

Article

Poplar Wood Pretreatment Using Deep Eutectic Solvents for Promoting Enzymatic Hydrolysis

Xuyang Zhao, Ying Zhan, Lihua Han, Xiaoran Sun, Tianyu Zhang and Zheng Zhao *

College of Chemical Engineering, North China University of Science and Technology, Tangshan 063210, China; a15736973753@163.com (X.Z.); zhanying0326@163.com (Y.Z.); tshlh@ncst.edu.cn (L.H.); xiaoranhb@yeah.net (X.S.); a13786654504@163.com (T.Z.)

* Correspondence: buctzhaozheng@163.com; Tel.: +86-315-8805-460

Abstract: Bioethanol produced from lignocellulose is a renewable energy substitute for traditional fossil fuels. Poplar wood as forest waste is popular in bioethanol production. Nonetheless, the complex structure of lignocellulose leads to low reducing sugar and ethanol yields. Thus, lignocellulose pretreatment is necessary to promote enzymatic hydrolysis. Deep eutectic solvents (DESs) have good dissolution capacity, low vapor pressure, a simple synthesis procedure, low synthesis cost and low toxicity. More and more researchers have begun paying attention to the application of DESs in lignocellulose pretreatment. In this work, poplar wood was pretreated using a series of basic DESs based on diol. The effects of the DES species, the basicity of the solvents, the pretreatment temperature and the pretreatment time on the effectiveness of pretreatment and enzyme hydrolysis for poplar wood were investigated, and characterization analysis (Fourier transform infrared spectroscopy, X-ray diffraction and scanning electron microscopy) of poplar wood was carried out to reveal the pretreatment mechanism. The best pretreatment effect was obtained from K: 1, 2-PG, which removed 89.2% and 71.6% of the lignin and hemicellulose, respectively, while preserving 97.5% of the cellulose at 130 °C for 7 h. This enhanced the reducing sugar yield to 82.5% relative to that of the raw sample (3.3%) after 72 h of hydrolysis. The results of the characterization analysis demonstrated that lignin and hemicellulose were removed. Therefore, the DES based on K: 1, 2-PG is a promising solvent for poplar wood pretreatment, and could improve the industrial production of reducing sugar and bioethanol.

Keywords: deep eutectic solvents; diol; poplar wood; pretreatment; enzymatic hydrolysis



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1. Introduction

Bioethanol, a source of renewable energy, can replace traditional fossil fuels and, thus, resolve two energy problems. The main sources of lignocellulosic biomass include agricultural and forestry wastes. In particular, a huge amount of forest waste originates from deforestation, timber processing and forest pruning, i.e., about 400 million tons of standard coal annually in China [1]. Nevertheless, the complex structure of lignocellulose hinders the transformation of cellulose into bioethanol. Lignocellulosic biomass contains lignin, hemicellulose and cellulose. Hemicelluloses adhere to the surface of cellulose through van der Waals forces and hydrogen bonds, and connect lignin through ether bonds and glycoside bonds to form a stable network structure [2]. Therefore, it is necessary to pretreat lignocellulosic biomass by removing lignin and hemicellulose in order to improve the reducing sugar and ethanol yields.

Many common traditional pretreatment methods, such as dilute acid, dilute alkali, organic solvent and biological methods [3–7], have one or several disadvantages, including high energy consumption, high toxicity, harsh conditions, corrosion equipment or a long pretreatment time. Therefore, the majority of scientific research workers have begun to look at green solvent ionic liquids (ILs). ILs have the characteristics of high thermal stability,

low steam pressure, non-flammability and non-volatilization [8], making them suitable for lignocellulose pretreatment. Nonetheless, the greenness of ILs has been questioned and the industrial application of IL pretreatment technology has been hindered because they possess several drawbacks, such as high synthetic cost, complex synthetic steps and high toxicity in ILs.

Recently, the application of deep eutectic solvents (DESs) in lignocellulosic biomass pretreatment has attracted extensive attention. DESs usually consist of two or more hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs). Otherwise, the melting points of DESs are lower than those of individual components. The most important point is that DESs have a few similar properties to ILs and possess merits such as a low cost of synthesis, a simple synthesis procedure, low toxicity and good biocompatibility, which compensate for the shortcomings of ILs. Lee et al. [9] used choline chloride: glycerol, choline chloride: lactic acid and choline chloride: urea to pretreat oil palm empty fruit bundles under ultrasound with 210 W at 50 °C for 15 min. As a result, reducing sugar yields of 35.3%, 36.7% and 35.8%, respectively, were obtained after the pretreated residues were enzymatically hydrolyzed for 72 h. Sai et al. [10] used chloride: lactic acid (molar ratio was 1:10) to pretreat empty fruit bunches at 100 °C for 1 h. After residue hydrolysis for 48 h, 51.1% of the reducing sugar yield was obtained. Li et al. [11] compared the pretreatment capacity of choline chloride: lactic acid and choline chloride: glycine. The research results showed that choline chloride: lactic acid removed 90.4% of the lignin, but choline chloride: glycine only removed 58.4% of the lignin during poplar wood pretreatment at suitable pretreatment conditions (temperature: 120 °C, time: 12 h). Wu et al. [12] reported that the continuous pretreatment of 0.75% (*w/w*) NaOH and choline chloride: lactic acid for sorghum straw exhibited high pretreatment efficiency. Firstly, 0.75% (*w/w*) NaOH pretreated sorghum straw at 121 °C for 1 h. Then, choline chloride: lactic acid was used to pretreat sorghum straw at 140 °C for 40 min. As a result, 78.4% lignin and 67.6% hemicellulose were removed, respectively. After the enzymatic hydrolysis of pretreated residue for 72 h, 94.9% of the reducing sugar yield was obtained. However, several problems, such as the larger proportions of cellulose loss and low reducing sugar yield, have yet to be resolved in the current DES pretreatment method. Our research group [13,14] has reported that basic DESs have the capacity to selectively remove the lignin and hemicellulose while retaining the majority of the cellulose in wheat straw. Otherwise, the basic DESs pretreatment method is less studied; in addition, the studies are not extensive and the pretreatment mechanism lacks depth, so it is necessary to conduct systematic research on basic DES pretreatment.

In this paper, a series of basic DESs based on diol (i.e., sodium hydroxide: ethylene, sodium hydroxide: polyethylene glycol-200, potassium hydroxide: polyethylene glycol-300, lithium hydroxide: polyethylene glycol-400, sodium hydroxide: polyethylene glycol-600, sodium hydroxide: 1,4-butyl glycol and potassium hydroxide: 1,2-propylene glycol) were screened to pretreat poplar wood. The effects of the DES species, the basicity of the solvents, the pretreatment temperature (i.e., 70 °C, 90 °C, 110 °C, 130 °C, 150 °C) and the pretreatment time (i.e., 7 h, 9 h, 11 h, 13 h, 15 h, 17 h) on the composition and reducing sugar yield of poplar wood were investigated. Moreover, the crystal structure, surface morphology and chemical structure of poplar biomass were also analyzed via Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) to clarify the mechanism of basic DESs based on diol pretreating poplar wood.

2. Materials and Methods

2.1. Materials

Sodium hydroxide, potassium hydroxide (purity: 95%), ethylene glycol, lithium hydroxide (purity: 98%), 1,2-propanediol, 1,4-butanediol (purity: 99%), polyethylene glycol-600, polyethylene glycol-400, polyethylene glycol-300, polyethylene glycol-200 and cellulase (enzymatic activity: 10,000 U·g⁻¹, C805042) were utilized. All of the aforementioned reagents were purchased from Maclean. Poplar wood was obtained from Shijiazhuang Farm. The poplar wood was milled using a pulverizer and then screened to

the desirable particles with a 50–80 mesh. After Soxhlet extraction was performed using ethanol and water for 24 h, the poplar wood particles was airdried and stored at 4 °C.

2.2. Preparation of DESs

The HBAs and HBDs were mixed with a specific molar ratio. Subsequently, the mixture was magnetically stirred at 80 °C until transparent and homogeneous solutions formed [15,16]. Table 1 lists the compositions of prepared DESs and the molar ratios of HBA to HBD in DESs.

Table 1. The preparation of DESs.

HBA	HBD	Molar Ratio (HBA:HBD)	Abbreviation
Sodium hydroxide	Ethylene glycol	1:4	Na: EG
Sodium hydroxide	Polyethylene glycol-200	1:2	Na: PEG-200
Potassium hydroxide	Polyethylene glycol-300	1:4	K: PEG-300
Lithium hydroxide	Polyethylene glycol-400	1:10	Li: PEG-400
Sodium hydroxide	Polyethylene glycol-600	1:4	Na: PEG-600
Sodium hydroxide	1,4-Butyl glycol	1:5	Na: 1,4-BDO
Potassium hydroxide	1,2-Propylene glycol	1:2	K: 1,2-PG

2.3. Pretreatment Process

Firstly, a 50–80 mesh of poplar wood was mixed with DESs at 1:20 (*w/w*), then stirred magnetically at a certain temperature for a set length of time. Next, the pretreatment liquid was diluted using hot water, then centrifuged (3000 r·s⁻¹) and filtered to separate the solid from the liquid. When the separation step was completed, the solid was washed further using deionized water. Centrifuging and washing were repeated until the scrubbing solution was neutral. In the end, the solid residue was freeze-dried for 72 h, then weighed and stored at 25 °C.

2.4. Enzymatic Hydrolysis

Firstly, the dipotassium hydrogen phosphate–citrate buffer solution was adjusted to pH = 4.8. Then, a mixture of 52.5 mL of ready-prepared buffer solution, 7.5 mL of cellulase solution and 0.4 g of the poplar wood sample was shaken in 72 h at 120 r/min and 45 °C. Additionally, 0.6 mL of hydrolysate was withdrawn at regular intervals, and the enzymatic hydrolysis reaction was terminated in a boiling water bath. According to the DNS method, the reducing sugar concentration was determined using the standard curve for reducing sugar ($y = 1.68x - 0.075$, $R^2 = 0.9998$). The reducing sugar yield was calculated based on the reducing sugar concentration [17].

2.5. Components Analysis

2.5.1. Determination of Lignin Content

Firstly, the 72% (*w/w*) sulfuric acid (10 mL) hydrolyzed a 0.3 g poplar wood sample for 1.5 h. Afterwards, the filtration of the suspension liquid was carried out to obtain liquids and solids (hydrolysis residue). The deionized water was used to wash the solid until the scrubbing solution was neutral. After filtering, the solid containing acid-insoluble lignin and ash was dried and calcined to determine the acid-insoluble lignin content [18]. The absorbance of the liquid was measured at 205 nm to determine the acid-soluble lignin content. The total lignin content in the poplar wood sample consisted of the acid-soluble lignin content and the acid-insoluble lignin content. According to Equation (1), the lignin removal was calculated.

$$R_l = [1 - (R \times w_{rl}) / w_l] \quad (1)$$

R_l —lignin removal (%), R —residue recovery (%), w_{rl} —lignin content of pretreated poplar wood (%) and w_l —lignin content of untreated poplar wood (%).

2.5.2. Determination of Cellulose and Hemicellulose Content

First, 5 mL of 4 mg·mL⁻¹ KOH solution (containing 1 mg·mL⁻¹ NaBH₄) was used to treat 0.1 g of the poplar wood sample for 1 h in a water bath shaker at 25 °C. Next, 10 mL of deionized water and an equal volume of 4 mg·mL⁻¹ KOH solution were added to the treatment liquid for washing and diluting. The supernatant and the absorbances were measured at 660 nm (xylose) and 620 nm (glucose), respectively. Then, the concentrations of xylose and glucose were calculated based on the absorbances and the standard curves of xylose ($y = 300.97x - 0.016$, $R^2 = 0.9994$) and glucose ($y = 102.75x + 0.0075$, $R^2 = 0.9992$) [19].

Afterwards, the 72% (*w/w*) sulfuric acid (4 mL) treated the residue, which was obtained from the above alkali treatment process, in a water bath shaker at 25 °C for 1 h. Then, the treatment liquid was filled to 30 mL by adding deionized water. The absorbances of the supernatant were also measured in order to calculate the concentrations of xylose and glucose, as described above.

Lastly, the cellulose content of the poplar wood sample was calculated according to the dehydration coefficient of glucose (0.90) and the glucose concentration obtained from the acid treatment process. The hemicellulose content in the poplar wood sample was determined according to the dehydration coefficient of glucose (0.90) and xylose (0.88), while the glucose and xylose concentrations were determined during the base treatment process and the xylose concentration was determined during the acid treatment process. According to Equations (2) and (3), the hemicellulose removal and cellulose reservation were calculated, respectively.

$$R_h = [1 - (R \times w_{rh}) / w_h] \quad (2)$$

R_h —hemicellulose removal (%), R —residue recovery (%), w_{rh} —hemicellulose content in poplar wood residue (%) and w_h —hemicellulose content in untreated poplar wood (%).

$$R_c = [1 - (R \times w_{rc}) / w_c] \quad (3)$$

R_c —cellulose reservation (%), R —residue recovery (%), w_{rc} —cellulose content in pretreated poplar wood (%) and w_c —lignin content in untreated poplar wood (%).

2.6. SEM Analysis

The crushed poplar wood sample was evenly dispersed on a disc coating with the conductive glue, and then spray-gold treatment was performed. Afterwards, the surface morphology of poplar wood samples before and after pretreatment by DESs was observed using a JSM-IT100 (A) type SEM [20].

2.7. FT-IR Analysis

Firstly, the mixture of the poplar wood sample and potassium bromide, with a mass ratio of 100:1, was pressed into a tablet. Then, a Vertex 80 V spectrometer was used to scan the tablet for 32 s (resolution: 2 cm⁻¹) in order to carry out the chemical structural analysis of the poplar wood sample. The wavenumber ranged from 4000 cm⁻¹ to 400 cm⁻¹ [21].

2.8. XRD Analysis

The poplar wood sample was scanned from 10° to 50° at a rate of 5°/min, using a Bruker D8 Advanced diffractometer to analyze the crystallinity in the poplar wood. The intensity of the spectrum peaks was used to calculate the crystallinity index, as shown in Equation (4) [21].

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

CrI —crystallinity index, I_{002} —peak intensity of crystalline region ($2\theta = 22.5^\circ$) and I_{am} —peak intensity of amorphous region ($2\theta = 18^\circ$).

3. Results and Analysis

3.1. Effect of DES Type on Pretreatment of Poplar Wood

Figure 1 shows the results of different DESs pretreating poplar wood at 90 °C for 11 h. As depicted in Figure 1, the pretreatment effects of diol monomer-based DESs were better than those of PEG-based DESs. The lignin and hemicellulose removal of Na: EG, Na: 1,4-BDO and K: 1,2-PG (diol monomer based DESs) pretreated residues ranged from 56.6% to 76.4% and from 54.1% to 69.5%, respectively, while Na: PEG-200, K: PEG-300, Li: PEG-400 and Na: PEG-600 (PEG based DESs) only removed 37.6–56.2% of the lignin and 24.3–60.9% of the hemicellulose. It is worth noting that K: 1,2-PG exhibited the best pretreatment capacity for poplar wood; for example, 76.4% lignin and 69.5% hemicellulose were removed, but 97.3% cellulose was preserved during K: 1,2-PG pretreatment of poplar wood.

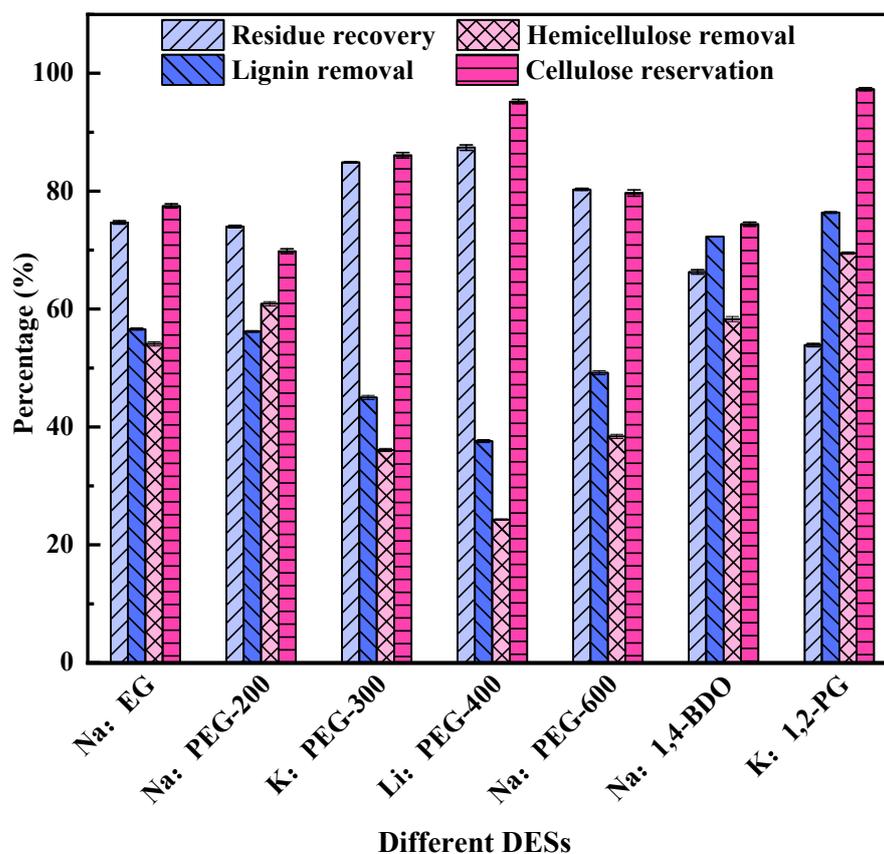


Figure 1. Effect of DES type on pretreatment of poplar wood (pretreatment conditions: 90 °C, 11 h).

The pH values of different DESs were measured as depicted in Figure 2. From Figure 2, we can observe a phenomenon: the pH values of the seven DESs were all greater than 11, which suggests that all of the DESs were basic solvents. In particular, K: 1,2-PG had the strongest basicity among these DESs, with the highest pH value being 13.3. Our previous studies [13,22] have proven that basic solvents can dissolve lignin and hemicellulose, but little cellulose. This explains the phenomenon by which a larger amount of lignin and hemicellulose than cellulose was removed by K: 1,2-PG.

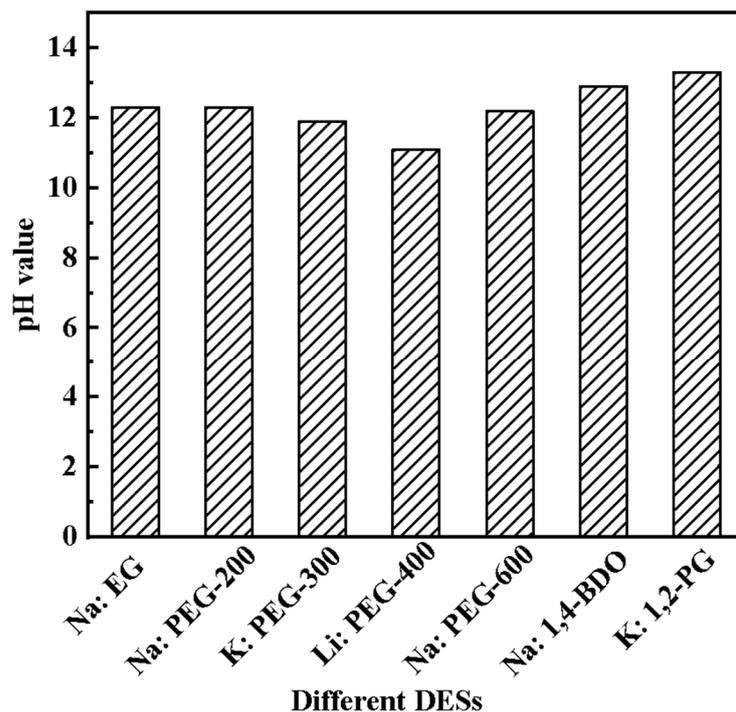


Figure 2. pH values of different DESs (the pH values were obtained by measuring 0.1 mol·L⁻¹ DES aqueous solutions).

In addition, the pH values of DESs influence the pretreatment effects. Figure 3 illustrates the relationship between the pH values of DESs and the results of lignin or hemicellulose removal. As shown in Figure 3, the higher the pH values of DESs, the greater the amount of lignin and hemicellulose that is removed. Therefore, K: 1,2-PG can remove more lignin and hemicellulose than other DESs, which can be explained by our previous study, in which the lignin–carbohydrate complex and deacetylation of hemicellulose were destroyed by removing the carbonyl group in hemicellulose and cutting off the ester bond between lignin and hemicellulose during basic DES pretreatment [13].

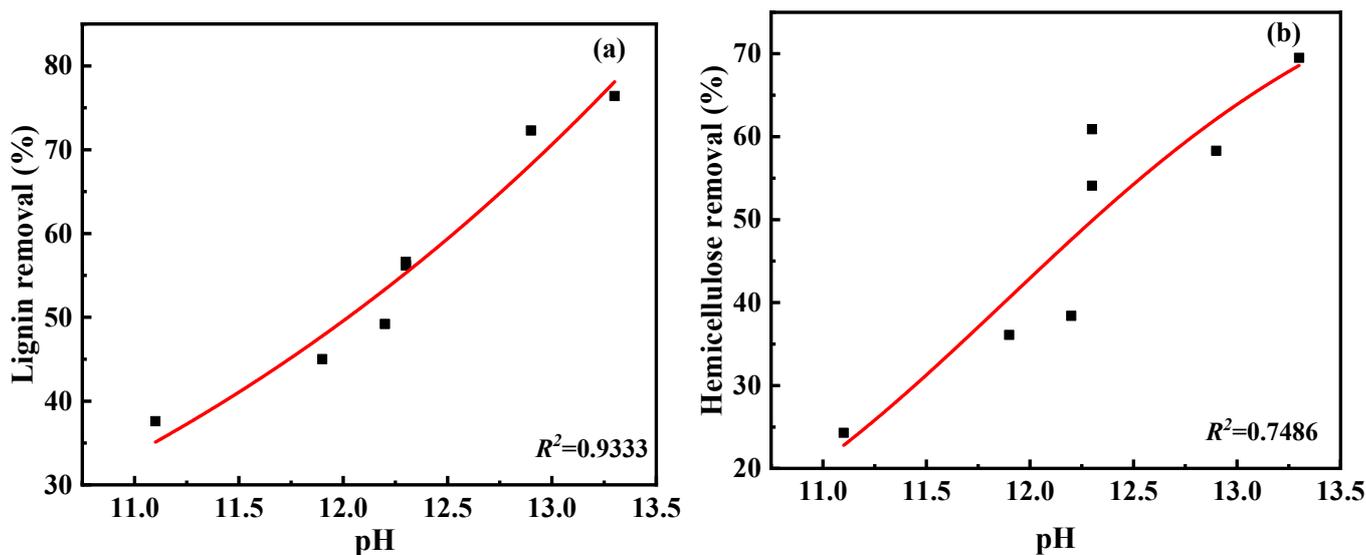


Figure 3. The relationship of lignin removal (a) and hemicellulose removal (b) with the pH values of different DESs.

Figure 4 shows the influence of DES species on the initial saccharification rate and the reducing sugar yield during the enzyme-catalyzed hydrolysis of poplar wood. According to Figure 4a, the initial saccharification rates of Na: EG-, Na: PEG-200-, K: PEG-300-, Li: PEG-400- and Na: PEG-600-pretreated residues were similar to that of untreated poplar wood ($2.7\text{--}3.6\text{ mg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$ vs. $2.6\text{ mg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) during enzymatic hydrolysis. However, the Na: 1,4-BDO and K: 1,2-PG pretreatment process for poplar wood can significantly increase the initial saccharification rate, i.e., $5.7\text{ mg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$ and $7.5\text{ mg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$. In particular, the initial saccharification rate of K: 1,2-PG-pretreated residues was its maximum, which is in agreement with the results obtained from pretreatment in Figure 1.

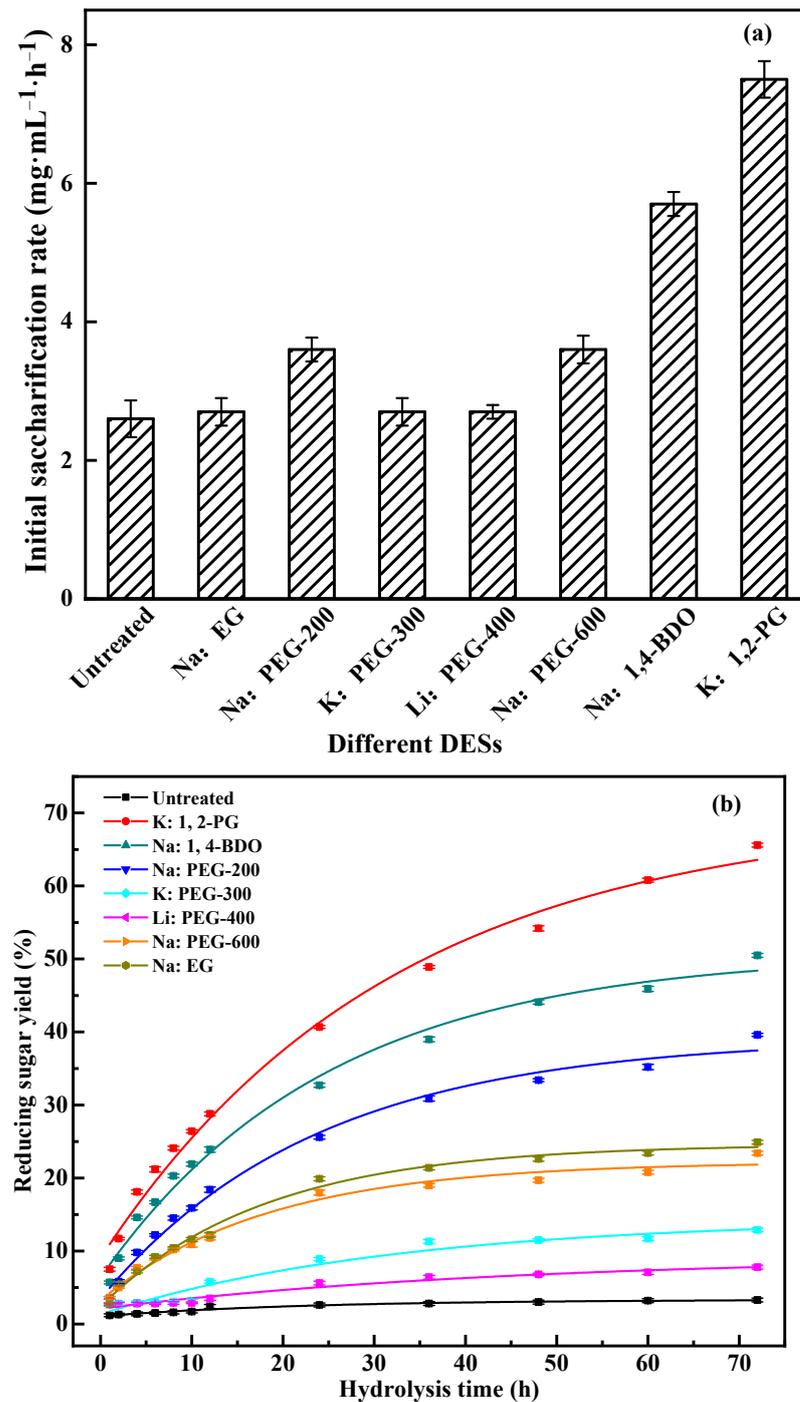


Figure 4. Effect of DES types on enzymatic hydrolysis: (a) initial saccharification rate; (b) reducing sugar yield.

As observed from Figure 4b, except for Na: EG, the other two diol-based DESs more effectively promoted the production of reducing sugar than PEG-based DESs did. For instance, compared with untreated poplar wood, the reducing sugar yield of Na: PEG-200-, K: PEG-300-, Li: PEG-400- and Na: PEG-600-pretreated residues did not increase significantly (3.3% vs. 7.8–39.6%), while the reducing sugar yields of Na: 1,4-BDO- and K: 1,2-PG-pretreated residues after hydrolysis for 72 h were 15.3 and 19.9 times higher, respectively, than that of untreated poplar wood (i.e., 3.3% vs. 50.5% and 65.6%). This is because the removal of both lignin and hemicellulose has a positive relationship with the reducing sugar yield, as shown in Figure 5. The more lignin and hemicellulose was removed, the higher the obtained reducing sugar yield was, indicating that the removal of lignin and hemicellulose is beneficial to enzyme hydrolysis. From Figures 1 and 4, the pretreatment and hydrolysis results of K: 1,2-PG-pretreated residue were the best of the seven DES-pretreated residues. The lignin and hemicellulose removal reached 76.4% and 69.5%, respectively. About 65.6% of reducing sugar was produced during hydrolysis for 72 h. As a consequence, K: 1,2-PG was determined to be the most effective solvent promoting enzymatic hydrolysis among these seven DESs.

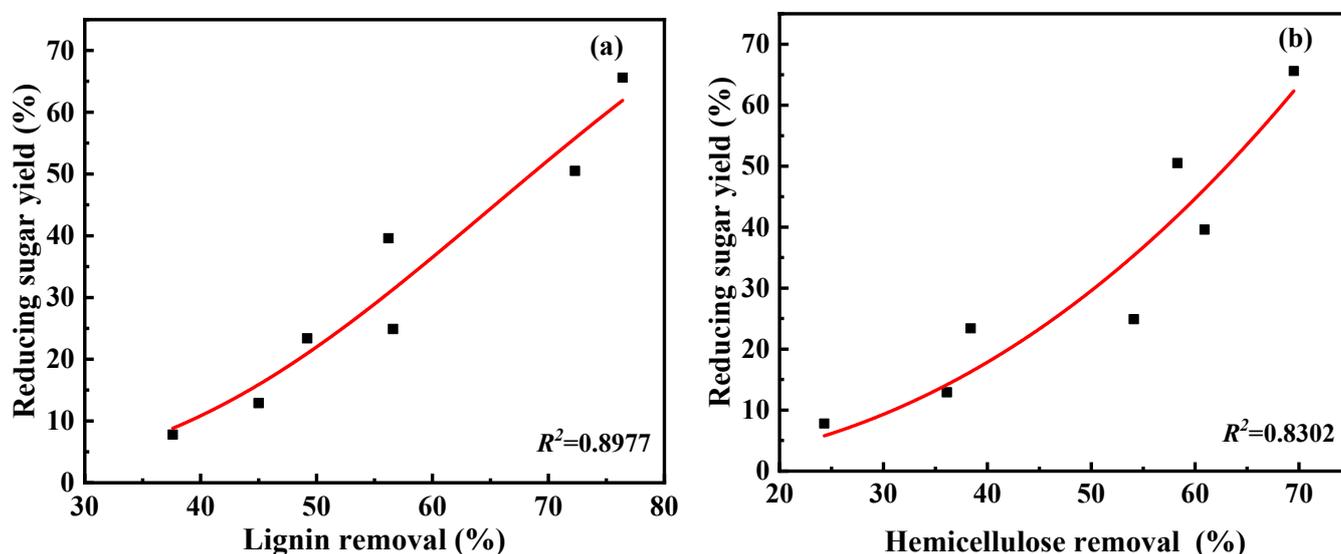


Figure 5. The relationship of reducing sugar yield (72 h) with lignin removal (a) or hemicellulose removal (b).

3.2. Influence of Pretreatment Temperature on Pretreatment of Poplar Wood

Figure 6 illustrates the effect of the pretreatment temperature on the pretreatment of poplar wood. It can be observed from Figure 6 that, as the temperature increased, the lignin and hemicellulose removal gradually increased from 48.1% to 97.4% and from 48.3% to 91.2%, respectively. This indicates that a high pretreatment temperature contributes to the removal of lignin and hemicellulose. The reason may be that the viscosity of K: 1,2-PG decreases with increasing temperature, and the lower the viscosity, the more diffusible the solute is in the solvent, increasing the contact opportunities between poplar wood and K: 1,2-PG. In addition, there was no obvious change in the lignin removal when the pretreatment temperature was elevated from 130 °C to 170 °C (e.g., 91.1–97.4%). However, cellulose preservation gradually decreased with the increase in the pretreatment temperature. Notably, there was a significant decrease in cellulose preservation from 93.8% to 39.2% when the pretreatment temperature increased from 130 °C to 170 °C, which indicates that an excessive pretreatment temperature causes the degradation of cellulose, resulting in a significant loss of cellulose. Therefore, the balance between the lignin and hemicellulose removal and the cellulose loss should be considered comprehensively. From the perspective of the pretreatment effect, 130 °C can be selected as the suitable pretreatment temperature.

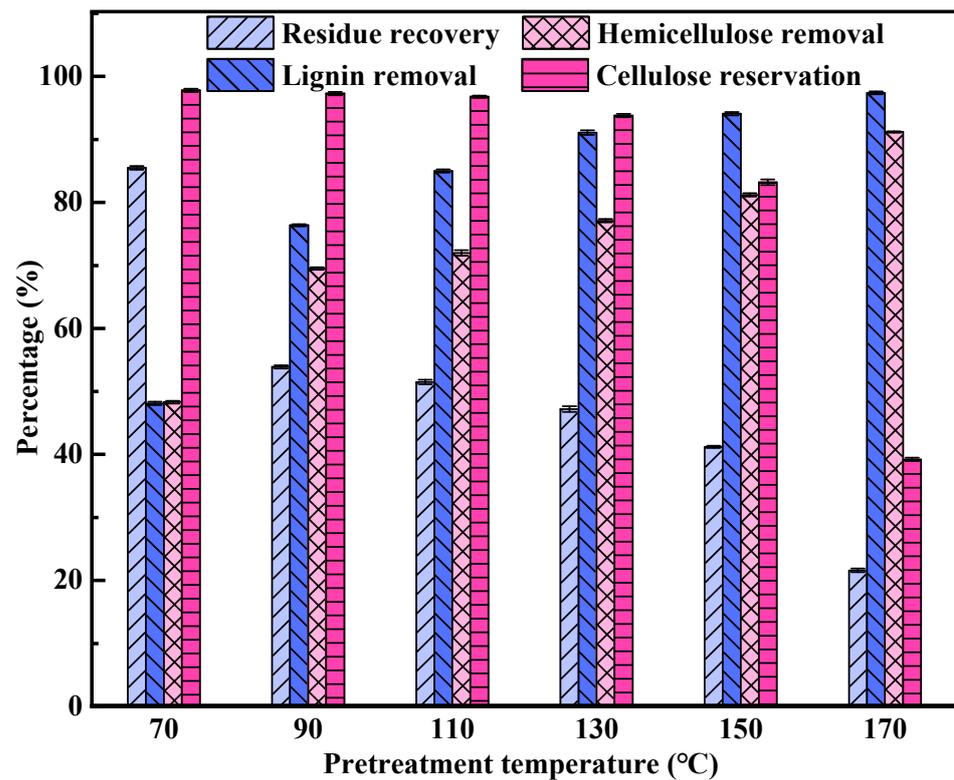


Figure 6. Influence of pretreatment temperature on pretreatment of poplar wood.

Figure 7 exhibits the influence of pretreatment temperature on the hydrolysis results. As shown in Figure 7, the reducing sugar yields and initial saccharification rates of residues obtained from pretreatment at different temperatures all increased in comparison with untreated poplar wood. With the increase in the pretreatment temperature, the reducing sugar yield and initial saccharification rate after hydrolysis for 72 h first gradually increased and then gradually decreased. When the pretreatment temperature was elevated from 70 °C to 130 °C, the reducing sugar yield (hydrolysis for 72 h) and initial saccharification rate increased from 34.1% to 82.6% and from 3.2 mg·mL⁻¹·h⁻¹ to 11.7 mg·mL⁻¹·h⁻¹, respectively. Nonetheless, when the pretreatment temperature increased from 130 °C to 170 °C, the reducing sugar yield and initial saccharification rate began to decrease gradually from 82.6% to 81.6% and from 11.7 mg·mL⁻¹·h⁻¹ to 4.9 mg·mL⁻¹·h⁻¹, respectively. This is because degradation products such as organic acids, furfural and phenolic compounds, which were produced during the pretreatment of poplar wood at an excessive pretreatment temperature, may inhibit the cellulase activity, resulting in a low reducing sugar yield and initial saccharification rate.

Consequently, 130 °C was chosen as the best pretreatment temperature for further investigation. As a result, 91.1% of lignin and 77.1% of hemicellulose were removed, and 93.8% of cellulose was preserved during the pretreatment of poplar wood using K: 1,2-PG at 130 °C for 11 h. The reducing sugar yield reached 82.6% after 72 h of enzymatic hydrolysis.

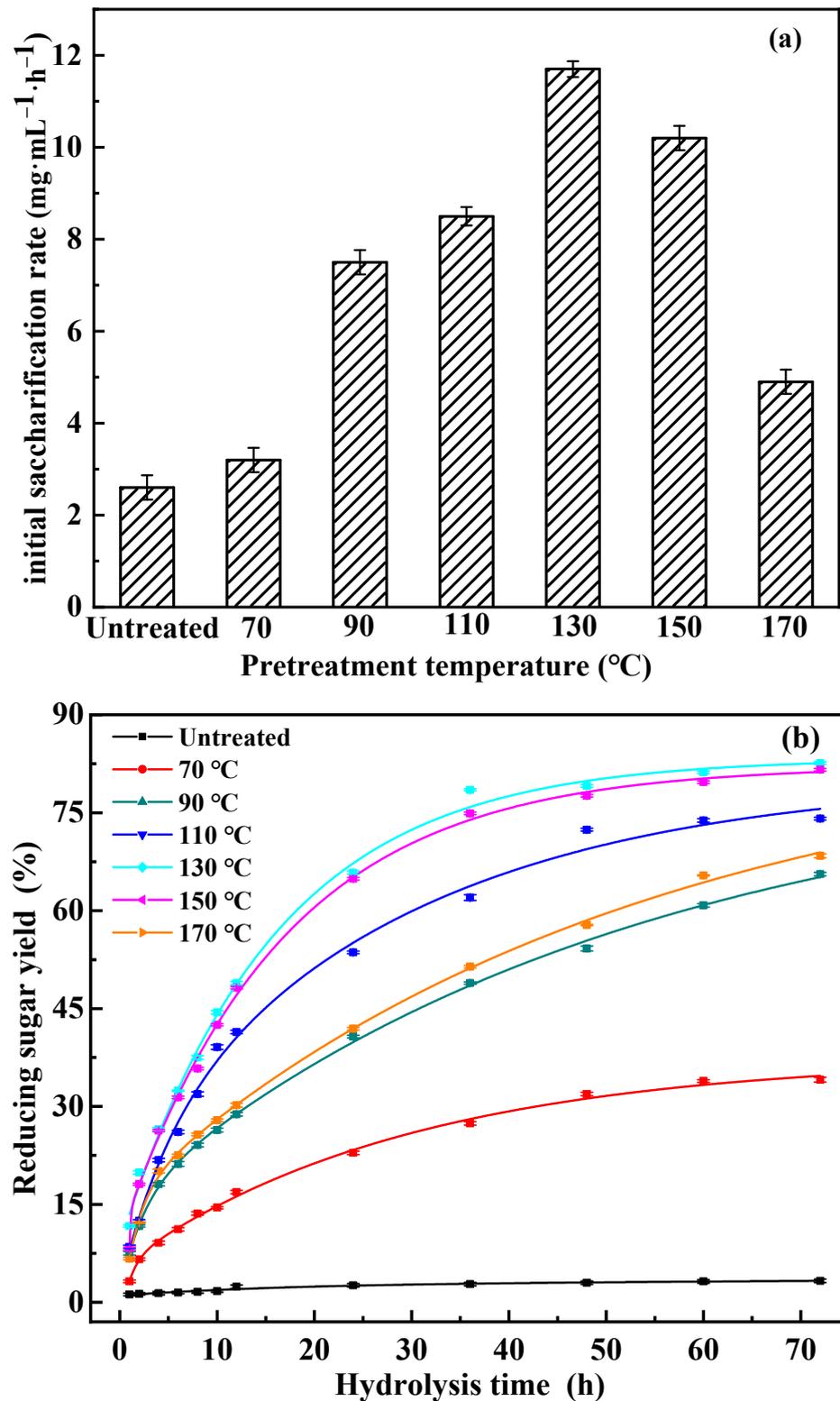


Figure 7. Influence of pretreatment temperature on enzymatic hydrolysis: (a) initial saccharification rate; (b) reducing sugar yield.

3.3. Influence of Pretreatment Time on Pretreatment of Poplar Wood

Figure 8 depicted the influence of pretreatment time on the residue recovery, lignin removal, hemicellulose removal and cellulose preservation of poplar wood. Figure 8 shows that the residue recovery and cellulose reservation slowly decreased from 50.6% to 46.3%

and from 97.5% to 92.7% with the increase in pretreatment time, respectively, while when the operation time increased, the lignin removal slowly increased from 89.2% to 93.9% and the hemicellulose removal increased from 71.6% to 77.1%. These results indicate that pretreatment time has little effect on poplar wood.

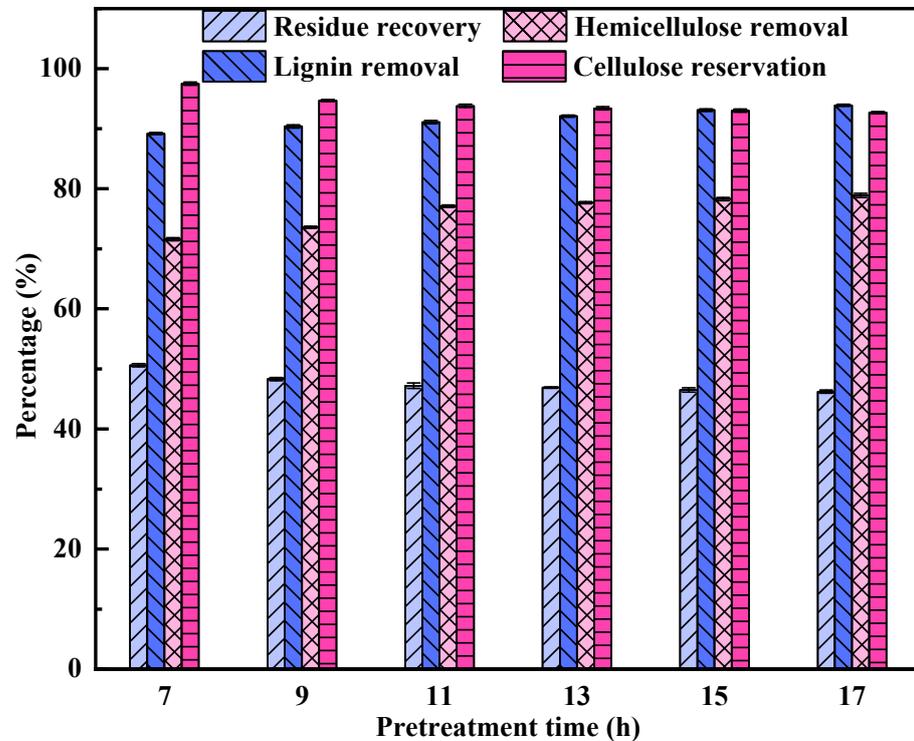


Figure 8. Influence of pretreatment time on the pretreatment of poplar wood.

Figure 9 shows the influence of pretreatment time on the hydrolysis efficiency of poplar wood. As observed from Figure 9, both the reducing sugar yield (72 h) and initial saccharification rate of poplar wood residue for enzymatic hydrolysis were higher than those of untreated poplar wood (82.5~87.3% vs. 3.3% and 10.2–13.2 $\text{mg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$ vs. 2.6 $\text{mg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$). These phenomena demonstrate that the removal of lignin and hemicellulose promoted the hydrolysis of poplar wood. Furthermore, the reducing sugar yield (72 h) and initial saccharification rate slowly increased from 82.5% to 87.3% and from 10.2 $\text{mg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$ to 13.2 $\text{mg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$ as the pretreatment time increased from 7 h to 17 h. The lower hydrolysis results indicate that enzymatic hydrolysis efficiency is not sensitive to pretreatment time.

Therefore, 7 h was chosen as the best pretreatment time. The lignin and hemicellulose removal rates were 89.2% and 71.6%, and the cellulose preservation rate was 97.5% when the poplar wood was pretreated with K: 1,2-PG at 130 °C for 7 h. While the pretreated poplar wood was hydrolyzed by enzymes for 72 h, the reducing sugar yield reached 82.5%.

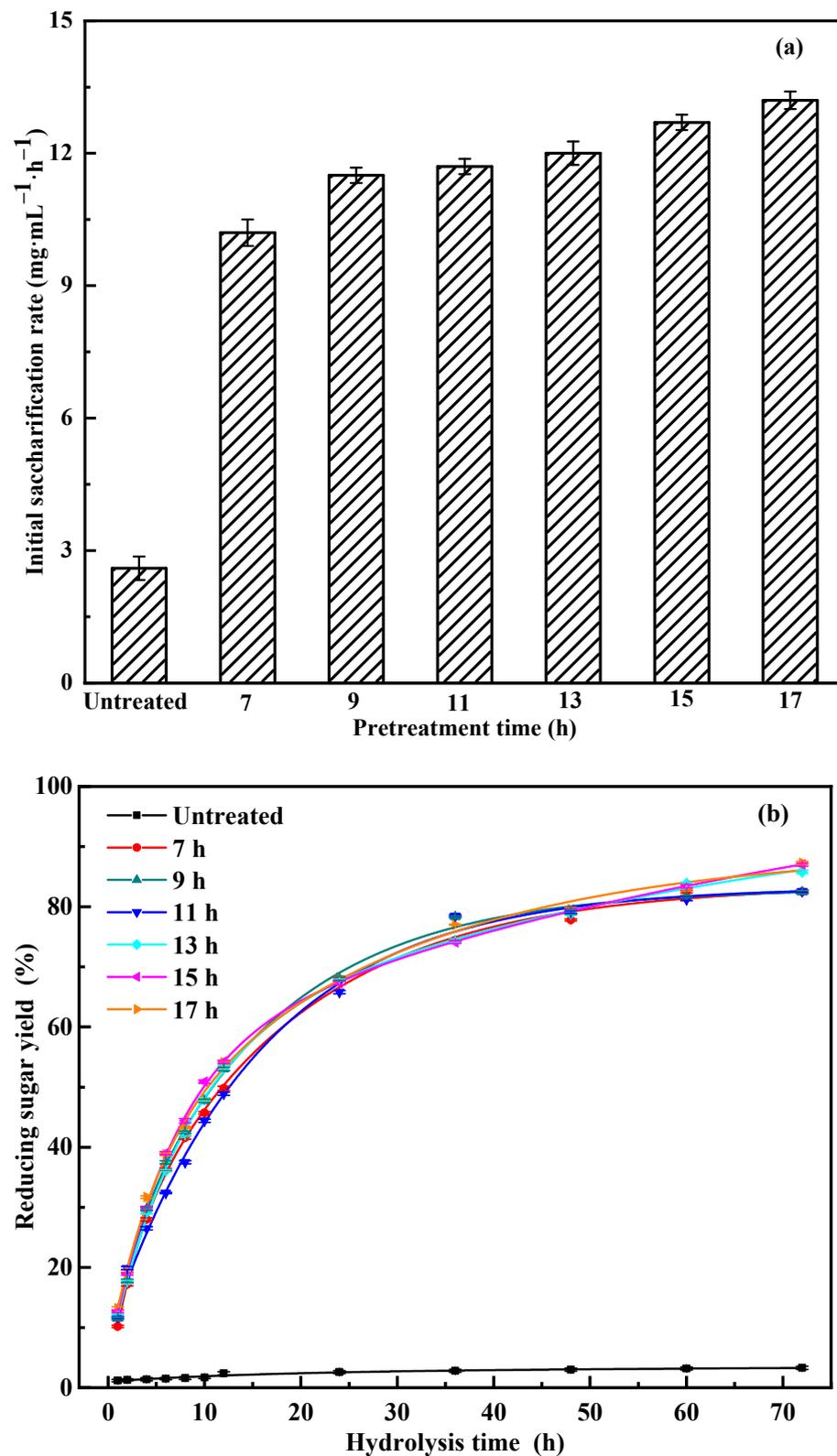


Figure 9. Influence of pretreatment time on enzymatic hydrolysis: (a) initial saccharification rate; (b) reducing sugar yield.

3.4. Comparison of Different Pretreatment Methods

Figure 10 compares the different methods of poplar wood pretreatment. The pretreatment efficiency of K: 1,2-PG was superior to that of choline chloride: guaiacol: aluminum

chloride (molar ratio 25: 50:1, C:G:A) [23], choline chloride:lactic acid (molar ratio 1:8, C:L) [24] and 1-ethyl-3-methyl-imidazolium acetate ([C₂mim][OAc]) [25]. For example, K: 1,2-PG removed the greatest amount of lignin and the lowest amount of cellulose of the four pretreatment solvents (89.2% vs. 58.0–88.0% and 97.5% vs. 87.5–94.1%). More significantly, the reducing sugar yield of poplar wood pretreated with K: 1,2-PG exhibited values higher than or similar to those of poplar wood pretreated with the other two DESs, or [C₂mim][OAc], after enzymatic hydrolysis for 72 h (82.5% vs. 32.6–88.2%).

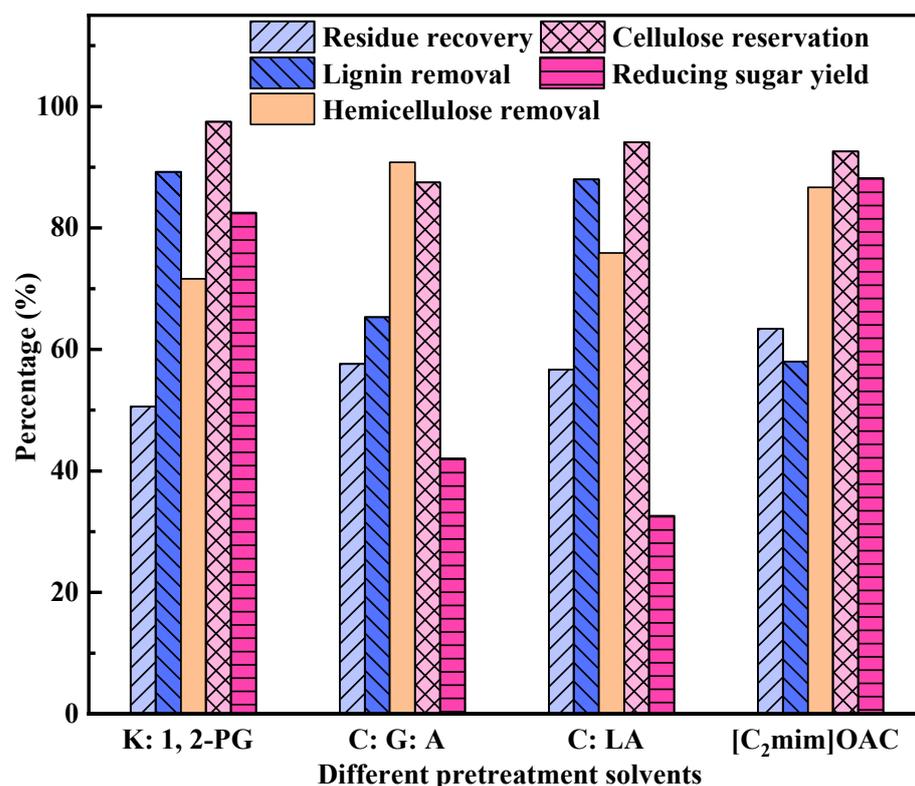


Figure 10. Comparison of different methods of poplar wood pretreatment.

3.5. Structural Characterization of Poplar Wood

3.5.1. XRD

Figure 11 shows the XRD pattern of poplar wood samples before and after pretreatment. From Figure 11, we can observe that the change in the cellulose crystal structure (CrI value) of poplar wood before and after pretreatment with K: 1,2-PG was investigated. The CrI value of K: 1,2-PG-pretreated poplar wood was significantly higher than that of untreated poplar wood (e.g., 57.1 vs. 48.3). This indicates that the crystallinity of pretreated poplar wood significantly increased compared with untreated poplar wood. The increased crystallinity was due to K: 1,2-PG, which has a stronger capacity to remove amorphous structures, i.e., lignin and hemicellulose, than that of structurally ordered cellulose. For example, 89.2% of lignin and 71.6% of hemicellulose were removed, while only 2.5% of cellulose was lost during the pretreatment process with K: 1,2-PG at 130 °C for 7 h.

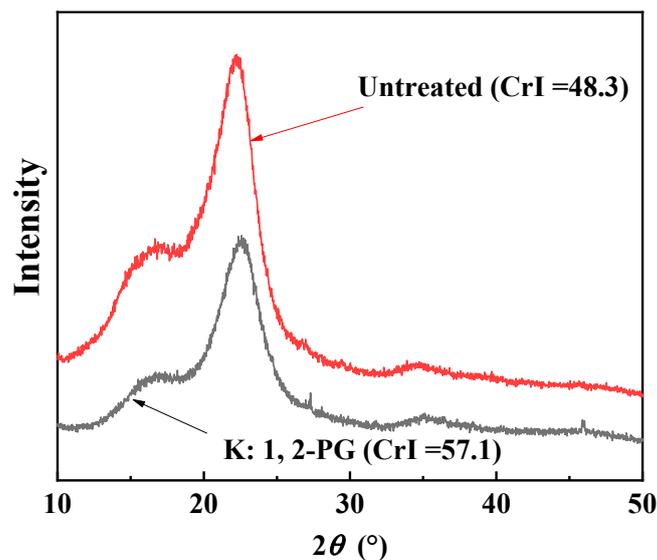


Figure 11. XRD pattern of poplar wood samples before and after K: 1,2-PG pretreatment (pretreatment conditions: 130 °C, 7 h).

3.5.2. FT-IR

Figure 12 depicts the FT-IR spectra of the poplar wood sample before and after K: 1,2-PG pretreatment. As shown in Figure 12, the peaks of K: 1,2-PG-pretreated poplar wood at 1737 cm^{-1} , 1602 cm^{-1} , 1510 cm^{-1} and 1245 cm^{-1} almost disappeared compared with untreated poplar wood. The peak at 1737 cm^{-1} represents the C=O stretching vibration peak in the carbonyl or ester groups. The disappearance of this peak demonstrates that K: 1,2-PG removed the carbonyl group in hemicellulose and cut off the ester bond between lignin and hemicellulose. The peaks at 1602 cm^{-1} , 1510 cm^{-1} and 1245 cm^{-1} represent the aromatic ring skeleton vibration peaks of lignin. The near-disappearance of these peaks illustrates that K: 1,2-PG destroyed the aromatic ring structure of lignin [26–29]. The results obtained from the FT-IR spectra, which were in accordance with the results of pretreatment, confirmed the removal of lignin and hemicellulose.

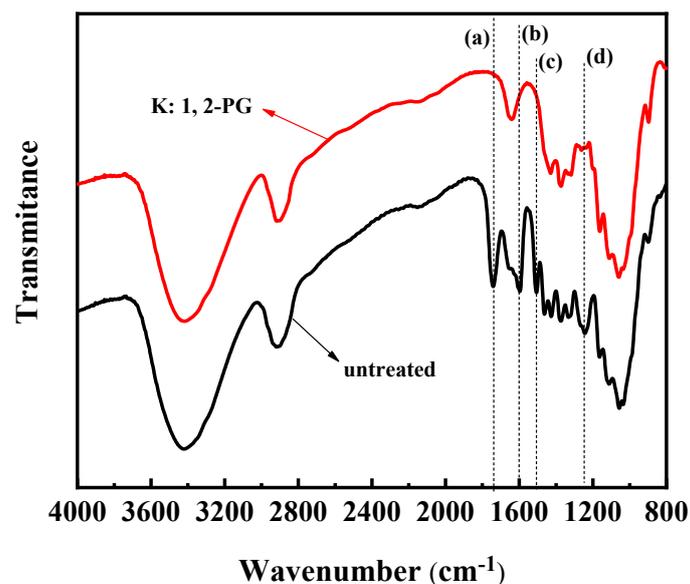


Figure 12. FT-IR spectra of poplar wood samples before and after K: 1,2-PG pretreatment: (a) 1737 cm^{-1} , (b) 1602 cm^{-1} , (c) 1510 cm^{-1} and (d) 1245 cm^{-1} .

3.5.3. SEM

Figure 13 illustrates the surface morphology of poplar wood before and after K: 1,2-PG pretreatment. As observed from Figure 13a,b, the intact surface of the untreated poplar wood was displayed, and as a result, it was difficult to see the internal cellulose structure. Figure 13c,d show that many pore structures appeared on the surface of K: 1,2-PG-pretreated residue, and a large number of fiber bundles were distributed in a disorganized manner, which greatly improved the contact area between the enzyme and the cellulose. The broken structure was caused by the removal of lignin and hemicellulose. Hence, the variations in the surface morphology between the untreated and pretreated poplar wood confirm that the removal of lignin and hemicellulose resulted from K: 1,2-PG pretreatment of poplar wood.

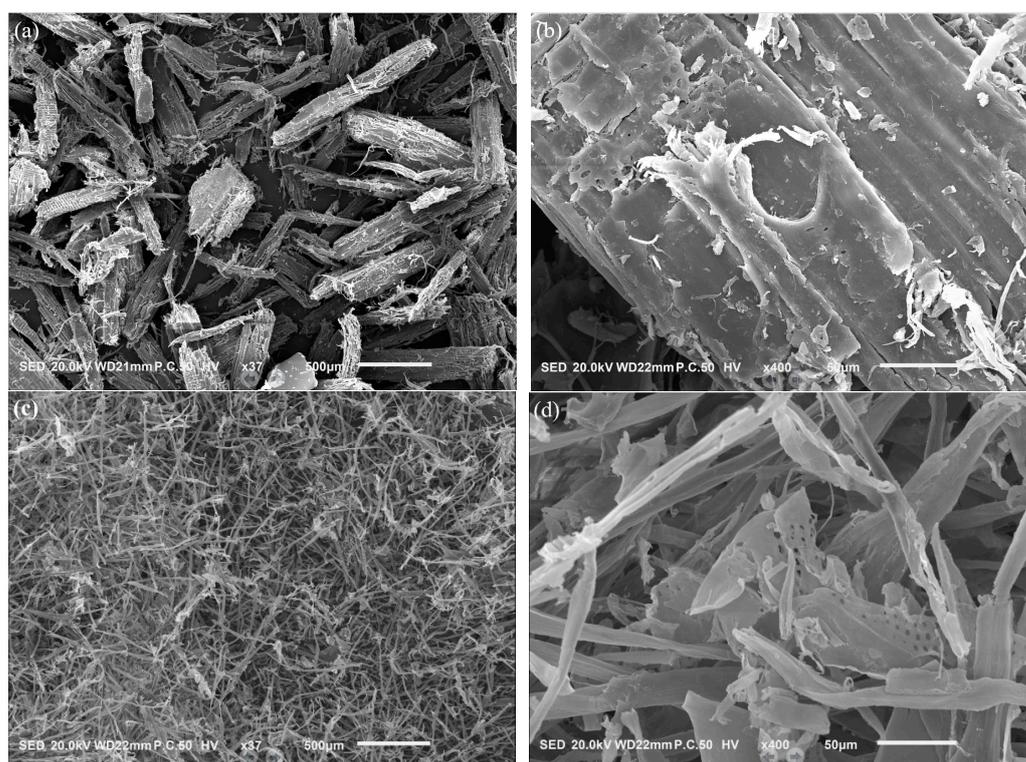


Figure 13. Surface morphology of poplar wood before and after K: 1,2-PG pretreatment: (a) untreated poplar wood $\times 37$, (b) untreated poplar wood $\times 400$, (c) K: 1,2-PG-pretreated poplar wood $\times 37$ and (d) K: 1,2-PG-pretreated poplar wood $\times 400$.

4. Conclusions

Seven basic DESs based on diol were screened for their ability to improve enzymatic hydrolysis in poplar wood pretreatment. Among these DESs, the pretreatment effects of diol monomer-based DESs were better than those of PEG-based DESs. The best pretreatment and hydrolysis results were obtained using a K: 1,2-PG pretreatment process (pretreatment conditions: 130 °C, 7 h), which furthered the enzymatic hydrolysis process (hydrolysis time: 72 h). As a result, the higher the pH of the DES, the greater the amounts of lignin and hemicellulose that were removed. For instance, the lignin and hemicellulose removal rates were 89.2% and 71.6%, the cellulose preservation rate was 97.5% and the reducing sugar yield reached 82.5%. Otherwise, the results of the FT-IR, XRD and SEM analyses all confirmed the removal of lignin and hemicellulose and revealed the pretreatment mechanism by which K: 1,2-PG removes lignin and hemicellulose by cutting the ester bond between lignin and hemicellulose, breaking the carbonyl group of hemicellulose and the aromatic ring structure of lignin. Thus, the establishment of this new pretreatment system will promote enzymatic hydrolysis in poplar wood.

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