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Synthesis of Green Deep Eutectic Solvents for Pretreatment Wheat Straw: Enhance the Solubility of Typical Lignocellulose

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Abstract: Deep eutectic solvents (DESs), a novel and environmentally-friendly solvent, have high potential for biomass pretreatment due to its advantages of low cost, low toxicity, strong solubility, excellent selectivity and biocompatibility. Two types of DES (binary and ternary) were synthesized and characterized, and optimized ternary DES was selected to pretreat wheat straw for enhancement of the solubility of lignocellulose. Moreover, enzymatic hydrolysis was tested to verify the performance of pretreatment. In addition, the changes in surface morphology, structure and crystallinity of wheat straw pretreated by DES were analyzed to reveal the pretreatment mechanism. Experimental results indicated that viscosity exhibited little difference in different types of DESs, and a declining trend as the temperature increases in same DES. The ternary DES pretreatment efficiently enhanced the solubility of typical lignocellulose, with the optimal removal rate of lignin at approximately 69.46%. Furthermore, the total sugar concentration of the residue was about 5.1 times more than that of untreated wheat straw after the pretreated samples were hydrolyzed by the cellulase for 24 h, indicating that DES has the unique ability to selectively extract lignin and hemicellulose from wheat straw while retaining cellulose, and thus enhanced the solubility of lignocellulose. The scanning electron microscope (SEM) observation and X-ray diffraction (XRD) determination showed that the surface of wheat straw suffered from serious erosion and the crystallinity index of wheat straw increased after DES5 pretreatment. Therefore, DES cleaves the covalent bond between lignin and cellulose and hemicellulose, and reduces the intractability of lignin resulting in the lignin dissolution. It suggests that DES can be used as a promising and biocompatible pretreatment way for the cost-effective conversion of lignocellulose biomass into biofuels.

Keywords: deep eutectic solvents; pretreatment; lignocellulose; enzymatic hydrolysis; total sugar

1. Introduction

Biobutanol, renewable carbon-neutral liquid fuel, is considered as an alternative to gasoline, and is expected to meet the demand of sustainable economic development without increasing emissions of greenhouse gas as fossil fuels [1,2]. Lignocellulosic biomass is an ideal raw material for the biobutanol production due to its merits of being renewable, cheap and easily accessible [3]. However, the complex structure of lignocellulose hinders its direct hydrolysis, and it is difficult to achieve component separation, hydrolysis and saccharification, making efficient utilization of lignocellulosic biomass challenging [4,5]. Therefore, pretreatment to dissolve or separate the lignin from the cellulose in lignocellulosic biomass removes the physical and chemical barriers that make it difficult to degrade

the native biomass and facilitates enzymatic hydrolysis of hemicellulose and cellulose [6], which is a key step in the biochemical processing of lignocelluose based on the concept of sugar platform [7,8].

Traditional pretreatment methods of lignocellulosic biomass include physical methods (e.g., mechanical comminution, microwave and ultrasonic) [9,10], physical and chemical methods (e.g., steam explosion and hydrothermal) [11], chemical processes (e.g., acid, alkali and ionic liquids) [12–15] and biological methods [16]. Although various traditional pretreatment technology can improve the hydrolysis rate through change the physical and chemical structure of lignocellulosic biomass [17], it tends to be harsh, yield lignocellulose-derived by-products and lead to downstream complex of the production process [18].

Recently, deep eutectic solvents (DESs) have been viewed as potential green solvents for extracting lignin from lignocellulose [19,20], which have great biodegradability, low-cost and easy synthesis compared to ionic liquids (ILs) [21,22]. Constitutionally, DESs are synthesized from renewable and natural components, with at least a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) (e.g., quaternary ammonium salts, alcohols and acid amides) [23]. The most common HBAs in DES are quaternary ammonium salts with choline chloride (ChCl). There is more variability in the choice of HBD such as polyols and carboxylic acids [24]. The complex hydrogen bond network structure in DES makes it as a special solvent for different applications [25–27]. The eutectic solvent technology has been successfully applied to the pretreatment of wheat straw, corn cob and switch grass [28,29]. Despite some unique merits for the separation of the three main components of lignocellulosic biomass, DESs still suffers from lengthy pretreatment time to reduce biomass recalcitrance so as to obtain a desirable outcome. In order to efficiently separate cellulose, hemicelluloses and lignin from lignocellulose, new DES and its synthesis strategy should be studied in order to achieve the overall biorefining of lignocellulosic biomass [30]. Previous works have shown that types of DESs are influential in biomass fractionation, i.e., acidic DESs are more effective in biomass deconstruction and the enhancement of enzymatic hydrolysis of cellulose [31,32]. Compared to acidic DES pretreatment, alkaline one can reduce equipment corrosion, which improves the economic and commercial viability of biofuel production [33].

Aimed to efficiently separate lignin, cellulose and hemicellulose from lignocellulosic biomass, this study first prepared six kinds of different eutectic solvents with two types (binary and ternary) and analyzed the changes in the composition before and after the pretreatment of wheat straw by the prepared EDS. Then, enzyme hydrolysis was explored to examine the pretreatment performance with different EDS. The pretreatment effect of DES at different temperatures was also investigated. Finally, the pretreatment mechanism of biomass with DES was analyzed by scanning electron microscope (SEM) and X-ray diffraction (XRD) characterization.

2. Materials and Methods

2.1. Synthesis and Characterization of DESs

2.1.1. Synthesis of Different Types of DES

The hydrogen bond acceptor (Choline chloride) and hydrogen bond donor (Urea, Ethanediol and Lactic acid) of deep eutectic solvent with different mole ratio were mixed in a beaker, and then the beaker was sealed with a film to reduce the volatilization of solvent during the preparation process. The mixture was heated to 60 °C with a constant temperature magnetic stirrer at a stirring speed of 200 rpm until a uniform transparent liquid was formed. After preparation, the mixture was stored at room temperature. Six eutectic solvents were synthesized in this study, and the composition was showed in Table 1.

Table 1. Composition of deep eutectic solvents.

DES	Composition (Mole Ratio)
DES1	Choline chloride + Urea (1:2)
DES2	Choline chloride + Ethanediol (1:2)
DES3	Choline chloride + Lactic acid (1:2)
DES4	Choline chloride + urea (1:2) + 10 wt%H_2O
DES5	Choline chloride + urea (1:2) + 10 wt%H_2O + 1 wt%NaOH
DES6	Choline chloride + Ethanediol + Lactic acid (1:1:1)

2.1.2. Viscosity and Density of Eutectic Solvent

The viscosimeter was cleaned with anhydrous ethanol, and then about 5 mL of the prepared deep eutectic solvent was injected into the instrument (DMA5000M-Lovis2000ME, Anton Paar, Wien, Austria) with a syringe. The viscosity and density of different deep eutectic solvents at 25, 55 and 85 °C were measured, respectively.

2.1.3. pH Value of Eutectic Solvent

The prepared deep eutectic solvent was cooled to room temperature and placed in a beaker. The pH meter electrode was washed with distilled water and gently dried with mirror paper. The pH meter electrode was placed in the deep eutectic solvent until the indicator is stable.

2.2. Pretreatment of Wheat Straw with DESs

2.2.1. Wheat Straw Materials

Wheat straw was collected from a farm in Jiangsu province, China. The sample of wheat straw was dried at room temperature for one week, and then was stored in sealed plastic bags at room temperature after passed through 40 mesh sieve.

2.2.2. Experimental Design of Pretreatment with DES

A mixture containing of 5% (*w/w*) wheat straw (WS) was prepared by combining 1 g of WS (particle size of 250–500 mm) with 20 g solvent in a 100 mL Teflon-sealed autoclave and put into a muffle furnace for 2 h at the defined temperature. After the pretreatment, the mixtures were cooled to room temperature. The precipitate was harvested by centrifuging at $10,000 \times g$ rpm and washing with deionized water until the supernatant was colorless followed by drying with a vacuum freeze dryer (FD-1A-50, Biocool, Beijing, China).

2.2.3. Assay of the Component Content of Wheat Straw Treated with DES

The specific steps of sample component determination before and after pretreatment were as follows: In the first step, 72% H_2SO_4 was used to hydrolyze the sample in a shaker at 30 °C for 1 h (solid-liquid ratio 1:10 *w/v*). In the second step, 72% H_2SO_4 in the first step was diluted to 4% H_2SO_4, and the system was hydrolyzed for 1 h in an air-dry oven at 121 °C. The hydrolyzed suspension was cooled naturally at room temperature, and the solid and liquid were separated by vacuum filtration device. The resulting supernatant was used to measure the contents of cellulose, hemicellulose and acid soluble lignin derived from wheat straw. Sediment was further used to determine the content of acid insoluble lignin.

2.2.4. Enzymatic Hydrolysis

Enzymatic saccharification of the pretreated wheat straw was described previously [34]. Briefly, 10 mg pretreated wheat straw, cellulase solution (add cellulase at a concentration of 50 mg protein/g solid) and citrate buffer solution (50 mM sodium citrate, pH 4.8) were successively added into 50 mL corked triangular flask. The blank samples were not added with wheat straw. The enzymatic reaction was carried out at 50 °C (at 180 rpm) and levels of the resultant sugar were estimated at various time intervals (1, 2, 4, 6 and 24 h) using the DNS method.

2.3. Morphology and Structure of Wheat Straw Pretreated with DESs

The surface morphology of wheat straw was observed by scanning electron microscopy (SEM, JSM-7610F, Tokyo, Japan). A multi-functional X-ray diffraction (XRD, Ultima IV, Tokyo, Japan) system was used to conduct X-ray diffraction analysis on the wheat straw samples under avoltage of 40 kV and current of 30 mA. The test range was 5–80°, and the test speed was 5°/min. The crystallinity index (*CrI*) was calculated by the following formula:

$$CrI = [(I_{002} - I_{amorphous})/I_{002}] \times 100\% \qquad (1)$$

where I_{002} is the diffraction intensity of crystalline structure ($2\theta = 22.5°$), and $I_{amorphous}$ is the diffraction intensity of amorphous fraction ($2\theta = 18.2°$).

3. Results and Discussion

3.1. Characterization of DESs

Manipulation of alkalinity and acidity levels of deep eutectic solvents as a potential tool to modulate hydrogen bond donor plays a key role in the dissolution capacity of lignocellulosic biomass. In the binary DES system, the pH value of DES1 was 8.55, showing weak alkalinity. While the pH values of DES2 and DES3 were 3.42 and 0.25, respectively (Figure 1). DES3 showed strong acidity, indicating that the pH value of the deep eutectic solvent is closely related to the hydrogen bond donor, this was similar to Hansen's research [26]. Based on the correlation, solvents with different pH values can be designed by changing the use of different hydrogen bond donors in the choline chloride-based deep eutectic solvent. In the ternary DES system, DES4 and DES5 were alkaline, and DES6 presented acidic. As shown in Figure 1, the pH value of DES2 did not change significantly compared to DES1, but the pH value increased to 13.41 after adding 1 wt% NaOH and 10 wt% H_2O to DES1, showing strong alkalinity. In acidic deep eutectic solvents, although the pH value of DES6 was between DES2 and DES3, its pH value was closer to DES3, showing strong acidity. Previous research showed that the pH of deep eutectic solvent had a great influence on the composition of residue, and the enhancement of acidity or alkalinity could improve the removal efficiency of lignin [35].
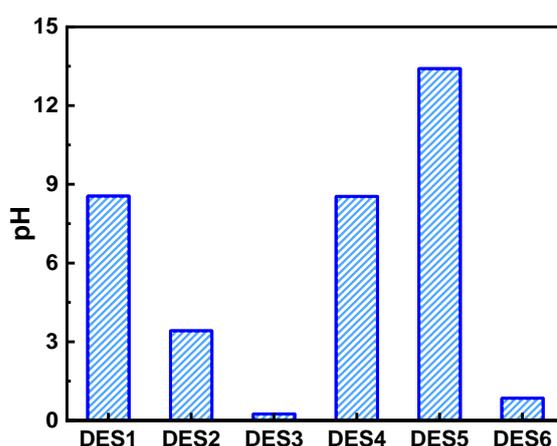

Figure 1. pH values of different deep eutectic solvents.

The synthesis of new DES with low viscosity is of potential significance because the hydrogen bond between components of DES makes it low fluidity and high viscosity, which affects its performance on pretreatment of lignocellulose. At room temperature, the viscosity of DES is range about 30–700 mPa·s, which reduces its fluidity to a large extent (Table 2). The reduction of viscosity can often improve the solvent's solubility and mass transfer performance, thus having a great impact on the pretreatment. Therefore, the synthesis of low viscosity deep eutectic solvent or reducing the viscosity of deep eutectic solvent is an important way to improve solvent solubility. As shown in Table 2, the viscosity of deep eutectic solvent is DES1 > DES5 > DES4 > DES2 in descending order at 25 °C. The

recent research showed that the increased temperature and water content effected on the physical properties and esterification of DES [36]. After adding 10 wt%H_2O into DES1 to form DES4, the viscosity of DES4 was only about 1/10 that of DES1. Although the viscosity of DES5 increased slightly after the addition of 1 wt% NaOH and 10 wt%H_2O, it was still far less than that of DES1. This could be attributed to that the low mole fraction of water did not affect the diffusion of ions in the deep eutectic solvent, but significantly reduced the viscosity of the solvent, thus enhancing the fluidity of the solution and improving the mass transfer performance and efficiency. However, at a high molar fraction, water molecules could form hydrogen bonds with anions (such as Cl^-) in hydrogen bond receptors and hydrogen bond donors, which inhibited intermolecular and intramolecular interactions in deep eutectic solvents and caused serious changes in their properties. Collectively, chemical properties of components, water content, temperature and other factors in deep eutectic solvents affected the interaction between molecules, and change the viscosity [37,38].

Table 2. Viscosity of deep eutectic solvents at different temperatures.

Treatment	Viscosity mPa·s (25 °C)	Viscosity mPa·s (55 °C)	Viscosity mPa·s (85 °C)
DES1	611.40	73.74	21.29
DES2	38.49	14.26	7.23
DES4	53.01	15.73	7.14
DES5	75.68	19.78	8.44

Density is another important property affecting the mass transfer in the pretreatment process of DES, which can also provide information about intermolecular interactions in a DES. As showing in Figure 2, the density of deep eutectic solvent varied as a function of temperature according to the hydrogen bond donor of DES. Ethanediol based DES have the lower density compared to urea–based DES. Typically, DESs have exhibited higher densities than that of water [26]. It can be seen that the addition of a small amount of water reduces the density of DES1. The density of deep eutectic solvent is DES1 > DES5 > DES4 > DES2 in descending order at 25–85 °C. With the increase of temperature, the density of deep eutectic solvent decreased, which was consistent with the change of liquid density with temperature [39].
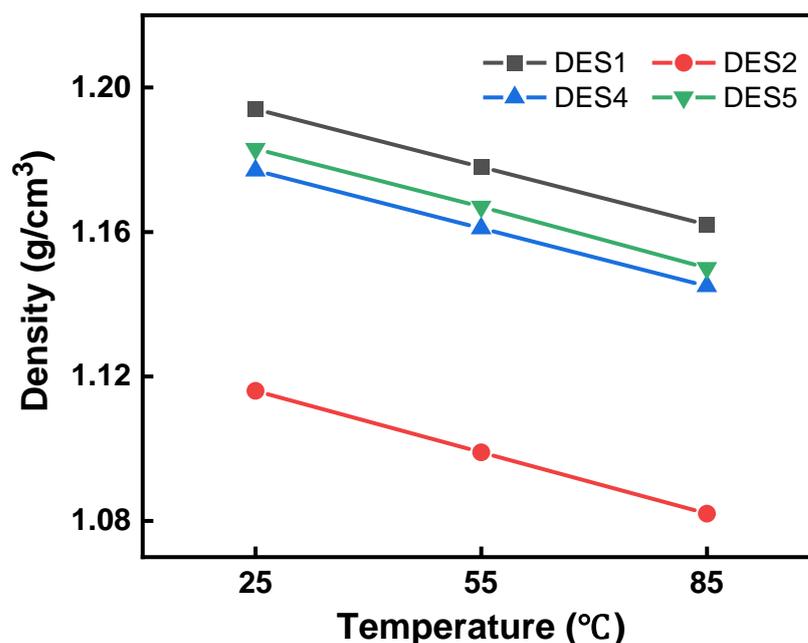

Figure 2. Density versus temperature curve of deep eutectic solvents.

3.2. Effect of DESs on Decomposition of Wheat Straw

The composition changes of wheat straw before and after various pretreatments were presented in Table 3. Obviously, in terms of lignin contents, the lignin content of residues of wheat straw pretreated by DES1, DES2 and DES4 were higher than that of untreated wheat straw, while it pretreated by DES3, DES5 and DES6 were lower than that of untreated wheat straw, indicating that DES3, DES5 and DES6 had high removal capacity of lignin. The lignin removal rates of DES3, DES5 and DES6 were 69.46%, 61.98% and 63.81%, respectively, whereas those of cellulose were 50.55%, 41.70% and 44.09%, respectively. It was noted that the dissolution efficiency of the three deep eutectic solvents for lignin was obviously higher than that for cellulose, and all of them showed moderately selective solubility of wheat straw components. In acidic deep eutectic solvent, the lignin contents of residues of wheat straw pretreated by DES3 and DES6 were lower than that of untreated wheat straw, in contrast, the lignin content of DES2 pretreated residues was higher than that of untreated wheat straw. Therefore, DES3 and DES6 had evident pretreatment effect on wheat straw. Although acidic solvent system was favorable for lignin fragmentation and subsequent solubilisation, the acidic conditions tended to cause severe hemicellulose degradation [11]. In alkaline deep eutectic solvent, the cellulose content of residue of wheat straw pretreated by DES5 increased slightly compared with DES1 and DES4, while hemicellulose content presented no significant difference. The results showed that DES5 with strong alkalinity could significantly improve the lignin removal rate and better preserve cellulose and hemicellulose compared to DES1 and DES4 with weak alkalinity. It was beneficial for sugar production since cellulose is the most important raw material used for reducing sugar production. The deep eutectic solvent with strong alkalinity had good selectivity in components of wheat straw and was a potentially high-quality solvent for pretreatment of lignocellulosic biomass to produce reducing sugars for biobutanol fermentation [33].

Table 3. Effect of different DES on the pretreatment of wheat straw.

Pretreatment Method	Pretreatment (%)				Composition (%)		
	Lignin Removal Rate	Hemicellulose Retention Rate	Cellulose Retention Rate	Solid Yield	Cellulose	Hemicellulose	Lignin
Control	-	-	-	-	28.95	15.30	20.72
DES1	36.98	62.47	83.33	50.71	37.80	26.02	25.95
DES2	27.26	36.16	91.75	56.07	37.64	13.62	27.09
DES3	69.46	13.62	80.95	36.83	50.55	7.81	17.32
DES4	41.37	65.36	84.70	51.87	37.56	26.61	23.60
DES5	61.98	57.06	81.88	45.16	41.70	26.68	17.58
DES6	63.81	21.14	81.27	42.39	44.09	10.53	17.83

3.3. Effect of DESs on Enzymatic Hydrolysis Efficiency

The total reducing sugar concentration was 0.296 g/L after 24 h of enzymatic hydrolysis of the residue from wheat straw pretreated by DES1 (Figure 3), and the initial hydrolysis rate of polysaccharide was 0.015 $g \cdot L^{-1} \cdot h^{-1}$ (Figure 4). Although the lignin removal rate with DES4 pretreatment was not significantly improved compared with that with DES1 pretreatment, the enzymatic hydrolysis yield was dramatically enhanced. The total reducing sugar concentration is about 1.6 times that with DES1 pretreatment, and the initial hydrolysis rate of polysaccharide is about 7.5 times that with DES1 pretreatment. An explanation for the improvement of enzymatic hydrolysis is that the addition of water reduces the viscosity of DES4 (the viscosity of DES4 is evidently lower than that of DES1 at room temperature), which makes the residue of wheat straw pretreated by DES4 easier to wash, with the weakened agglomeration. Compared with DES4, the enzymatic hydrolysis effect of the residue from wheat straw after DES5 pretreatment was further significantly improved. After 24 h of enzymatic hydrolysis, the total sugar concentration the residue with DES5 pretreatment was 1.547 g/L, which was about 3 times of that with DES4 pre-

treatment. Moreover, initial hydrolysis rate of polysaccharides was 0.704 $g\cdot L^{-1}\cdot h^{-1}$, which was about 6 times of that with DES4 pretreatment. This could be attributed to that most of the lignin in wheat straw was removed by DES5 pretreatment, resulting in the accessibility of enzyme to polysaccharide in the residue. The previous study indicated that the large removal of hemicelluloses and high delignification efficiency could be elevated after DES treatment, thus obtaining the reduced nonproductive absorption and the diminished coating efficiency [20].
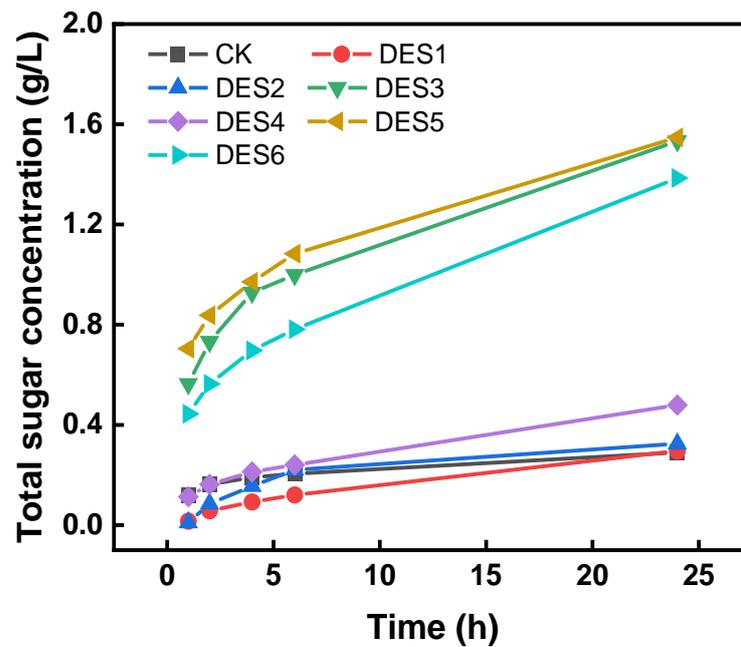

Figure 3. Total sugar concentration of enzymatic hydrolysis of wheat straw before and after pretreatment.
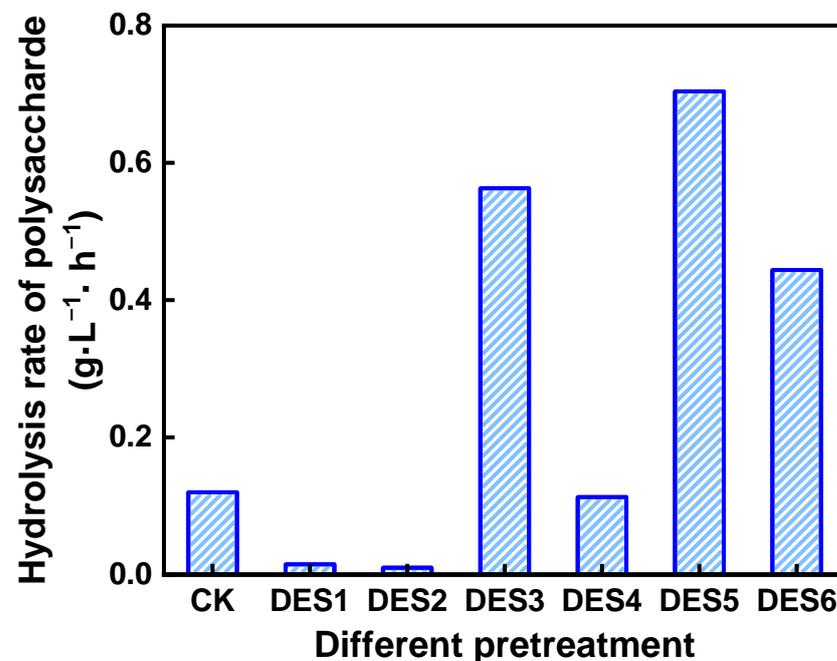

Figure 4. Initial hydrolysis rate of polysaccharide before and after pretreatment.

In conclusion, DES5 pretreatment achieved superior enzymatic hydrolysis yield, indicating that the enhancement of alkalinity of deep eutectic solvent could improve the lignin removal rate, and subsequently remarkably promote the enzymatic hydrolysis of

the residue derived from lignocellulosic biomass with DES pretreatment [40]. In view of the less processing time and high enzymatic digestibility, it could be concluded that the pretreatment using DES was a desirable approach for a cost-effective processing and dissolution of lignoellulosic biomass.

3.4. Mechanism of Pretreatment by DESs

DES pretreatment can cleave the structure of wheat straw and bring in the impact of lignin relocation, biocompatibility and biomass components on enzymatic accessibility. The morphological changes induced by DES5 pretreatment were observed by SEM (Figure 5). The wheat straw had relatively smooth surface without any erosion trace. After DES5 pretreatment, the fiber bundles of wheat straw became uneven, loose and disordered, with more fragmented structures, and the specific surface area increased obviously. The explanation might be that DES5 pretreatment caused the release of lignin and hemicellulose, forming the porosity and channel of the biomass. The interior of biomass becomes fluffy, which facilitates the enzyme molecules to enter the interior of biomass and effectively improves the enzyme hydrolysis efficiency [41]. Consequently, the fragmentation of the fiber bundle is due to the destruction of a large number of special bond-bridge structures connecting lignin and hemicellulose, which is consistent with the experimental results of large amounts of dissolved lignin and hemicellulose. Both the enhancement of enzymatic accessibility to cellulose and rupture of wheat straw fibers contributed to the increase of total sugar yield during enzymatic hydrolysis [10].
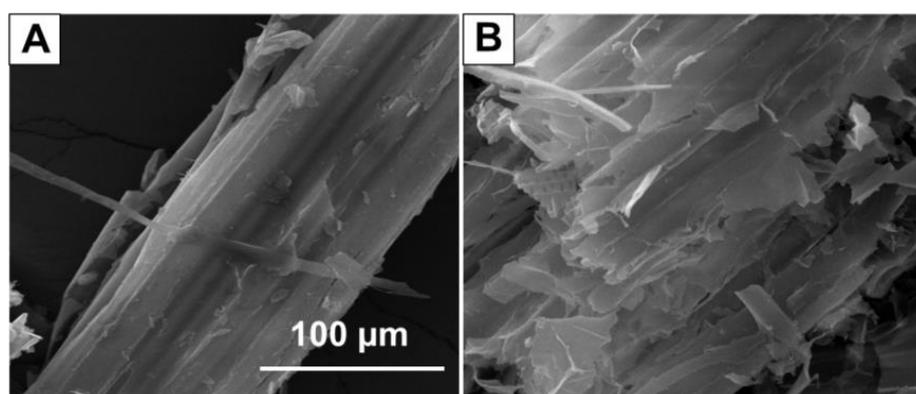

Figure 5. SEM images of the wheat straw before and after pretreatment: (**A**) untreated wheat; (**B**) the residue with DES5 pretreatment.

CrI, the ratio of crystalline cellulose among the lignocellulosic biomass, is a significant factor affecting the enzymatic hydrolysis. If the aim of pretreatment is to remove lignin and hemicellulose, higher crystallinity would show better pretreatment performance. However, if the aim is to break the cellulose crystallization zone, lower crystallinity would indicate better pretreatment performance. In this study, the former is what we need, thus higher crystallinity means superior pretreatment performance (Figure 6). X-ray diffraction measurement of *CrI* is the appropriate way to estimate the impact of the pretreatment on biomass crystallinity [42]. The crystallinity of wheat straw pretreated with DES5 (36.8%) (DES5 is alkaline deep eutectic solvent synthesized in this study) was significantly higher than that of untreated wheat straw (23.7%) (Table 4). The results showed that the removal rate of disordered structures (lignin and hemicellulose) in wheat straw was higher than that of cellulose after pretreatment with alkaline deep eutectic solvent, which was consistent with the results of selective solubility of wheat straw pretreated with deep eutectic solvent mentioned above. As reported previously, similar attempts on the increase of *CrI* of lignocellulosic biomass have also been made by introducing alkaline pretreatment [43].
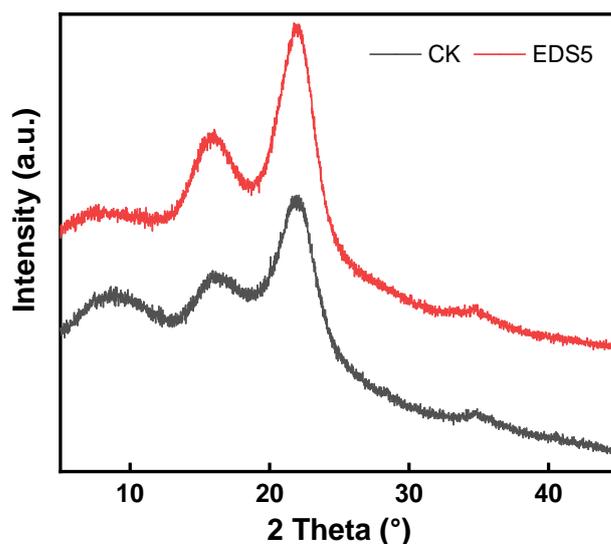
Figure 6. XRD of wheat straw before and after pretreatment.

Table 4. The crystallinity of wheat straw pretreated with DES5.

Treatment	I_{am} (18.2)	I_{002} (22.5)	Crystallinity (%)
CK	4299	5635	23.7
DES5	4284	6780	36.8

4. Conclusions

Deep eutectic solvents have been viewed as potential green solvents for extracting lignin from lignocellulose. The DESs synthesized in this study have the unique ability to selectively extract lignin and hemicellulose from wheat straw, while retaining cellulose, and thus enhanced the solubility of lignocellulose. The pH of deep eutectic solvent has a great influence on the composition of residue. The enhancement of acidity or alkalinity is helpful to improve the removal efficiency of lignin, but too low pH value of deep eutectic solvent caused the loss of a large amount of xylan and part of cellulose, resulting in low content of fermentable sugar. Under the condition of the same lignin removal rate, alkaline DES5 can preserve relatively more xylan besides cellulose, thus increasing the total sugar concentration as the substrate for subsequent fermentation. Moreover, the DES pretreatments significantly improved solubilization of lignin, and crystallinity index (*CrI*) of residual cellulose. It suggests that combining DES pretreatment with cellulase contributes novel green and effective approach in lignocelluloses hydrolysis for bioenergy production.

Author Contributions: Data curation, B.H. and Y.Y.; writing—original draft preparation, Z.T.; writing—review and editing, L.W., T.L. and J.L. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dou, J.Z.; Chandgude, V.; Vuorinen, T.; Bankar, S.; Hietala, S.; Lê, H.Q. Enhancing Biobutanol Production from biomass willow by pre-removal of water extracts or bark. *J. Clean. Prod.* **2021**, *327*, 129432. [CrossRef]
2. Hong, Y.; Wu, Y.R. Acidolysis as a biorefnery approach to producing advanced bioenergy from macroalgal biomass: A state-of-the-art review. *Bioresour. Technol.* **2020**, *318*, 124080. [CrossRef] [PubMed]
3. Xia, Q.; Liu, Y.; Meng, J.; Cheng, W.; Chen, W.; Liu, S.; Liu, Y.; Li, J.; Yu, H. Multiple hydrogen bond coordination in three-constituent deep eutectic solvents enhances lignin fractionation from biomass. *Green Chem.* **2018**, *20*, 2711–2721. [CrossRef]
4. Pang, B.; Sun, Z.H.; Wang, L.; Chen, W.J.; Sun, Q.; Cao, X.F.; Shen, X.J.; Xiao, L.; Yan, J.L.; Deuss, P.J.; et al. Improved value and carbon footprint by complete utilization of corncob lignocellulose. *Chem. Eng. J.* **2021**, *419*, 129565. [CrossRef]
5. Zhao, X.; Zhang, L.; Liu, D. Biomass recalcitrance. Part I: The chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels Bioprod. Biorefin.* **2012**, *6*, 465–482. [CrossRef]
6. Liu, X.M.; Wang, M.C.; Zhang, X.; Sun, Y.; Song, W.; Liu, Y. The correlation between the physicochemical properties of water-based deep eutectic solvents and catalytic activity of lipase Novozym 435. *J. Mol. Liq.* **2021**, *325*, 115200. [CrossRef]
7. Krishania, M.; Kumar, V.; Vijay, V.K.; Malik, A. Analysis of different techniques used for improvement of biomethanation process: A review. *Fuel* **2013**, *106*, 1–9. [CrossRef]
8. Zhu, Z.; Liu, Z.; Zhang, Y.; Li, B.; Lu, H.; Duan, N.; Si, B.C.; Shen, R.X.; Lu, J.W. Recovery of reducing sugars and volatile fatty acids from cornstalk at different hydrothermal treatment severity. *Bioresour. Technol.* **2016**, *199*, 220–227. [CrossRef]
9. Hoang, A.T.; Nižetić, S.; Ong, H.C.; Mofijur, M.; Ahmed, S.F.; Ashok, B.; Bui, V.T.V.; Chau, M.Q. Insight into the recent advances of microwave pretreatment technologies for the conversion of lignocellulosic biomass into sustainable biofuel. *Chemosphere* **2021**, *281*, 130878. [CrossRef] [PubMed]
10. Wang, S.Q.; Li, F.; Zhang, P.Y.; Jin, S.G.; Tao, X.; Tang, X.; Ye, J.P.; Nabi, M.; Wang, H.J. Ultrasound assisted alkaline pretreatment to enhance enzymatic saccharification of grass clipping. *Energy Convers. Manag.* **2017**, *149*, 409–415. [CrossRef]
11. Tian, C.; Li, B.; Liu, Z.; Zhang, Y.; Lu, H. Hydrothermal liquefaction for algal biorefinery: A critical review. *Renew. Sustain. Energy Rev.* **2014**, *38*, 933–950. [CrossRef]
12. Fang, W.; Zhang, P.Y.; Zhang, G.M.; Jin, S.G.; Li, D.; Zhang, M. Effect of alkaline addition on anaerobic sludge digestion with combined pretreatment of alkaline and high pressure homogenization. *Bioresour. Technol.* **2014**, *168*, 167–172. [CrossRef]
13. Uju; Wijayanta, A.T.; Goto, M.; Kamiy, N. Great potency of seaweed waste biomass from the carrageenan industry for bioethanol production by peracetic acideionic liquid pretreatment. *Biomass Bioenergy* **2015**, *81*, 63–69. [CrossRef]
14. Uju; Wijayanta, A.T.; Goto, M.; Kamiya, N. High yield hydrolysis of seaweed-waste biomass using peracetic acid and ionic liquid treatments. *AIP Conf. Proc.* **2018**, *1931*, 020004.
15. Uju; Goto, M.; Kamiya, N. Powerful peracetic acid–ionic liquid pretreatment process for the efficient chemical hydrolysis of lignocellulosic biomass. *Bioresour. Technol.* **2016**, *214*, 487–495. [CrossRef]
16. Wang, S.Q.; Li, F.; Wu, D.; Zhang, P.Y.; Wang, H.J.; Tao, X.; Ye, J.P.; Nabi, M. Enzyme pretreatment enhancing biogas yield from corn stover: Feasibility, optimization, and mechanism analysis. *J. Agric. Food Chem.* **2018**, *66*, 10026–10032. [CrossRef]
17. Sun, X.; Liu, S.; Zhang, X.Y.; Tao, Y.; Boczkaj, G.; Yoon, J.Y.; Xuan, X.X. Recent advances in hydrodynamic cavitation-based pretreatments of lignocellulosic biomass for valorization. *Bioresour. Technol.* **2021**, 126251. [CrossRef]
18. Zhang, Q.; Ma, R.T.; Ma, L.S.; Zhang, L.L.; Fan, Y.M.; Wang, Z.G. Contribution of lignin in esterifed lignocellulose nanofbers (LCNFs) prepared by deep eutectic solvent treatment to the interface compatibility of LCNF/PLA composites. *Ind. Crops Prod.* **2021**, *166*, 113460. [CrossRef]
19. Zhang, H.Y.; Han, L.J.; Dong, H.M. An insight to pretreatment, enzyme adsorption and enzymatic hydrolysis of lignocellulosic biomass: Experimental and modeling studies. *Renew. Sustain. Energy Rev.* **2021**, *140*, 10758. [CrossRef]
20. Xie, J.X.; Chen, J.J.; Cheng, Z.; Zhu, S.Y.; Xu, J. Pretreatment of pine lignocelluloses by recyclable deep eutectic solvent for elevated enzymatic saccharification and lignin nanoparticles extraction. *Carbohydr. Polym.* **2021**, *269*, 118321. [CrossRef] [PubMed]
21. 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*, 2032–2050. [CrossRef]
22. Tang, X.; Zuo, M.; Li, Z.; Liu, H.; Xiong, C.; Zeng, X.; Sun, Y.; Hu, L.; Liu, S.; Lei, T.; et al. Green processing of lignocellulosic biomass and its derivatives in deep eutectic solvents. *ChemSusChem* **2017**, *10*, 2696–2706. [CrossRef] [PubMed]
23. Hossain, M.A.; Rahaman, M.S.; Yelle, D.; Shang, H.; Sun, Z.H.; Renneckar, S.; Dong, J.; Tulaphol, S.; Sathitsuksanoh, N. Effects of polyol-based deep eutectic solvents on the efficiency of rice straw enzymatic hydrolysis. *Ind. Crops Prod.* **2021**, *167*, 113480. [CrossRef]
24. Li, Q.W.; Dong, Y.; Hammond, K.D.; Wan, C.X. Revealing the role of hydrogen bonding interactions and supramolecular complexes in lignin dissolution by deep eutectic solvents. *J. Mol. Liq.* **2021**, *344*, 117779. [CrossRef]
25. Smith, E.L.; Abbott, A.P.; Ryder, K.S. Deep eutectic solvents (DESs) and their applications. *Chem. Rev.* **2014**, *114*, 11060–11082. [CrossRef]
26. Hansen, B.B.; Spittle, S.; Chen, B.; Poe, D.; Zhang, Y.; Klein, J.M.; Horton, A.; Adhikari, L.; Zelovich, T.; Doherty, B.W.; et al. Deep eutectic solvents: A review of fundamentals and applications. *Chem. Rev.* **2020**, *121*, 1232–1285. [CrossRef]
27. Svigelj, R.; Dossi, N.; Grazioli, C.; Toniolo, R. Deep eutectic solvents (DESs) and their application in biosensor development. *Sensors* **2021**, *21*, 4263. [CrossRef]

28. Yang, J.Y.; Wang, Y.; Zhang, W.J.; Li, M.F.; Peng, F.; Bian, J. Alkaline deep eutectic solvents as novel and effective pretreatment media for hemicellulose dissociation and enzymatic hydrolysis enhancement. *Int. J. Biol. Macromol.* **2021**, *193*, 1610–1616. [CrossRef]

29. Xu, H.F.; Peng, J.J.; Kong, Y.; Liu, Y.Z.; Su, Z.N.; Li, B.; Song, X.M.; Liu, S.W.; Tian, W.D. Key process parameters for deep eutectic solvents pretreatment of lignocellulosic biomass materials: A review. *Bioresour. Technol.* **2020**, *310*, 123416. [CrossRef]

30. Fakayode, O.A.; Akpabli-Tsigbe, N.D.K.; Wahia, H.; Tu, S.S.; Ren, M.; Zhou, C.; Ma, H. Integrated bioprocess for bio-ethanol production from watermelon rind biomass: Ultrasound-assisted deep eutectic solvent pretreatment, enzymatic hydrolysis and fermentation. *Renew. Energy* **2021**, *180*, 258–270. [CrossRef]

31. Thi, S.; Lee, K.M. Comparison of deep eutectic solvents (DES) on pretreatment of oil palm empty fruit bunch (OPEFB): Cellulose digestibility, structural and morphology changes. *Bioresour. Technol.* **2019**, *282*, 525–529. [CrossRef] [PubMed]

32. 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.

33. Schutyser, W.; Renders, T.; Van den Bosch, S.; Koelewijn, S.F.; Beckham, G.T.; Sels, B.F. Chemicals from lignin: An interplay of lignocellulose fractionation, depolymerisation, and upgrading. *Chem. Soc. Rev.* **2018**, *47*, 852–908. [CrossRef] [PubMed]

34. Resch, M.G.; Baker, K.O.; Decker, S.R. *Low Solid Enzymatic Saccharification of Lignocellulosic Biomass Renewable Energy Laboratory*; NREL/TP-5100-63351; NREL: Golden, CO, USA, 2015.

35. Singh, M.B.; Kumar, V.S.; Chaudhary, M.; Singh, P. A mini review on synthesis, properties and applications of deep eutectic solvents. *J. Indian Chem. Soc.* **2021**, *98*, 100210. [CrossRef]

36. Liu, Z.L.; Wan, X.; Wang, Q.; Tian, D.; Hu, J.G.; Huang, M.; Shen, F.; Zeng, Y.M. Performances of a multi-product strategy for bioethanol, lignin, and ultra-high surface area carbon from lignocellulose by PHP (phosphoric acid plus hydrogen peroxide) pretreatment platform. *Renew. Sustain. Energy Rev.* **2021**, *150*, 111503. [CrossRef]

37. Amphlett, J.T.M.; Choi, S. The effect of increasing water content on transition metal speciation in deep eutectic solvents. *J. Mol. Liq.* **2021**, *332*, 115845. [CrossRef]

38. Fan, C.; Sebbah, T.; Liu, Y.; Cao, X.L. Terpenoid-capric acid based natural deep eutectic solvent: Insight into the nature of low viscosity. *Clean. Eng. Technol.* **2021**, *3*, 100116. [CrossRef]

39. Zhao, B.; Xu, P.; Yang, F. Biocompatible deep eutectic solvents based on choline chloride: Characterization and application to the extraction of rutin from Sophora japonica. *ACS Sustain. Chem. Eng.* **2015**, *3*, 2746–2755. [CrossRef]

40. Zheng, Y.; Zhao, J.; Xu, F.; Li, Y. Pretreatment of lignocellulosic biomass for enhanced biogas production. *Prog. Energy Combust.* **2014**, *42*, 35–53. [CrossRef]

41. Ramadoss, G.; Muthukumar, K. Mechanistic study on ultrasound assisted pretreatment of sugarcane bagasse using metal salt with hydrogen peroxide for bioethanol production. *Ultrason. Sonochem.* **2016**, *28*, 207–217. [CrossRef]

42. Kumar, R.; Mago, G.; Balan, V.; Wyman, C.E. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresour. Technol.* **2009**, *100*, 3948–3962. [CrossRef] [PubMed]

43. Gu, Y.; Zhang, Y.; Zhou, X. Effect of Ca(OH)$_2$ pretreatment on extruded rice straw anaerobic digestion. *Bioresour. Technol.* **2015**, *196*, 116–122. [CrossRef] [PubMed]