



# A novel deep eutectic solvent–mediated Fenton-like system for pretreatment of water hyacinth and biobutanol production

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## Abstract

To facilitate depolymerization of cellulose into fermentable sugars, a novel deep eutectic solvent (DES)–mediated Fenton-like system was developed for efficient removal of hemicellulose and lignin components from lignocellulosic biomass. Water hyacinth (WH) was treated with the newly synthesized DES (choline chloride–ethylene glycol–FeCl<sub>3</sub>; ChCl–EG–FeCl<sub>3</sub>) at 140 °C for 2 h and then subjected to treatment with 32 mL 30 wt% H<sub>2</sub>O<sub>2</sub> (Fe<sup>3+</sup>: H<sub>2</sub>O<sub>2</sub> = 1:50 mol·mol<sup>-1</sup>) for 1 h in a Fenton-like reaction system (DES/H<sub>2</sub>O<sub>2</sub>). Hydrolysate containing 28 g·L<sup>-1</sup> glucose was obtained, which was 19.6- and 1.6-fold of raw WH and WH pretreated by DES, respectively. The DES/H<sub>2</sub>O<sub>2</sub>-WH hydrolysate was further evaluated for butanol fermentation; no significant inhibition was observed on butanol production, giving butanol titer of 8.3 g·L<sup>-1</sup>, along with a yield of 0.18 g·g<sup>-1</sup> glucose and a productivity of 0.12 g·L<sup>-1</sup>·h<sup>-1</sup>. Remarkably, DES could be recovered and recycled for five consecutive batches.

**Keywords** Water hyacinths · Deep eutectic solvent · Fenton-like system · Pretreatment · Biobutanol

## 1 Introduction

Under the circumstance of rapid consumption of non-renewable fossil energy, as well as the intensification of the greenhouse effect, the development of green and renewable energy draws increasingly attentions [1]. Lignocellulosic biomass is one of the most abundant renewable resources on earth. Moreover, it has been recognized as an alternative source for the production of second-generation biofuels such as bioethanol and biobutanol [2, 3].

Water hyacinth (WH), an aquatic invasive weed, could infest various water bodies and consume dissolved oxygen in water during its growth spurts [4]. However, WH is a potential renewable resource and promising lignocellulosic biomass for biofuel production due to its high carbohydrate percentage (mostly cellulose) and reproduction rate [5, 6]. Transforming of invasive WH into renewable energies is of special interest, because it could not only solve the environmental problems caused by WH but also develop clean and renewable biomass-based energies [7]. However, lignocellulosic cellulose is highly indigestible due to its rigid structure of crystalline fibrils embedded in a matrix of hemicellulose and lignin, which is hardly accessible by cellulase. To reduce the recalcitrance, various lignocellulose pretreatment methods have been developed, including physical (ball milling, microwave and high-energy radiation, etc.), chemical (acid, alkali, organic solvent, chlorine salt, ionic liquid, deep eutectic solvent and Fenton reagent, etc.), physical–chemical (hot water, dilute-acid steam and ammonia fiber explosion, etc.), and biological pretreatments (rot fungus and termites) [8, 9]. However, it is still difficult to maximize the utilization of lignocelluloses via one-stage pretreatment method [10]. Hence, combinatorial pretreatment methods should be developed in order to full destruction of the dense structure

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of lignocelluloses by removing the hemicellulose and lignin, featuring with high glucose yield at low enzyme dosage and short saccharification time [10, 11]. Practical application of pretreatment technologies is often limited by high cost and energy consumption. As a result, establishing efficient pretreatment processes of high environmental friendliness, easy operation as well as cost and energy savings is of great importance.

Deep eutectic solvent (DES) has been considered as a new type of economical and environmentally friendly solvents. DES usually consists of quaternary ammonium salt as a hydrogen bond acceptor (HBA) with a hydrogen bond donors (HBD), thereby generating a eutectic complex [12]. DES possesses physicochemical properties similar to ionic liquids, in addition to low cost, easy preparation, and good renewability. Therefore, DES is regarded as potential solvents in biomass utilization [13–18]. Lin and coworkers [19] used TEBA-LAC-based DES to pretreat corn straw at 120 °C for 90 min, the removal of xylan and lignin reached 57.58% and 61.40%, respectively. The pretreatment of sugar cane bagasse with TEBA-LA DES at 120 °C for 4 h with 1:15 (solid to liquid ratio) resulted in the best cellulose digestibility 88.23% [20]. DES pretreatment with different molar ratios of choline chloride–lactic acid was carried out on bamboo residues, enzymatic digestibility of 76.9% was achieved [21]. ChCl-EG is obtained by mixing choline chloride with ethylene glycol, which is applied in delignification of various lignocellulosic biomass [22–25]. However, ChCl-EG displayed insufficient efficiency in biomass fractionation. In addition, due to the absence of active protons and acidic sites, ChCl-EG cannot adequately cleave the chemical bonds between cellulose, hemicellulose, and lignin. As common Lewis acids, metal chlorides such as  $\text{FeCl}_3$  have been applied in biomass fractionation [26–29]. A three-constituent DES (ChCl-EG- $\text{FeCl}_3$ ) was designed by coordinating  $\text{FeCl}_3$  in ChCl-EG based on acidic multisite coordination theory.  $\text{FeCl}_3$  was complexed with ChCl-EG to form a ternary stable and homogeneous solution, which could enhance the digestibility of lignocellulosic biomass in addition to the recycle of  $\text{FeCl}_3$  [30]. This suggests that pretreatment effectiveness will be greatly promoted by combining the pretreatment with a  $\text{FeCl}_3$  and ChCl-EG system.

Fenton reagents have been proved effective in the deconstruction of lignocellulosic biomass to facilitate enzymatic hydrolysis [32, 33]. In Fenton reaction, ferrous iron ( $\text{Fe}^{2+}$ ) salt reacts with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) at pH 2.8–3.0 to produce hydroxyl radical ( $\cdot\text{OH}$ ). The generated  $\cdot\text{OH}$  is a strong oxidant that could break lignin in the lignocellulose and improve cellulose accessibility [33], which is favorable for the

enzymatic saccharification [8, 34]. Nevertheless, the application of Fenton reagents is restricted by strict acidic conditions, leakage of iron oxide, and non-recovery. As a result,  $\text{Fe}^{3+}$  was introduced by confinement in a host matrix to generate  $\cdot\text{OH}$  at uncontrolled pH, which reduced the leakage of  $\text{Fe}^{3+}$  [35]. A variety of catalytic iron species, including  $\text{Fe}^{3+}$  impregnated on different supporting materials (zeolites, resins, pumice particles, etc.), iron oxide minerals ( $\text{Fe}_3\text{O}_4$ ,  $\alpha\text{-FeOOH}$ ,  $\alpha\text{-Fe}_2\text{O}_3$ ,  $\gamma\text{-Fe}_2\text{O}_3$ , etc.) and iron chelates ( $\text{Fe}^{3+}$ -NTA,  $\text{Fe}^{3+}$ -EDTA, etc.) have been exploited for Fenton oxidation [32, 36–41]. To the best of our knowledge, DES-mediated Fenton-like oxidation pretreatment that has not been applied to the depolymerization of lignocellulosic biomass.

In this study, a novel DES (ChCl-EG- $\text{FeCl}_3$ ) and a  $\text{Fe}^{3+}$ -complexation-mediated Fenton-like system (DES/ $\text{H}_2\text{O}_2$ ) were developed and applied in the utilization of harmful aquatic plant WH, with significantly increased enzymatic saccharification. The composition, morphology, structural properties, crystallinity, and pore structure of WH during pretreatment process were analyzed. In addition, recycle of this novel Fenton-like system was evaluated. The potential application of the DES/ $\text{H}_2\text{O}_2$ -WH hydrolysate was explored in the fermentative production of biobutanol using *Clostridium saccharobutylicum* DSM13864. Finally, the mechanism of pretreatment and the process of mass balance were investigated in detail.

## 2 Materials and methods

### 2.1 Feedstock, strains, and chemicals

WH was salvaged in a river in Binzhou, Shandong Province, China. The root and leaf were removed, and further dried in the sun for several days. The resultant biomass was smashed to 40–60 mesh for further used. The cultivation conditions of *C. saccharobutylicum* DSM13864 were described in previous reports [42]. Choline chloride (ChCl,  $\geq 98\%$ ), iron trichloride ( $\text{FeCl}_3$ ,  $\geq 98\%$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ , 30 wt%), ethylene glycol, and other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cellulase was supplied by Qingdao Vland Biotech Inc (Qingdao, China).

### 2.2 Synthesis of deep eutectic solvent

ChCl was recrystallized from anhydrous ethanol, filtered and dried under vacuum before use [23]. ChCl-EG (pH 4.8) was synthesized by simply mixing ChCl and EG with a molar ratio of 1:2 at 80 °C and 180 rpm. The synthesis of ChCl-EG- $\text{FeCl}_3$  (pH 3.8) was prepared by mixing of ChCl, EG,

and  $\text{FeCl}_3$  in a molar ratio of 1:2:0.025 at 80 °C and 180 rpm until dark brown clear liquid formed. The synthesized DES was stored under vacuum in a desiccator for further use.

### 2.3 One-pot sequential pretreatment of WH with DES-mediated Fenton system

Firstly, 4.0 g of WH and 60.0 g of  $\text{ChCl-EG-FeCl}_3$  were placed in a 250-mL three-port flask with a mechanical stirrer. The flask was immersed in a bath of oil, and the internal temperature was maintained at 80–160 °C for 0–5.0 h. After pretreatment, distilled water was added into the mixture, stirred for 10 min to regenerate the pretreated WH. The regenerated residue (DES-WH) was filtered and rinsed with distilled water, and dried in an oven at 60 °C. Then, 6.4–64 mL 30 wt%  $\text{H}_2\text{O}_2$  ( $\text{Fe}^{3+}$  to  $\text{H}_2\text{O}_2$  of 1:10–1:100, mol/mol) was slowly dropped into the DES pretreatment solution with drip funnel, and the stirring was continued for 5–240 min at 30 °C under the optimal DES pretreatment conditions. To prevent loss of liquid, the flask was sealed with a plastic film. The DES/ $\text{H}_2\text{O}_2$ -WH was isolated as above mentioned.

### 2.4 Enzymatic hydrolysis of WH

Pretreated or raw WH (1.0 g) was mixed with 20 mL of 50 mM sodium acetate (pH 4.8), 100  $\mu\text{L}$  of 1.0  $\text{g}\cdot\text{L}^{-1}$  ampicillin and appropriate amount of cellulase (40 FPU $\cdot\text{g}^{-1}$  biomass), at 50 °C shaken for 48 h in 50-mL conical flask with plug. The supernatant (200  $\mu\text{L}$ ) was sampled periodically during enzymatic hydrolysis to monitor the process status. For all enzymatic hydrolysis, triplicate reactions were performed separately, and the results were averaged. The relative standard deviation (RSD) was less than 5%.

### 2.5 Biobutanol fermentation using DES/ $\text{H}_2\text{O}_2$ -WH hydrolysate by *C. saccharobutylicum* DSM 13,864

The DES/ $\text{H}_2\text{O}_2$ -WH hydrolysate was prepared by enzymatic hydrolysis of WH under the optimized pretreatment conditions and condensed under vacuum to concentration of 50  $\text{g}\cdot\text{L}^{-1}$  glucose, and further used as carbon source for acetone-butanol-ethanol (ABE) fermentation by *C. saccharobutylicum* DSM 13,864 [43]. Control experiment was performed with 50  $\text{g}\cdot\text{L}^{-1}$  glucose solution as carbon source. Biobutanol fermentation experiments were performed independently in triplicate. The relative standard deviation (RSD) was less than 5%.

### 2.6 Evaluation of DES recycling

The DES was collected by filtration and evaporation, and the recovered DES was further utilized in pretreatment of WH. At the end of each batch of pretreatment, the DES was recovered for the next round of pretreatment. The reusability of recycled DES was evaluated for at least five batches under DES-mediated Fenton-like system conditions.

### 2.7 Characterization methods and composition analysis

The constituent of raw and pretreated WHs was determined according to the literature [44]. SEM, FTIR, XRD, and BET techniques were employed to analyze the changes in WH before and after pretreatment. Sugar (glucose, xylose, and arabinose) concentrations were analyzed by HPLC as previously described [45]. The contents of acetone, butanol, and ethanol were analyzed by gas chromatography (GC) as previously described [42].

Glucose yield after enzymatic saccharification of WH samples was calculated according to the following formula:

$$\text{Glucose yield (\%)} = \frac{\text{concentration of glucose in hydrolysate} \times 20 \text{ mL}}{\text{weight of cellulose in WH samples}} \times 0.9 \times 100\% \quad (1)$$

Removal rates of hemicellulose and lignin were calculated according to the formulas as follows:

$$\text{Solid recovery (\%)} = \frac{\text{weight of pretreated WH}}{\text{weight of raw WH}} \times 100\% \quad (2)$$

$$\text{Hemicellulose removal (\%)} = \left( 1 - \frac{\text{weight of hemicellulose in pretreated WH}}{\text{weight of hemicellulose in raw WH}} \times \text{solid recovery} \right) \times 100\% \quad (3)$$

$$\text{Delignification (\%)} = \left( 1 - \frac{\text{weight of lignin in pretreated WH}}{\text{weight of lignin in raw WH}} \times \text{solid recovery} \right) \times 100\% \quad (4)$$

The crystallinity index (CrI) was calculated by following formula:

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

where  $I_{002}$  is the maximum intensity of the 002 lattice diffraction at  $2\theta = 23\text{--}24^\circ$  and  $I_{\text{am}}$  is the minimum intensity of peak near  $2\theta = 18.0^\circ$  corresponding to the amorphous region of cellulose.  $(I_{002} - I_{\text{am}})$  represents the intensity of the crystalline peak.

## 3 Results and discussion

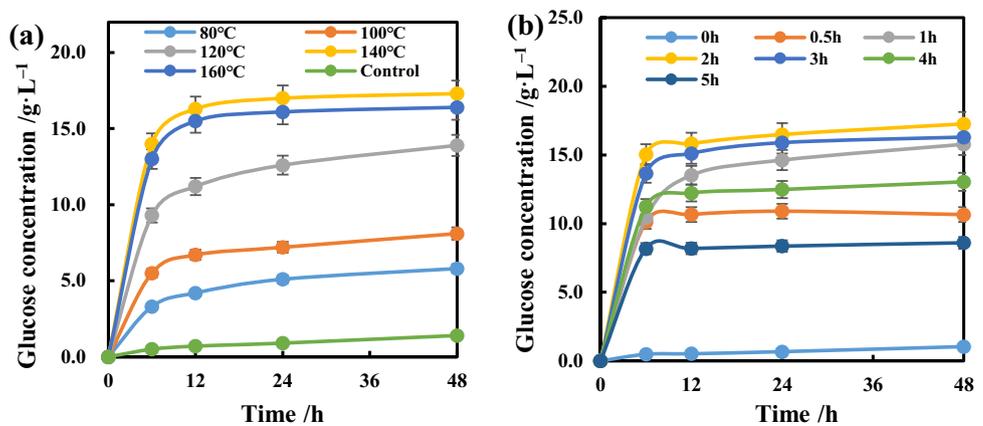
### 3.1 DES pretreatment of WH

A novel DES ChCl-EG-FeCl<sub>3</sub> composed of polyol and metal ion was synthesized and attempted in the pretreatment of

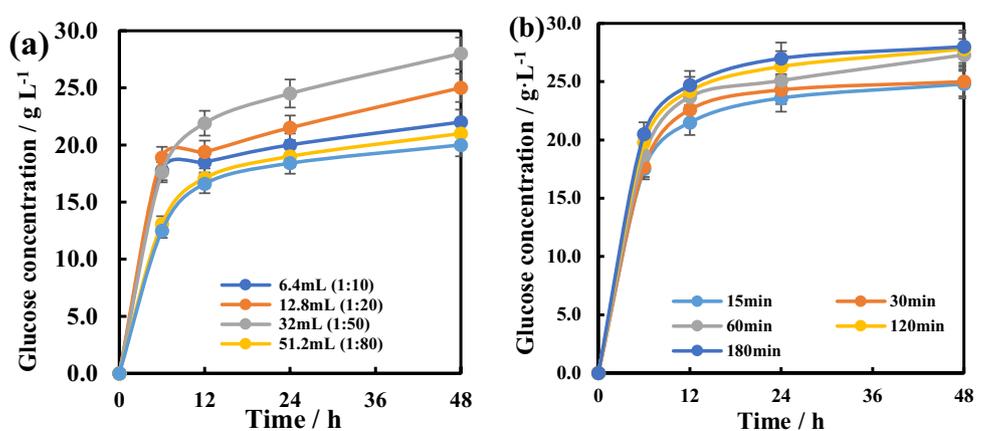
WH. The pretreated WH samples were subsequently saccharified, and the glucose concentrations were determined. The glucose concentration of ChCl-EG-FeCl<sub>3</sub> pretreatment reached 17.3 g·L<sup>-1</sup> after enzymatic hydrolysis for 48 h, which was 12.4- and 1.4-fold of that of WH and WH pretreated by ChCl-EG respectively. FeCl<sub>3</sub> plays important roles in coordinating ChCl and EG to form stable ternary DES. In ChCl-EG-FeCl<sub>3</sub>, the acidity and hydrogen bond interactions were strengthened, leading to the increased removal rates of lignin and hemicellulose in DES system [46].

In order to achieve efficient pretreatment of WH, factors influencing the pretreatment including temperature and reaction durations were investigated. As presented in Fig. 1a, glucose concentration after 48 h hydrolysis increased from 5.8 to 17.3 g·L<sup>-1</sup> alongside with the temperature increased from 80 to 140 °C. When pretreatment temperature further increased to 160 °C, there were no significant changes in glucose concentration. The hydrolysis rates were positively correlated with temperatures in the

**Fig. 1** Glucose concentrations of enzymatic hydrolysis of raw and DES-WH at different pretreatment temperatures (a) and time (b)



**Fig. 2** Glucose concentrations of enzymatic hydrolysis of Fenton-like pretreated DES-WH with different amounts of H<sub>2</sub>O<sub>2</sub> (a) and pretreatment time (b)



initial 6.0 h, which could be attributed to the depolymerization of crystalline cellulose and accelerate accessibility of enzymes to cellulose [47]. In the view of higher fermentation sugars and relative low energy consuming, 140 °C was regarded as the optimal temperature for further experiment. Furthermore, different pretreatment time was explored at 140 °C. As shown in Fig. 1b, the glucose concentration increased to 17.3 g·L<sup>-1</sup> (66% glucose yield) after 2 h of pretreatment, followed by a gradual decrease to 8.6 g·L<sup>-1</sup> after 5 h. Longer time of pretreatment with ChCl-EG-FeCl<sub>3</sub> might lead to degradation of cellulose to some extent, similar as previously reported [46].

### 3.2 One-pot sequential pretreatment of WH with DES-mediated Fenton-like system

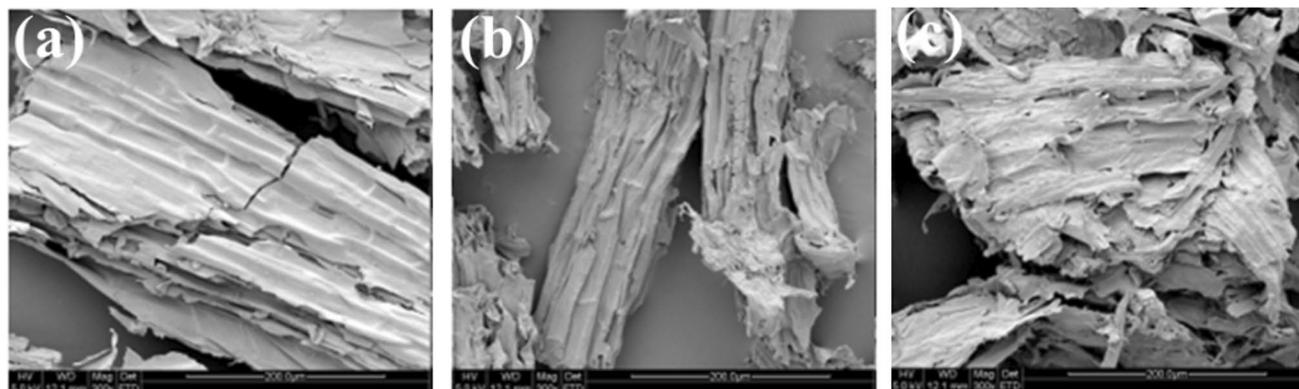
After pretreated with ChCl-EG-FeCl<sub>3</sub> at 140 °C for 2 h, H<sub>2</sub>O<sub>2</sub> was dropwise added into the mixture to form the DES/H<sub>2</sub>O<sub>2</sub> pretreatment system, which was further conducted at 30 °C. Factors of the Fenton-like

reaction including the amount of H<sub>2</sub>O<sub>2</sub> (molar ratio of Fe<sup>3+</sup> to H<sub>2</sub>O<sub>2</sub>) and the pretreatment time were explored. As shown in Fig. 2a, when 6.4 mL of 30 wt% H<sub>2</sub>O<sub>2</sub> (Fe<sup>3+</sup>: H<sub>2</sub>O<sub>2</sub> = 1:10 mol/mol) was supplemented, there was no influence on the glucose concentration and the delignification of WH, indicating the amount of H<sub>2</sub>O<sub>2</sub> might not be adequate to form hydroxyl radicals. Furthermore, addition of 30 wt% H<sub>2</sub>O<sub>2</sub> was increased to 32 mL (Fe<sup>3+</sup>: H<sub>2</sub>O<sub>2</sub> = 1:50 mol/mol) to generate more hydroxyl radicals, resulting in increased delignification of WH and the highest glucose concentration. However, excessive amount of H<sub>2</sub>O<sub>2</sub> was also unfavorable for the pretreatment of WH, since excessive active hydroxyl radicals might cause further destruction of lignin, which resulted in the collapse of the crystalline structure of cellulose. Furthermore, WH was pretreated in DES/H<sub>2</sub>O<sub>2</sub> pretreatment system for different times, and the glucose concentrations were investigated. As shown in Fig. 2b, the glucose concentration reached the highest level of 28 g·L<sup>-1</sup> (86% glucose yield) after 1.0 h of pretreatment, which was 19.6- and 1.6-fold of raw WH and WH pretreated by DES (DES-WH). Therefore, a novel DES-mediated Fenton-like system was successfully established for the pretreatment of WH. WH was pretreated by steam explosion at 190 °C for 10 min, and the reducing sugar was 15.5 g/L by enzymatic hydrolysis [48]. The organic solvent pretreatment of WH with glycerol and FeCl<sub>3</sub>, resulting 51% release of sugars [49]. Compared with the recent pretreatment methods of WH, DES/H<sub>2</sub>O<sub>2</sub> system has high efficiency and recyclability, which can save the pretreatment cost. In addition, compared with the two-step pretreatment method [50], this one-pot sequential pretreatment system eliminated the filtration, washing and drying steps of intermediate residues, and achieved energy saving and emission reduction. In conclusion, this method is efficient, green, and economical in the pretreatment of biomass.

**Table 1** Chemical compositions of raw and pretreated WHs

Composition /%	WH	DES-WH	DES/H <sub>2</sub> O <sub>2</sub> -WH
Cellulose	38.17	47.03	58.66
Hemicellulose	9.39	2.22	0.19
Acid-soluble lignin	19.62	12.16	14.90
Acid-insoluble lignin	1.45	1.84	1.47
Ash	0.85	0.84	1.13
Solid recovery	–	67.42*	46.97*
Cellulose retention	–	83.07*	72.18*
Hemicellulose removal	–	84.20*	98.65*
Delignification	–	55.50*	63.50*

*T* test was conducted, and *P* value < 0.05 was denoted as “\*”



**Fig. 3** SEM images of WH (a), DES-WH (b), and DES/H<sub>2</sub>O<sub>2</sub>-WH (c)

### 3.3 Analysis of chemical compositions of raw and pretreated WH

Changes in chemical compositions of WH before and after pretreatment were summarized in Table 1. Cellulose content increased from 38.2% of raw WH to 47.0% of WH pretreated by DES system and 58.7% of WH after pretreatment employing DES/H<sub>2</sub>O<sub>2</sub> system (DES/H<sub>2</sub>O<sub>2</sub>-WH). The higher cellulose content in pretreated WH agrees with the high glucose concentrations obtained by enzymatic hydrolysis. It is remarkable that DES/H<sub>2</sub>O<sub>2</sub> system can effectively remove hemicellulose, leaving only 0.19% hemicellulose in DES/H<sub>2</sub>O<sub>2</sub>-WH with a removal rate of 98.65%. Moreover, DES/H<sub>2</sub>O<sub>2</sub> system displays better performance in removal of lignin with removal rate of 63.5%, which was 8.0% higher than 55.5% of single DES. Therefore, DES/H<sub>2</sub>O<sub>2</sub> system was favorable for the removal of hemicellulose and lignin, resulting in a final solid recovery of 46.97%. Chemical composition analysis indicated that DES/H<sub>2</sub>O<sub>2</sub> system is highly efficient in removing hemicellulose and lignin, and can be used in the pretreatment of lignocellulosic biomass.

### 3.4 Physical characterization of raw and pretreated WH

In order to monitor the structural changes of WH surface before and after pretreatment, the microstructures of WH, DES-WH and DES/H<sub>2</sub>O<sub>2</sub>-WH were analyzed by SEM (Fig. 3). For the WH, rigid fiber bundles with smooth and dense surface were observed. While in DES-WH, the crystalline structure of WH was destroyed, and the fiber bundle showed collapse and shrinkage to certain extent, with many ravines. The surface structure of WH pretreated by DES/H<sub>2</sub>O<sub>2</sub> was damaged more seriously. The surface becomes

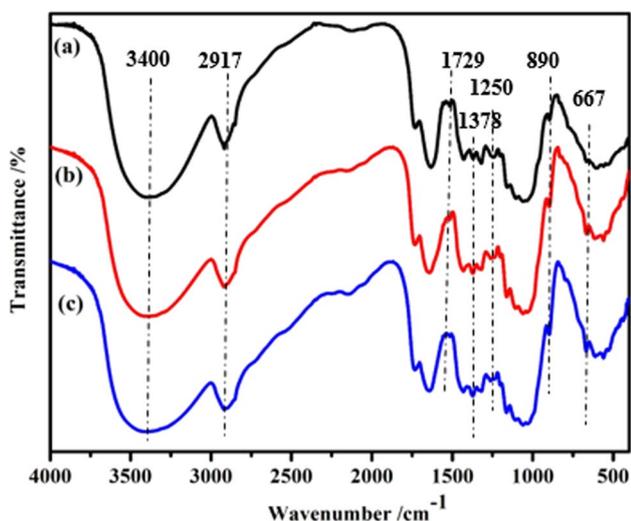


Fig. 4 FTIR images of WH (a), DES-WH (b), and DES/H<sub>2</sub>O<sub>2</sub>-WH (c)

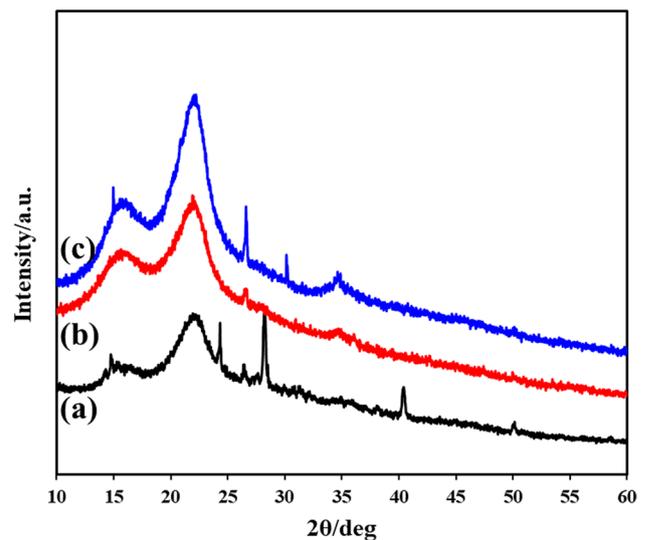


Fig. 5 XRD images of WH (a), DES-WH (b), and DES/H<sub>2</sub>O<sub>2</sub>-WH (c)

disordered, loose, and porous, which might be related to the removal of large amounts of hemicellulose and lignin, as well as the breakdown of hydrogen bonds on the crystal surface. These changes led to the increase in the accessibility to cellulase, which are favorable for subsequent enzymatic saccharification.

Moreover, FTIR spectrum analysis was used to determine the changes of major functional groups after WH pretreatment (Fig. 4). The bands at around 3400 and 2917 cm<sup>-1</sup>, corresponding to the absorption peak of -OH groups linked by intramolecular and intermolecular hydrogen bonds respectively, were weaker in the spectrum of pretreated WH compared with WH. These changes suggest the intermolecular and intramolecular hydrogen bonds of cellulose molecules were broken down, the highly ordered cellulose crystalline structure could be destroyed. A decrease in the band intensity at 1729 cm<sup>-1</sup> after pretreatment reflects the breakdown of acetyl group in hemicellulose. The band near 1378 cm<sup>-1</sup> was CH-bending vibrations of cellulose and hemicellulose, which was slightly increased after pretreatment. Additionally, the peak at 1250 cm<sup>-1</sup> was attributed to the C-O stretching in lignin, reduced after pretreatment, which might be attributed to the efficient delignification of WH. The bands at 890 cm<sup>-1</sup> were the vibration absorption

Table 2 Pore characterizations of WH and DES/H<sub>2</sub>O<sub>2</sub>-WH

Sample	BET surface area (m <sup>2</sup> ·g <sup>-1</sup> )	Pore volume (cm <sup>3</sup> ·g <sup>-1</sup> )	Pore size (nm)
WH	0.0734	0.0003	18.7215
DES/H <sub>2</sub> O <sub>2</sub> -WH	0.3959*	0.0030*	30.4542*

*T* test was conducted, and *P* value < 0.05 was denoted as “\*\*”

peak of  $\beta$ -(1-4)-glycosidic linkages, and  $667\text{ cm}^{-1}$  was related with the C-OH out-of-plane bending. Both of them were increased to some extent in the pretreated WH, due to the increase of glucan proportion, which agreed with the chemical component analysis.

The cellulose crystallinity index (CrI) of WH, DES-WH, and DES/H<sub>2</sub>O<sub>2</sub>-WH was determined by X-ray diffractometry (XRD) (Fig. 5). Two major peaks at around  $17.6^\circ$  and  $22.5^\circ$  were assigned to the cellulose I (101 and 002) crystalline structures. XRD curves revealed that the positions of diffraction peaks were unchanged after pretreatment, indicating there was no transformation of crystalline cellulose. The CrI value of WH was 39.3%. After WH was pretreated by DES/H<sub>2</sub>O<sub>2</sub> system, the crystallinity eventually decreased by 11.3%. The results indicated that the cellulose crystallinity structure of WH was damaged to a certain extent. The

significantly decreased crystallinity intensity of the pretreated WH proved that this newly developed DES-mediated Fenton-like system was effective in removal of hemicellulose and lignin.

The pore properties of raw and pretreated WH were investigated using BET analysis. The BET surface area of the WH pretreated with DES/H<sub>2</sub>O<sub>2</sub> system was  $0.3959\text{ m}^2\cdot\text{g}^{-1}$ , which was 5.4 times of that of raw WH (Table 2). In addition, the volume and size of pores were also significantly increased in the pretreated WH. These results were consistent with the rough surface and notable tiny dots observed in SEM images. The structural changes after pretreatment allow the cellulase to penetrate, absorb, and hydrolyze WH more easily, resulting in remarkably increased enzymatic saccharification efficiency.

### 3.5 Biobutanol fermentation using DES/H<sub>2</sub>O<sub>2</sub>-WH hydrolysate

Lignocellulose materials have attracted increasing attention as a new biomass for biobutanol fermentation. In the practical application, lignin exhibits ineffective adsorption with cellulase and acts as a main physical barrier to hinder the adsorption of cellulase on cellulose. Here, DES/H<sub>2</sub>O<sub>2</sub> system can efficiently remove lignin to obtain high sugar hydrolysate for butanol fermentation. In this study, the fermentability of DES/H<sub>2</sub>O<sub>2</sub>-WH hydrolysates was applied for butanol fermentation by *C. saccharobutylicum* DSM13864. Glucose consumption and solvents (acetone-butanol-ethanol; ABE) production were monitored (Fig. 6). In the initial

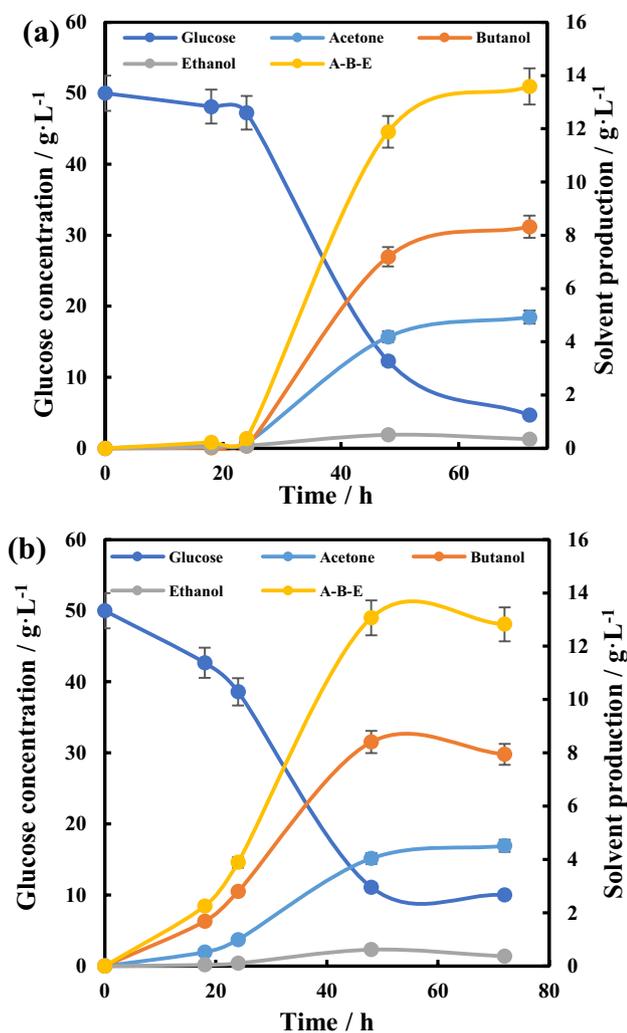


Fig. 6 Biobutanol fermentation using DES/H<sub>2</sub>O<sub>2</sub>-WH hydrolysate (a) and glucose solution as carbon sources (b) for *C. saccharobutylicum* DSM 13,864

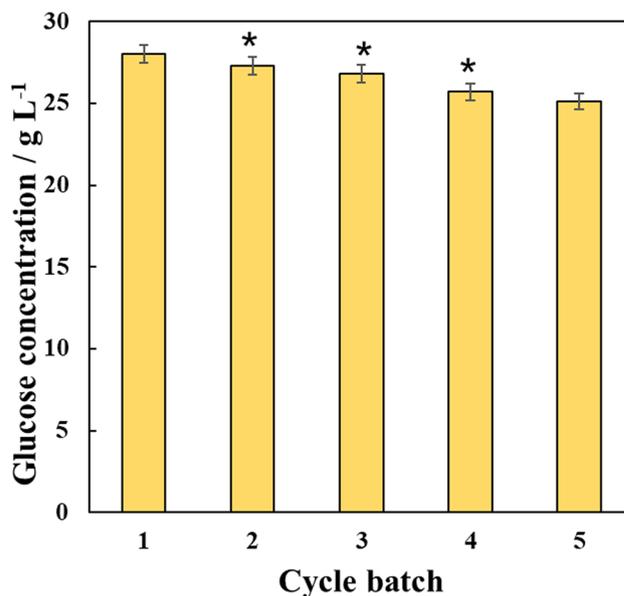


Fig. 7 Glucose concentrations of enzymatic hydrolysis of DES/H<sub>2</sub>O<sub>2</sub>-WH from different pretreatment batches. Note: *T* test was conducted and *P* value < 0.05 was denoted as “\*\*”

24 h, *C. saccharobutylicum* DSM13864 grew slowly with few bubbles, hinting low consumption of glucose and low production of ABE. During 24 h to 48 h, elevated glucose consumption associated with increased ABE production was observed. At 72 h, the highest butanol titer of 8.3 g·L<sup>-1</sup> was achieved, with consumption of about 45.7 g·L<sup>-1</sup> glucose (Fig. 6b). The butanol yield and productivity of DES/H<sub>2</sub>O<sub>2</sub>-WH hydrolysate were calculated to be 0.18 g·g<sup>-1</sup> glucose and 0.12 g·L<sup>-1</sup>·h<sup>-1</sup>, which were close to those of the control (0.20 g·g<sup>-1</sup> glucose and 0.11 g·L<sup>-1</sup>·h<sup>-1</sup>) (Fig. 6a). The yield and productivity of total solvent in DES/H<sub>2</sub>O<sub>2</sub>-WH hydrolysate were 0.30 g·g<sup>-1</sup> glucose and 0.19 g·L<sup>-1</sup>·h<sup>-1</sup> respectively. Consequently, there was no obvious inhibitory effect of DES/H<sub>2</sub>O<sub>2</sub>-WH hydrolysate on the biobutanol production of *C. saccharobutylicum* DSM13864.

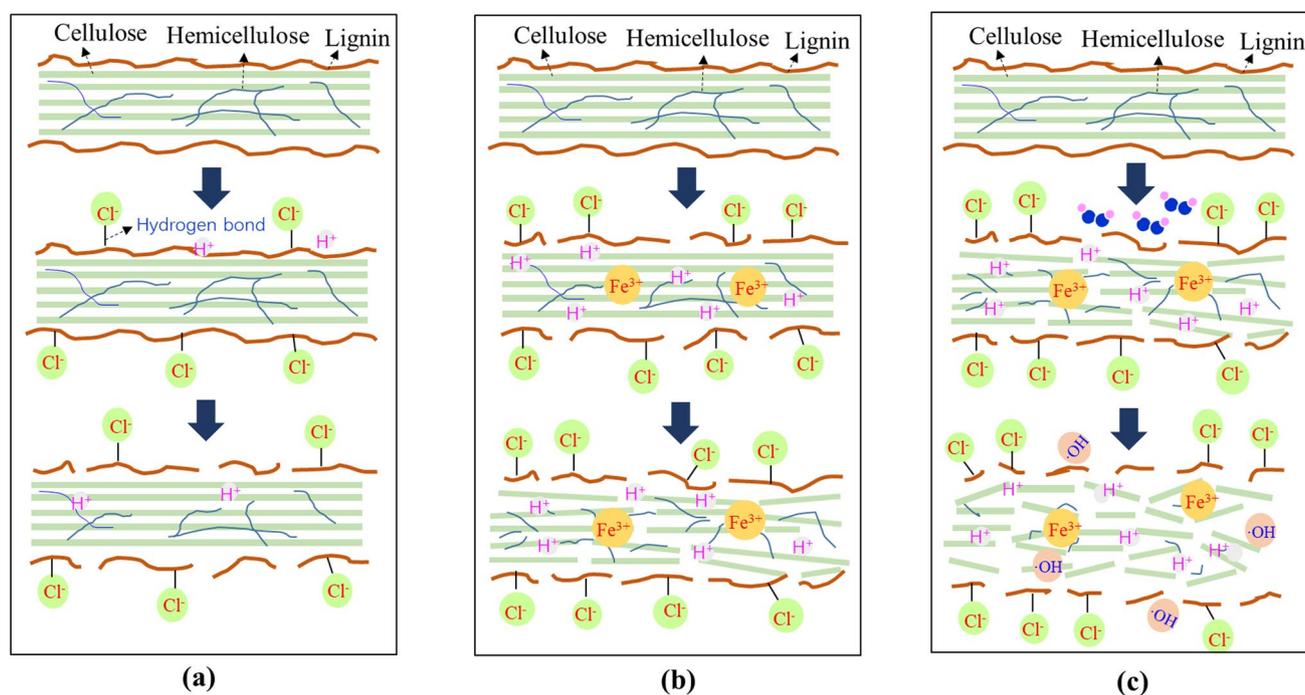
### 3.6 DES recovery and recycling

The recovery and recycling of DESs are essential for scale-up applications in biomass pretreatment processing [51], considering the low cost and consumption of metal ions. ChCl-EG-FeCl<sub>3</sub> was isolated and purified by filtration and evaporation, and further used for pretreatment in fed-batch model. As shown in Fig. 7, ChCl-EG-FeCl<sub>3</sub> could be reused for at least 5 times, with only slightly decreased glucose yield. This could be the possible reason for the degradation of cellulose to HMF during pretreatment [52]. Moreover, the DES has good recyclability, and its stable structure and

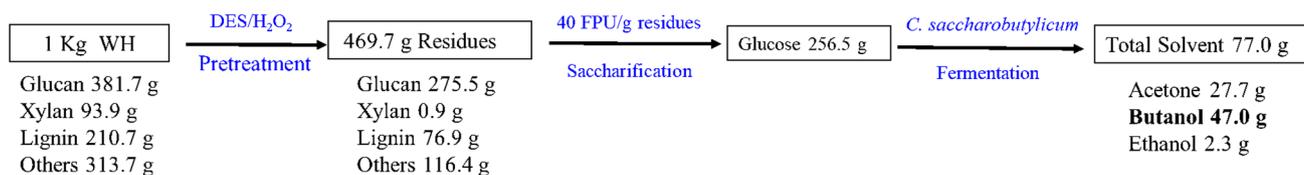
excellent pretreatment efficiency. All above demonstrate that this renewable DES could be recycled in biomass pretreatment, and has great potential in cost-competitive biorefinery processes.

### 3.7 Proposed pretreatment mechanism

ChCl-EG is a lignin-dissolving solvent for extraction of lignin component from lignocellulosic biomass [53]. As illustrated in Fig. 8a, Cl<sup>-</sup> from ChCl could form hydrogen bonds with the hydroxyl group of lignin [54]. Moreover, strong hydrogen-bonding interactions between EG and free hydroxyl groups in lignin could enhance dissolution of lignin. However, this performance was less efficient than acid pretreatment, owing to the lack of acidity, which resulted in less removal of xylan and lignin. Combination of ChCl-EG and Lewis acid (FeCl<sub>3</sub>) could improve the degradation of hemicellulose, and the formation of acidic DES (ChCl-EG-FeCl<sub>3</sub>). From Fig. 8b, the addition of FeCl<sub>3</sub> promoted hydrogen bond interactions between Cl<sup>-</sup> and hydroxyl group of lignin. On the other hand, the acidity produced by Fe<sup>3+</sup> could promote the rupture of chemical bonds (ester- and ether bond) between hemicellulose and lignin. In addition, hemicellulose also degraded spontaneously under acidic conditions. Thus, the synergistic effects of ChCl-EG and FeCl<sub>3</sub> provide evidence for the combinatory pretreatment for removal of lignin and hemicellulose. As shown in Fig. 8c, with the addition of H<sub>2</sub>O<sub>2</sub>, a Fenton-like



**Fig. 8** The schematic figure of the pretreatment mechanism for **a** ChCl-EG, **b** ChCl-EG-FeCl<sub>3</sub>, **c** ChCl-EG-FeCl<sub>3</sub>/H<sub>2</sub>O<sub>2</sub>



**Fig. 9** Material balance diagram. Using 1-kg WH as starting material

reaction occurred with DES. The Fe<sup>3+</sup> initially reacted with H<sub>2</sub>O<sub>2</sub> to produce Fe<sup>2+</sup> (Fe<sup>3+</sup> + H<sub>2</sub>O<sub>2</sub> → Fe<sup>2+</sup> + H<sup>+</sup> + •HO<sub>2</sub>), and then the generated Fe<sup>2+</sup> capable of decomposing H<sub>2</sub>O<sub>2</sub> to produce •OH (Fe<sup>2+</sup> + H<sub>2</sub>O<sub>2</sub> → Fe<sup>3+</sup> + •OH + OH<sup>-</sup>), which can degrade carbohydrates and lignin in WH and provide more exposed cellulose in enzymatic hydrolysis [32]. Consequently, the DES/H<sub>2</sub>O<sub>2</sub> system pretreated WH produced higher saccharification concentration than WHs pretreated by ChCl-EG and ChCl-EG-FeCl<sub>3</sub>. It is worth noting that the DES/H<sub>2</sub>O<sub>2</sub> system did not require pH control and was easy to be recycled and reused.

### 3.8 Process mass balance

Accurate quality analysis is necessary to further evaluate the applicability of DES/H<sub>2</sub>O<sub>2</sub> system in synergistic pretreatment of WH. The overall mass balance of DES/H<sub>2</sub>O<sub>2</sub> system pretreatment of WH, enzymatic saccharification, and ABE fermentation is summarized in Fig. 9. About 469.7 g highly degradable biomass, rich in glucan, could be obtained from 1 kg WH by DES/H<sub>2</sub>O<sub>2</sub> system pretreatment. Moreover, due to the massive removal of xylan and lignin, the relative content of glucan in the residue was greatly increased. After saccharification and fermentation, 77-g total solvent was obtained, including 47-g biobutanol. The results showed that DES/H<sub>2</sub>O<sub>2</sub> system pretreatment is a promising procedure for reducing the recalcitrance and producing fermentable sugars from lignocellulosic biomass. In addition, DES could be recovered and recycled without complicated purification steps. Consequently, this collaborative bioprocess could transform invasive and useless WH into biobutanol, and has great potential in biorefineries.

## 4 Conclusions

Here, DES-mediated Fenton-like system was successfully developed for sequential pretreatment of invasive WH, with remarkable hemicellulose and lignin removal rates and high biocompatibility for further enzymatic saccharification. The DES/H<sub>2</sub>O<sub>2</sub>-WH hydrolysate could be used for biobutanol fermentation, achieving high butanol titer and desirable fermentation efficiency. Moreover, the DES could be recovered and reused for at least 5 times. This newly established

DES-mediated Fenton-like system is a promising strategy for delignification of lignocellulosic biomass, production of fermentable sugars, and biofuel production.

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### Declarations

**Conflict of interest** The authors declare no competing interests.

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