

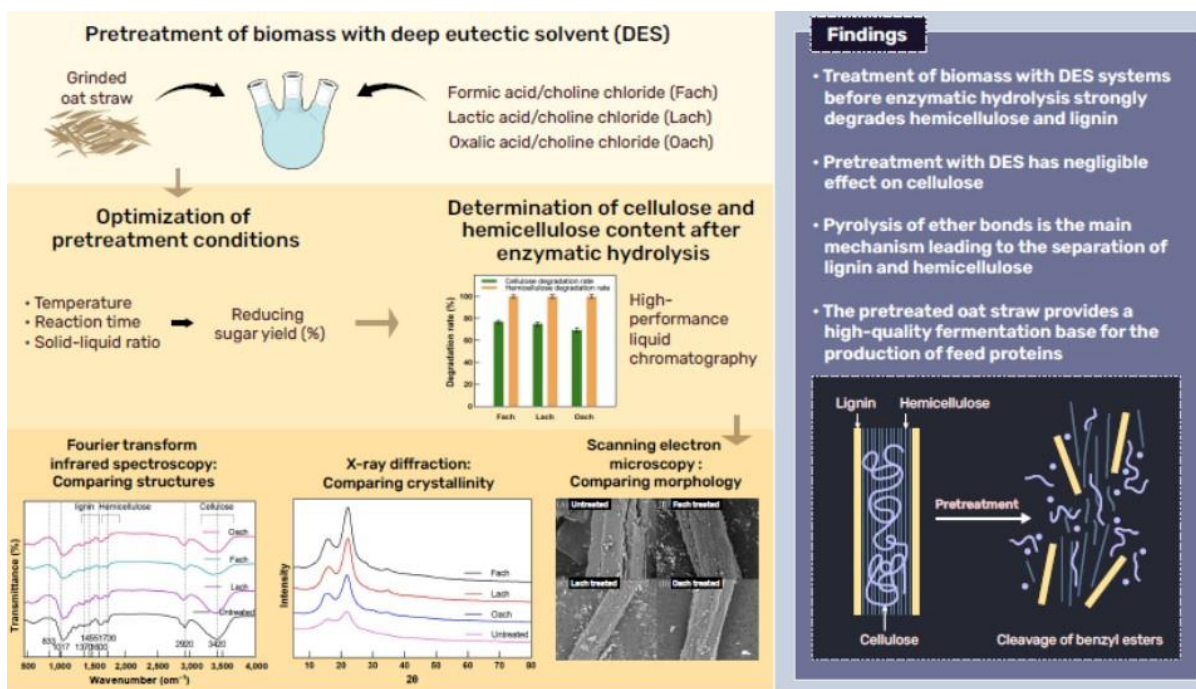
Unlocking the Potential of Oat Straw: Efficient Pretreatment Methods for Enhanced Glucose Production

Mingda Zhu,^{a,b} Xuejiao Qiu,^{a,b} Jingyun Hao,^c Jianhua Hu,^{a,b} and Zhanying Liu^{a,b,*}

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GRAPHIC ABSTRACT



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The excellent physical and chemical properties of deep eutectic solvents, especially the ability of some of them to dissolve biomass, make them broadly applicable in biomass pretreatment. In this study, oat straw was pretreated with deep eutectic solvents composed of choline chloride and an acid (formic, lactic, or oxalic acid). The highest reducing sugar yield was obtained for the formic acid/choline chloride mixture. Using a pretreatment temperature of 110 °C, a reaction time of 2 h, and a solid-liquid ratio of 1:20, the reducing sugar yield obtained by cellulase hydrolysis was 23.5%, the degradation rate of cellulose reached 76.9%, and hemicellulose was completely degraded. The pretreated oat straw was then analyzed by scanning electron microscopy, Fourier transform infrared spectroscopy, and X-ray diffraction. The changes in its crystallinity and lignin content compared to the untreated specimen were determined. A preliminary mechanism for the pretreatment of oat straw with formic acid/choline chloride was revealed. The study could provide an opportunity to expand the application in biochemicals or biological feed processes.

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Keywords: Deep eutectic solvents; Lignocellulose; Oat straw; Pretreatment

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INTRODUCTION

Oats are one of the most cultivated cereals worldwide. The planting area of oats in Inner Mongolia, China, accounts for more than 35% of the domestic production and is the main food crop in this region (Wang 2020). After removing the oat grains, some of the leftover oat straw is used as animal feed or burned. To avoid resource waste and environmental pollution, many countries have banned the burning of agricultural waste and are promoting the comprehensive utilization of the biomass for energy generation, livestock feed, insulation, and fertilizer (Borrega *et al.* 2022). New applications are actively sought after, and straw is seen to have potential as a raw material to produce biochemicals or biological feed. Among them, single cell protein feedstuff with fermentation technology is an effective way to utilize and add value to straw resources, while alleviating feed shortages. However, due to the complex natural structure of lignocellulosic biomass, it is difficult to degrade directly using yeast fermentation or cellulase enzymolysis. To efficiently utilize the fermentable sugars present in lignocellulosic biomass, pretreatment must be performed before enzymatic degradation. An efficient and feasible lignocellulosic pretreatment method can reduce the cost of fermentation of a biological feed and provide

environmental benefits (Xu *et al.* 2020).

Many studies have shown that ionic liquids have broad application prospects in biomass pretreatment because of their excellent physical and chemical properties, especially their ability to act as good solvents for both organic and inorganic compounds (Shill *et al.* 2011). Ionic liquids can improve the accessibility of polysaccharides by effectively destroying the microstructure of lignocellulose. However, the industrial applicability of ionic liquids is limited by their high cost and significant toxicity (Zhu *et al.* 2006). Abbott *et al.* (2003) first reported deep eutectic solvents (DESs) as emerging green solvents for replacing ionic liquids. Following a common convention, the term DES is used in this article to refer to systems that may or may not be at the eutectic ratio of ingredients. DESs comprise two or three components with certain proportions of hydrogen-bond donors and acceptors. DESs have similar physical and chemical properties to ionic liquids, and they have further advantages such as a low cost, simple preparation methods, and good biodegradability and biocompatibility (Gorke *et al.* 2008). Francisco *et al.* (2012) applied DESs to the pretreatment of biomass for the first time and found that DESs can convert biomass into reducing sugars. Since then, many studies have investigated DES pretreatment of various lignocellulosic biomasses, such as corn cobs, corn stalks, and straw (Procentese *et al.* 2015; Kumar *et al.* 2016; Xu *et al.* 2016), although no relevant reports of oat-straw pretreatment exist. Therefore, the investigation of the DES pretreatment of oat straw before enzymolysis is of particular interest, especially for regions with extensive oat plantations.

Various DESs comprising different quaternary ammonium salts and hydrogen donors have been designed to increase the number of accessible cellulose binding sites. Furthermore, organic acids, including formic acid (Zhang *et al.* 2010), oxalic acid (Kundu *et al.* 2015), and acetic acid (Trzcinski and Stuckey 2015), have been introduced as pretreatment reagents to break the recalcitrant structures of lignocellulosic materials *via* a mechanism similar to that of diluted acids. Therefore, we speculate that DESs with acidic hydrogen donors may be more effective at improving the cellulose accessibility of lignocellulosic materials. This study investigated the effects of three types of DESs (*i.e.*, formic acid/choline chloride, lactic acid/choline chloride, and oxalic acid/choline chloride) on the pretreatment of oat straw under different conditions to optimize the reaction conditions. Subsequently, the reducing sugar yields obtained by enzymatic hydrolysis using cellulase were determined. Finally, the structural and morphological changes resulting from pretreatment of the straw with the three different solvent systems were evaluated by X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, and scanning electron microscopy (SEM).

EXPERIMENTAL

Materials

Oat straw raw material was collected from the suburbs of Hohhot, Inner Mongolia Province, China, in the autumn after harvesting the oats. The oat straw was dried to a water mass fraction of 8% at 60 °C, cut into 3- to 5-cm pieces, crushed, and then screened with a 40-mesh sieve before use. Choline chloride was selected as the hydrogen bond acceptor, while formic acid, lactic acid, and oxalic acid were selected as hydrogen bond donors. All chemicals were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. The Cellic® Ctec3 cellulose enzyme was purchased from Gaoruisen Technology Co., Ltd (Beijing, China).

Preparation of DESs

Three different DESs were prepared: formic acid/choline chloride (Fach), lactic acid/choline chloride (Lach), and oxalic acid/choline chloride (Oach). Specifically, choline chloride was mixed with formic acid, lactic acid, or oxalic acid in molar ratios of 1:2, 1:2, and 1:1, respectively. Each mixture was stirred in an oil bath at 60 °C and 180 rpm to produce uniform solutions.

Pretreatment of Oat Straw

Each DES was mixed separately with 5.0 g oat straw powder in a 1 L three-necked flask and subjected to magnetic stirring (180 rpm) in an oil bath for 10 to 15 min until a clear liquid solution was formed, as described by Francisco *et al.* (2013). The effects of different operating temperatures (*i.e.*, 110, 120, 130, and 140 °C), reaction times (1, 2, 3, and 4 h), and solid-liquid ratios (1:5, 1:10, 1:15, 1:20, and 1:25) were determined. The slurry was washed with an anti-solvent (water, ethanol), and then dried at 38 °C. The dinitrosalicylic acid (DNS) method was used to determine the reducing sugar content of the liquid (Thi and Lee 2019). The solid residues were washed with deionized water several times and dried in a vacuum drying oven at 60 °C for 24 h. The dried solids were then subjected to enzyme hydrolysis and characterized by FT-IR, SEM, and XRD.

Determination of Cellulose and Hemicellulose

The method published by the National Renewable Energy Laboratory (NREL) (Sluiter *et al.* 2008) was used to determine the content of cellulose and hemicellulose in the samples. Specifically, 0.3 g of the sample was accurately weighed in a test tube, then 3 mL of 7.34 mol/L sulfuric acid was added, followed by reaction in a constant temperature water bath at 30 °C for 1 h under continuous stirring with a glass rod. After this time, the tube was immediately placed into the prepared ice bath to terminate the reaction. The liquid in the roller tube was transferred to a Cillin bottle, and then 84 mL of deionized water was added and mixed evenly to reduce the sulfuric acid concentration to 4%. The bottle was placed in an autoclave at 121 °C for 1 h, and then was quickly removed when the temperature dropped to 100 °C to prevent the sugar from being over-decomposed. After cooling, the supernatant and the solid-liquid residue were separated. The composition of the supernatant (20 µL samples) was determined by high performance liquid chromatography (HPLC) to obtain the concentrations of glucose and xylose and calculate the contents of cellulose and hemicellulose, respectively. HPLC was performed using a Bio-rad Aminex HPX-87H column (300 mm × 7.8 mm id, 9 µm) and Waters 2414 RID detector at 50 °C with 5 mmol/L H₂SO₄ as the mobile phase and a flow rate of 0.5 mL/min.

Cellulase Enzymatic Hydrolysis

Pretreated oat straw samples (1 g) were mixed with 70 BHU(2) cellulase in a sodium citrate buffer (10 mL, pH = 5), sealed in a bottle, and incubated at 50 °C for 36 h on a shaking table. After this time, the sample was treated with boiling water for 5 min to inactivate the enzyme. After cooling to 25 °C, the sample was subjected to centrifugation at 3000 rpm for 10 min. The filtered solids were washed with deionized water 3 times, and the degradation rate of cellulose and hemicellulose was measured by drying. The reducing sugar yield (Eq. 1) (Miller 1959), and the degradation rates of cellulose (Eq. 2) and hemicellulose (Eq. 3) were calculated according to the DNS method (Thi *et al.* 2019).

$$\text{Reducing sugar yield(\%)} = \frac{\text{Reducing sugar weight}}{\text{Pretreated biomass weight}} \times 100(\%) \quad (1)$$

$$\text{Cellulose degrad. (\%)} = \left(1 - \frac{\text{Cellulose content of treated samples}}{\text{Cellulose content of untreated samples}} \times \text{Solid yield}\right) \times 100(\%) \quad (2)$$

$$\text{Hemicellulose degrad. (\%)} = \left(1 - \frac{\text{Hemicellulose content of treated samples}}{\text{Hemicellulose content of untreated samples}} \times \text{Solid yield}\right) \times 100(\%) \quad (3)$$

FT-IR Spectroscopy

Fourier transform infrared (FT-IR) spectral analysis of the samples was performed using an IRTracer-100 spectrometer (Shimadzu, Japan), and the degree of pretreatment was determined by comparing the presence of important functional groups in the original and three DES-pretreated oat straw samples. Each sample for analysis was prepared using the KBr method, and the spectra were obtained by scanning in the range of 400 to 4000 cm^{-1} at a spectral resolution of 4 cm^{-1} .

XRD

A SmartLab X-ray diffractometer (Rigaku, Japan) was used to scan the sample between 5 and 90° at a rate of 5°/min. To determine the crystallinity index, *CrI*, Eq. (4) was employed, where I_{002} and I_{am} are the intensity of the peaks at 2θ of near 22.0° and 17.8°, respectively.

$$CrI = (I_{002} - I_{am}) / I_{002} \times 100\% \quad (4)$$

SEM

Before the observation, the gold spraying instrument was used to spray 40 s gold to the samples, and the gold spraying current was 10 mA. After pretreatment, the sample was placed in an SU8020 (Hitachi, Japan) field emission SEM at an acceleration voltage of 3.0 kV to obtain images for analysis.

Statistical Analysis

The presented results are quoted as averages from measurements performed in triplicate. Significance analysis of the experimental data was performed using SAS version 8.2 software to analyze the variance of the data, while the ANOVA module was used to test whether the differences between the mean values were significant according to $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

Optimization of Oat Straw Pretreatment Conditions

Figure 1 shows the reducing sugar contents obtained after the pretreatment of oat straw using the three DES systems at various temperatures. Under the condition of time 2 h and solid-liquid ratio 1:20. In all cases, the reducing sugar yields initially increased and then decreased with increasing temperature. The low reducing sugar yield at 90 °C indicates that the reaction was not complete; *i.e.*, the energy provided was not sufficient to completely break the covalent bonds between the lignocellulose molecules. Upon increasing the temperature to 100 °C, the Oach DES gave the highest sugar yield (12.3%) of all systems, reaching its maximum performance at this temperature. Similarly, the optimal temperature for Fach was 110 °C (14.6%), while that of Lach was 130 °C (11.2%).

In all cases, further increases in temperature after the performance maximum had been reached resulted in the reducing sugars present in the reaction solution being degraded into furfural and other substances, thereby lowering the reducing sugar yield.

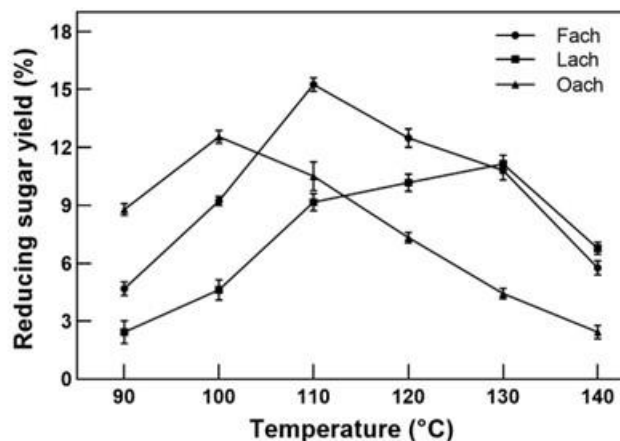


Fig. 1. Reducing sugar yield obtained after the pretreatment of oat straw with the three DES systems at various temperatures

The effect of the oat straw pretreatment time on the sugar yield was then investigated, under the conditions of solid-liquid ratio 1:20 and temperature 110 °C. Figure 2 shows that for all three DES systems, the reducing sugar yields initially increased and then decreased over time. The Oach pretreatment reached a maximum reducing sugar yield of 12.1% after 1 h of reaction, and then it decreased rapidly. This finding is attributed to the increasing amounts of degradation products produced by the thermal decomposition of Oach over time, which made the pretreatment increasingly less effective. The optimal reaction time was 2 h for both the Fach and Lach DES systems, giving reducing sugar yields of 13.7% and 9.2%, respectively. At longer reaction times, the yield decreases significantly, possibly because the solvent concentration decreases over time as it is volatilized during long-term heating.

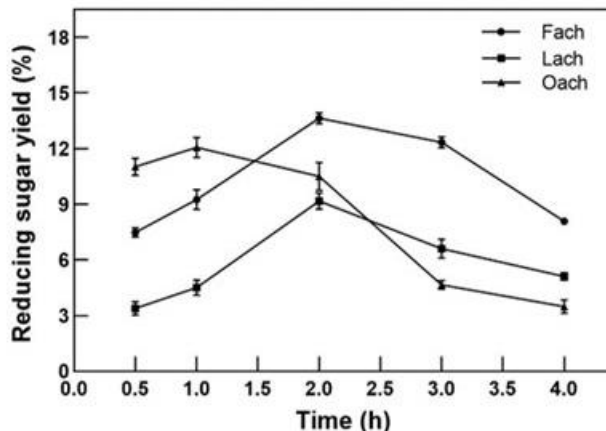


Fig. 2. Effect of time on the reducing sugar yield during the pretreatment of oat straw with the three DES systems

The effect of the solid-liquid ratio on the oat straw pretreatment process was then investigated. These tests involved a time of 2 hours and a temperature of 130 °C. Figure 3

shows that the reducing sugar yields obtained after all DES pretreatments slightly increase with increasing solid-liquid ratio. However, as the solid-liquid ratio (1:20) further increased, the reducing sugar yields increased more and more slowly. Large amounts of the DES were consumed in the reaction, which makes the process uneconomical. Thus, the optimal solid-liquid ratio was 1:20, giving reducing sugar yields of 10.8%, 11.2%, and 4.43% for the Oach, Fach, and Lach systems, respectively. The lower yield obtained for the Oach DES is attributed to the fact that this experiment was carried out at 130 °C, which exceeds the optimal temperature for treatment with this DES due to solvent decomposition.

After the determination of the optimal pretreatment conditions for the three DES systems, the effect of pretreatment on the enzymatic hydrolysis of the reducing sugars by cellulase was examined. The optimal conditions for oat straw DES pretreatment and the resulting yields of reducing sugars obtained by cellulase enzymatic hydrolysis are shown in Table 1. Higher yields of reducing sugars resulted in greater enzymatic hydrolysis efficiencies due to the effective degradation of lignocellulose. Among the tested DES systems, the enzymatic hydrolysis of the oat straw sample pretreated with Fach gave the optimal results, with a reducing sugar yield of 23.5% being obtained.

Table 1. Optimal DES Pretreatment Conditions and Resulting Reducing Sugar Yields After Enzymatic Hydrolysis

DESs	Temperature (°C)	Time (h)	Solid-Liquid Ratio (W/V)	Reducing Sugar Yield (%)
Fach	110	2	1:20	23.5±0.5 ^a
Lach	130	2	1:20	22.7±0.4 ^b
Oach	100	1	1:20	22.4±0.4 ^b

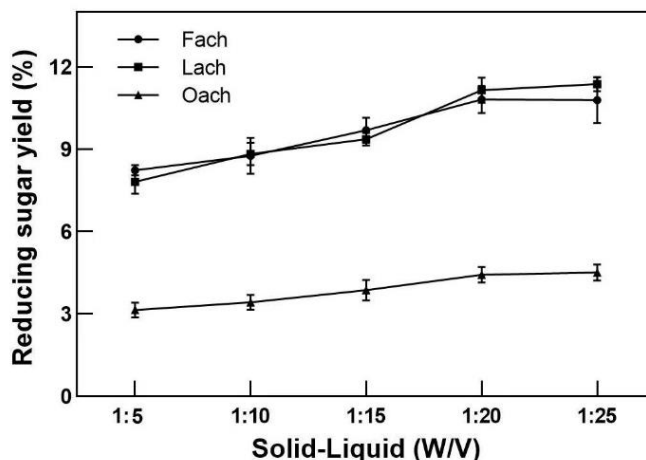


Fig. 3. Effect of the solid-liquid ratio on the reducing sugar yield during the pretreatment of oat straw with the three DES systems

The chemical composition of hemicellulose and cellulose on the feedstock were 30.8±1.6% and 25.1±0.8%, respectively. DES pretreatment substantially enhanced the digestibility of cellulose and hemicellulose in oat straw. Figure 4 shows the degradation rates of the oat straw cellulose and hemicellulose under the optimal enzymatic hydrolysis conditions following DES pretreatment. The cellulose present in the pretreated oat straw underwent efficient enzymatic hydrolysis, with Fach pretreatment giving the highest degradation rate of 76.9%. For all three systems, the hemicellulose components underwent complete degradation.

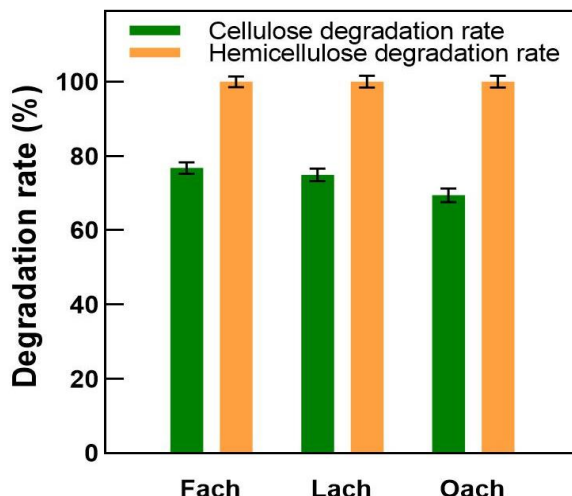


Fig. 4. Degradation rates of the oat straw cellulose and hemicellulose following enzymatic hydrolysis

Comparison of the Structures of the Original and Pretreated Samples

FT-IR was initially employed to analyze the structural changes occurring in the lignocellulose during the enzymatic hydrolysis of oat straw.

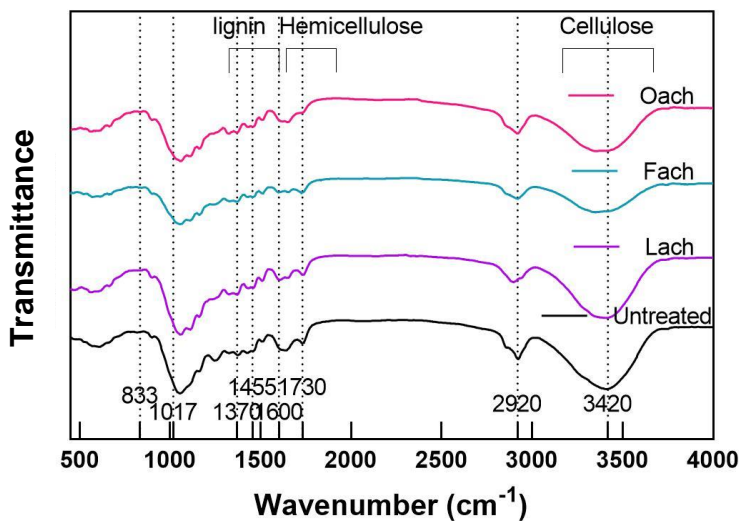


Fig. 5. FT-IR spectra of the untreated and DES-treated oat straw samples

Figure 5 shows the characteristic infrared peaks of the oat straw pretreated with the three DES systems, where peaks corresponding to the stretching vibrations of the O–H groups in cellulose and hemicellulose were observed at 3300 to 3500 cm^{-1} , while the peaks at 2920, 1370, and 1017 cm^{-1} correspond to the stretching vibrations, bending vibrations, and stretching vibrations of the C–H and C–O bonds of cellulose, respectively (Zhang *et al.* 2023). The changes observed at 2920 cm^{-1} after DES pretreatment indicated that some rupture might have occurred in the methyl and methylene groups of cellulose (Kumar *et al.* 2016). Although the characteristic peaks of cellulose became weaker after pretreatment of the oat straw, the peak shape was maintained, indicating that the amorphous region of cellulose was partially degraded while the crystalline region remained unchanged. The vibration peak of C=O bond expansion in the carbonyl or ester group of hemicellulose was

observed at 1730 cm^{-1} (Song *et al.* 2020). Compared with the original oat straw, this peak was significantly weakened after pretreatment, which confirmed that most hemicellulose was removed. The characteristic absorption peaks at 1600 , 1512 , and 1455 cm^{-1} correspond to the benzene ring in lignin, and that at 833 cm^{-1} is the vibration absorption peak of the C–H bond connected to the benzene ring (Liu *et al.* 2020; Zhang *et al.* 2023). The absorption peak was significantly weakened or disappeared after pretreatment, consistent with a very low or negligible lignin content in the oat straw.

Crystallinities of the Original and Pretreated Samples

XRD patterns provide information regarding the crystal phases and degree of crystallinity of the constituent materials. The *CrI* is an important parameter for biomass treatment as the removal of amorphous hemicellulose and lignin components increase or decrease this value, respectively (Chourasia *et al.* 2022). Thus, this technique was used to examine the crystallinity of the oat straw samples before and after DES pretreatment. The XRD patterns in Fig. 6 show similar peak positions for the original and treated samples, indicating that DES pretreatment did not change the crystal form of the cellulose. The observation of diffraction peaks at $\sim 18^\circ$, 22° , and 35° , in addition to a unimodal peak at 22° , indicates that the cellulose component of these samples exhibited an I-type crystal structure (Smirnov *et al.* 2020). The *CrI* of the untreated oat straw was 30.05%, which increased after treatment with Fach, Lach, and Oach to 49.33%, 47.30%, and 40.81%, respectively (Table 2). Although all DES pretreatments increased the crystallinity of the oat straw, Fach pretreatment resulted in the most significant enhancement. These results further confirm that DES pretreatment enhances the connection between lignocellulose moieties, while degrading cellulose and some amorphous hemicellulose and lignin, thereby increasing the relative degree of cellulose crystallinity. It is important to note that the increase in *CrI* does not reflect an increase in the amount of crystalline cellulose, but rather an increase in the fraction of cellulose in the total biomass sample due to the removal of amorphous hemicellulose and lignin.

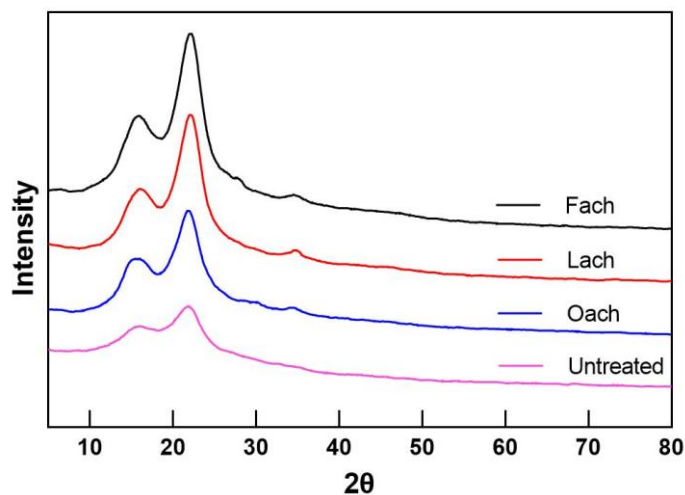
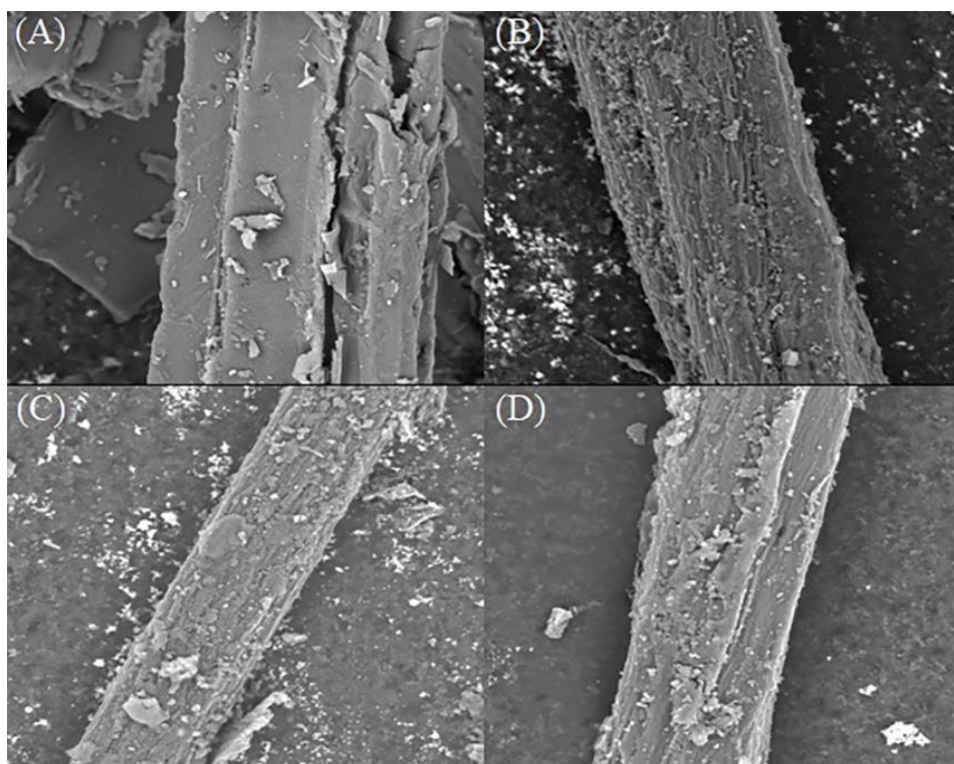


Fig. 6. XRD analysis patterns of oat straw

Table 2. Crystallinity Indices of the Untreated and Pretreated Oat Straw Samples

Lignocellulose	Crystallinity (%)
Untreated oat straw	30.05
Fach pretreated oat straw	49.33
Lach pretreated oat straw	47.30
Oach pretreated oat straw	40.81

**Fig. 7.** SEM images of the oat straw samples: (A) untreated; treated with (B) Fach, (C) Lach, or (D) Oach

Morphology of the Original and Pretreated Samples

To further explore the physicochemical properties of the DES-pretreated oat straw, the surface morphologies of the samples before and after pretreatment with the three DES systems were observed by SEM. Figure 7(A) shows that the surface of the untreated oat straw is smooth and dense, with an orderly multi-layered three-dimensional composite structure. After pretreatment with Fach, Lach, and Oach, Figs. 7(B), (C), and (D), respectively, show separation of the fibers and small cracks. In particular, the straw pretreated with Fach (Fig. 7(B)) showed the roughest surface, where the originally dense fiber bundles became looser and more disordered due to the removal of lignin and hemicellulose. These results are consistent with the FT-IR and XRD results, and confirm that the lignocellulosic structure that is tightly bound by hemicellulose and lignin can break upon DES pretreatment, resulting in partial degradation of hemicellulose and lignin components, leaving the cellulose exposed. This microstructure is beneficial for enzyme penetration in the subsequent enzymatic hydrolysis of oat straw by cellulase.

Mechanism Analysis

Based on the results presented here and in the literature, the degradation process occurring during the pretreatment of oat straw with the studied DES systems is summarized in Fig. 8. The characterization results and analysis described above confirm that DES pretreatment of oat straw results in the degradation of the complex three-dimensional lignocellulose structure, followed by the complete removal of the lignin and hemicellulose components through the complexation of cellulose and cellulase on the surface of the active center. This results in a catalytic hydrolysis mechanism that is conducive to the enzymatic degradation of cellulose.

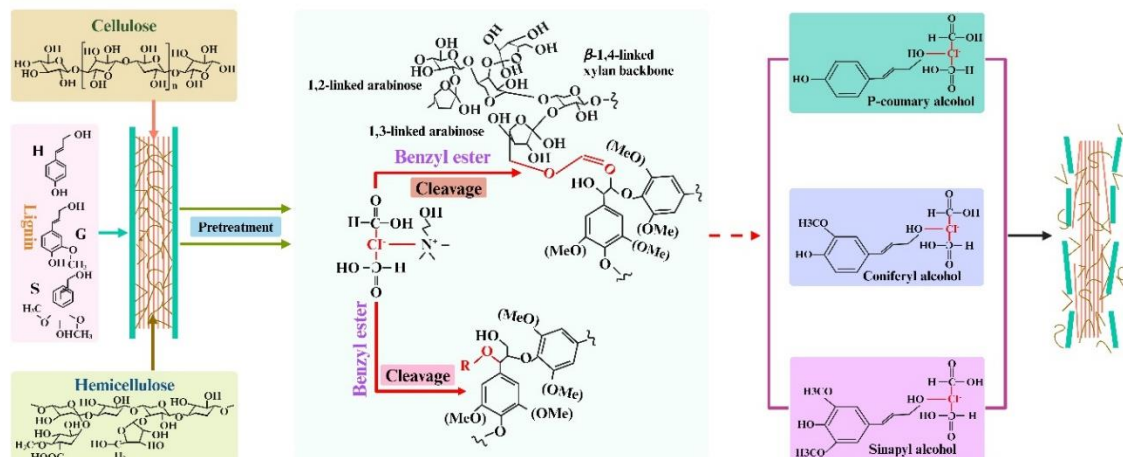


Fig. 8. Reaction scheme for the pretreatment of lignocellulose by the studied DES systems

The three types of DES evaluated in this study contain large quantities of chloride ions and asymmetric carboxylic acid molecules, which form competing hydrogen bonds between the oat straw carbohydrates and chloride ions, thereby weakening the original lignocellulose covalent bonding interactions and van der Waals forces. This, in turn, damages the internal hydrogen bonding network and breaks the three-dimensional lignocellulose structure. The dissolution of cellulose in the DES systems requires the dissociation of two hydrogen bond networks to form a thermodynamically stable system, which is difficult to achieve because of the high cohesive energy of cellulose (Alvarez-Vasco *et al.* 2016). Although the chloride ions present in the DES systems can break the hydrogen bonds of cellulose, expand the long chain of the fiber, and reduce the degree of polymerization, the presence of hydroxyl groups in the choline cation hinders the interactions between the chloride ions and cellulose (Kumari and Singh 2018). This is consistent with the weak cellulose degradation abilities of the three DES systems evaluated here. Francisco *et al.* (2012) also confirmed that cellulose was poorly or negligibly soluble in the DES systems used to process biopolymers. However, the hemicellulose present in oat straw contains relatively short amorphous chains, a low degree of polymerization, and a highly branched structure than cellulose (Lynam *et al.* 2017). As a result, the DESs can easily break C–O–C bonds in hemicellulose and degrade it into oligosaccharides and monosaccharides. In the three-dimensional structure of oat straw lignocellulose, the lignin and hemicellulose molecules are connected to form complexes through hydrogen and ether bonds, with the number of ether bonds accounting for 40 to 60% of the total phenylpropane unit bonds present in lignin. Thus, the pyrolysis of ether bonds is the main mechanism leading to the depolymerization or separation of lignin and hemicellulose (Chen *et al.*

2019). Yu *et al.* (2018) reported a similar mechanism upon the pretreatment of cellulose from Chinese herbs with other DESs. DES pretreatment can result in the formation of hydrogen bonds between the chloride ions of the DES with the hydroxyl groups of the lignin structure, which results in cleavage of the lignin aryl ether bond of the phenylpropane unit, thereby enhancing lignin removal. This is enabled by the good solubilities of lignin and hemicellulose in DESs. Zulkefli *et al.* (2017) also reached the same conclusion following the pretreatment of oil palm stem with DESs, wherein the removal efficiencies of lignin and hemicellulose reached 42% and 83%, respectively, and the dissolution of oil palm dry biomass reached 58%. Overall, the results indicate that DESs strongly degrade hemicellulose and lignin, but they have little effect on cellulose.

CONCLUSIONS

1. The treatment of biomass with deep eutectic solvent (DES) systems before enzymatic hydrolysis enhances the saccharification process by removing lignin and hemicellulose, which improves the cellulose accessibility by enzymes, leading to significant glucose recovery.
2. The Fach, Lach, and Oach DES systems used to pretreat the oat straw before enzymatic hydrolysis gave optimal reducing sugar yields of 23.5%, 22.7%, and 22.4%. Fach pretreatment achieved the highest cellulose degradation percentage (76.9%) and complete hemicellulose degradation.
3. The highest fraction of initially available carbohydrates was converted to fermentable sugars after Fach pretreatment. A preliminary mechanism for the pretreatment of oat straw with Fach was revealed.
4. The use of DES systems as a green and environmentally friendly pretreatment method is of great significance for the high-value utilization of all components of lignocellulosic biomass and is expected to be of particular interest in areas where oat straw is a main food crop.
5. The pretreated oat straw provides a high-quality fermentation base for the production of feed proteins. This study provides basic data and a theoretical basis for future research on single-cell protein.

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Conflict of Interest

The authors confirm that this manuscript is not submitted or under consideration in any other journal. The manuscript is prepared strictly according to the journal format as provided in the instruction to authors; and there is no conflict of interest.

Author Contributions

Mingda Zhu and Xuejiao Qiu contributed equally to this work and should be considered co-first authors. Zhanying Liu conceived and designed research. All authors have approved the manuscript and agree with its submission to *BioResources*.

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