 3.6. The degree of cellulose polymerization decreased substantially after all three DES pretreatments. ChCl-HBA pretreatment resulted in the lowest cellulose DP_w (1834). ChCl-VA and ChCl-SA also decreased cellulose DP_w to 2829 and 3376, respectively. These results indicated that the phenolic aldehyde DESs not only affected delignification but also had interactions with cellulose, resulting in different cellulose DP.

3.8. Structural properties of recovered lignin from phenolic aldehyde DES pretreatment

Table 2. Number-average molecular weight (M_n), weight-average molecular weight (M_w), and dispersity ($\bar{D}$) of recovered lignin after phenolic-aldehyde DESs pretreatment.

Lignin	M_n (g/mol)	M_w (g/mol)	$\bar{D}$
CEL	1468	4339	2.9
ChCl-HBA Lignin	1482	2077	1.4
ChCl-VA Lignin	966	1356	1.4
ChCl-SA Lignin	1040	1594	1.5

In addition to the abovementioned fermentable sugars, fractionated lignin can also be used for the production of sustainable biofuels and biochemicals through several approaches such as hydrothermal carbonization, pyrolysis, biomass liquefaction, and gasification.⁵³ For instance, Lyu et al. extracted lignin from the willow using a ChCl-lactic acid DES and then processed it further via pyrolysis.⁵⁴ The authors reported that the relatively low molecular weight of lignin (1810–1040 g/mol) would facilitate lignin intermolecular linkage cleavage during pyrolysis and result in a high yield of phenol and guaiacol production. This implies that the characteristics of the recovered lignin, like molecular weight, are critical factors for its valorization. **Table 2** presents the number-average molecular weight (M_n), weight-average molecular weight (M_w), and dispersity ($\bar{D}$) of phenolic aldehyde DES-extracted lignins and CEL. The M_w of CEL was 4339 g/mol and its $\bar{D}$ was 2.9. After the pretreatment, the M_w of lignin decreased substantially, ranging from 1500 to 2100 g/mol, while the dispersity decreased to 1.4–1.5, indicating improved homogeneity. Among the three phenolic aldehyde DESs, ChCl-VA resulted in the lowest M_w of lignin of 1356 g/mol, followed by ChCl-SA lignin (1594 g/mol) and ChCl-HBA lignin (2077 g/mol). It is interesting to note that ChCl-HBA, with no methoxy groups in the phenolic aldehyde hydrogen bond donor structure, resulted in the most significant delignification (**Figure 3**) while generating lignin with

the highest molecular weight. ChCl-HBA predominantly cleaved β -O-4 linkages (**Figure S9**), potentially causing condensation between the extracted lignin fragments, leading to the higher molecular weight, related to the amount of C-O-C linkages in recovered lignin.⁵⁵ ChCl-VA and ChCl-SA, however, resulted in lower β -O-4 linkage cleavage (**Figure S9**). The evaluation of the chemical structural properties in lignin such as inter-unit linkages and aromatic units is available. The remained hydrogen bond donors in the recovered lignin can be extracted through simple hydrothermal treatment and recycled in a biorefinery system. Wang et al. have synthesized lignin-based DES consisting of *p*-hydroxybenzoic acid as hydrogen bond donor and successfully provide ways to obtain sustainable biorefinery processes.³² Also, many studies have been trying to verify the lignin modification in material applications (e.g., phenolation).^{56, 57} Furthermore, the incorporation of functional groups like phenolic aldehydes benefits application in various ways, such as lignin-containing hydrogel, composite, and polymer.⁵⁸ In the aromatic region of the HSQC spectra, the DES fractionated lignin showed some interactions with applied hydrogen bond donors. However, further study is needed to explain their interactions and potential applications (**Figures S10 and S11**).

Conclusion

Three phenolic aldehyde DESs, ChCl-HBA, ChCl-VA, and ChCl-SA, were prepared to understand the impact of the methoxy group on DES formation and biomass pretreatment. The formation of DESs was confirmed with the significantly lowered phase transition temperature of ChCl-HBA, ChCl-VA, and ChCl-SA compared with their individual components. The studied phenolic aldehyde DESs showed over 225 °C of onset thermal degradation temperature, making them biomass processing solvents with a wide range of operational temperatures. Pretreatment with ChCl-HBA resulted in the highest xylan removal and delignification, and the lowest cellulose

DP_w, leading to 83.4% glucan and 50.1% xylan conversion yields. The presence of methoxy groups in hydrogen bond donors negatively influenced biomass pretreatment and fermentable sugar production. These results provide insight into the impact of functional groups of hydrogen bond donor structures on biomass pretreatment using DES, which would facilitate understanding the performance during the pretreatment process and the exploitation of DES as a designer solvent.

AUTHOR INFORMATION

Corresponding Authors

Chang Geun Yoo- Department of Chemical Engineering, State University of New York Environmental Science and Forestry, Syracuse, NY 13210, United States; <https://orcid.org/0000-0002-6179-2414>; Email: cyoo05@esf.edu

Authors

Jiae Ryu- Department of Chemical Engineering, State University of New York Environmental Science and Forestry, Syracuse, NY 13210, United States; <https://orcid.org/0009-0001-2875-2023>

Mairui Zhang- Department of Chemical Engineering, State University of New York Environmental Science and Forestry, Syracuse, NY 13210, United States; <https://orcid.org/0000-0001-6565-0571>

Yunxuan Wang- Department of Chemical & Biomolecular Engineering, University of Tennessee-Knoxville, Knoxville, TN 37996, United States; <https://orcid.org/0000-0001-8095-0484>

Ruoqian Li- Department of Chemical Engineering, State University of New York Environmental Science and Forestry, Syracuse, NY 13210, United States

Kwang Ho Kim- Department of Wood Science, University of British Columbia, Vancouver BC V6T 1Z4, Canada; <https://orcid.org/0000-0003-3943-1927>

Arthur J. Ragauskas- Department of Chemical & Biomolecular Engineering, University of Tennessee-Knoxville, Knoxville, TN 37996, United States; <https://orcid.org/0000-0002-3536-554X>

Gyu Leem- Department of Chemistry, State University of New York Environmental Science and Forestry, Syracuse, NY 13210, United States; <https://orcid.org/0000-0003-0169-1096>

Min Bum Park- Department of Energy and Chemical Engineering, Incheon National University, Incheon 22012, Korea; <https://orcid.org/0000-0003-0788-1130>

ACKNOWLEDGMENT

This project was supported by the USDA National Institute of Food and Agriculture, McIntire Stennis project 7002095 and the National Science Foundation grant CBET 2239299.

Supporting Information Available: additional figures, and tables including in the result and discussion section, images of phenolic aldehyde DESs mixture (Figure S1), heating-cooling-heating cycled thermogram of phase transition of phenolic aldehyde DESs (Figure S2), FT-IR spectrum of phenolic aldehyde DESs (Figure S3), thermal degradation behaviors of individual DESs constituents (Figure S4), enzymatic hydrolysis of phenolic aldehyde DES pretreated biomass for 72 hours (Figure S5), chemical compositions and their retention and removal of phenolic aldehyde DES pretreated biomass (Figure S6), enzymatic hydrolysis of phenolic aldehyde DES pretreated biomass with post-processing for 72 hours (Figure S7), chemical compositions and their retention and removal of phenolic aldehyde DES pretreated biomass with post-processing (Figure S8), aliphatic region of 2D HSQC NMR spectra of CEL and recovered lignins (Figure S9), aromatic region of 2D HSQC NMR spectra of CEL and recovered lignins (Figure S10), 2D HSQC of pure phenolic aldehyde (Figure S11), phase transition temperatures of

the aldehyde DESs (Table S1), chemical compositions of untreated and DES pretreated sugar maple (Table S2), cellulose retention and removal of xylan and lignin of pretreated sugar maple (Table S3), glucan and xylan conversions of untreated and pretreated biomass after 72h of enzymatic hydrolysis (Table S4), chemical composition of untreated, pretreated, and post-processed sugar maple (Table S5), cellulose retention and removal of xylan and lignin of pretreated sugar maple with post processing (Table S6), glucan and xylan conversions of untreated and pretreated biomass with post-processing, after 72h of enzymatic hydrolysis (Table S7), degree of polymerization of cellulose in untreated and phenolic aldehyde DES pretreated sugar maple (Table S8) (PDF)

REFERENCES

1. Zhao, L.; Sun, Z.-F.; Zhang, C.-C.; Nan, J.; Ren, N.-Q.; Lee, D.-J.; Chen, C., Advances in pretreatment of lignocellulosic biomass for bioenergy production: Challenges and perspectives. *Bioresour. Technol.* **2022**, *343*, 126123.
2. Singh, N.; Singhania, R. R.; Nigam, P. S.; Dong, C.-D.; Patel, A. K.; Puri, M., Global status of lignocellulosic biorefinery: Challenges and perspectives. *Bioresour. Technol.* **2022**, *344*, 126415.
3. Zhong, L.; Wang, C.; Xu, M.; Ji, X.; Yang, G.; Chen, J.; Janaswamy, S.; Lyu, G., Alkali-catalyzed organosolv pretreatment of lignocellulose enhances enzymatic hydrolysis and results in highly antioxidative lignin. *Energy Fuels* **2021**, *35* (6), 5039-5048.
4. Zhao, J.; Lee, J.; Weiss, T.; Wang, D., Technoeconomic analysis of multiple-stream ethanol and lignin production from lignocellulosic biomass: insights into the chemical selection and process integration. *ACS Sustainable Chem. Eng.* **2021**, *9* (40), 13640-13652.
5. Alper, K.; Tekin, K.; Karagöz, S.; Ragauskas, A. J., Sustainable energy and fuels from biomass: a review focusing on hydrothermal biomass processing. *Sustainable Energy Fuels* **2020**, *4* (9), 4390-4414.
6. Yoo, C. G.; Meng, X.; Pu, Y.; Ragauskas, A. J., The critical role of lignin in lignocellulosic biomass conversion and recent pretreatment strategies: A comprehensive review. *Bioresour. Technol.* **2020**, *301*, 122784.
7. Yang, M.; Gao, X.; Lan, M.; Dou, Y.; Zhang, X., Rapid fractionation of lignocellulosic biomass by *p*-TsOH pretreatment. *Energy fuels* **2019**, *33* (3), 2258-2264.
8. Bharadwaj, A. S.; Dev, S.; Zhuang, J.; Wang, Y.; Yoo, C. G.; Jeon, B.-H.; Aggarwal, S.; Park, S. H.; Kim, T. H., Review of chemical pretreatment of lignocellulosic biomass using low-liquid and low-chemical catalysts for effective bioconversion. *Bioresour. Technol.* **2023**, *368*, 128339.

9. Meng, X.; Wang, Y.; Conte, A. J.; Zhang, S.; Ryu, J.; Wie, J. J.; Pu, Y.; Davison, B. H.; Yoo, C. G.; Ragauskas, A. J., Applications of biomass-derived solvents in biomass pretreatment—Strategies, challenges, and prospects. *Bioresour. Technol.* **2023**, *368*, 128280.
10. Xu, P.; Zheng, G.-W.; Zong, M.-H.; Li, N.; Lou, W.-Y., Recent progress on deep eutectic solvents in biocatalysis. *Bioresour. Bioprocess.* **2017**, *4*, 1-18.
11. Ashworth, C. R.; Matthews, R. P.; Welton, T.; Hunt, P. A., Doubly ionic hydrogen bond interactions within the choline chloride–urea deep eutectic solvent. *Phys. Chem. Chem. Phys.* **2016**, *18* (27), 18145-18160.
12. Andruch, V.; Makoś-Chelstowska, P.; Plotka-Wasyłka, J., Remarks on use of the term “deep eutectic solvent” in analytical chemistry. *Microchem. J.* **2022**, *179*, 107498.
13. Yu, D.; Xue, Z.; Mu, T., Eutectics: formation, properties, and applications. *Chem. Soc. Rev.* **2021**, *50* (15), 8596-8638.
14. Vanda, H.; Dai, Y.; Wilson, E. G.; Verpoorte, R.; Choi, Y. H., Green solvents from ionic liquids and deep eutectic solvents to natural deep eutectic solvents. *C.R. Chim.* **2018**, *21* (6), 628-638.
15. Wang, Y.; Kim, K. H.; Jeong, K.; Kim, N.-K.; Yoo, C. G., Sustainable biorefinery processes using renewable deep eutectic solvents. *Curr. Opin. Green Sustainable Chem.* **2021**, *27*, 100396.
16. Mamilla, J. L.; Novak, U.; Grilc, M.; Likozar, B., Natural deep eutectic solvents (DES) for fractionation of waste lignocellulosic biomass and its cascade conversion to value-added bio-based chemicals. *Biomass Bioenergy* **2019**, *120*, 417-425.
17. Satlewal, A.; Agrawal, R.; Bhagia, S.; Sangoro, J.; Ragauskas, A. J., Natural deep eutectic solvents for lignocellulosic biomass pretreatment: recent developments, challenges and novel opportunities. *Biotechnol. Adv.* **2018**, *36* (8), 2032-2050.
18. Huang, C.; Cheng, J.; Zhan, Y.; Liu, X.; Wang, J.; Wang, Y.; Yoo, C. G.; Fang, G.; Meng, X.; Ragauskas, A. J., Utilization of guaiacol-based deep eutectic solvent for achieving a sustainable biorefinery. *Bioresour. Technol.* **2022**, *362*, 127771.
19. Wang, Y.; Meng, X.; Tian, Y.; Kim, K. H.; Jia, L.; Pu, Y.; Leem, G.; Kumar, D.; Eudes, A.; Ragauskas, A. J., Engineered Sorghum Bagasse Enables a Sustainable Biorefinery with *p*-Hydroxybenzoic Acid-Based Deep Eutectic Solvent. *ChemSusChem* **2021**, *14* (23), 5235-5244.
20. Wang, Y.; Ryu, J.; Kim, K. H.; Meng, X.; Pu, Y.; Tian, Y.; Eudes, A.; Leem, G.; Ragauskas, A. J.; Yoo, C. G., Investigation of the effects of ternary deep eutectic solvent composition on pretreatment of sorghum stover. *AIChE J.* **2023**, *69* (12), e18227.
21. Kim, K. H.; Dutta, T.; Sun, J.; Simmons, B.; Singh, S., Biomass pretreatment using deep eutectic solvents from lignin derived phenols. *Green Chem.* **2018**, *20* (4), 809-815.
22. Yu, H.; Xue, Z.; Shi, R.; Zhou, F.; Mu, T., Lignin dissolution and lignocellulose pretreatment by carboxylic acid based deep eutectic solvents. *Ind. Crops Prod.* **2022**, *184*, 115049.
23. Han, H.; Chen, L.; Zhao, J.; Yu, H.; Wang, Y.; Yan, H.; Wang, Y.; Xue, Z.; Mu, T., Biomass-based acidic deep eutectic solvents for efficient dissolution of lignin: Towards performance and mechanism elucidation. *Acta Phys.-Chim. Sin* **2023**, *39*, 2212043.
24. Perkins, S. L.; Painter, P.; Colina, C. M., Experimental and computational studies of choline chloride-based deep eutectic solvents. *J. Chem. Eng. Data* **2014**, *59* (11), 3652-3662.

25. Hou, X.-D.; Li, A.-L.; Lin, K.-P.; Wang, Y.-Y.; Kuang, Z.-Y.; Cao, S.-L., Insight into the structure-function relationships of deep eutectic solvents during rice straw pretreatment. *Bioresour. Technol.* **2018**, *249*, 261-267.
26. Kim, K. H.; Yoo, C. G., Challenges and perspective of recent biomass pretreatment solvents. *Front. Chem. Eng.* **2021**, *3*, 785709.
27. Alherech, M.; Omolabake, S.; Holland, C. M.; Klinger, G. E.; Hegg, E. L.; Stahl, S. S., From lignin to valuable aromatic chemicals: Lignin depolymerization and monomer separation via centrifugal partition chromatography. *ACS Cent. Sci.* **2021**, *7* (11), 1831-1837.
28. Zhou, N.; Thilakarathna, W. W.; He, Q. S.; Rupasinghe, H. V., A review: depolymerization of lignin to generate high-value bio-products: opportunities, challenges, and prospects. *Front. Energy Res.* **2022**, *9*, 758744.
29. Kim, K. H.; Eudes, A.; Jeong, K.; Yoo, C. G.; Kim, C. S.; Ragauskas, A., Integration of renewable deep eutectic solvents with engineered biomass to achieve a closed-loop biorefinery. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116* (28), 13816-13824.
30. Kim, K. H.; Eudes, A.; Yoo, C. G.; Kim, C. S., Deep eutectic solvent pretreatment of transgenic biomass with increased C₆C₁ lignin monomers. *Front. Plant Sci.* **2020**, *10*, 505993.
31. Kim, K. H.; Mottiar, Y.; Jeong, K.; Tran, P. H. N.; Tran, N. T.; Zhuang, J.; Kim, C. S.; Lee, H.; Gong, G.; Ko, J. K., One-pot conversion of engineered poplar into biochemicals and biofuels using biocompatible deep eutectic solvents. *Green Chem.* **2022**, *24* (23), 9055-9068.
32. Wang, Y.; Meng, X.; Jeong, K.; Li, S.; Leem, G.; Kim, K. H.; Pu, Y.; Ragauskas, A. J.; Yoo, C. G., Investigation of a lignin-based deep eutectic solvent using *p*-hydroxybenzoic acid for efficient woody biomass conversion. *ACS Sustainable Chem. Eng.* **2020**, *8* (33), 12542-12553.
33. Zhou, M.; Fakayode, O. A.; Yagoub, A. E. A.; Ji, Q.; Zhou, C., Lignin fractionation from lignocellulosic biomass using deep eutectic solvents and its valorization. *Renewable Sustainable Energy Rev.* **2022**, *156*, 111986.
34. Ha, J.-M.; Hwang, K.-R.; Kim, Y.-M.; Jae, J.; Kim, K. H.; Lee, H. W.; Kim, J.-Y.; Park, Y.-K., Recent progress in the thermal and catalytic conversion of lignin. *Renewable Sustainable Energy Rev.* **2019**, *111*, 422-441.
35. Paiva, A.; Craveiro, R.; Aroso, I.; Martins, M.; Reis, R. L.; Duarte, A. R. C., Natural deep eutectic solvents—solvents for the 21st century. *ACS Sustainable Chem. Eng.* **2014**, *2* (5), 1063-1071.
36. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of structural carbohydrates and lignin in biomass*; NREL/TP-510-42618; National Renewable Energy Laboratory: Golden, Colorado, 2012
<https://www.nrel.gov/docs/gen/fy13/42618.pdf> (accessed 2024-07-09).
37. Meng, X.; Pu, Y.; Yoo, C. G.; Li, M.; Bali, G.; Park, D. Y.; Gjersing, E.; Davis, M. F.; Muchero, W.; Tuskan, G. A., An in-depth understanding of biomass recalcitrance using natural poplar variants as the feedstock. *ChemSusChem* **2017**, *10* (1), 139-150.
38. He, D.; Wang, Y.; Yoo, C. G.; Chen, Q.-J.; Yang, Q., The fractionation of woody biomass under mild conditions using bifunctional phenol-4-sulfonic acid as a catalyst and lignin solvent. *Green Chem.* **2020**, *22* (16), 5414-5422.
39. Nguyen, H. V. D.; De Vries, R.; Stoyanov, S. D., Natural deep eutectics as a “green” cellulose cosolvent. *ACS Sustainable Chem. Eng.* **2020**, *8* (37), 14166-14178.

40. Gilmore, M.; Swadzba-Kwasny, M.; Holbrey, J. D., Thermal properties of choline chloride/urea system studied under moisture-free atmosphere. *J. Chem. Eng. Data* **2019**, *64* (12), 5248-5255.
41. Nowak, P.; Sikorski, A., Structural diversity of cocrystals formed from acridine and two isomers of hydroxybenzaldehyde: 3-hydroxybenzaldehyde and 4-hydroxybenzaldehyde. *RSC adv.* **2023**, *13* (29), 20105-20112.
42. Costa, C. A.; Casimiro, F. M.; Manrique, Y. A.; Rodrigues, A. E., From sulfite liquor to vanillin: effect of crystallization process on the yield, purity, and polymorphic form of crystals. *Sep. Sci. Technol.* **2024**, 1-12.
43. Ibrahim, M. N. M.; Balakrishnan, R. S.; Shamsudeen, S.; Bahwani, S. A.; Adam, F., A concise review of the natural existence, synthesis, properties, and applications of syringaldehyde. *BioResources* **2012**, *7* (3), 4377-4399.
44. Zhu, S.; Li, H.; Zhu, W.; Jiang, W.; Wang, C.; Wu, P.; Zhang, Q.; Li, H., Vibrational analysis and formation mechanism of typical deep eutectic solvents: An experimental and theoretical study. *J. Mol. Graphics Modell.* **2016**, *68*, 158-175.
45. Wang, H.; Liu, S.; Zhao, Y.; Wang, J.; Yu, Z., Insights into the hydrogen bond interactions in deep eutectic solvents composed of choline chloride and polyols. *ACS Sustainable Chem. Eng.* **2019**, *7* (8), 7760-7767.
46. Yiin, C. L.; Yap, K. L.; Ku, A. Z. E.; Chin, B. L. F.; Lock, S. S. M.; Cheah, K. W.; Loy, A. C. M.; Chan, Y. H., Recent advances in green solvents for lignocellulosic biomass pretreatment: potential of choline chloride (ChCl) based solvents. *Bioresour. Technol.* **2021**, *333*, 125195.
47. Delgado-Mellado, N.; Larriba, M.; Navarro, P.; Rigual, V.; Ayuso, M.; García, J.; Rodríguez, F., Thermal stability of choline chloride deep eutectic solvents by TGA/FTIR-ATR analysis. *J. Mol. Liq.* **2018**, *260*, 37-43.
48. Liu, S.; Yu, D.; Chen, Y.; Shi, R.; Zhou, F.; Mu, T., High-resolution thermogravimetric analysis is required for evaluating the thermal stability of deep eutectic solvents. *Ind. Eng. Chem. Res.* **2022**, *61* (38), 14347-14354.
49. Zhang, K.; Hou, Y.; Wang, Y.; Wang, K.; Ren, S.; Wu, W., Efficient and reversible absorption of CO₂ by functional deep eutectic solvents. *Energy Fuels* **2018**, *32* (7), 7727-7733.
50. Li, P.; Zhang, Q.; Zhang, X.; Zhang, X.; Pan, X.; Xu, F., Subcellular dissolution of xylan and lignin for enhancing enzymatic hydrolysis of microwave assisted deep eutectic solvent pretreated *Pinus bungeana* Zucc. *Bioresour. Technol.* **2019**, *288*, 121475.
51. Sharma, V.; Nargotra, P.; Sharma, S.; Bajaj, B. K., Efficacy and functional mechanisms of a novel combinatorial pretreatment approach based on deep eutectic solvent and ultrasonic waves for bioconversion of sugarcane bagasse. *Renewable Energy* **2021**, *163*, 1910-1922.
52. Yoo, C. G.; Yang, Y.; Pu, Y.; Meng, X.; Muchero, W.; Yee, K. L.; Thompson, O. A.; Rodriguez, M.; Bali, G.; Engle, N. L., Insights of biomass recalcitrance in natural *Populus trichocarpa* variants for biomass conversion. *Green Chem.* **2017**, *19* (22), 5467-5478.
53. Li, C.; Zhao, X.; Wang, A.; Huber, G. W.; Zhang, T., Catalytic transformation of lignin for the production of chemicals and fuels. *Chem. Rev.* **2015**, *115* (21), 11559-11624.
54. Lyu, G.; Wu, Q.; Li, T.; Jiang, W.; Ji, X.; Yang, G., Thermochemical properties of lignin extracted from willow by deep eutectic solvents (DES). *Cellulose* **2019**, *26*, 8501-8511.
55. Shen, X.-J.; Wen, J.-L.; Mei, Q.-Q.; Chen, X.; Sun, D.; Yuan, T.-Q.; Sun, R.-C., Facile fractionation of lignocelluloses by biomass-derived deep eutectic solvent (DES)

pretreatment for cellulose enzymatic hydrolysis and lignin valorization. *Green chem.* **2019**, *21* (2), 275-283.

56. Jiang, X.; Liu, J.; Du, X.; Hu, Z.; Chang, H.-m.; Jameel, H., Phenolation to improve lignin reactivity toward thermosets application. *ACS Sustainable Chem. Eng.* **2018**, *6* (4), 5504-5512.

57. Bertella, S.; Luterbacher, J. S., Simultaneous extraction and controlled chemical functionalization of hardwood lignin for improved phenolation. *Green Chem.* **2021**, *23*(9), 3459-3467.

58. Yu, X.; Yang, B.; Zhu, W.; Deng, T.; Pu, Y.; Ragauskas, A.; Wang, H., Towards functionalized lignin and its derivatives for high-value material applications. *Ind. Crops Prod.* **2023**, *200*, 116824.

TOC

