

# Production of Furfuryl Alcohol from Corncob Catalyzed By CCZU-KF Cell Via Chemoenzymatic Approach

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**Abstract:** In this work, the hybrid route of chemo-catalysis and bio-catalysis were used to chemoenzymatically catalyze corncob to produce furfuryl alcohol via sequential conversion with solid acid catalyst at 180 °C for 10 min, and *E. coli* CCZU-KF whole-cell biocatalyst at 35 °C for 72 h in 10 vol% choline chloride system. The yield of furfuryl alcohol was 97.7%. This work successfully demonstrated the green and efficient synthesis of furfuryl alcohol production from biomass via chemoenzymatic approach.

**Keywords:** Furfuryl alcohol, Furfural, CCZU-KF, Sn-CFMS, Chemoenzymatic synthesis.

## 1. Introduction

Furfuryl alcohol (FOL) is a key furan-based chemical, which has a wide range of applications, such as pharmaceuticals, perfumes, adhesives, polymers, plastics, surfactants, furan resins and biofuels [1]. Typically, biomass or biomass derived *D*-xylose produces furfural (FAL) through an acid-catalyzed dehydration reaction [2]. As an important FAL derivative, FOL can be manufactured by chemical reduction hydrogenation of FAL under high temperature and pressure [3]. Metal catalysts, such as Cu/MgO-Al<sub>2</sub>O<sub>3</sub> [4], Co/SBA-15 [5] and Au/Cu-Al<sub>2</sub>O<sub>3</sub> [6], can chemically catalyze FAL to synthesize FOL, and obtain higher FOL yield and selectivity. While these chemical hydrogenation methods have some problems, such as high energy consumption, high catalyst cost and the formation of inhibitory by-products [7]. Hence, it is necessary to develop a green and sustainable process to transform FAL derived from biomass into FOL. Due to its simple operation, low cost, high catalytic activity and environmental friendliness, bio-catalysis has attracted great attention in recent years [8]. The production of FOL from FAL by microorganism or enzyme has become a new research hotspot. Some recombinant *Escherichia coli* bacteria containing NADH-dependent reductase have good alcohol selectivity and can biotransform carbonyl compounds to corresponding alcohols [9]. Xylose-derived FAL was converted to FOL with 100% yield by recombinant *Escherichia coli* CCZU-T15 in a toluene-water system containing OP-10 [10]. The FAL solution was completely reduced to FOL within 2.5 h in dibutyl phthalate water system by recombinant *Escherichia coli* CCZU-Y10 containing reductase PgCR [11].

FAL is a key platform molecule for the synthesis of high-value furan chemicals, widely used in the food, pharmaceutical, agricultural and other industries [12, 13]. In recent years, the conversion of cheap biomass into FAL, an intermediate product with many uses, has attracted wide attention [14]. In industrial production, inorganic homogeneous acids (HCl, H<sub>2</sub>SO<sub>4</sub>, etc.) are usually used as chemical catalysts to catalyze FAL production from biomass. Under the catalysis of acid, the hemicellulose in biomass depolymerizes to xylose, and then the xylose is dehydrated

and cyclized to form furfural [15]. However, the traditional homogeneous catalysts often have the defects of high cost, strong corrosion, difficult separation and recovery and environmental pollution [16]. Compared with homogeneous catalysts, heterogeneous catalysts have been gradually used in practical production in recent years due to their good catalytic performance, easy recovery, excellent stability and environmental friendliness. The commonly used heterogeneous catalysts are modified zeolite [17], montmorillonite [18], metal oxide [19] and sulfonated biochar catalysts [20], which play a great role in promoting the synthesis of FAL. In the H<sub>2</sub>O/CO<sub>2</sub> system, ion exchange resin Amberlyst 70 produced FAL from xylose at 150 °C for 16 h in a yield of 52% [21]. The SO<sub>4</sub><sup>2-</sup>/Sn-TRP was used to produce FAL from corn stover in toluene/H<sub>2</sub>O system at 190 °C for 180 min in a yield of 78% [22].

In addition to the catalyst required for the reaction, the choice of reaction medium is also very important for the synthesis and biotransformation of FAL [23]. The stability and solubility of FAL can be improved by suitable reaction medium, and FOL can be produced more effectively. The solubility of FAL in a single aqueous phase system is low, and it will further degrade in water for a long time [24], resulting in a decrease of FAL yield. Ionic liquids (ILs) and deep eutectic solvents (DESs) have been prepared as reaction media and solvents for bio-catalysis. DES is an unconventional non-aqueous solvent obtained by the complex of hydrogen-bond-acceptor (such as choline chloride) and hydrogen-bond-donor (such as transition metals, organic acids or polyols) at 100 °C [25]. DES is not only similar to IL, but also has unique properties such as non-toxicity, simple preparation, high reaction selectivity and good biocompatibility, so it is greatly favored by researchers [26]. As an environmentally friendly solvent, DES can dissolve lignocellulosic biomass and FAL, and reduce the side reactions during the preparation of FAL, thus improving the FAL productivity. Therefore, DES has potential applications in the conversion of biomass into furan-based chemicals [27].

In this study, in the eco-friendly DES ChCl:MA-water system, a chemical catalyst (biomass based solid acid Sn-CFMS) and a bio-catalyst (recombinant *Escherichia coli* CCZU-KF cell) were combined to efficiently catalyze the

formation of FOL from biomass via chemoenzymatic approach. Currently, most shells, which make up 75 percent of the total weight of shellfish, go directly to landfills or into seawater [28]. Mussel shell can be used as a carrier to prepare solid acid catalysts. Sn-CFMS, a new type of Sn-based multiphase catalyst, was prepared from mussel shells after pretreatment (acid-base and ultrasonic pretreatment). FAL was catalyzed by solid acid catalyst Sn-CFMS in an optimized ChCl:MA-water system using corn cob (CB) as raw materials. Using recombinant *Escherichia coli* CCZU-KF containing reductase as biocatalyst, the effects of various reaction parameters (such as ChCl:MA, loading, glucose dosage, bioreaction temperature, medium pH, chloride salts and substrate FAL loading, etc.) on the biological reduction of FAL to FOL were investigated. Finally, under optimal parameter conditions, corncob (CB) was converted to FOL through the tandem reaction with solid catalyst Sn-CFMS and recombinant *Escherichia coli* CCZU-KF whole-cell biocatalyst. The established hybrid catalytic strategy provides a novel idea for the efficient conversion of CB into valuable furan chemical FOL, thus realizing the value upgrade of cheap biomass resources.

## 2. Materials and Methods

### 2.1. Chemicals and materials.

Corn cob (CB) (40-60 mesh) was obtained from Lianyungang city (Jiangsu province, China). Mussel shell (MS) was purchased from Bozhou City (Anhui Province, China). SnCl<sub>4</sub>·5H<sub>2</sub>O, ethanol, malic acid (MA) and choline chloride (ChCl) were purchased from Energy Chemical Co. (Anhui Province, China). Sodium hydroxide (NaOH), ammonia water, NADH, FAL, β-D-1-thiopyranogalactoside (IPTG), 5-hydroxymethylfurfural (5-HMF), levulinic acid (LA), formic acid (FA), glucose, calcium chloride (CaCl<sub>2</sub>) and other chemicals were purchased from China National Pharmaceutical Group Co.

### 2.2. Preparation of chemocatalyst Sn-CFMS using MS as carrier

The MS was washed with water to remove impurities, and crushed after drying. The MS powder (40–60 mesh) was treated with 4% HCl at a ratio of 1:20 (w/v), stirred continuously, and allowed to stand for 24 h to remove minerals (mainly CaCO<sub>3</sub>). The acid-treated MS was pretreated by ultrasonic (200 W) for 1 h at the ambient condition, and then solid was filtered and washed several times with distilled water (to neutral pH) and dried under oven at 80 °C. The proteins in treating MS powder were removed by 4% NaOH solution at a ratio of 1:20 (w/v) in an oil bath at 100 °C for 3 h. The experiment was followed by ultrasound treatment for 1 h, the alkali-soaked MS powder was filtered and washed to neutral with distilled water. Fresh chitin was obtained by drying in an oven at 80 °C, namely CFMS. SnCl<sub>4</sub>·5H<sub>2</sub>O (194 g), ethanol (800 mL) and CFMS (420 g) were mixed, and the generated liquor was regulated to 6.0 with ammonia water. The generated mixture was placed in an oven (70 °C) for 48 h and then dried at 90 °C for 24 h. The formed CFMS solid was sulfonated with H<sub>2</sub>SO<sub>4</sub> (4 M) at a ratio of 1:20 (w/v) for 3 h, and then filtered and washed several times with distilled water (to neutral pH). The solid was oven-dried under the temperature of 80 °C for 12 h followed by calcination under a muffle furnace (550 °C) for 240 min. The sulfonated catalyst Sn-CFMS was collected and

used to catalyze the transformation of CB into FAL.

### 2.3. Synthesis of FAL from biomass catalyzed by Sn-CFMS in DES system

In a 250 mL flask, ChCl (as HBA) was mixed with MA (as HBD) in a molar ratio of 1:1 under the agitation (200 rpm) for 2 h at 80 °C until a homogeneous DES was produced.

FAL was prepared in a 10 L stainless steel high-pressure reactor (Jingkou Dandu Global Electromechanical Components Factory, Zhenjiang City, China) with a rotor placed for magnetic stirring. FAL was prepared from CB in DES (ChCl:MA)-water system with solid acid Sn-CFMS as catalyst. The solid acid catalyst Sn-CFMS (2.4 wt%) and 100 g of dried biomasses mentioned above were added to a 125 mL of DES, with 50 mM CaCl<sub>2</sub>. Transformation at 180 °C for 10 min, the FAL in the reaction product was analyzed by High Performance Liquid Chromatography (HPLC).

### 2.4. Culture of *E. coli* strains

*E. coli* CCZU-KF contains reductase, which can be used to bioconvert furfural to furfuryl alcohol [29]. CCZU-KF cells were inoculated into Luria-Bertani medium containing kanamycin (100 mg/L) and incubated in a constant temperature shaker (180 rpm, 35 °C) for 10 hours. Activated CCZU-KF cells were transferred to Terrific medium (TB) containing kanamycin (100 mg/L) and incubated in a constant temperature shaker (180 rpm, 35 °C) until OD<sub>600</sub> grew to 0.60. Isopropyl β-D-1-thiopyranolactose (IPTG) was then placed in a constant temperature shaker (180 rpm, 25 °C) for 12-16 hours. Finally, cells were harvested by washing with a phosphate buffered solution (pH 7.0) and centrifugation (8000 rpm, 5 min) and stored in a 4 °C refrigerator.

### 2.5. Biological transformation of FAL into FOL

In order to study the optimal biological reaction conditions for the synthesis of FOL in ChCl:MA (0-50 vol%)-water, the pH of the solution was adjusted from 6-8, and the CCZU-KF cells (0.025-0.15 g/mL), cosubstrate (glucose/FAL = 0-2 mol/mol), NAD<sup>+</sup> (NAD<sup>+</sup>/FAL = 0-3 μM/ mM) and chlorate salts (eg. BaCl<sub>2</sub>, ZnCl<sub>2</sub>, LiCl, MgCl<sub>2</sub>, CuCl<sub>2</sub>, CoCl<sub>2</sub>, KCl, CaCl<sub>2</sub>, FeCl<sub>3</sub>, AlCl<sub>3</sub>, NH<sub>4</sub>Cl, CrCl<sub>3</sub>, 1 mM) were supplemented for the transformation of FAL (50 mM) in a shaker (25 to 45 °C, 180 rpm) and incubated for 0-72 h. FAL and FOL were analyzed by HPLC.

To detect FAL tolerance to *E. coli* CCZU-KF cells, a biological reduction process was performed with commercial FAL at 25-400 mM. In ChCl:MA(10 vol%)-water system, CCZU-KF cell (0.1 g/mL), glucose (1.5 mol glucose /mol FAL) NAD<sup>+</sup> (1 μM NAD<sup>+</sup>/mM FAL), BaCl<sub>2</sub> (1 mM) and FAL (25-400 mM) were incubated on a shaker (200 rpm, 35 °C) for 0-72 h. The samples were extracted and tested by HPLC.

### 2.6. Analytical methods

FAL and FOL were monitored by HPLC with Athena C18-WP column (4.6 mm × 250 mm, 5 μm) [30].

The yield of FAL produced from CB was defined as below equation:

$$\text{Yield of FAL} = \frac{\text{FAL produced (g)} \times 0.88}{\text{CB (g)} \times 0.341} \times \frac{150}{96} \times 100\%$$

The yield of FOL was defined as follows:

$$\text{Yield of FOL} = \frac{\text{FOL produced (mM)}}{\text{Initial FAL (mM)}} \times 100\%$$

### 3. Results and Discussion

#### 3.1. Effect of solvent system on bio-catalyzed FAL to FOL

DES is a kind of non-toxic, recyclable, inexpensive solvent and reaction medium [31]. Because of its good thermal stability and biodegradability, it is often used for FAL formation and biocatalytic conversion [32]. Because FAL is slightly soluble in water and easily degraded, DES is usually added to the biological reduction reaction to improve the solubility of FAL and inhibit the formation of byproducts, thus increasing the conversion rate of FAL [33]. In the previous studies, the FAL catalyzed by ChCl:MA has the highest yield, and the viscosity is smaller than other DES, which is conducive to proton transfer in the biological reduction reaction. Therefore, ChCl:MA-water is selected as the appropriate reaction medium. In this study, FAL was reduced to FOL by recombinant *E. coli* CCZU-KF in the system of ChCl:MA and water, and the effect of ChCl:MA addition on biocatalytic reduction was investigated.

As shown in Figure 1, when the volume fraction of ChCl:MA increased from 0 to 10%, the reducing activity increased significantly. When the volume fraction was 10%, the maximum reducing activity reached 145.9%, after which the reducing activity gradually decreased. The reason for the decrease in the reduction activity might be that the excess ChCl:MA increased the viscosity of the reaction system, resulting in poor proton mobility, which slowed down the FAL conversion. The results showed that the reaction was inhibited when the dosage of ChCl:MA exceeded the tolerance range of *E. coli* CCZU-KF. Therefore, the optimum amount of ChCl:MA was 10 vol%.

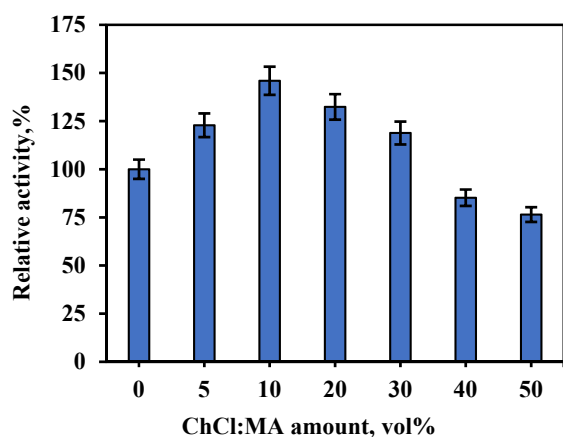


Figure 1. The dose of DES(ChCl:MA) on relative activity.

#### 3.2. Effect of cosubstrate on bio-catalyzed FAL to FOL

Through the cosubstrate (glucose, formic acid, etc.) to achieve repeated generation of coenzymes, thus reducing the cost of biological reduction reaction [34]. Because glucose is cheap, readily available and non-toxic, it is often used as a hydrogen donor in biological reduction reactions. Different glucose concentration will affect the recycling of coenzyme and thus change the time course of biological reduction reaction. In this study, 100 mM commercial FAL was used as substrate, and different concentrations of glucose (the molar ratio of glucose to FAL, 0:1-2:1) were added to investigate the

effect of glucose dosage on the bio-reduction activity in the ChCl:MA-water system. In Fig. 2, the FAL-reducing activity of CCZU-KF cell increased as the molar fraction of glucose to FAL increased from 0:1 to 1.5:1. But when the molar fraction of glucose to FAL was greater than 1.5:1, the reducing activity was weakened gradually. This suggested that excessive glucose concentrations might have damaged the cells of CCZU-KF. Therefore, the optimal dosage of glucose in the ChCl:MA-water system was the molar ratio of glucose to FAL of 1.5:1.

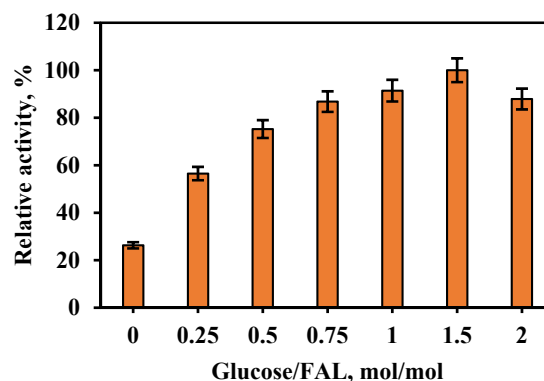


Figure 2. Effect of glucose-to-FAL molar ratio on relative activity

#### 3.3. Effect of reaction temperature and pH on bio-catalyzed FAL to FOL

Different reaction temperature and pH have great influence on the rate of biological reduction reaction. Because *Escherichia coli* can be genetically engineered, it is often used as a catalyst for biological reduction reactions. Appropriate reaction temperature and pH will accelerate the binding efficiency of reductase and substrate in recombinant *Escherichia coli*, thus improving the activity of biological reduction reaction [35]. In order to further increase FOL yield, the effects of different reaction temperatures on biological reduction reactivity were investigated. Fig. 3 showed that the obtained reductase activity gradually increased from 25 °C to 35 °C, while the relative activity gradually dropped after 35 °C. This indicated that the activity and stability of reductase in *E. coli* CCZU-KF were deteriorated at high reaction temperature, thus limiting the formation of FOL. In addition, the effects of different pH (6-8) of the reaction system on the bioreduction activity at 35 °C were investigated. Under the condition of 35 °C, Fig. 4 showed that the reactivity continued to increase when the pH of the reaction system increases from 6 to 7. As the pH of the reaction system was 7, the reactivity reached the maximum value. When the pH of the reaction system exceeds 7, the reactivity gradually decreased. It might be ascribed to that the non-neutral environment affected the structure of reductase, weakened the stability of the enzyme, and thus reduced the CCZU-KF reaction activity of recombinant *E. coli* containing reductase. The results showed that the enzyme activity was the best at neutral pH, and the enzyme stability and selectivity were the highest at this time. Therefore, the optimal reaction temperature and pH were 35 °C and 7.0, respectively.

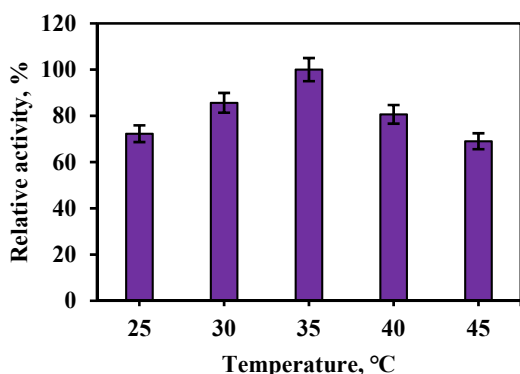


Figure 3. Effect of temperature on relative activity

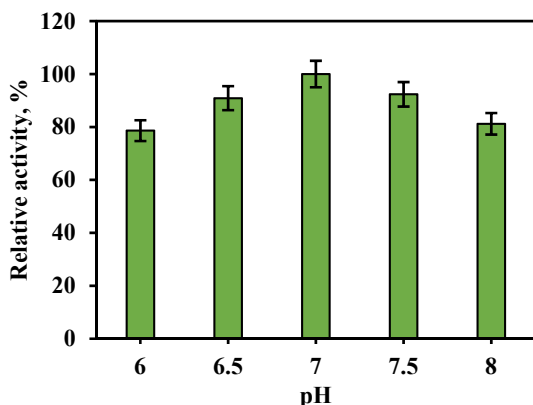


Figure 4. Effect of pH on relative activity

### 3.4. Effect of Cell concentration on bio-catalyzed FAL to FOL

Selecting the appropriate catalyst type and dosage in the catalytic reaction can promote the reaction and increase the production of FOL [36]. In this study, *E. coli* CCZU-KF was used for catalytic hydrogenation of FAL, and the effects of different cell additions (0.025-0.15 g/mL) on bio-reduction activity were investigated. As shown in Fig. 5, when the addition of CCZU-KF cells increased from 0.025 g/mL to 0.1 g/mL, the reduction activity was apparently improved. When the dosage of cells was 0.1 g/mL, the reduction activity reached the highest level, and then the amount of CCZU-KF cells was increased, but the reduction activity was weakened. This might be ascribed to that the high concentration of CCZU-KF cells would increase the viscosity of the bioreaction system and weaken the transfer ability of protons, thus leading to the decrease of biological reduction reaction activity. Hence, the optimal addition amount of CCZU-KF cells was 0.1 g/mL (wet weight).

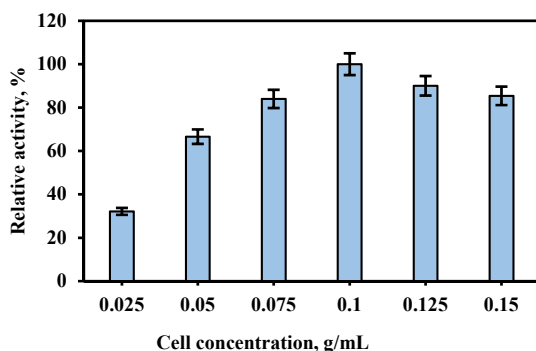


Figure 5. Cell concentration on relative activity

### 3.5. Effect of NAD<sup>+</sup> on bio-catalyzed FAL to FOL

In the biological reduction reaction system, oxidizing coenzyme NAD<sup>+</sup> participates in the enzymatic reaction of reductase, and generates NADH through hydrogen transfer of glucose as hydrogen donor, so as to realize the regeneration cycle of coenzyme factors. In this study, different concentrations of NAD<sup>+</sup> (0-3 μM NAD<sup>+</sup>/mM FAL) were added to the catalytic reaction system to reduce FAL to FOL, and the effect of the amount of NAD<sup>+</sup> on the activity of biological reduction was investigated. As displayed in Fig. 6, when the ratio of NAD<sup>+</sup> and FAL increases from 0:1 to 1:1 (μM:mM), the biocatalytic activity was gradually raised. As the ratio of NAD<sup>+</sup> and FAL increased from 0:1 to 1:1 (μM:mM), the biocatalytic activity was the strongest. When the ratio of NAD<sup>+</sup>-to-FAL was greater than 1:1 (μM:mM), the biocatalytic activity gradually dropped. The results showed that the addition of NAD<sup>+</sup> exceeded the tolerance range of CCZU-KF cells, and would inhibit the activity of reductase. Therefore, the optimum dosage of NAD<sup>+</sup> was 1 μM NAD<sup>+</sup>/mM FAL.

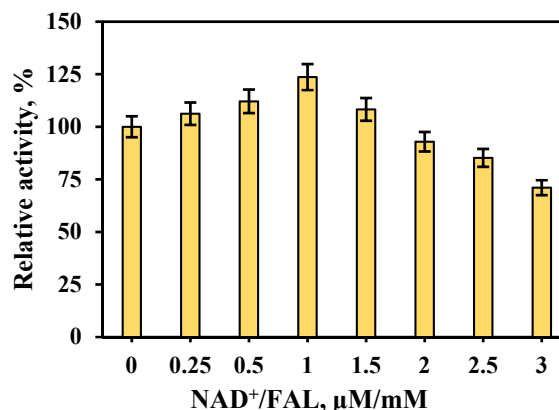


Figure 6. Effect of NAD<sup>+</sup> on relative activity

### 3.6. Effect of chloride salts on bio-catalyzed FAL to FOL

According to previous reports, the type and amount of chloride salts in biocatalytic system are important factors affecting the activity of enzyme reactions [37]. In this study, various chloride salts (BaCl<sub>2</sub>, ZnCl<sub>2</sub>, LiCl, MgCl<sub>2</sub>, CuCl<sub>2</sub>, CoCl<sub>2</sub>, KCl, CaCl<sub>2</sub>, FeCl<sub>3</sub>, AlCl<sub>3</sub>, NH<sub>4</sub>Cl, and CrCl<sub>3</sub>; 1 mM) were supplemented to the ChCl:MA-water system to explore the effects of chloride salt types on the biological reduction activity. As displayed in Fig. 7, compared with the blank control, BaCl<sub>2</sub>, ZnCl<sub>2</sub> and LiCl significantly improved the reducing activity. MgCl<sub>2</sub> and CuCl<sub>2</sub> had no significant promoting or inhibiting effect on the reducing activity. While CaCl<sub>2</sub>, FeCl<sub>3</sub>, AlCl<sub>3</sub>, NH<sub>4</sub>Cl and CrCl<sub>3</sub> had significant inhibition effect on the reducing activity. Among them, BaCl<sub>2</sub> had the highest promoting effect on the FAL-reducing activity, with the relative activity reaching 125%. This might be attributed to the high selectivity of BaCl<sub>2</sub> for compounds containing alcohols in DES-water system. In addition, in order to explore the effect of BaCl<sub>2</sub> concentration on reduction activity, different concentrations of BaCl<sub>2</sub> (0-3 mM) were tested. As illustrated in Fig. 8, when the concentration of BaCl<sub>2</sub> increased from 0 mM to 1 mM, the reduction activity gradually was raised. While the concentration of BaCl<sub>2</sub> was greater than 1 mM, the reduction activity was declined

gradually. The results showed that excessive  $\text{BaCl}_2$  could inhibit the reductase in CCZU-KF cells. It could be observed that the optimal chloride salts and their concentrations were  $\text{BaCl}_2$  and 1 mM, respectively.

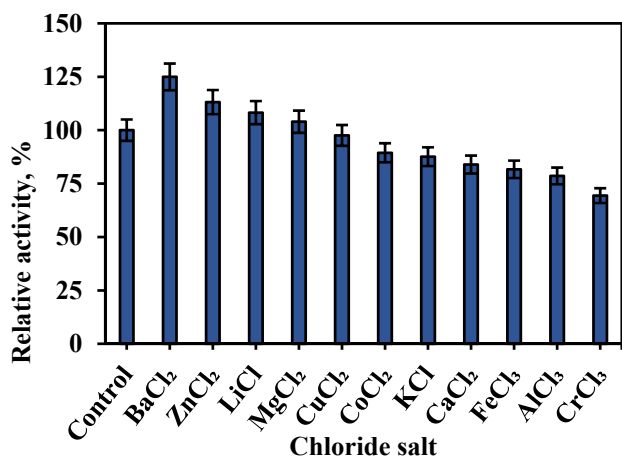


Figure 7. Effect of chloride salts on relative activity

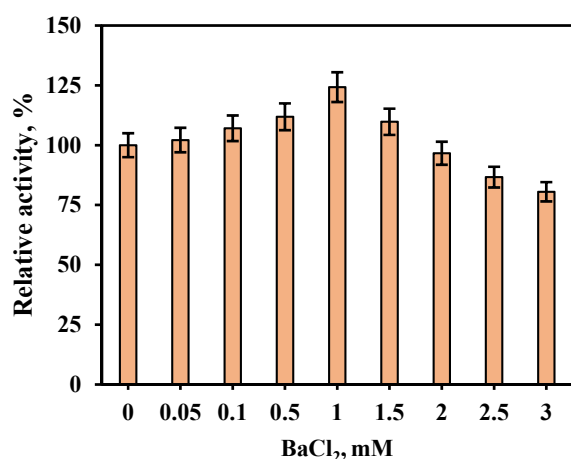


Figure 8. Effect of  $\text{BaCl}_2$  dose on relative activity

### 3.7. Effect of concentration of FAL on biocatalyzed FAL to FOL

In biocatalytic system, the reaction time required for FAL reduction to FOL depends on the concentration of FAL substrate [38]. This is because recombinant *Escherichia coli* CCZU-KF is tolerant to the substrate FAL. When the concentration of FAL exceeded the tolerance range of CCZU-KF cell, the enzyme activity decreased, thus reducing the FOL production and generation rate. In order to investigate the concentration tolerance range of CCZU-KF cell for FAL substrate, the conversion process of FAL into FOL at different concentrations (25-400 mM) was investigated under optimal conditions. As displayed in Fig. 9, the conversion rate of FAL within 10 h was relatively fast. After 72 h of reaction, 200 mM FAL could be fully converted. 300 mM FAL was could be also efficiently converted into FOL, with a conversion rate of 91%. The bioconversion of 400 mM FAL was incomplete, with a conversion rate of only 30%. Xue et al. converted xylose-derived FAL to FOL by recombinant *E. coli* CCZU-K14 [39], and investigated the relationship between FAL concentration of different substrates and FAL conversion. It was found that FAL of 200 mM could be completely converted to FAL within 24 h, while FAL of 300 mM could not be completely converted, and the resulting FAL conversion rate was only

67%. In contrast, in this study, the conversion efficiency of 300 mM FAL catalyzed by recombinant *Escherichia coli* was higher in the  $\text{ChCl}:\text{MA}$ -water system, thus confirming the excellent biocatalytic activity of CCZU-KF cells. Therefore, the concentration tolerance range of CCZU-KF cells to the substrate FAL in the  $\text{ChCl}:\text{MA}$ -water system was not more than 300 mM.

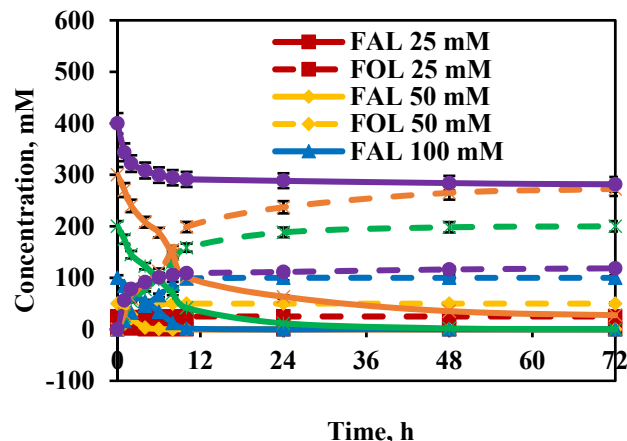


Figure 9. Biological conversion of FAL by whole cells

### 3.8. The bioproduction of FOL from corn-cob-derived FAL with CCZU-KF

In this study, the biological reduction reaction of FAL solution derived from CB was carried out by recombinant *E. coli* CCZU-KF in  $\text{ChCl}:\text{MA}$ -water system. When the pH of FAL solution derived from CB was adjusted to 7.0, the FAL generated by CB was 105.9 mM. At 35 °C, CCZU-KF cell (0.1 g/mL), coenzyme  $\text{NAD}^+$  (1  $\mu\text{M}$   $\text{NAD}^+/\text{mM}$  FAL), co-substrate glucose (1.5 mol glucose/mol FAL) and  $\text{Ba}^{2+}$  (1 mM) were added to FAL solution. The biological reduction reaction of FAL was conducted through the catalysis with recombinant *E. coli* CCZU-KF. As displayed in Fig. 10, the transformation of FOL occurred rapidly within 2 hours of the reaction. In the subsequent reaction, the rate of FOL formation gradually slowed down, but the transformation of FOL could reach 97.7% in 72 h. The FOL yield was higher above 97.7% after 72 h of reaction. The FAL derived from CB could be completely and efficiently converted to FOL, which verified the feasibility of the strategy of chemical-biological catalytic synthesis of FOL from CB.

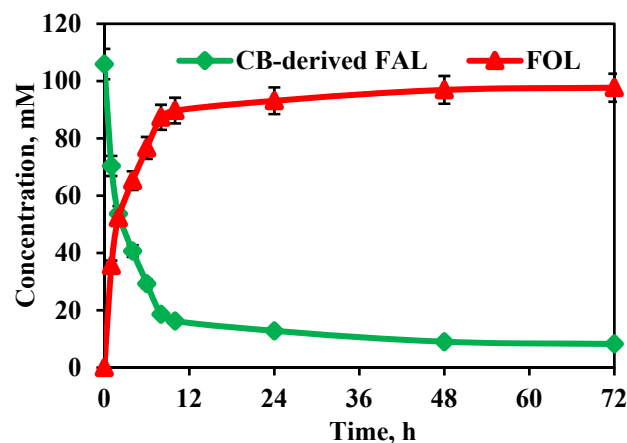


Figure 10. Conversion of CB-derived FAL to FOL via tandem catalysis

### 3.9. Mass balance from corn cob to FOL

Fig. 11 summarized the CB to FAL mass balance in the ChCl:MA-water system. 100 g CB was composed of 31.73 g glucan, 31.47 g xylan, and 12.20 g lignin. The preparation of FAL was generally carried out in a 10 L stainless steel reactor, in which 100 g CB, 32 g Sn-CFMS, 125 ml ChCl:MA and 7.4

g CaCl<sub>2</sub> were added, and then stirred at 170 °C for 15 min at 500 rpm to react evenly mixed. 100 g CB was catalyzed to 1.25 L FAL solution by Sn-CFMS. Then the pH of the obtained FAL derivative was adjusted to 7, and 142.67 g CCZU-KF was added according to the optimal reaction conditions. After 72 h, 14.0 g furfuryl alcohol was obtained at 35 °C when 0.11 g NAD and 16.53 g glucose were added.

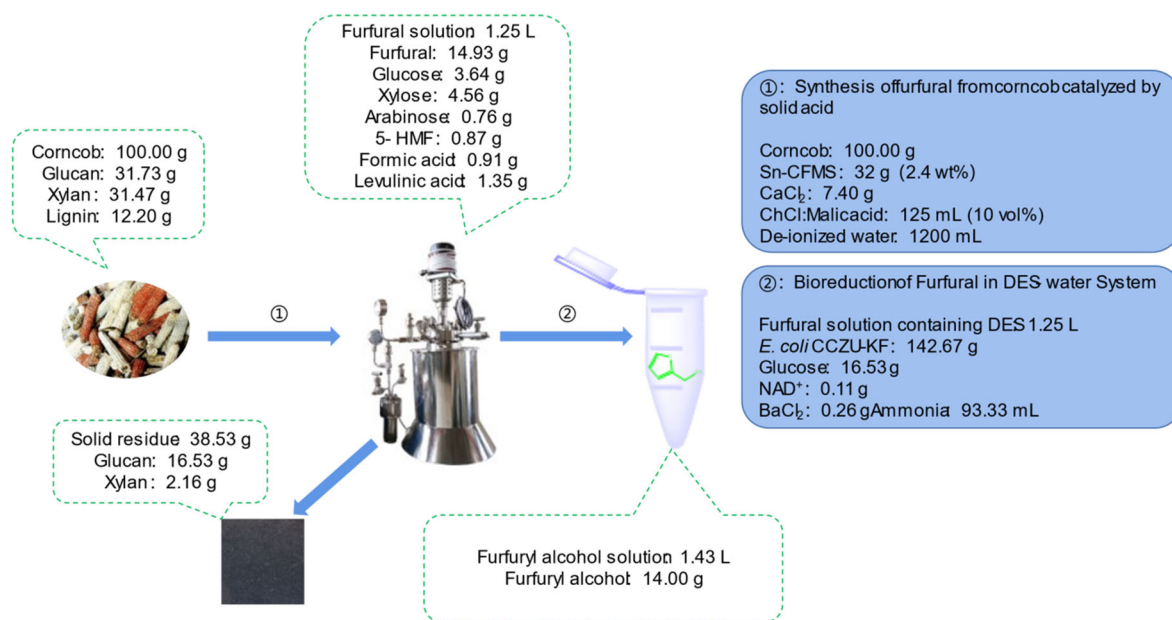


Figure 11. Mass balance from corn cob to FOL

## 4. Conclusion

In this study, CB was catalyzed to produce FAL, and then CCZU-KF was used to convert the CB-derived FAL into FOL. In the 10 vol% ChCl:MA system containing NAD<sup>+</sup> (1 mM NAD<sup>+</sup>/mM FAL), glucose (1.5 mol glucose/mol FAL), and BaCl<sub>2</sub> (1 mM), the whole-cell of CCZU-KF (0.1 g/mL) catalyzed the CB-derived FAL at 35 °C and pH 7, and the bioconversion of FAL to FOL resulted in a yield of 97.7% for 72 h. By combining chemo-catalysis and bio-catalysis, corncob was efficiently catalyzed to FOL, and its feasibility was verified in this work. This pathway provides a new idea for the green production of FOL from lignocellulose.

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