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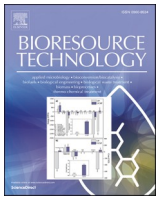

Efficient extraction of lignin from moso bamboo by microwave-assisted ternary deep eutectic solvent pretreatment for enhanced enzymatic hydrolysis

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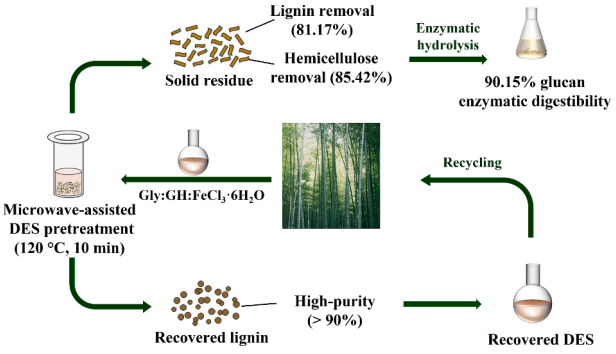
HIGHLIGHTS

- Novel ternary Lewis acid-based deep eutectic solvent (DES) systems were developed.
- Microwave-assisted DES pretreatment effectively removed lignin (81.17%) and hemicellulose (85.42%) from bamboo.
- Enzymatic digestibility after $FeCl_3$-based DES pretreatment was up to 90%.
- Recovered lignin had well-preserved structure and high purity (>90 %).
- Recyclability shown for DES systems supports sustainability for biomass refining.

GRAPHICAL ABSTRACT



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ABSTRACT

Applications of deep eutectic solvent (DES) systems to separate lignocellulosic components are of interest to develop environmentally friendly processes and achieve efficient utilization of biomass. To enhance the performance of a binary neutral DES (glycerol:guanidine hydrochloride), various Lewis acids (e.g., $AlCl_3{\cdot}6H_2O$, $FeCl_3{\cdot}6H_2O$, etc.) were introduced to synthesize a series of ternary DES systems; these were coupled with microwave heating and applied to moso bamboo. Among the ternary DES systems evaluated, the $FeCl_3$-based DES effectively removed lignin (81.17%) and xylan (85.42%), significantly improving enzymatic digestibility of the residual glucan and xylan (90.15% and 99.51%, respectively). Furthermore, 50.74% of the lignin, with high purity and a well-preserved structure, was recovered. A recyclability experiment showed that the pretreatment performance of the $FeCl_3$-based DES was still basically maintained after five cycles. Overall, the microwave-assisted ternary DES pretreatment approach proposed in this study appears to be a promising option for sustainable biorefinery operations.

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1. Introduction

Due to unprecedented environmental and energy concerns caused by continuous economic development, there is an urgent need to promote the utilization of chemicals and products derived from renewable resources (Sun et al., 2023). Lignocellulosic biomass is considered to have the most potential to replace fossil-based resources; its efficient development and application are needed to alleviate the current energy shortage and also address environmental problems (Zhai et al., 2020). Both the dense structure of crystalline cellulose and the presence lignin-carbohydrate complexes gives lignocellulosic biomass its high degree of recalcitrance, thereby inhibiting decomposition by enzymes and chemicals. The pretreatment of biomass is typically the first and most determinative operation in the integrated biorefinery process (Galbe et al., 2019; Zhang et al., 2023) and is used to improve the accessibility of cellulose and hemicellulose for producing chemicals and materials (Zhang et al., 2020b). In addition to the utilization of carbohydrates, deriving value from the lignin is also regarded as the key to achieving the economic and environmental feasibility of biorefineries (Mankar et al., 2022).

At present, the pretreatment methods mainly include mechanical, alkali, dilute acid, organic solvent, and ionic liquid treatments (Haldar et al., 2021). The practical application of each is subject to various advantages and shortcomings. Therefore, there still remains a need to explore environmentally-friendly alternatives for the pretreatment of lignocellulosic biomass. A deep eutectic solvent (DES) is a new type of solvent composed of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA), which are driven by hydrogen bonding, resulting in a melting point that is significantly lower than the melting points of its individual pure components. First proposed by Abbott et al. in 2003, DES pretreatments are relatively new, having almost all the advantages of ionic liquids (Saini et al., 2022). In addition, DES systems have the characteristics of low toxicity, low cost, biodegradability, easy synthesis, and recyclability. A large number of previous studies have found that DES systems can have an excellent ability to dissolve lignin from lignocellulosic biomass, and DES pretreatments can improve the yield of the subsequent enzymatic hydrolysis (Sharma et al., 2022).

A considerable number of different DES systems have been designed and investigated in previous studies, such as those synthesized using carboxylic acids, amines/amides, alcohols, and more. Polyol-based DES systems are among the most frequently studied DES systems in recent years. Despite this, numerous studies have reported that neutral DES systems synthesized with polyols as the HBD were unsatisfactory for removing lignin and promoting enzymatic hydrolysis of polysaccharides through the mild pretreatments (Cheng et al., 2022). Thus, harsh conditions appear to be required in the pretreatment process to achieve the desired results, including high temperatures or prolonged pretreatment times.

In recent years, the synthesis of ternary DES systems and their application in the pretreatment of lignocellulose have garnered widespread attention. For instance, excellent lignin removal and hydrolytic digestibility were obtained by adding Lewis acids to polyol-based DES systems to provide acidic conditions during pretreatment. However, choline chloride was mostly used as the HBA in polyol-based ternary DES systems; studies with other HBAs (e.g., guanidine hydrochloride), and their effects on the pretreatment of lignocellulosic biomass are still rare. Microwave-assisted DES systems have also been employed for biomass pretreatments, resulting in improved saccharification performance and lignin extraction (Zhang et al., 2020a). In addition to directly interacting with the polar molecules in the DES, microwave energy can selectively heat the polar portions of biomass. Meanwhile, it improves the interaction between the DES and lignin, and microwave-induced stirring and mixing at the molecular scale increases the mass transfer rate, allowing for easier extraction and dissolution of lignin (Zou et al., 2024).

In this study, a series of Lewis acids were used to synthesize glycerol-based ternary DES systems for the pretreatment of moso bamboo under microwave-assisted heating. The pretreatment effect of the DES was evaluated by measuring changes in chemical composition and enzymatic digestibility after pretreatment; the reasons for the varying effects of the DES pretreatments are discussed. The effects of Lewis acid dosage, temperature, and time on the performance of the optimal $FeCl_3$-based DES pretreatment were comprehensively investigated, as were the yield and purity of the recovered lignins obtained from using different DES systems. Finally, the recyclability of a $FeCl_3$-based DES was also assessed. Altogether, the results of this study provide valuable insight into the application of DES systems in the field of lignocellulosic biomass refining, thereby holding potential significance in enhancing both biomass conversion efficiency and promoting the sustainable utilization of biomass resources.

2. Materials and methods

2.1. Materials

Moso bamboo powder was provided by a processing factory in Sichuan Province, China. Before use, it was passed through a 40–60 mesh sieve, then dried overnight in an oven at 105 °C, placed in a sealed bag and stored until needed. $MnCl_2{\cdot}4H_2O$, $MgCl_2{\cdot}4H_2O$, $CrCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$ $FeCl_3{\cdot}6H_2O$, $AlCl_3{\cdot}6H_2O$, glycerol, and guanidinium chloride were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Acetone and H_2SO_4 were purchased by Nanjing Chemical Reagent Co., Ltd. The cellulase enzyme mixture (Cellic CTec3) was obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd., and the cellulase activity was determined as 220 FPU/mL.

2.2. Synthesis of ternary DES systems

The binary neutral DES (referred to as GG) was prepared by mixing glycerol (Gly) with guanidine hydrochloride (GH) in a molar ratio of 2:1, and was magnetically stirred in an oil bath at 80 °C until a clear and homogeneous solution was formed. Each Lewis acid-based ternary mixture, Gly:GH:Lewis acid (using $MnCl_2{\cdot}4H_2O$, $MgCl_2{\cdot}4H_2O$, $CrCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, $FeCl_3{\cdot}6H_2O$, or $AlCl_3{\cdot}6H_2O$) with a molar ratio of 2:1:0.1, was synthesized using the preparation method of the binary neutral DES mentioned above. The six synthesized DES systems were abbreviated as GG-Mn, GG-Mg, GG-Cr, GG-Cu, GG-Fe, and GG-Al, respectively. In addition, the synthesized Gly:GH:$FeCl_3{\cdot}6H_2O$ DES, with the molar ratio of 2:1:0.2, was abbreviated as GG-Fe-2. The mixtures were dried in a vacuum drying oven at 80 °C until a constant weight was achieved; free of moisture, the mixtures were stored in sealed containers.

2.3. Microwave-assisted DES pretreatment and lignin recovery

Microwave-assisted heating is characterized by immediacy, integrality, selectivity, safety, and high efficiency, which can obviously shorten the reaction time of the biomass refining process and improve the pretreatment efficiency (Zhang et al., 2020a). A 1:20 ratio (w/w) of biomass to DES was chosen mainly because of the viscous nature of the glycerol component in the DES. In preliminary experiments, we found both 1:15 and 1:10 biomass to DES ratios (w/w) would make it difficult for the solids and liquids to fully react, and would be prone to agglomeration in the rapid heating process using microwaves; also, the vacuum filtration and washing time would be prolonged after the pretreatment. This study was conducted using a microwave extraction system (Milestone Ethos Ex, Italy) equipped with fiber optic temperature probes (ATC-400FO, Milestone, Italy). The microwave system consists of ten Teflon vessels, each with a capacity of 100 mL; a magnetic stirrer provided constant mixing. The sample holder was rotated around the central axis of the turntable, which minimized temperature inconsistencies.

Thus, into 1.5 g of dry bamboo powder was mixed 30 g of DES in a 100 mL Teflon vessel with magnetic stirring that was then placed in the microwave extraction system. The temperature was raised to 120 °C using up to 350 W of power for 2 min and maintained at up to 150 W of power for 10 min (Fig. 1). In the process of heating and maintaining temperature, the wattage was automatically adjusted in the ranges of 0–350 W and 0–150 W, respectively, according to the programmed temperature curve. Each pretreatment condition was repeated 3 times in the microwave, and the preparation of different DES samples was repeated 3 times. The reported values were all averages. In the experiment whereby the conditions were varied, the reaction system was heated to 100–140 °C with microwave assistance and kept in this temperature range for 5–20 min under stirring.

Once the reaction was complete, the reaction vessel was removed and cooled to room temperature. Next, the reaction vessel contents were transferred to a 250 mL beaker containing 50 mL of acetone/water (1:1, v/v). The suspension was magnetically stirred for 2 h, then vacuum-filtered and subsequently washed with fresh acetone/water (v/v, 1:1) until the filtrate was colorless; the retentate was subsequently washed with distilled water until neutral. All filtrates were collected and then rotary evaporated at 50 °C under -0.1 MPa to remove the acetone. The volume of the DES solution was then adjusted to 500 mL with distilled water to partially precipitate the dissolved lignin. Finally, the precipitated lignin was separated by centrifugation (10278 × *g*, 5 min, at room temperature), washed three times with distilled water, and freeze-dried. The dried lignin was sealed in a sample vial and stored in a desiccator for further analysis. Following lignin recovery, the DES was recovered by rotary evaporation at 70 °C under -0.1 MPa, then placed in an 80 °C vacuum oven under -0.1 MPa for 12 h to remove the remaining moisture. The weight of collected DES was measured to calculate the recovery efficiency.

2.4. Chemical compositions of the pretreatment residues and recovered DES

The glucan, xylan and lignin contents of the raw bamboo, and pretreatment residues generated in this study, were analyzed according to the standard procedures from the National Renewable Energy Laboratory (NREL) of the US Department of Energy (Cassoni et al., 2022). Hydrolyses were carried out using 0.3 g sample and 3 mL 72 % H_2SO_4 introduced into a 100 mL flask. The mixture was kept in a water bath at approximately 30 °C for 1 h with stirring every 5–10 min. After cooling, the volume of the partially hydrolyzed sample was adjusted to 87 mL with distilled water. The diluted sample was heated in an autoclave for 1 h at 121 °C to complete sample hydrolysis. All analyses were conducted in triplicate. The acid-insoluble lignin was recovered by vacuum filtration, washed with distilled water, then dried and weighed to give a value for the amount of Klason lignin. All sugars were analyzed by high performance liquid chromatography system (HPLC) equipped with an Aminex HPX-87H column. The content of acid-soluble lignin was determined using an ultraviolet spectrometer (UV-2550, Shimadzu, Japan) at a wavelength of 205 nm at room temperature. The solid yield of pretreated bamboo, recovery yield of cellulose, removal of lignin and hemicellulose were calculated using Eqs. (1) to (4) below (Wang et al., 2018):

$$Solid\ \ yield\ (\%) = \frac{Pretreated\ bamboo\ (g)}{Raw\ bamboo\ (g)} \times 100 \quad (1)$$

$$Cellulose\ recovery\ yield\ (\%) = \frac{Cellulose\ in\ pretreated\ bamboo\ (g)}{Cellulose\ in\ raw\ bamboo\ (g)} \times 100 \quad (2)$$

$$Lignin\ \ removal\ (\%) = \left(1 - \frac{Lignin\ \ in\ \ pretreated\ \ bamboo(g)}{Lignin\ \ in\ \ raw\ \ bamboo(g)}\right) \times 100 \quad (3)$$

$$Hemicellulose\ \ removal\ (\%) = \left(1 - \frac{Hemicellulose\ \ in\ \ pretreated\ \ bamboo(g)}{Hemicellulose\ \ in\ \ raw\ \ bamboo(g)}\right) \times 100 \quad (4)$$

The sugars content in the recovered DES were analyzed by high performance liquid chromatography system (HPLC) equipped with an Aminex HPX-87H column.

2.5. Enzymatic hydrolysis

Raw bamboo and pretreatment residues were subjected to enzymatic hydrolysis in sodium citrate buffer (pH 4.8) at 5 % substrate concentration (w/v). The enzyme loading was 10 FPU/g substrate; all samples were hydrolyzed in a thermostatic shaking chamber at 50 °C and 150 rpm for 72 h. The supernatants were taken from the hydrolysis solution at regular intervals and analyzed by HPLC as described above to determine the amounts of released glucose and xylose.

2.6. Characterizations of pretreatment residues and recovered lignin

Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS) were employed for the analysis of the raw bamboo and pretreatment residues. A Quanta 200 microscope (FEI, USA) was employed for SEM analysis. The FT-IR analyses were conducted using a Nicolet 380 spectrometer (Thermo, USA) with 32 scans and a spectral resolution of 2 cm^{-1} over a spectral range of 4000 and 500 cm^{-1}; the sample percentage in KBr pellets was 1 %, and the prepared samples were analyzed in transmission (Wu et al., 2023). The XRD analysis was performed on an Ultima IV (Rigaku, Japan) instrument equipped with a D/teX-Ultra diffractometer. The measurements were executed in the 2θ range from 5° to 40° at a 5°/ min scanning rate. The crystallinity index (CrI) of each sample was calculated using the following equation:

$$CrI\ (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (5)$$

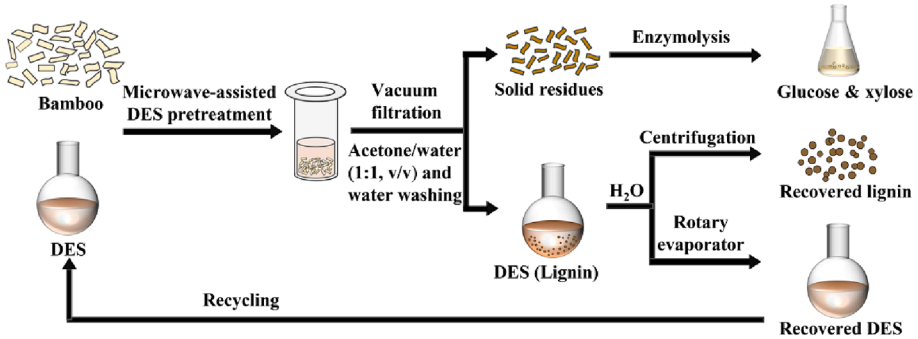

Fig. 1. The process flow chart of DES pretreatment.

where I_{am} is the diffraction intensity of amorphous cellulose at $2\theta \approx 18^{\circ}$ and I_{002} is the scattered intensity of crystalline cellulose peak at $2\theta \approx 22.5^{\circ}$ (Li et al., 2023). The XPS analysis was performed on an AXIS UltraDLD diffractometer (Shimadzu, Japan). The surface coverage by lignin (S_{lig}) and carbohydrates (S_{carb}) on the samples were calculated according to the average O/C values according to the following equations:

$$S_{lig}\ (\%) = \frac{O/C_{pretreated} - O/C_{carbohydrate}}{O/C_{lignin} O/C_{carbohydrate}} \times 100 \quad (6)$$

$$S_{carb} = -S_{lig} \quad (7)$$

where O/C_{lignin} is 0.33, $O/C_{carbohydrate}$ is 0.83.

The carbohydrate content of recovered lignin was analyzed using the standard procedures of NREL (Cassoni et al., 2022) to determine its purity. The classic method proposed by Björkman was used to prepare milled wood lignin (MWL) from raw bamboo (Björkman, 1956). The number average (Mn) and weight average (Mw) molecular weights of MWL and recovered lignins were determined using an Agilent GPC/HPLC 1200 system equipped with two Jordi Gel DVB HPLC columns in series. All lignin samples were acetylated before GPC analysis by dissolving 100 mg of lignin in 6 mL of acetic anhydride and pyridine (1:1, v/v) at room temperature. After continuous stirring for 48 h, the resulting acetylated lignins were recovered using a rotary evaporator, dissolved in THF and then analyzed by GPC (Wang et al., 2020a); tetrahydrofuran (THF) was used as the mobile phase with a flow rate of 1.0 mL/min. The chemical structure of recovered lignin was assessed by two-dimensional ^{13}C–^{1}H heteronuclear single quantum correlation nuclear magnetic resonance (2D-HSQC) using a Bruker Avance-400 spectrometer; samples were dissolved in DMSO-d_6 at 25°C.

2.7. Statistical analysis

All laboratory analyses were conducted in triplicate to ensure the reproducibility of our findings. All results are represented as 1 mean ± standard deviation (error bars) in the figures.

3. Results and discussion

3.1. Microwave-assisted pretreatment of bamboo by different DES systems

Different metal salts with different Lewis acidities were selected to synthesize a series of ternary DES systems; the influence the Lewis acid component on the pretreatment of the moso bamboo biomass was then investigated. Fig. 2a shows the solid yield and the chemical compositions of bamboo samples treated with the synthesized DES systems. The raw bamboo contained 39.59 % of cellulose, 19.95 % of hemicellulose, and 28.52 % of lignin. It can be seen that the solid yield decreased only slightly with the binary GG DES pretreatment, coinciding with the very limited removal of lignin and hemicellulose. Likewise, no obvious components change was observed after the GG-Mn and GG-Mg pretreatments. However, the pretreatments with GG-Cr, GG-Cu, GG-Fe, and GG-Al gave significantly lower solid yields of 55.15 %, 54.54 %, 53.97 %, and 47.55 %, respectively. For these four DES systems, increased lignin and hemicellulose removal were clearly observed, up to 80.84 % and 88.27 %, respectively; the majority of cellulose was retained as shown by the cellulose recovery yields. This may be due to the enhanced hydrogen bond acceptance and acidity of the four DES systems obtained with these particular Lewis acids (Xia et al., 2018). For all the ternary DES systems, the metal chloride salts (i.e., Cl^{-}) could provide active protons for the reactions and compete with guanidine hydrochloride for the formation of stronger hydrogen bonds to cleave the ether bonds in lignin-carbohydrate complexes (LCC) (Huo et al., 2023).

One of the main purposes of lignocellulosic biomass pretreatment is to break its tight physical and chemical structures and thereby promote the production of fermentable sugars through enzymatic hydrolysis (Xie et al., 2022). Accordingly, the enzymatic digestibility of the pretreated bamboo solid was evaluated. Fig. 3 shows the enzymatic digestibility of raw bamboo to be low as expected. Specifically, the values for glucan and xylan enzymatic digestibility after 72 h were 5.96 % and 4.02 %, respectively. These results are consistent with the component analyses in Fig. 2, and can be attributed to the recalcitrant cell wall structure (Pandey et al., 2014). There was no significant difference in enzymatic hydrolysis yield for the bamboo pretreated with GG, GG-Mn, and GG-Mg, which might be explained by the slight removal of lignin. However, the enzymatic hydrolysis efficiency was remarkably improved through the remaining four ternary DES systems (GG-Cr, GG-Cu, GG-Fe, and GG-Al), attributable to the relatively high delignification efficiencies shown in Fig. 2; the effective removal of lignin, and hemicellulose, increases the accessibility of the cellulose and thereby increases the fermentable sugar yields (Tang et al., 2023). Surprisingly, glucan and xylan enzymatic digestibility reached 79.12 % and 82.70 %, respectively, when using GG-Fe DES, which were both higher values than those obtained when the GG-Al DES (61.59 % and 60.64 %, respectively) was used.

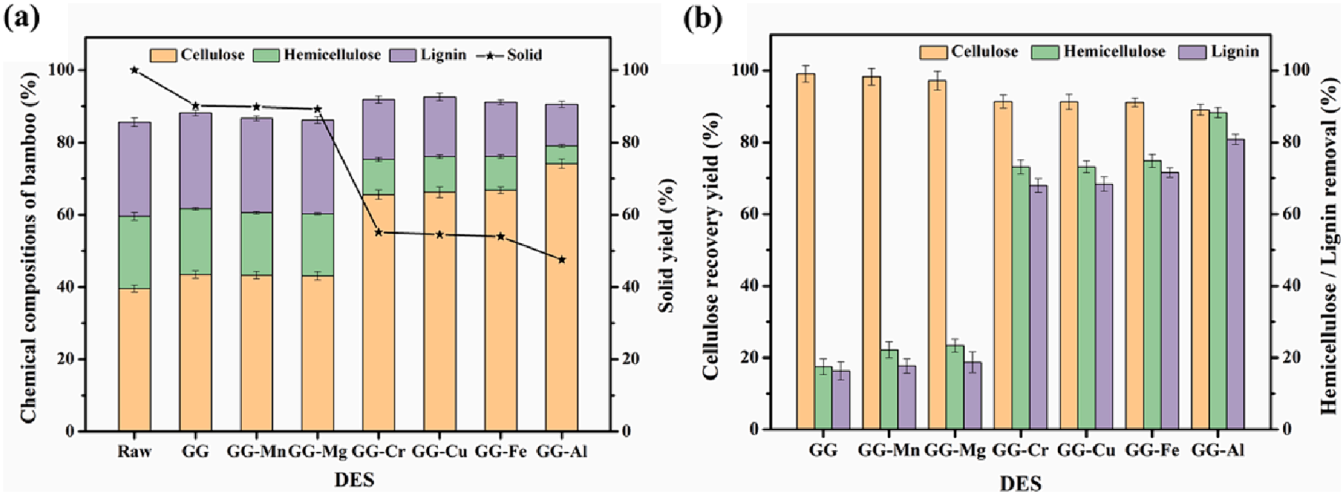

Fig. 2. (a) The solid yield, chemical compositions of raw bamboo and pretreatment residues, and (b) cellulose recovery, and hemicellulose and lignin removal of the pretreatment residues obtained using different DES systems (at 120 °C for 10 min).

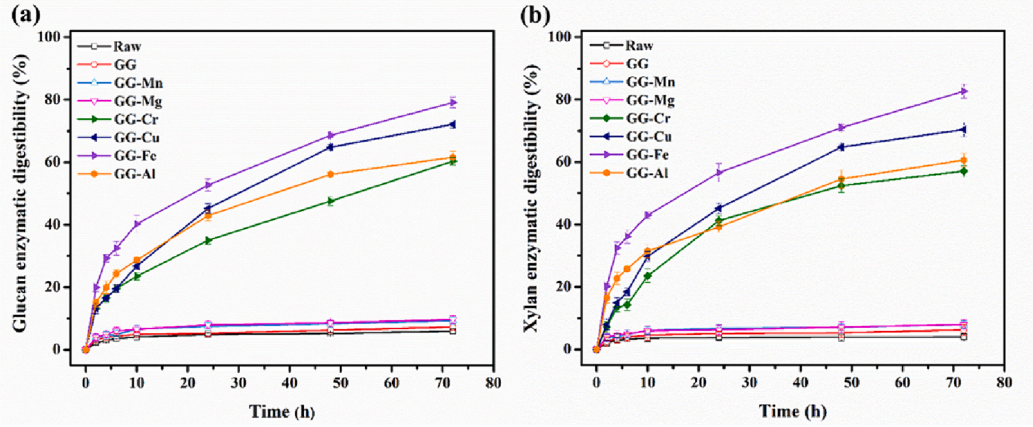

Fig. 3. The enzymatic digestibility of (a) glucan and (b) xylan of the raw bamboo and the pretreatment residues obtained using different DES systems (at 120 °C for 10 min).

3.2. Characterizations of raw bamboo and pretreatment residues

The surface morphologies of raw bamboo and the pretreatment residues using different DES systems were characterized using SEM analysis (see supplementary materials). It can be observed that untreated bamboo exhibited continuous and smooth fiber surfaces, and these may impede the entry of enzyme molecules into the cell wall as needed for enzymatic hydrolysis. Significant changes in fiber morphology and particle size were apparent after selected ternary DES (GG-Fe and GG-Al) pretreatments. Along with the deconstruction of the surface structure, the fiber size of the pretreated bamboo decreased, and the particle surfaces were rougher. Additionally, the appearance of some cracks, wrinkled texture, and a partial layered structure were consistent for other pretreated substrates (Zhang et al., 2022). These changes coincide with the effective destruction and removal of hemicellulose and lignin during the microwave-assisted ternary DES pretreatments. It has been widely reported that all these changes to surface morphology can lead to the generation of a larger number of active sites on the substrate surface, conducive to improving the accessibility of a lignocellulose and promoting the production of glucose and xylose.

The changes in the chemical functional groups of the bamboo after DES treatment were examined by FTIR analysis (see supplementary materials). For raw bamboo, the peaks at 2925 cm^{-1} and 1375 cm^{-1} are assigned to C-H stretching related to cellulose, while the absorption band at 3433 cm^{-1} is ascribed to the intramolecular hydrogen bond O-H stretching vibration of cellulose. The peaks at 1056 cm^{-1} and 897 cm^{-1} are assigned to C-O stretching and C-O-C stretching in amorphous cellulose, respectively. The peak at 1730 cm^{-1} is associated with carbonyl groups in branched hemicellulose. The peaks at 1510 cm^{-1} and 1460 cm^{-1} are assigned to the aromatic skeletal vibrations of lignin. The spectra of the pretreatment residues after DES pretreatment showed essentially the same pattern as the raw bamboo. However, the characteristic peaks of cellulose increased slightly in intensity, indicating the enrichment of cellulose after the pretreatment as shown with most DES systems in this study (Xie et al., 2022). Furthermore, the peak at 1730 cm^{-1} was observed to be weakened or even disappeared after ternary DES pretreatment. Similarly, the FTIR peaks at 1510 cm^{-1} and 1460 cm^{-1} (related to the lignin) were significantly reduced in all pretreatment samples. These results revealed the removal of hemicellulose and lignin, and thus the apparent degradation of the LCC (lignin-carbohydrate complex) structure occurred during the pretreatment process, which was also consistent with the results from the chemical composition analysis exhibited in Fig. 2 (Cheng et al., 2022).

The XRD patterns and crystallinity index (CrI) values of raw bamboo and the pretreatment residues were analyzed (see supplementary materials). It can be seen that the characteristic diffraction peaks at 18.0°, 22.4°, and 33.4° are exhibited in the spectra of all pretreated samples, corresponding to the (101), (002) and (040) crystalline planes of cellulose I. This indicates that the crystalline phases of the cellulose in the bamboo powder remained as cellulose I after the pretreatments with all the DES systems prepared in this study (Guo et al., 2019). Nevertheless, the crystallinity of the cellulose rich residue samples after DES treatment all increased to varying degrees compared to the raw bamboo, which was consistent with the percentage of removed lignin and hemicellulose as shown in Fig. 2. Specifically, these changes should be due to the removal of amorphous hemicellulose and lignin in the raw bamboo. When using the ternary DES with the molar ratio of 2:1:0.1, as the crystallinity of the pretreated sample increased, an increase of enzymatic digestibility could also be observed (Fig. 3). It should be noticed that although the sample treated with GG-Al achieved a remarkable crystallinity index of 60.8 %, its enzymatic digestibility was lower than that of the sample treated with GG-Fe. Previous studies have indicated that the residual lignin content in the pretreated samples, including the content of surface lignin, impacts the saccharification efficiency (Wang et al., 2022). Thus, the low enzymatic digestibility of the sample treated with GG-Al may be attributed to the excessive deposition of lignin on the surface.

The raw bamboo and pretreatment residues were further analyzed by XPS (see supplementary materials), and the O/C ratio, chemical bond types, and surface lignin coverage were calculated; the results are listed in Table 1. The O/C ratio and surface lignin coverage of the raw bamboo were 0.42 and 82 %, respectively. This indicated that the cellulose surface of the raw bamboo sample was mostly covered by lignin, which was also related to the low saccharification efficiency (Meng et al., 2022). As shown in Table 1, the surface lignin coverage values of the samples were lower after the ternary DES pretreatments. Although the sample pretreated by GG-Al had the better delignification efficiency compared with the sample pretreated by GG-Fe, the surface lignin coverage was still at a high level of 68 %. The high surface coverage of lignin might be caused by the redeposition of lignin during the pretreatment and washing steps (Yan et al., 2013). The presence of lignin on the surface of lignocellulose has been reported to negatively affect enzymatic digestion. More specifically, lignin impedes enzymatic action through two competitive binding processes, including binding to the surface of cellulose and binding to enzymes that recognize and bind cellulose. The irreversible binding of surface lignin in lignocellulose to the enzyme, as well as presenting a physical barrier to the enzyme, may seriously hinder the hydrolysis of glucan and xylan in the biological process of enzymatic saccharification (Vermaas et al., 2015; Liu et al., 2017). Indeed, the content and properties of surface lignin might play a more critical role than the total content of lignin during the enzymatic hydrolysis process (Chen et al., 2023). This result was consistent with

Table 1
Oxygen-to-carbon ratios, surface coverage by lignin (S_{lig}) and carbohydrates (S_{carb}) of the raw bamboo and pretreatment residues by XPS analysis.

DES	O/C ratio	S_{lig} (%)	S_{carb} (%)	C-C, C-H (284.6, eV) (%)	C-O (286.6, eV) (%)	C = O (287.6, eV) (%)	O-C = O (289, eV) (%)
Raw	0.42	82	18	23.22	52.44	11.28	13.07
GG-Fe	0.65	36	64	22.31	39.30	17.36	21.03
GG-Al	0.49	68	32	36.12	42.20	9.77	11.92
GG-Fe-2	0.67	32	68	17.05	32.56	23.75	26.64

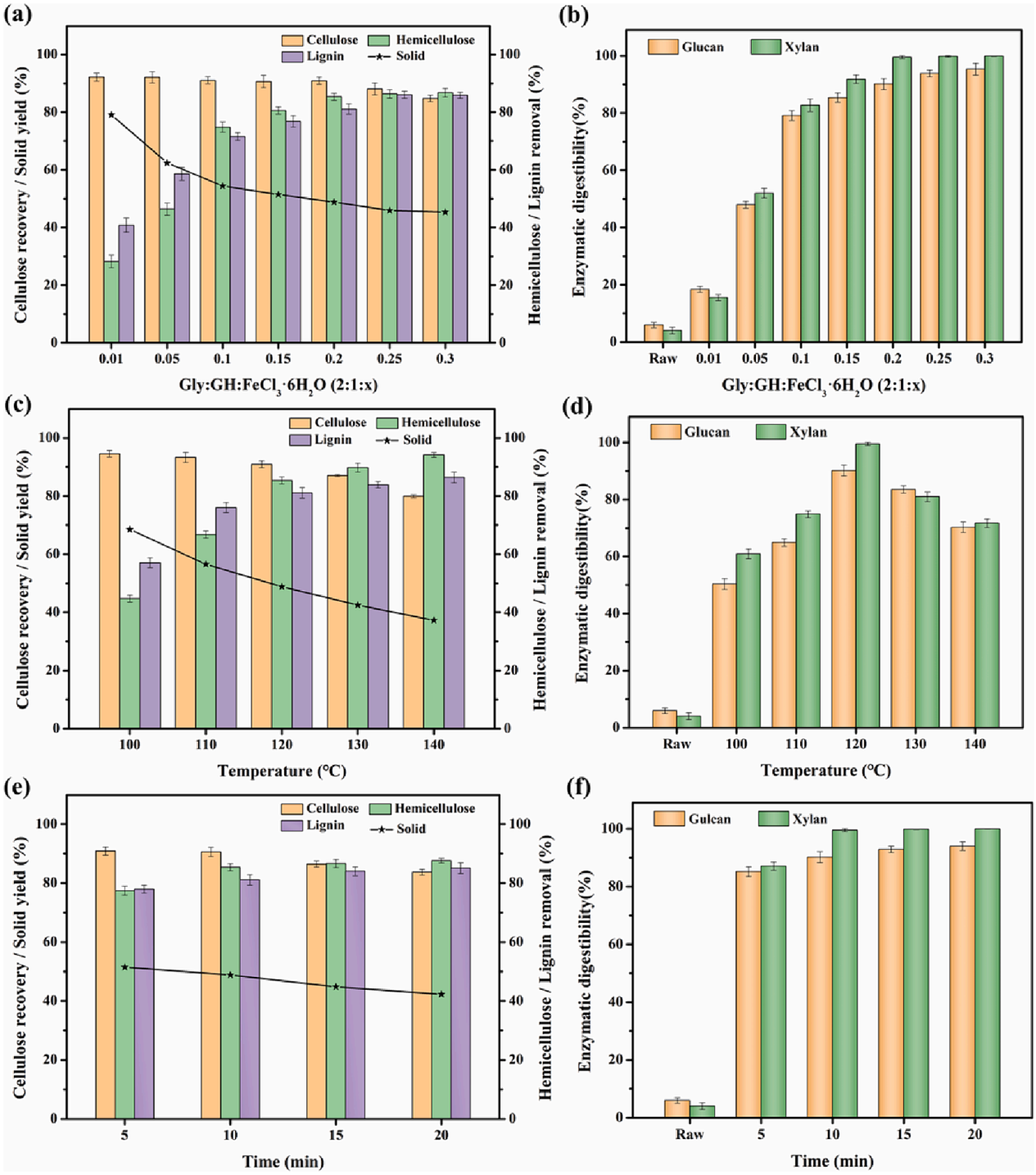

Fig. 4. Solid yield, cellulose recovery yield, hemicellulose and lignin removal, and glucan and xylan enzymatic digestibility for $FeCl_3$-based DES pretreatments under different conditions (a and b: molar ratio of Gly:GH:$FeCl_3$·$6H_2O$ at 2:1:x, x = 0.01–0.3 (120 °C, 10 min); c and d: temperature from 100 °C to 140 °C(2:1:0.2, 10 min); e and f: time from 5 min to 20 min (2:1:0.2, 120 °C)).

the low enzymatic hydrolysis yield of the samples pretreated with the GG-Al DES.

On the other hand, the O/C ratios of the samples increased to 0.65 and the surface lignin coverage of the sample reduced to 36 % after the pretreatment with the GG-Fe DES. This result suggested that this pretreatment was more effective in removing the lignin covering the surface of the cellulose rich residues and allowed more fibers to be exposed to the enzyme, therefore improving the efficiency of subsequent enzymatic hydrolysis (Yang et al., 2022a).

3.3. Optimization of the pretreatment molar ratio, temperature, and time

Based on the results from DES systems synthesized with different metal salts, the effects of the proportion of different components in the DES, pretreatment temperature and time on the percentage of lignin removal and enzymatic digestibility were investigated to optimize the pretreatment conditions; $FeCl_3$-based pretreatments were used given that the GG-Fe system showed the highest enzymatic digestibility values (Fig. 3). As shown in Fig. 4a, as the gradual adding of $FeCl_3{\cdot}6H_2O$ in the DES, the solid yield of pretreated bamboo decreased from 78.46 % to 44.97 %, indicating increased dissolution and degradation of the lignocellulose (Cheng et al., 2022). The decline in the solid yield tapered off as the percentage of lignin and hemicellulose removal both reached a plateau. It can be observed that the enzymatic digestibility increased rapidly with the increase in $FeCl_3{\cdot}6H_2O$ until an apparent saturation, which showed the same increasing trend as the percentage of lignin removal. When the molar ratio of Gly:GH:$FeCl_3{\cdot}6H_2O$ changed to 2:1:0.2, the removal of lignin and hemicellulose were 81.17 % and 85.42 %, respectively, and coinciding with glucan and xylan enzymatic digestibility of 90.15 % and 99.51 %, respectively. However, with the molar ratio of 2:1:0.3, the delignification efficiency and the yield of enzymatic hydrolysis showed no apparent improvement. Taking these results into consideration, the optimal molar ratio for Gly:GH:$FeCl_3{\cdot}6H_2O$ pretreatment was 2:1:0.2 (GG-Fe-2).

It is commonly known that temperature is a key factor for the pretreatment of lignocellulosic biomass. As shown in Fig. 4c, the solid yield of the bamboo decreased from 67.95 % to 36.95 % when the temperature increased from 100 °C to 140 °C. This result was similar to those reported in recent years on the pretreatment of lignocellulose with acidic DES (Liu et al., 2019). At the same time, the interaction between ions and hydrogen bonds of DES was strengthened at higher temperature, which effectively removed 44.75 %-94.15 % of hemicellulose and 57.05 %-86.40 % of lignin. The experimental results show that the pretreatment effects improved to a temperature of 120 °C, with 81.17 % of lignin and 85.42 % of hemicellulose removal efficiencies. In addition, enzymatic digestibility of the pretreatment residue was used to evaluate the effect of temperature on the pretreatment performance. As shown in Fig. 4d, the enzymatic digestibility of pretreated samples increased with the pretreatment temperature in the range of 100 °C to 120 °C. Interestingly, the glucan and xylan enzymatic digestibility of the pretreated sample at 120 °C (90.15 % and 99.51 %) were higher than those of the pretreated samples at 130 °C and 140 °C, although the former exhibited a slightly lower lignin removal. The possible reason was that some dissolved lignin may still adhered or reprecipitated to the surface of the pretreated solid under the excessive temperature condition, which in turn, limited the enzyme accessibility (Lou et al., 2019). Based on the results presented, GG-Fe-2 DES with the pretreatment temperature of 120 °C was selected for further experiments to assess the pretreatment condition of time.

As shown in Fig. 4e, the pretreatment time also affected the composition and properties of bamboo after treatment, but only to a limited extent. With the extension of pretreatment time, the promotion effect on the removal of lignin and hemicellulose were not significant, especially after 10 min. As shown in Fig. 4f, when the microwave reaction time was extended to 15 min and 20 min, the enzymatic digestibility remained almost unchanged. Therefore, the pretreatment time of 10 min was chosen as the optimal time the GG-Fe-2 DES, with longer pretreatment times providing little or no benefit, but greater energy use and thus less favorable economics.

3.4. Characterization of the recovered lignin

The molecular weight and polydispersity index (PDI) of the recovered lignin samples were examined by gel permeation chromatography analysis (GPC); the results are shown in Fig. 5. The Mw and Mn values for the recovered lignins were in the ranges of 5154–6958 and 1942–2320 g/mol, respectively, which are lower than values obtained for a MWL (Mw = 9411, Mn = 3170) analyzed for comparison. This result indicated that lignin depolymerization, and the dissociation of lignin from carbohydrates in the raw material, both occurred during the Lewis acid enhanced Gly:GH fractionation process (Wang et al., 2020b). The recovered lignin from GG-Al pretreated bamboo exhibited the lowest molecular weight, which is likely due to the strong acidity of $AlCl_3{\cdot}6H_2O$ that further facilitated the breakage of ether bonds in the lignin and the cleavage of the LCC (Yang et al., 2022b). From this, it can be inferred that smaller lignin fragments were more likely to be adsorbed on the surface of the pretreated residues, which was associated with the higher surface lignin coverage in Table 1, leading to the enzyme digestion efficiency being still at a lower level when more lignin was removed. Recovered lignins with higher molecular weights were obtained after the other ternary DES pretreatments, especially in the use of GG-Fe-2. This means that lignin with different molecular weights can be obtained from different DES systems, thus having the potential impact the value of the lignin and the applications for it.

Chemical properties of the recovered lignins obtained from using different DES systems are shown in Table 2. The microwave-assisted GG-Cr, GG-Cu, GG-Fe, and GG-Al pretreatments resulted in 3.81 %, 3.32 %, 3.09 %, and 2.38 % of total associated sugars being in the recovered lignins, respectively. The above results indicate that the ternary DES system has the ability to efficiently separate lignin and polysaccharide components, resulting in the cleavage of the LCC structure (Guo et al., 2020). This can be attributed to the strong hydrogen bond acceptance ability and high acidic strength characteristics of these chlorides (Wang et al., 2020b). Meanwhile, the yields of recovered lignin from the GG-Cr, GG-Cu, GG-Fe, and GG-Al systems were 28.81 %, 33.05 %, 36.52 %, and 42.95 %, respectively, which were directly associated with their corresponding lignin removal percentage as shown in Fig. 2. Although similar amount of lignins were removed after GG-Al and GG-Fe-2 pretreatments (compare between Fig. 2b and Fig. 4a), the recovered lignin yield after the GG-Fe-2 pretreatment was significantly higher, reaching 50.74 % (Table 2). This may be due to the recovered lignin after GG-Al pretreatment having a lower molecular weight (Fig. 5), and thereby, impacting its precipitation and collection by centrifugation (Alvarez-Vasco et al., 2016). Overall, recovered lignins with high yields and purities could be obtained during the microwave-assisted ternary DES pretreatment processes, which should be beneficial for subsequent high-value utilization (Chen et al., 2019b; Cronin et al., 2020).

Given a higher enzymatic digestibility of the bamboo pretreated with the $FeCl_3$-based DES systems, the chemical structures of the recovered lignins after GG-Fe and GG-Fe-2 pretreatments were subjected to 2D-HSQC NMR analysis (see supplementary materials). The NMR spectra of the side chain region (δC/δH 46–86/2.9–5.1) and the aromatic region (δC/δH 96–139/5.9–8.1) of the two lignin samples and the assignments of the NMR signals were based on the previous study (Xu et al., 2023b). Structural studies on recovered lignins have demonstrated that β-O-4 (A) interunit linkages predominate in the side chain region, whereas β-β (B) and β-5 (C) bonds are less present. Numerous reports suggested that the presence of high boiling alcohols such as glycerol could protect the benzyl group of lignin when using acidic DES to pretreat herbaceous lignocellulose, thereby effectively preventing the condensation of lignin (Zheng et al., 2023). The $FeCl_3$-based DES systems containing glycerol, used herein, may protect the structure of lignin during the pretreatment

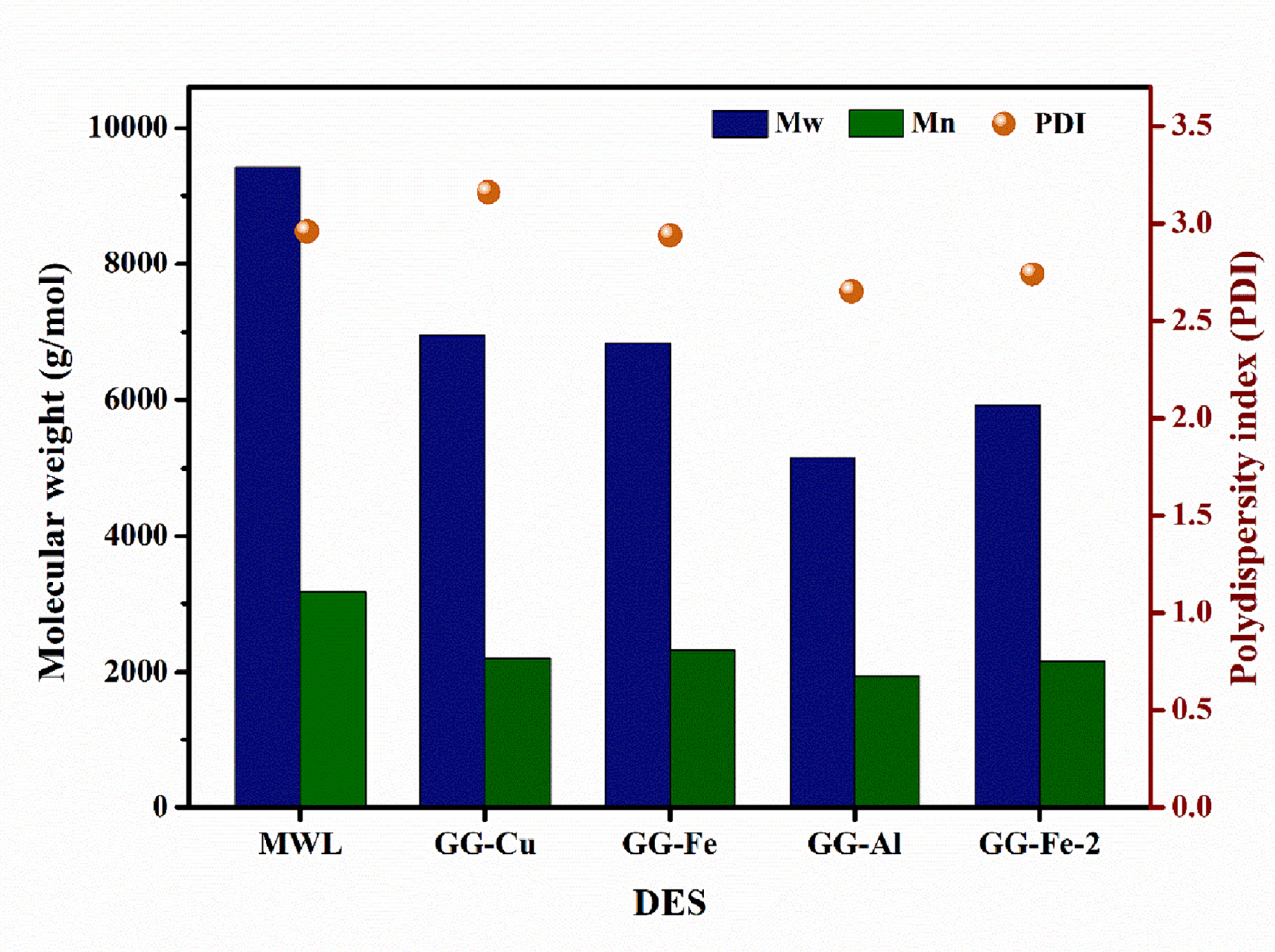

Fig. 5. Molecular weights and polydispersity index of MWL and recovered lignins obtained from pretreatments with different DES.

Table 2
Contents of associated carbohydrates, purity, and yield of the recovered lignin obtained from different DES systems.

DES	Associated carbohydrates (%)				Purity (%)	Yield (%)
	Arabinose	Glucose	Xylose	Total		
GG-Cr	0.74	1.26	1.81	3.81	96.19	28.81 ± 1.15
GG-Cu	0.53	1.19	1.60	3.32	96.18	33.05 ± 0.93
GG-Fe	0.49	1.15	1.45	3.09	96.91	40.52 ± 1.33
GG-Al	0.34	0.95	1.09	2.38	97.62	42.95 ± 1.44
GG-Fe-2	0.45	0.93	0.98	2.36	97.64	50.74 ± 1.10

processing and thereby retain the β-O-4 bonding environment. Interestingly, Hibbert's ketone (HK), as a lignin-derived compound, was detected after GG-Fe-2 pretreatment. The α-OH in the β-O-4 structure is dehydrated into enol ethers, which is usually cleaved (hydrolyzed) to produce HK in the presence of an acid. The structure of HK has also been found to exist in other microwave-assisted DES delignification processes, suggesting the potential occurrence of this mechanism during microwave heating (Muley et al., 2019). The appearance of the lignin derivative was mainly due to the degradation of β-O-4 linkages in lignin, which can also inhibit the condensation of lignin (Dong et al., 2019; Liu et al., 2021).

In the aromatic region, lignin NMR signals for syringyl (S), guaiacyl (G), *p*-coumaric acid (PCE) and ferulic acid (FA) were unambiguously identified. Between the $FeCl_3$-based DES pretreatments, the structures of recovered lignins were somewhat different. For example, it is known that in herbaceous plants, FA connects lignin and hemicellulose via ether and ester bonds (Chen et al., 2019a); it can be observed that compared with the lignin recovered by GG-Fe pretreatment, the NMR signals correlating to FA_2 unit disappeared in the recovered lignin after GG-Fe-2 pretreatment, which could be related to the greater removal percentage of lignin and hemicellulose (Fig. 4a) (Chen et al., 2019b). Meanwhile, it is worth noting that the signal intensity of the G-unit decreased more significantly than that of the S-unit, indicating that G-type lignin was more susceptible to be degraded than S-type lignin during the DES pretreatment (Zhang et al., 2022).

3.5. *The recyclability of GG-Fe-2 DES*

The recovery and reuse of the DES is an important issue for the successful application of DES-based systems in the integrated biomass refinery. Therefore, the recyclability of GG-Fe-2 DES was evaluated under optimized conditions. A slight loss of DES after each cycle was observed due to the post reaction processing. Nonetheless, over 90 % of the starting DES could be recovered and reused over five pretreatment cycles (see supplementary materials). With the increase of the number of pretreatment cycles, the solid yield increased slightly as shown in Fig. 6; the removal of hemicellulose and lignin still reached to 72.65 % and 66.13 %, respectively, after five cycles. The XRD analysis showed that the sample obtained after five cycles exhibited lower crystallinity than that treated with fresh DES (see supplementary materials). This may be

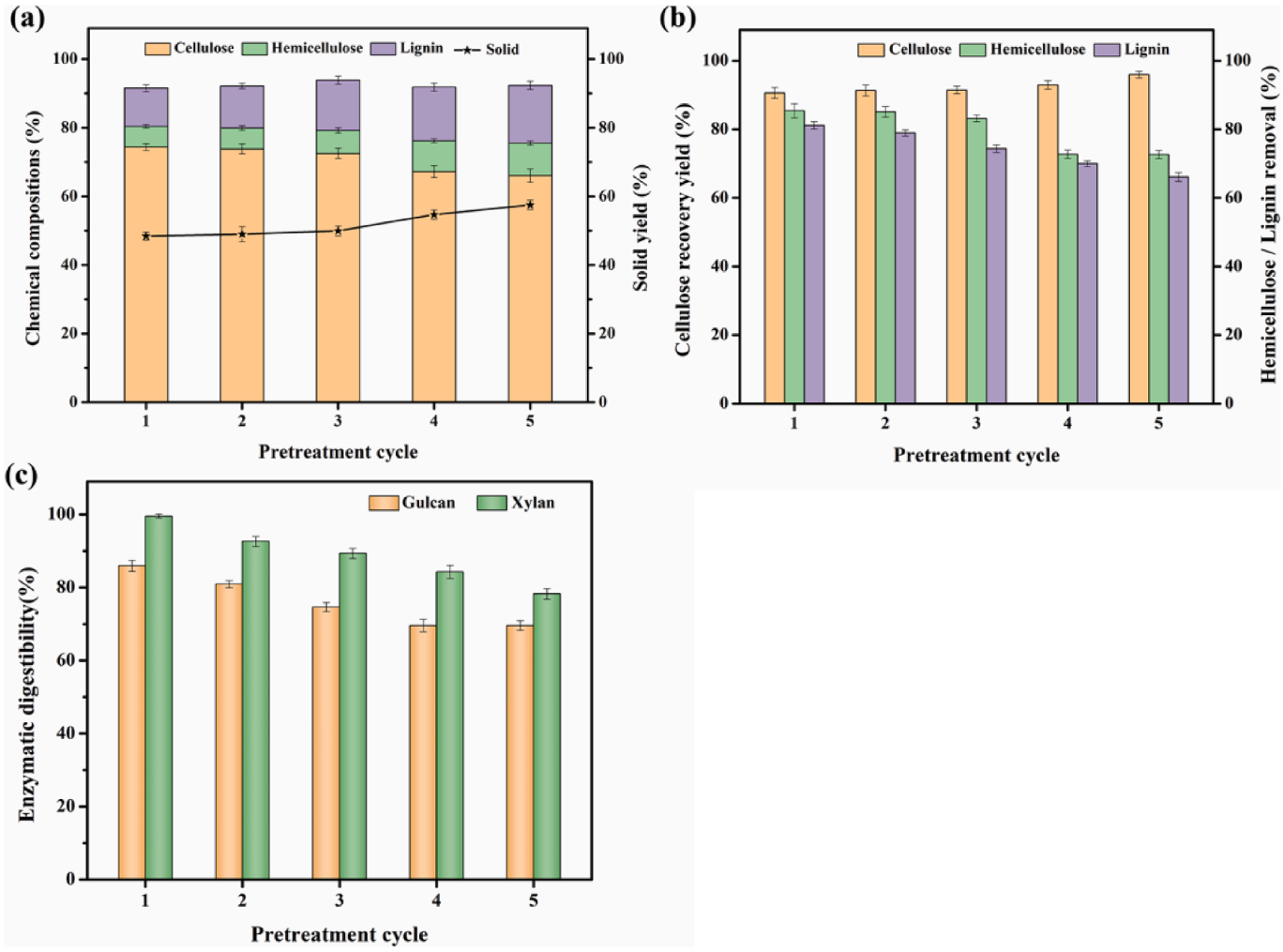

Fig. 6. (a) The solid yield, chemical compositions, (b) cellulose recovery yields and hemicellulose and lignin removal, and (c) enzymatic digestibility results when recycling the DES (GG-Fe-2) over several pretreatment cycles.

due to degradation of the DES system after five cycles, resulting in less removal of amorphous components, lignin and hemicellulose, from the lignocellulosic biomass. This could be caused by the continuous accumulation of the degradation products from hemicellulose and lignin in the DES, which would reduce the efficiency of the DES in the pretreatment (Huang et al., 2022). Besides that, the loss of DES throughout the entire experimental process should be an inevitable issue in most solvent recovery processes (Chen et al., 2018). Furthermore, the enzymatic digestibility of glucan and xylan decreased gradually as the number of pretreatment cycles increased. This is attributed to the same

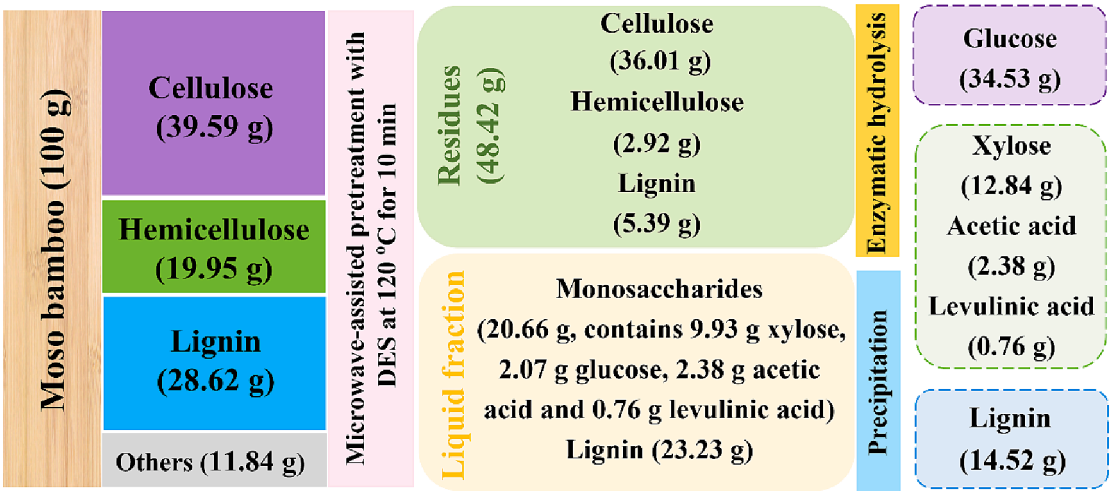

Fig. 7. Mass balance of the microwave-assisted DES pretreatment process (120 °C, 10 min) and subsequent enzymatic hydrolysis (10 FPU/g, 72 h).

reason mentioned above for the decrease in crystallinity of the samples with the recovery and reuse of the DES.

3.6. Mass balance

The mass balance of the microwave-assisted DES pretreatment process with GG-Fe-2 DES and the subsequent enzymatic saccharification (based on 100 g raw bamboo) is shown in Fig. 7. In brief, 100 g bamboo was pretreated with GG-Fe-2 (Gly:GH:$FeCl_3 \cdot 6H_2O$ at 2:1:0.2 M ratio) at a solid–liquid ratio of 1:20 for 10 min at 120 °C, then 48.42 g of pretreatment residue was recovered; said residue contained 36.01 g glucan, 2.92 g xylan and 5.39 g lignin. During the enzymatic hydrolysis process, cellulose was hydrolyzed by the cellulase enzyme mixture and 32.46 g of glucose was released into the system. The residual hemicellulose was also broken down, converting almost the entire amount to xylose (2.92 g) at the same time (Tang et al., 2023). After precipitation and centrifugal separation, 14.52 g (50.74 % of the total lignin content in the feedstock) of recovered lignin could be collected for further high-value utilization. Additionally, the recovered DES after DES pretreatment was analyzed by HPLC to determine the hydrolysis products of lignocellulosic biomass, which presented 20.66 g degradation products of cellulose and hemicellulose, mainly 9.93 g xylose, 2.07 g glucose, 2.38 g acetic acid and 0.76 g levulinic acid, with a small amount of furfural and 5-hydroxymethyl furfural. The mass balance results showed that the utilization rate of bamboo obtained with this DES was 65.03 % and the total conversion ratio of carbohydrates to monosaccharides in the feedstock was 87.00 %. These monosaccharides hold great potential for further conversion into various fuels and chemicals (Xu et al., 2023a).

4. Conclusions

This study has shown that microwave-assisted DES pretreatments can rapidly and selectively dissolve lignin and hemicellulose during biomass fractionation. Meanwhile, the destruction of lignocellulosic biomass structure increases the saccharification efficiency of glucan and xylan, which is beneficial for the value-added conversion of the carbohydrates. GG-Fe-2 DES is considered as the best suited for this feedstock for its ability to effectively remove 81.10 % lignin and 85.36 % hemicellulose under mild conditions (120 °C, 10 min), achieving enzymatic hydrolysis yield of over 90 %. In addition, the recovered lignin had the characteristics of high-purity, well-preserved structure, and seemingly practical molecular weight and PDI values which would be conducive to its downstream utilization. Notably, the synthesized GG-Fe-2 DES showed satisfactory pretreatment performance after several cycles. This work provides further insight needed to achieve sustainable and comprehensive applications of DES-based systems for biomass refining, which can be used for the production of biofuels, bio-based materials, and biochemicals.

CRediT authorship contribution statement

Yingying Feng: Writing – original draft. **Thomas L. Eberhardt:** Writing – review & editing. **Fanyang Meng:** Formal analysis. **Chen Xu:** Visualization. **Hui Pan:** Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.130666.

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