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Application of Aromatic Ring Quaternary Ammonium and Phosphonium Salts–Carboxylic Acids-Based Deep Eutectic Solvent for Enhanced Sugarcane Bagasse Pretreatment, Enzymatic Hydrolysis, and Cellulosic Ethanol Production

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Abstract: Deep eutectic solvents (DESs) with a hydrophobic aromatic ring structure offer a promising pretreatment method for the selective delignification of lignocellulosic biomass, thereby enhancing enzymatic hydrolysis. Further investigation is needed to determine whether the increased presence of aromatic rings in hydrogen bond receptors leads to a more pronounced enhancement of lignin removal. In this study, six DES systems were prepared using lactic acid (LA)/acetic acid (AA)/levulinic acid (LEA) as hydrogen bond donors (HBD), along with two independent hydrogen bond acceptors (HBA) (benzyl triethyl ammonium chloride (TEBAC)/benzyl triphenyl phosphonium chloride (BPP)) to evaluate their ability to break down sugarcane bagasse (SCB). The pretreatment of the SCB (raw material) was carried out with the above DESs at 120 °C for 90 min with a solid–liquid ratio of 1:15. The results indicated that an increase in the number of aromatic rings may result in steric hindrance during DES pretreatment, potentially diminishing the efficacy of delignification. Notably, the use of the TEBAC:LA-based DES under mild operating conditions proved highly efficient in lignin removal, achieving 85.33 ± 0.52% for lignin removal and 98.67 ± 2.84% for cellulose recovery, respectively. The maximum digestibilities of glucan (56.85 ± 0.73%) and xylan (66.41 ± 3.06%) were attained after TEBAC:LA pretreatment. Furthermore, the maximum ethanol concentration and productivity attained from TEBAC:LA-based DES-pretreated SCB were 24.50 g/L and 0.68 g/(L·h), respectively. Finally, the comprehensive structural analyses of SCB, employing X-rays, FT-IR, and SEM techniques, provided valuable insights into the deconstruction process facilitated by different combinations of HBDs and HBAs within the DES pretreatment.

Keywords: aromatic ring quaternary ammonium and phosphonium salts; sugarcane bagasse; carboxylic acids; deep eutectic solvents; pretreatment

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1. Introduction

The prevailing energy system, heavily reliant on fossil fuels, not only faces vulnerability to a potential "energy crisis", it also poses significant environmental risks, including global warming and the emissions of greenhouse gases [1–3]. Therefore, there exists an urgent imperative to prioritize the development of renewable, low-carbon energy sources as a viable replacement for fossil fuels [4]. In response to this imperative, there has been a notable surge in global attention towards the conversion of biomass into high-value platform chemicals or biofuels, driven by the pressing need to mitigate greenhouse gas emissions and reduce dependence on fossil fuels [5,6]. Among the array of biofuels, cellulosic ethanol is expected as the potential candidate for the transportation sector. This is attributed to its

ability to not only bypass competition with food production for sugar/starch feedstocks but also to alleviate disruptions to soil microorganisms caused by the direct combustion of agricultural residues [7,8]. During the process, the second-generation sugars obtained by the hydrolysis of lignocellulosic biomass can also be used to produce a wide range of bioproducts [9].

China, ranked as the world's third-largest cultivator of sugarcane, follows Brazil and India in this regard. Extensive cultivation of sugarcane is observed in southern China, and it serves as a pivotal raw material for the sugar industry [10,11]. After sugar production, approximately 30% of the sugarcane remains as a residue in the fields [12]. Currently, sugarcane bagasse (SCB) is predominantly incinerated in factories for energy generation [13]. However, due to its high moisture content, the combustion of SCB often proves to be inefficient [14]. In addition, the smoke emitted from burning discarded SCB and the unpleasant odor from open dumping can lead to serious environmental pollution and pose risks to public health [15]. Hence, the conversion of SCB to bioethanol presents a viable solution to address the primary issues associated with its combustion.

Compared with other agriculture residues, SCB stands out for its notably high carbohydrate content, primarily comprised 42–46% of cellulose, 23–30% of hemicellulose, and 21–26% of lignin [16,17]. However, the presence of lignin creates a formidable barrier, impeding enzyme access to cellulose and restricting the efficient utilization of lignocellulosic biomass. Consequently, there is an urgent need to develop pretreatment methods capable of breaking down the recalcitrant structure of lignocellulose to enhance enzymatic hydrolysis. Various pretreatment techniques, including ball milling [18], hydrothermal pretreatment [19,20], steam explosion [21], dilute acid [22,23], alkaline [24], organic solvents [25], and ionic liquids [26], have been employed to deconstruct SCB and improve enzymatic hydrolysis efficiency. Ambye-Jensen et al. employed hydrothermal pretreatment in combination with ensiling to treat SCB, resulting in a glucose yield of 0.30 g/g and an ethanol yield of 0.14 g/g, based on dry SCB. Nonetheless, this method demands high temperature and pressure conditions, leading to substantial energy consumption [27]. In addition, the use of hydrothermal or dilute acid pretreatment may introduce toxic inhibitors, such as phenols, furans, and carboxylic acids [28]. Xian et al. successfully employed hypercross-linked adsorption resin to detoxify the hydrolysate, achieving the highest ethanol concentration of 30.94 $\pm$ 0.13 g/L [29]. Ionic liquids (ILs) pretreatment has recently proven effective in significantly increasing fermentable sugar and ethanol yields [30,31]. However, concerns persist regarding the high cost of ILs, as well as the challenge of recovering or separating degradation products from the pretreatment solution [32,33]. Fortunately, deep eutectic solvents (DESs), emerging as viable alternatives to traditional ILs, have shown promise for lignocellulose pretreatment [34]. The lignin-rich stream can be first separated by DES systems, which is beneficial for the subsequent lignin valorization [35]. Their widespread adoption is fueled by their low raw material cost, green, environmentally friendly nature, and biocompatibility [36]. Furthermore, they exhibit characteristics of easy synthesis and recyclability [37].

In our previous work, we observed that pretreatment with benzyl triethyl ammonium chloride (TEBAC):lactic acid (LA) resulted in an outstanding delignification efficiency of 79.73 $\pm$ 0.93% for wheat straw (WS) [38]. TEBAC, classified as an aromatic quaternary ammonium salt, exhibits a superior phase transfer catalytic capacity, compared to choline chloride (ChCl), one of the most employed hydrogen bond acceptors (HBAs). This characteristic significantly enhances the solubility of lignin in the TEBAC-based DES. Similarly, the application of the TEBAC:LA DES for the deconstruction of corn straw (CS) yielded a remarkable lignin removal of 61.40% [39]. This underscores the notable effectiveness of TEBAC:LA pretreatment in eliminating lignin, a known impediment to the enzymatic hydrolysis of lignocellulose. Building on these insights, our current investigation is focused on determining whether an increased presence of aromatic rings in hydrogen-bonded receptors could lead to further improvements in lignin removal efficiency. Additionally, we are probing into the potential existence of a specific correlation between the quantity of

aromatic rings and both the rates of lignin removal and enzymatic conversion. To broaden the potential applications of DESs based on aromatic ring quaternary salt–carboxylic acids for pretreatment, this study synthesized six unique DES formulations to pretreat sugarcane bagasse (SCB). These formulations incorporated TEBAC and benzyl triphenyl phosphonium chloride (BPP) as HBAs in conjunction with lactic acid (LA), acetic acid (AA), and levulinic acid (LEA) as hydrogen bond donors (HBDs). Additionally, the structural and morphological alterations in SCB during pretreatment were characterized using scanning electron microscopy (SEM), Fourier-transform infrared (FT-IR) spectrometry, and X-ray diffraction (XRD). Finally, a detailed investigation into the impact of pretreatment was conducted through enzymatic hydrolysis and separate hydrolysis and fermentation (SHF).

2. Materials and Methods

2.1. Materials

SCB was kindly provided from Zhanjiang Junshi Group Co., Ltd. (Guangdong, China). The raw material underwent a thorough washing with water, subsequent drying, and milling to achieve a particle size of 60 mesh for use in the experiments. The chemical composition of SCB was analyzed using the NREL method [40]. The contents of cellulose, hemicellulose, and lignin in the raw SCB were 40.46 ± 1.79, 24.76 ± 0.78, and 25.16 ± 1.05%, respectively. Celluclast 2.0 L (75 filter paper unit (FPU)/mL) was purchased from Novozymes (Tianjin, China) Investment Co. Ltd. and then used for subsequent enzymatic saccharification. The microorganism used in this study was alcohol producing active dry yeast, which was kindly supplied by Angel Yeast, Co., Ltd. (Hubei, China). Analytical-grade TEBAC, BPP, AA, and LEA (see Figure 1) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Analytical-grade LA was purchased from Tianjin Kemi Ou Chemical Reagent Co., Ltd. (Tianjin, China). All other chemical reagents used in this work were of analytical grade.

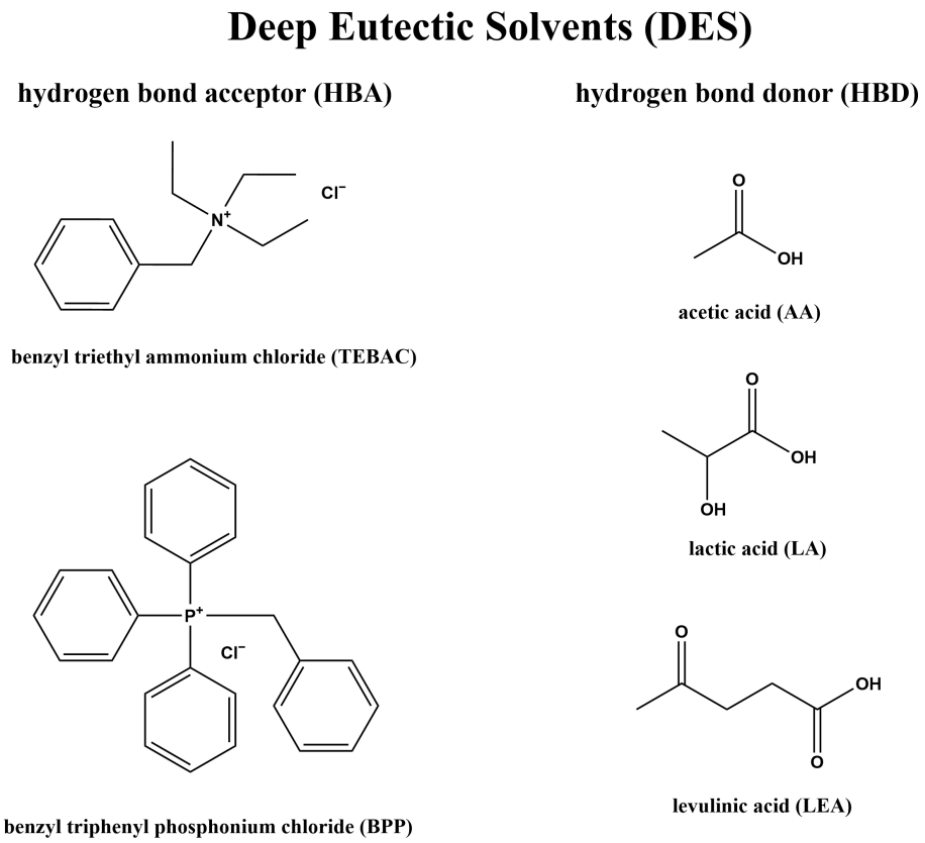

Figure 1. Structures of HBAs and HBDs used in this work.

2.2. Preparation of DESs

DESs were formulated with TEBAC or BPP as the HBA and with LA, AA, and LEA as HBDs. The condition of molar ratio 1:7 (HBA/HBD) was used for this study. The mixture was stirred at 80 °C until a transparent liquid was formed. Finally, all DESs were stored at room temperature (25 °C) for subsequent SCB pretreatment.

2.3. DES Pretreatment

The DES pretreatment of SCB was carried out in a three-neck bottle with a solid–liquid ratio (SLR) of 1:15 (*w*/*v*). The process took place over 90 min at 120 °C in a constant temperature oil bath, with stirring maintained at 200 rpm. Upon completion of the reaction, the reactor was immediately moved to a constant temperature oil bath set at 60 °C. Subsequently, ethanol, approximately three times the volume of the system, was added to wash the reactants for 1 h. Following this, the solid was washed with deionized water until the washing liquid appeared clear, after which it was dried for subsequent experiments (SCB composition analysis, characterization of solid fraction, enzymatic saccharification, and fermentation).

2.4. Characterization of Solid Fraction

SEM analysis was carried out to examine the morphological changes in SCB by using a scanning electron microscope (SU8100, Hitachi High Technology Co., Ltd., Tokyo, Japan). FT-IR measurements were conducted by a Nicolet 6700 spectrometer (Thermo Fisher Scientific, Dreieich, Germany) employing the potassium bromide method. Samples were collected within the range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. XRD analysis was executed on a Panalytical X'pert Pro diffractometer to assess the crystallinity of the SCB samples. The radiation source of XRD was Cu Ka, with an accelerating voltage of 40 kV and a power of 40 mA. Each diffraction pattern was acquired in the angles (2θ) spanning from 5° to 50° at a rotation speed of 80 rpm. The crystallinity index (CrI) of cellulose was determined using the following equation.

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

where I_{002} is the diffraction intensity from the 002 crystal plane at about $2\theta \approx 22.5°$, and I_{am} is the amorphous diffraction intensity at $2\theta \approx 18.2°$.

2.5. Enzymatic Saccharification and Fermentation

A 1.5 g pretreated biomass sample was added in a 50 mL Erlenmeyer flask containing 15 mL of citrate buffer (50 mM, pH = 5.0). The cellulase loading was set at 25 FPU/g SCB. The entire process was performed at 50 °C in a shaking incubator (ZQZY-80BS, Shanghai Zhichu Instrument Co., Ltd., Shanghai, China) operating at a speed of 150 rpm.

$$\text{Glucose digestibility}\ (\%) = \frac{c_1 \times V_1}{m_1 \times 1.111} \times 100\% \quad (1)$$

$$\text{Xylose digestibility}\ (\%) = \frac{c_2 \times V_1}{m_2 \times 1.136} \times 100\% \quad (2)$$

$$\text{Total sugar digestibility}\ (\%) = \frac{(c_1 + c_2) \times V_1}{m_1 \times 1.111 + m_2 \times 1.136} \times 100\% \quad (3)$$

where c_1 and c_2 (g/L) are the concentrations of glucose and xylose in the enzymatic hydrolysate, respectively; V_1 (L) is the volume of the hydrolysate; and m_1 and m_2 (g) are the masses of cellulose and xylan in the substrate, respectively.

Fermentation experiments of the pretreated SCB were implemented using separate hydrolysis and fermentation (SHF).

$$\text{Ethanol conversion } (\%) = \frac{c_3}{c_4 \times 0.51 + c_5 \times 0.46} \times 100\% \quad (4)$$

$$\text{Ethanol productivity } (g/(L \cdot h)) = \frac{c_3}{t} \quad (5)$$

where c_3 (g/L) represents the ethanol concentration, while c_4 and c_5 (g/L) denote the glucose and xylose consumptions during fermentation (initial to finial), respectively. t (h) signifies the duration of the fermentation process.

2.6. Analysis Method

The contents of cellulose, xylan, and lignin in both untreated and pretreated SCBs were determined using the NREL method. The concentrations of fermentable sugars and ethanol were determined by high-performance liquid chromatography (HPLC), with a refractive index detector (RID) from Agilent Technologies (1200 Series, Santa Clara, CA, USA). An Aminex HPX-87H anion exchange column (300 mm × 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) was used, maintaining a temperature of 55 °C. Diluted sulfuric acid (5 mM) was used as the mobile phase, and the flow rate was 0.5 mL/min.

3. Results and Discussion

3.1. Effect of DES Pretreatment on the Component Content of SCB

Six DES systems were formulated, utilizing three types of HBDs (LA/AA/LEA) in combination with two distinct HBAs (TEBAC/BPP), to assess their effectiveness in deconstructing SCB. The initial composition of raw SCB revealed cellulose, hemicellulose, and lignin contents of 40.46 ± 1.79, 24.76 ± 0.78, and 25.16 ± 1.05%, respectively. The alterations in SCB composition after pretreatment are presented in Table 1. Evidently, the contents of hemicellulose and lignin in SCB exhibited marked decreases, while the cellulose content saw a significant increase after the pretreatment process. The sequence of glucose content in SCB after DES pretreatment was as follows: TEBAC:LA > BPP:LA > BPP:AA > TEBAC:LEA > BPP:LEA > TEBAC:AA. Specifically, the glucan contents in the TEBAC:LA-pretreated SCB and BPP:LA-pretreated SCB were increased to 81.74 ± 2.35% and 74.43 ± 2.19%, respectively. Significantly, the xylan contents in the TEBAC:LA-pretreated SCB and BPP:LA-pretreated SCB were decreased to 12.93 ± 1.24% and 14.84 ± 0.50%, respectively. Additionally, the total lignin contents of the TEBAC:LA-pretreated SCB and BPP:LA-pretreated SCB were decreased to 7.56 ± 0.27% and 9.66 ± 0.15%, respectively. These findings strongly suggest that DES pretreatment effectively removed hemicellulose and lignin from SCB, allowing for the successful recovery of nearly all cellulose.

Table 1. Chemical compositions of untreated and DES-pretreated SCB at 120 °C for 90 min.

Pretreatment	Content (%)			Recovery Yield (%)			Removal Yield (%)
	Glucan	Xylan	Lignin	Solid	Glucan	Xylan	Lignin
SCB	40.46 ± 1.79	24.76 ± 0.78	25.16 ± 1.05	/	/	/	/
TEBAC:LA	81.74 ± 2.35	12.93 ± 1.24	7.56 ± 0.27	48.84 ± 1.97	98.67 ± 2.84	25.51 ± 2.45	85.33 ± 0.52
TEBAC:AA	46.55 ± 0.81	12.27 ± 0.71	21.20 ± 0.60	66.63 ± 0.46	103.99 ± 1.34	33.03 ± 1.92	44.78 ± 0.05
TEBAC:LEA	57.08 ± 1.23	20.26 ± 0.84	23.30 ± 0.53	69.77 ± 1.27	98.43 ± 2.12	57.09 ± 2.35	36.11 ± 1.13
BPP:LA	74.43 ± 2.19	14.84 ± 0.50	9.66 ± 0.15	53.75 ± 1.34	98.88 ± 4.34	32.21 ± 1.08	79.36 ± 0.32
BPP:AA	60.19 ± 0.40	15.13 ± 1.05	19.55 ± 0.34	66.05 ± 0.56	98.26 ± 0.65	40.36 ± 2.80	48.67 ± 0.90
BPP:LEA	53.17 ± 1.60	19.69 ± 0.91	24.53 ± 0.57	77.32 ± 1.12	101.60 ± 3.05	61.50 ± 2.83	24.62 ± 1.75

TEBAC:LA: benzyl triethyl ammonium chloride and lactic acid; TEBAC:AA: benzyl triethyl ammonium chloride and acetic acid; TEBAC:LEA: benzyl triethyl ammonium chloride and levulinic acid; BPP:LA: benzyl triphenyl phosphonium chloride and lactic acid; BPP:AA: benzyl triphenyl phosphonium chloride and acetic acid; BPP:LEA: benzyl triphenyl phosphonium chloride and levulinic acid.

Following pretreatment with all six DES systems, the recovery of cellulose from SCB exceeded 98%, demonstrating an exceptionally high success rate in cellulose recovery. Guo et al. also employed a TEBAC:LA-based DES for corncob deconstruction, reporting a relatively low cellulose loss [41]. However, Mor'an-Aguilar et al. observed a significantly lower cellulose recovery yield of only 31.83 ± 2.15% when utilizing a DES system prepared with ChCl as HBA and LA as HBD for SCB pretreatment [42]. This underscores the influential role of the HBA selection in DES on pretreatment selectivity. In the case of lignin removal from SCB pretreated with DES using TEBAC as the HBA, the following sequence was observed: TEBAC:LA (85.33 ± 0.52%) > TEBAC:AA (44.78 ± 0.05%) > TEBAC:LEA (36.11 ± 1.13%). Similarly, when BPP was employed as the HBA, a comparable trend was noted: BPP:LA (79.36 ± 0.32%) > BPP:AA (48.67 ± 0.90%) > BPP:LEA (24.62 ± 1.75%). These findings provide crucial insights into the impact of HBA selection within DES systems on lignin removal efficiency during SCB pretreatment.

When utilizing LA as the HBD, regardless of whether TEBAC or BPP served as the HBA, the DES exhibited remarkable efficacy in lignin removal, achieving removal yields of 85.33 ± 0.52% and 79.36 ± 0.32%, respectively. Additionally, the recoveries of xylan for TEBAC:LA- and BPP:LA-pretreated SCBs were 25.51 ± 2.45% and 32.21 ± 1.08%, respectively. Tan et al. emphasized the influential role of hydroxyl groups in HBDs on lignin extraction efficacy [43]. The presence of these hydroxyl groups enhances the effectiveness of DES pretreatment, a correlation supported by the findings of our study. All three HBDs in our study contained a carboxyl group, with LA additionally featuring a hydroxyl group (see Figure 1). In comparison to the performances of other HBDs, it became evident that LA, as an HBD, exhibited the highest efficiency in lignin removal. The efficiency of delignification would be affected by interactions between the hydroxyl groups in the HBD and the free and etherified hydroxyl groups in lignin. Furthermore, Tan et al. highlighted that the efficiency of delignification could be influenced by the acid strength of HBD [43]. However, irrespective of whether TEBAC or BPP served as the HBA, AA as the HBD (pKa = 4.75) exhibited a superior delignification ability compared to LEA as the HBD (pKa = 4.65). Formic acid (FA) as an HBD (pKa = 3.75) was also evaluated in a preliminary experiment. Lignin removal for TEBAC:FA- and BPP:FA-pretreated SCBs yielded 78.77 ± 0.65% and 75.38 ± 0.65%, respectively. These findings unmistakably demonstrated that, regardless of the HBA used, LA as the HBD (pKa = 3.86) outperformed FA as the HBD. These results indicated that the efficiency of delignification does not necessarily correlate with acid strength. The reduced delignification performance of LEA as an HBD may stem from the excessive length of its alkyl chain, which diminishes the strength of hydrogen bonds. Furthermore, HBAs, a crucial component of DESs, exert a significant influence on the pretreatment effectiveness of DESs. Yu et al. reported that the removals of the lignin of the ChCl:FA-pretreated herbal residues (HR) and pure FA-pretreated HR were 40.7% and 9.0%, respectively [44]. Notably, the lignin removal for the TEBAC:LA-based DES exceeded that of the BPP:LA-based DES, indicating that an increase in the benzene ring count might lead to steric hindrance in DES pretreatment, potentially reducing the effectiveness of delignification. This observation aligns with the findings reported by Guo et al., who explained that a decrease in alkyl chain length may result in less steric hindrance in lignin removal, compared to TEBAC [41].

3.2. Effect of DES Pretreatment on SCB Structure

3.2.1. Morphological Analysis

The morphological changes in the SCB surface before and after pretreatment are depicted in Figures 2 and 3. In its untreated state, the SCB displayed a smooth, intact, and well-organized outer fiber, characterized by a high degree of inherent fibrous structure. However, this structure was not conducive to enzyme penetration for cellulose access. Conversely, the treated SCB showcased a notable shift in structure, presenting a roughened surface with numerous pores, rendering it more favorable for subsequent enzymatic hydrolysis.

Micrographs employing LA as the HBD exhibited a pleated and rugged surface, primarily indicative of structural damage, regardless of whether TEBAC or BPP served as

the HBA. In contrast, micrographs applying AA or LEA as the HBD presented a relatively smoother surface. However, when compared, micrographs utilizing AA as the HBD displayed a structure with more pronounced perforations. This discrepancy is likely attributed to the removal of lignin and hemicellulose. These observations corroborate the compositional changes outlined in Table 1. Furthermore, it can be inferred that with DES employing LA as the HBD, a significant portion of the hemicellulose and lignin are removed, exposing more cellulose on the surface. This substantial enhancement greatly augments the performance of enzymatic hydrolysis.

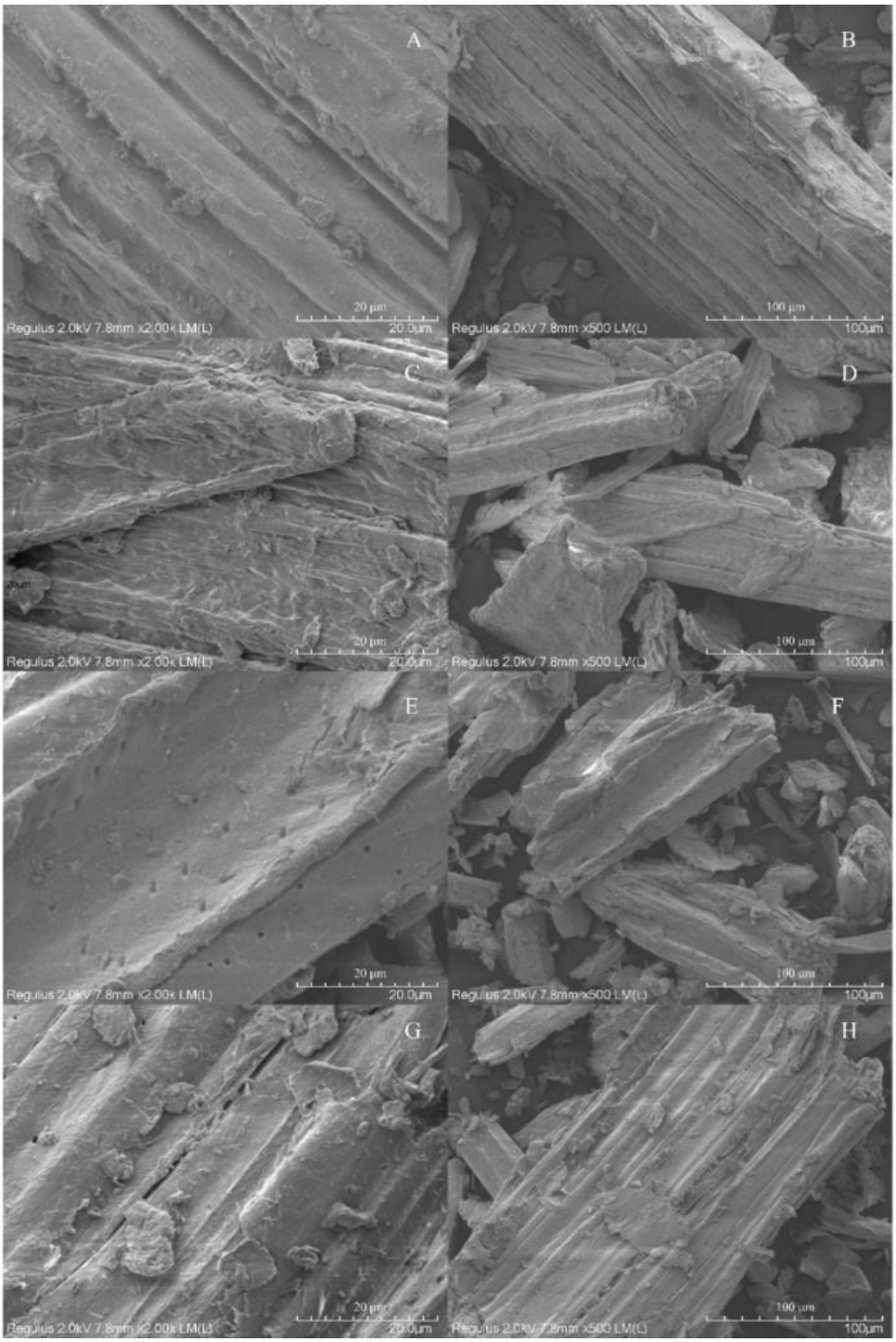

Figure 2. SEM images of the untreated (**A**,**B**) and pretreated SCBs: TEBAC:LA (**C**,**D**), TEBAC:AA (**E**,**F**), and TEBAC:LEA (**G**,**H**).

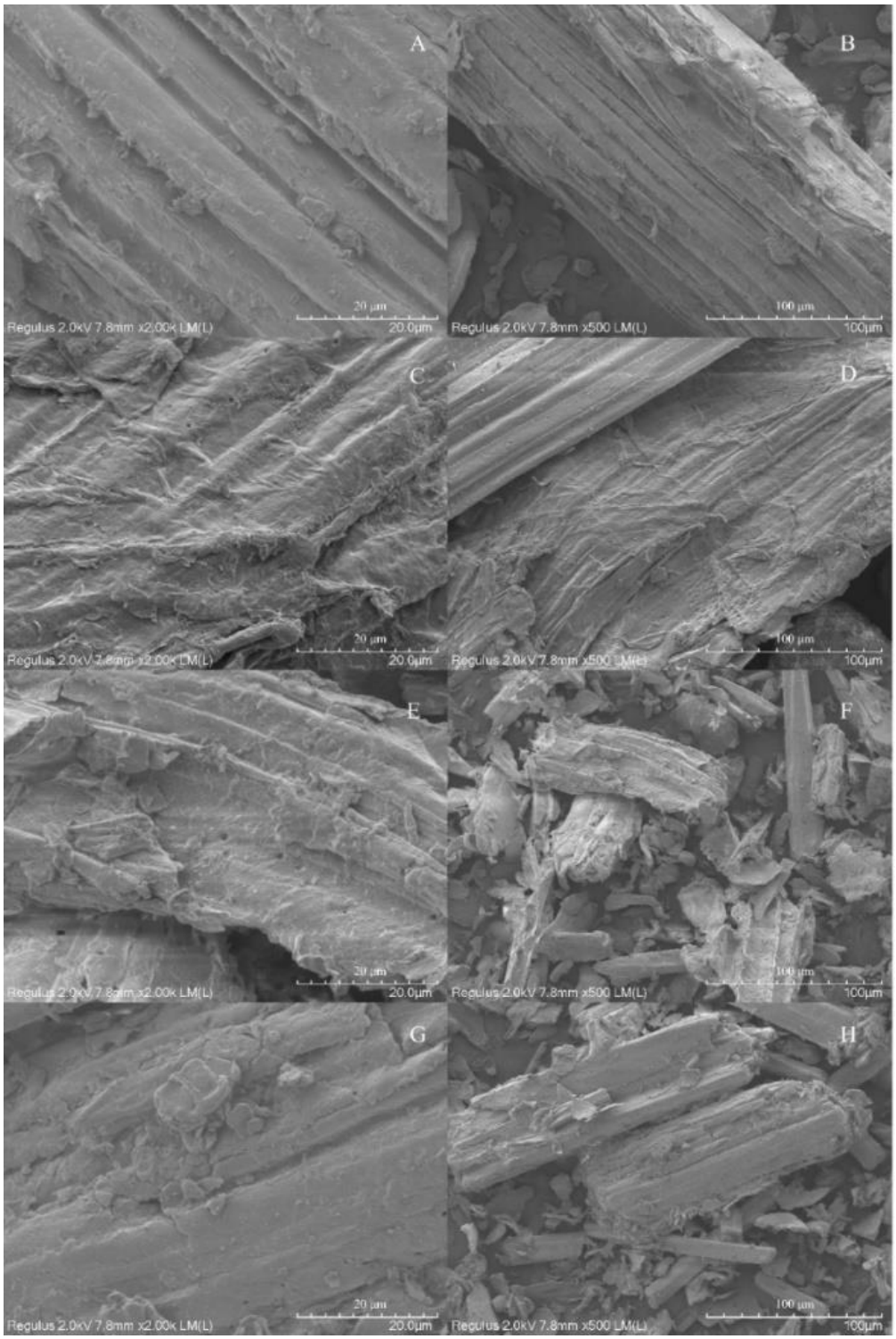

Figure 3. SEM images of the untreated (**A**,**B**) and pretreated SCBs: BPP:LA (**C**,**D**), BPP:AA (**E**,**F**), and BPP:LEA (**G**,**H**).

3.2.2. FT-IR Analysis

FT-IR analysis was conducted to evaluate alterations in the functional groups of SCB after DES pretreatment, as depicted in Figures 4 and 5. An increase was noted in the peak at approximately 902 cm^{-1} (associated with the β-(1-4) glycosidic bond in cellulose) when SCB underwent pretreatment with DES utilizing AA or LEA as the HBD. This suggests a substantial formation of amorphous cellulose after pretreatment. However, this peak

experienced a marked decrease when SCB was subjected to DES pretreatment with LA as the HBD, potentially indicating a higher content of crystalline cellulose after pretreatment. It is noteworthy that the peak remained, implying that cellulose in SCB underwent minimal removal. This phenomenon may be attributed to DES's capacity to prevent cellulose loss through hydrogen bond interactions, in line with the high cellulose recovery rates detailed in Table 1.

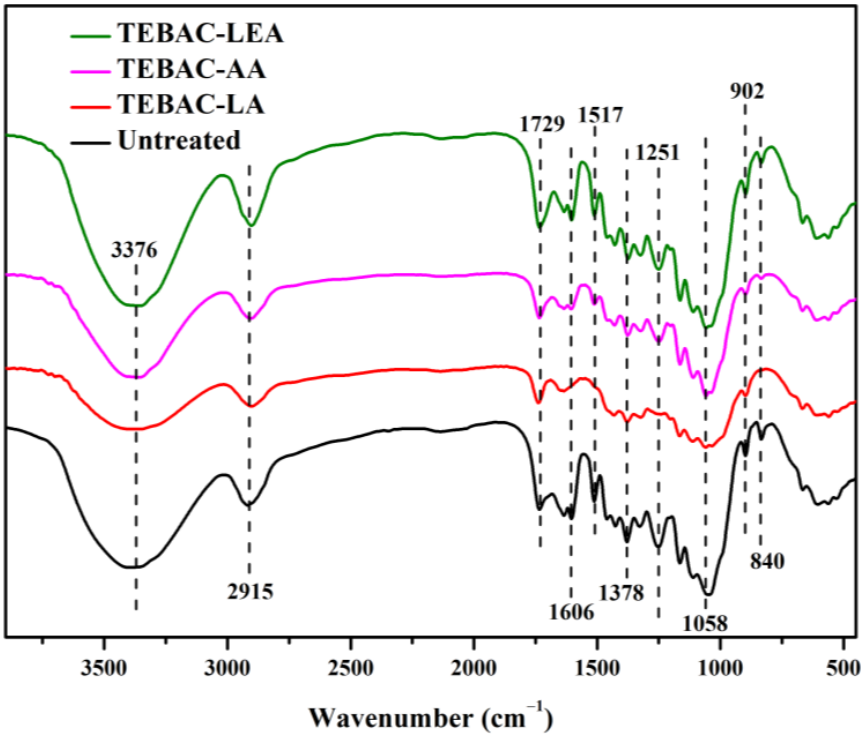

Figure 4. FT-IR spectra of the untreated SCB and pretreated SCB: TEBAC:LA, TEBAC:AA, and TEBAC:LEA.

More notably, there were significant alterations in the peaks associated with hemicellulose and lignin. The absorption peak at 1729 cm^{-1} (or 1741 cm^{-1}) is indicative of hemicellulose (specifically, the C=O bond) [45]. Strikingly, when TEBAC served as the HBA, this peak demonstrated a notable decrease after SCB underwent DES pretreatment with AA or LA as the HBD. Conversely, when LEA was used as the HBD, the peak remained basically unchanged. The above results indicated that less hemicellulose was retained after SCB was pretreated with DES employing AA or LA as the HBD. These findings are in line with the hemicellulose content variations observed in SCB treated with different DES formulations, as detailed in Table 1. In addition, the absorption peaks at 1606 cm^{-1} (or 1641 cm^{-1}) and 1517 cm^{-1} (or 1511 cm^{-1}), which are indicative of lignin content [46], displayed a decrease following DES pretreatment. Among them, after pretreatment with TEBAC:LA- and BPP:LA-based DESs, the absorption peaks associated with SCBs essentially vanished. In comparison to the absorption bands at approximately 1328 cm^{-1} (representing a bending C−O bond or −OH bond in lignin) and 838 cm^{-1} (related to the C−H bond in lignin) observed in untreated SCB, these bands were significantly weakened after DES pretreatment. In particular, the absorption peaks of SCBs almost disappeared after pretreatment with TEBAC:LA- and BPP:LA-based DESs. This reveals that LA as the HBD proved to be more effective in lignin removal. Thus, based on the FT-IR spectral outcomes and the observed compositional changes, the mechanism of DES pretreatment on SCB can be delineated: DES is capable of forming hydrogen bonds with hemicellulose and lignin constituents. This leads to an effective delignification process, enabling enzymes to efficiently permeate cellulose and hemicellulose. As a result, this enhances the enzymatic digestibility of carbohydrates.

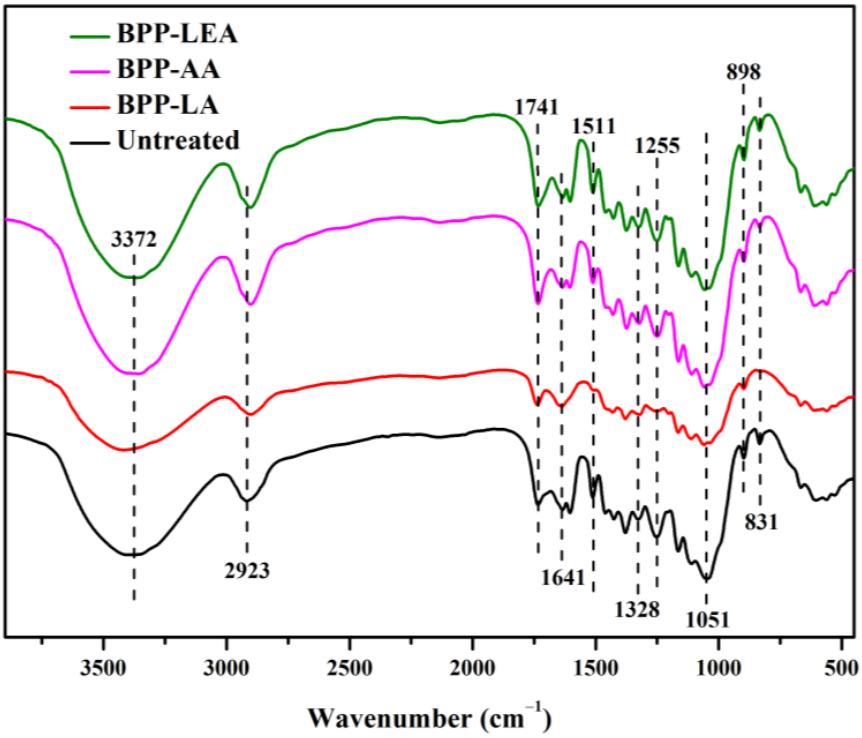

Figure 5. FT-IR spectra of the untreated SCB and pretreated SCB: BPP:LA, BPP:AA, and BPP:LEA.

3.2.3. X-ray Analysis

The crystallinity of lignocellulose is widely recognized as a crucial factor influencing the efficiency of enzymatic hydrolysis. Therefore, the crystallinity index (CrI) of both untreated and DES-treated SCBs were compared to assess enzymatic performance. The XRD patterns depicted in Figures 6 and 7 offer insights into the alterations in SCB's crystallinity. Notably, at approximately $2\theta \approx 22.5^{\circ}$, we observe the diffraction peaks corresponding to the cellulose crystallographic plane, while at $2\theta \approx 18.2^{\circ}$, the diffraction peaks pertain to the cellulose amorphous phase. After DES pretreatment, the positions of the characteristic peaks of cellulose remained unchanged. This indicates that the aromatic ring quaternary salt–carboxylic acid-based DESs used in this work did not change the crystal morphology of cellulose. It is noteworthy that these findings diverge from those reported by Morán-Aguilar et al. when using different acid-based deep eutectic solvents on SCB. In their work, a reduction in CrI values was observed using ChCl: citric acid (CA). Mor'an-Aguilar et al. attributed this phenomenon to the expansion and dissolution of cellulose and hemicellulose in biomass residues. According to the compositional changes detailed in Table 1 and the FT-IR analysis illustrated in Figures 4 and 5, it can be deduced that the removal of amorphous lignin and hemicellulose leads to an increase in crystallinity. In the case of SCB pretreated with DES using TEBAC as the HBA, the following sequence of CrI values was observed: TEBAC:LA (73.76%) > TEBAC:AA (64.27%) > TEBAC:LEA (62.32%). Likewise, when BPP was used as the HBA, a similar trend was observed: BPP:LA (71.69%) > BPP:AA (64.97%) > BPP:LEA (60.34%). Notably, the CrI of pretreated SCB exhibited a significant increase, especially with TE-BAC:LA (73.76%) and BPP:LA (71.69%), in contrast to untreated SCB (51.44%). These results suggest that DES pretreatment effectively removes amorphous hemicellulose and lignin, consequently enhancing the CrI value. A higher CrI value indicates a greater exposure of cellulose, which is a favorable condition for subsequent enzymatic saccharification.

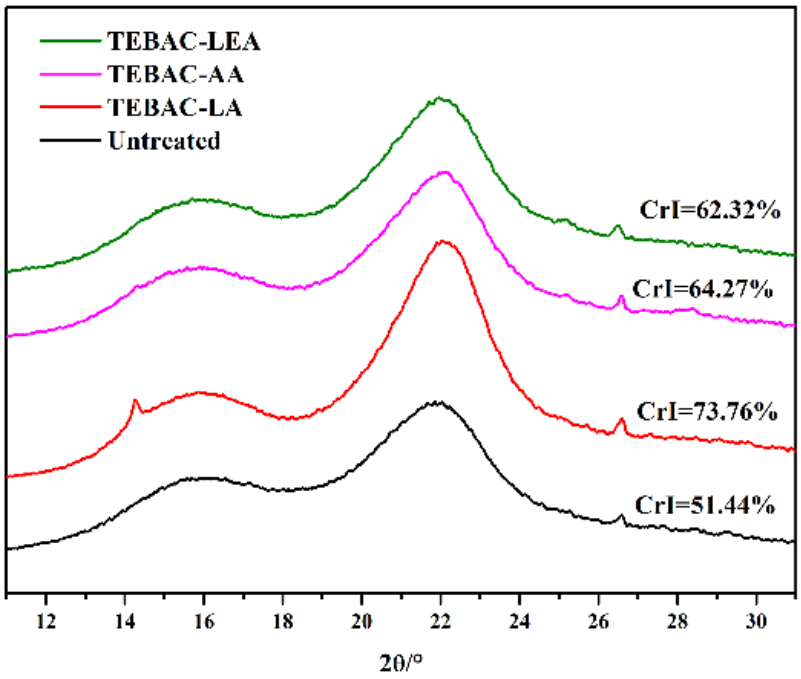

Figure 6. XRD diffractograms of the untreated SCB and pretreated SCB: TEBAC:LA, TEBAC:AA, and TEBAC:LEA.

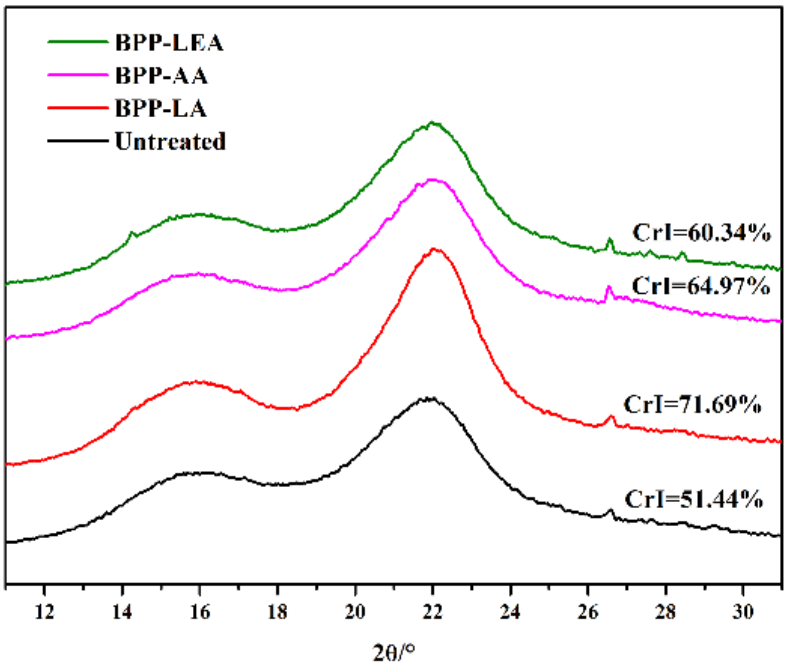

Figure 7. XRD diffractograms of the untreated SCB and pretreated SCB: BPP:LA, BPP:AA, and BPP:LEA.

3.3. Effect of DES Pretreatment on Enzymatic Saccharification

The enzymatic saccharification was carried out to evaluate the behaviors of DES pretreatment, where a higher yield of fermentable sugars indicates improved enzyme accessibility to carbohydrates. As shown in Figure 8A, SCB pretreated with DES using TEBAC as the HBA exhibited the following sequence in the glucose concentration from enzymatic saccharification: TEBAC:LA (46.81 ± 0.60 g/L) > TEBAC:AA (18.78 ± 0.91 g/L) > TEBAC:LEA (13.35 ± 0.51 g/L). Likewise, when DES systems were formulated with BPP as the HBA, a similar trend was observed: BPP:LA (42.19 ± 0.08 g/L) > BPP:AA

(17.17 ± 0.72 g/L) > BPP:LEA (12.87 ± 0.32 g/L) (Figure 8A). Thus, DES pretreatment can break the stubborn structure of biomass and then improve the performance of enzymatic hydrolysis. Figure 8B further illustrates consistent trends in sugar concentrations and enzymatic digestibility. The maximum digestibilities of glucan (56.85 ± 0.73%) and xylan (66.41 ± 3.06%) were attained after TEBAC:LA pretreatment, while the second was obtained after BPP:LA pretreatment. On the contrary, SCB pretreated with DES using LEA as the HBD displayed the lowest digestibility for fermentable sugars, regardless of whether TEBAC or BPP was employed as the HBA. The reasons for the above results can be attributed to the fact that compared with LA ($C_3H_6O_3$) and AA ($C_2H_4O_2$), the longer alkyl chains in LEA ($C_5H_8O_3$) lead to increased viscosity and steric hindrance. This, in turn, reduces the removal of lignin and hemicellulose, thereby inhibiting enzymatic hydrolysis. The above trends could be related to the chemical composition of SCB after the DES pretreatment reported in Table 1, which also corresponds with the SEM images, FT-IR, and X-ray results demonstrating an effective destruction of SCB after TEBAC:LA pretreatment.

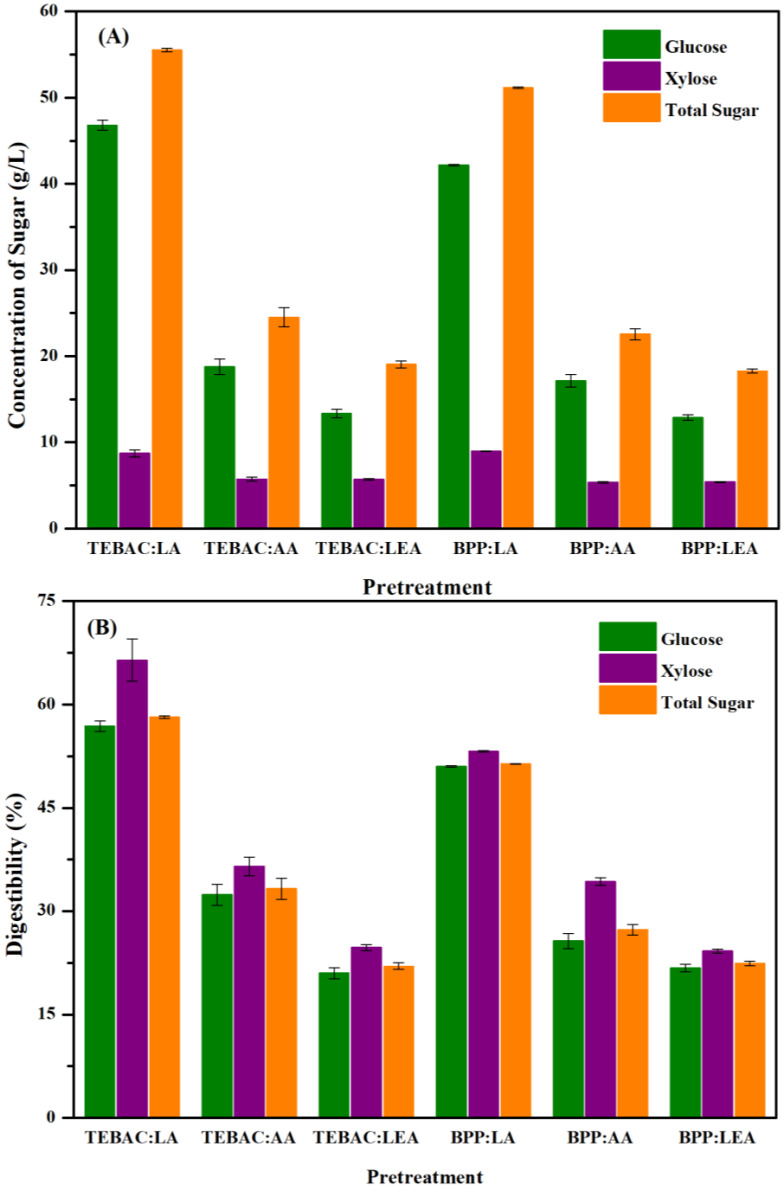

Figure 8. The concentration of sugar (**A**) and digestibility (**B**) obtained after the enzymatic hydrolysis of SCB pretreated with different DESs at 120 °C and 90 min. As shown in Tables S1 and S2, effects of pretreatment with different HBDs have significant differences statistically (one-way ANOVA, Tukey's test; $p < 0.05$).

3.4. Separate Hydrolysis and Fermentation (SHF) on DES-Treated SCB

The curves of ethanol production and sugar consumption are presented in Figure 9. It is evident from Figure 9 that a separate hydrolysis and fermentation (SHF) process for all SCBs pretreated with different DESs was completed within 36 h due to the low initial xylose content and the absence of inhibitory components in the fermentation solution. Notably, among the six DES systems, the maximum ethanol concentration and productivity attained from the TEBAC:LA-based DES-pretreated SCB were 21.76 g/L and 0.68 g/(L·h), respectively. Importantly, it is worth highlighting that the ethanol conversion surpassed 90% after DES pretreatment, indicating a highly effective conversion of fermentable sugars into bioethanol.

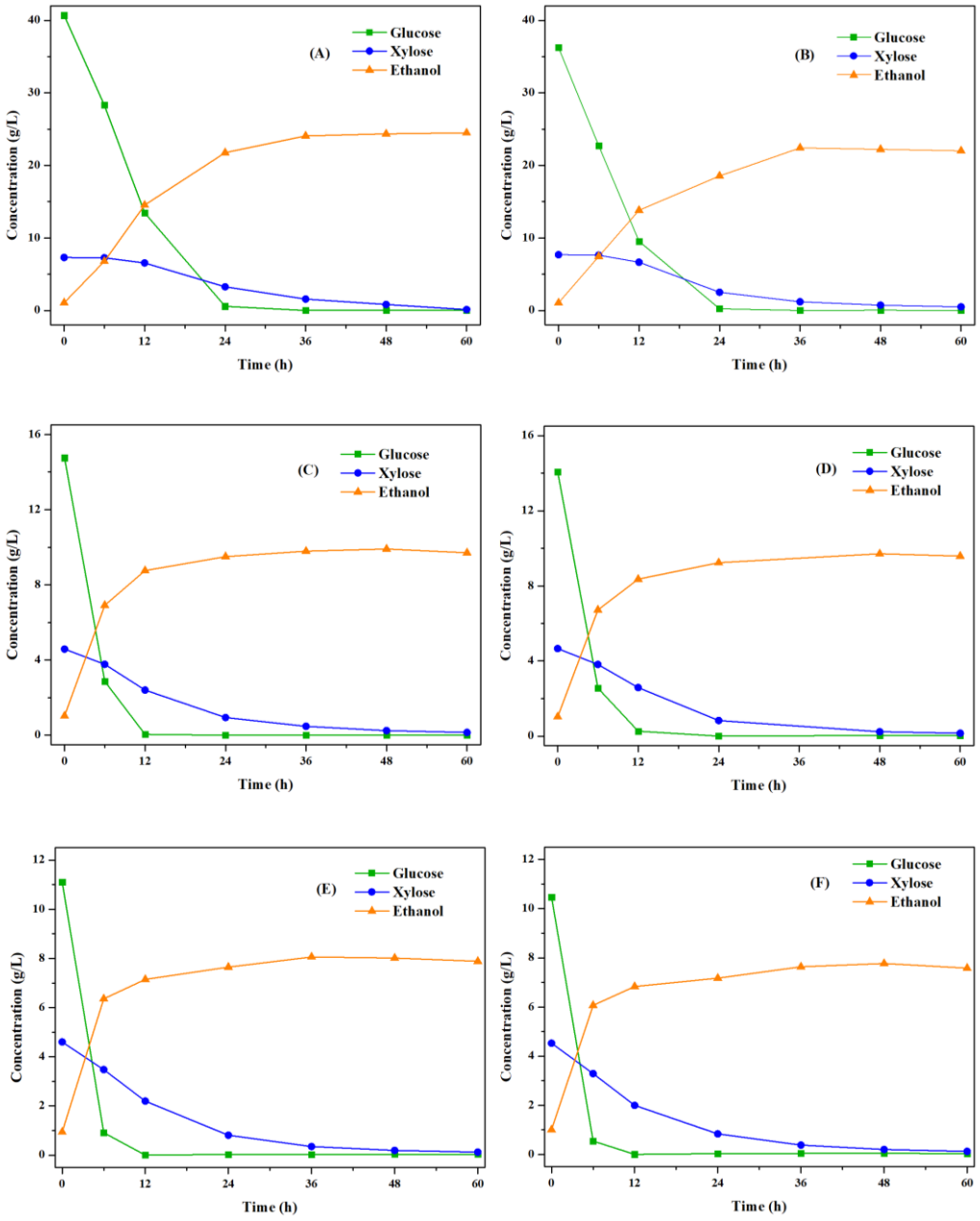

Figure 9. Changes in sugar and ethanol contents in the SHF process of pretreated SCB: TEBAC:LA (**A**), BPP:LA (**B**), TEBAC:AA (**C**), BPP:AA (**D**), TEBAC:LEA (**E**), and BPP:LEA (**F**).

In summary, when employing the same HBD, the TEBAC-based DES exhibited superior pretreatment efficacy, compared to the BPP-based DES. This difference could be attributed to the steric hindrance in DES pretreatment stemming from an increase in the

benzene ring count. Moreover, regardless of whether TEBAC or BPP was used as the HBA, SCB pretreated with the DES followed this trend: LA > AA > LEA. This is likely because LA contains hydroxyl groups, which enhance the efficiency of lignin extraction. Throughout this study, the TEBAC:LA-based DES demonstrated notable selectivity in delignification, facilitating the efficient conversion of biomass to bioethanol.

4. Conclusions

In this work, six DES systems were prepared using LA/AA/LEA as the HBD with two independent HBAs (TEBAC/BPP) to assess their abilities to deconstruct SCB. The investigation comprehensively analyzed the impact of DES pretreatment on SCB composition. Notably, the use of the TEBAC:LA-based DES under mild operating conditions proved highly efficient in lignin removal, achieving 85.33 ± 0.52% for lignin removal and 98.67 ± 2.84% for cellulose recovery, respectively. Furthermore, the TEBAC:LA-based DES exhibited a superior digestibility of fermentable sugar during enzymatic hydrolysis. This is attributed to the disruptive effect of DES pretreatment on the dense SCB structure, enhancing the accessibility of enzymes to carbohydrates. Moreover, it yielded the highest bioethanol concentration of 24.50 g/L, with a productivity rate of 0.68 g/(L·h). Therefore, the TEBAC:LA-based DES provides a promising pretreatment technique for selective delignification and the conversion of SCB to bioethanol.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9110981/s1, Table S1: ANOVA analysis and principal effects for HBDs (H), with TEBAC as HBA; Table S2: ANOVA analysis and principal effects for HBDs (H) with BPP as the HBA.

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