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Using deep eutectic solvents to improve the biocatalytic reduction of 2-hydroxyacetophenone to (*R*)-1-phenyl-1,2-ethanediol by *Kurthia gibsonii* SC0312

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ABSTRACT

The effects of five deep eutectic solvents (DESs) on the production of (*R*)-1-phenyl-1,2-ethanediol from 2-hydroxyacetophenone catalyzed by *Kurthia gibsonii* SC0312 were investigated in this study. Of these, choline chloride/1,4-butanediol (ChCl/Bd) showed excellent biocompatibility and suitably increased the cell membrane permeability while having a little impact on the structure of DNA. Indeed, ChCl/Bd at the concentration of 2 % increased the catalytic rate of the cells by 22 %. The other DESs did not stimulate the catalytic capacity of the cells, despite some increases in the cell membrane permeability. Additionally, the conformation of DNA was visibly changed when adding the other examined DESs except for choline chloride/triethylene glycol. The DESs modified the fatty acid composition of cellular membrane, decreased the relative amount of *iso*-C14:0 and increased the relative amount of normal C15:0. Meanwhile, the DESs were able to improve the relative ratio of normal fatty acids to branched fatty acids. Finally, a highly efficient reduction of 80 mM 2-hydroxyacetophenone by *K. gibsonii* SC0312 in the ChCl/Bd-containing system was established, affording (*R*)-1-phenyl-1,2-ethanediol in 80 % yield and optical purity > 99 % at 30 mg/mL wet cells. This work offers a promising approach for the preparation of (*R*)-1-phenyl-1,2-ethanediol from 2-hydroxyacetophenone using *K. gibsonii* SC0312.

1. Introduction

(*R*)-1-phenyl-1,2-ethanediol (PED) serves as a pivotal synthon for obtaining enantiopure medicines such as β-adrenergic blocking agents [1], which are suitable for treating cardiovascular disease and sympathetic nervous system disorder [2–4]. Up to date, some researchers have directed considerable attention towards biocatalytic approach for preparing enantiomeric PED owing to mild reaction conditions, high selectivity and environmental friendliness [5,6]. Enantiomerically pure (*R*)-PED is typically fabricated by asymmetric hydrolysis of epoxides, asymmetric resolution of racemic PED, or asymmetric reduction of prochiral ketones. Compared with enzymatic biocatalysis, whole cell biocatalytic reactions have characterized as extraordinarily superior features, including easy accessibility, strong resistance and cofactor regeneration [7,8]. Therefore, utilization of whole cell biocatalytic reaction for chiral PED production becomes potential and meaningful.

Deep eutectic solvents (DESs) generally encompass two or three structural components, which are usually sustainable, biodegradable or inexpensive, and form a eutectic mixture characterized by low toxicity, negligible volatility and biodegradation compared with conversional solvents, organic solvents and ionic liquids [9–12]. Since the beginning of the 21th century, DESs [12,13] have been applied as novel green solvents in the bioconversion either as the main reaction medium [14,15] or as a co-solvent [16–18]. Some studies have introduced DESs as co-solvents to promote reaction efficiency [19], as exemplified by the use of choline chloride/urea (ChCl/U) and choline chloride/glycerol (ChCl/Gly) [17] to increase the initial reaction rate or the biocatalytic stability [20], or even to change the stereoselectivity of biocatalyst [21]. A moderate amount of DESs can stimulate biocatalytic reaction efficiency chiefly through increasing the solubility of a substrate [22], or changing enzymatic structure [23]. In comparison to enzyme biocatalysts, the cell membrane of whole cell biocatalysts protects intracellular enzyme from potential harm and hinders the contact between intracellular enzyme and extracellular substance. The impact of none-aqueous solvents (organic solvents, ionic liquids, and DESs, etc.) on whole cell biocatalysts is therefore more complex than on the enzyme biocatalysts. Prior researches have reported the effect of ionic liquids or DESs on the cellular membrane and confirmed that ionic liquids could pass through cellular membrane and interact with intercellular enzymes [24–26]. Some studies also found that DESs as a co-

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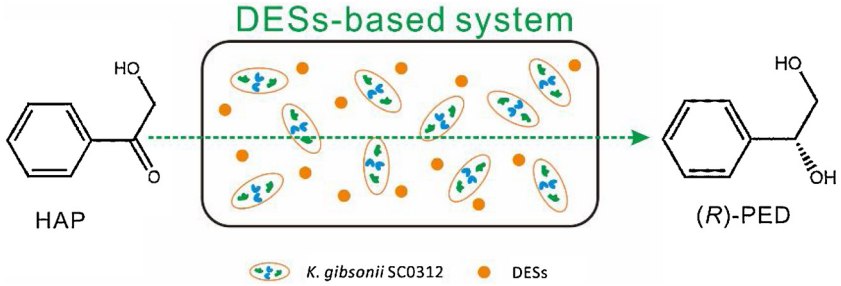

Scheme 1. The reduction of HAP to (*R*)-PED by K. gibsonii SC0312 in the system containing deep eutectic solvents.

solvent would benefit the expansion of cellular membrane to enhance catalytic efficiency [27–29]. To our knowledge, however, little information is available regarding the change of fatty acid components in microbial cell membrane within DESs-containing systems. DNA is an important carrier of genetic information and the maintenance of its structure is of great significance to life activities. Calf thymus DNA (ctDNA) has often employed in studying the interactions with molecules, such as farrerol [30] and isoxazolcurcumin [31]. There were reports indicating the structure of DNA would be changed by DESs [32,33]. However, few studies have addressed the relationship between the DNA structure and the catalytic properties of whole cells. Moreover, the DESs for different biocatalytic reactions may be diverse and little information concerning the application of DESs in the reduction of 2-hydroxyacetophenone (HAP) to (*R*)-PED has hitherto been published.

We previously isolated a strain of *Kurthia gibsonii* SC0312 (*K. gibsonii* SC0312) from soil, which exhibited high enantioselectivity in the synthesis of chiral PED [34]. In this study, our aims were to investigate the impact of DESs on whole-cell catalytic properties on the reduction of HAP to (*R*)-PED by *K. gibsonii* SC0312, and establish an approach for preparing chiral PED based on the system containing a DES (Scheme 1). Firstly, the effects of DESs on the catalytic activity of the cells, metabolic activity of the cells, cell membrane permeability, fatty acid compositions of cellular membrane and DNA were investigated. Subsequently, we selected a DES as the co-solvent and tested the variation in catalytic properties with various reaction factors for asymmetric reduction of HAP by *K. gibsonii* SC0312.

2. Materials and methods

2.1. Materials and strain

HAP (purity 97 %) was purchased from Acros Organics Technology Co., Ltd., China. (*S/R*)-PED (purity 97 %) was purchased from Guangzhou Qiyun Bioscience Co., Ltd., China. Choline chloride (purity 98 %) and 1,4-butanediol (purity 99 %) were purchased from Sinopharm Chemical Reagent Co., Ltd., China and Tianjin Kermel Chemical Reagent Co., Ltd., China, respectively. ctDNA was obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Other reagents were from commercial sources and were of analytical grade.

The DESs used in this study (Table 1) were prepared according to a previous work [35] and are all water-soluble. In a typical preparation, choline chloride and triethylene glycol were first mixed and stirred by a magnetic agitator at 80 °C for approximately 2 h until a stable homogeneous liquid formed. ChCl/Teg was subsequently dried in a vacuum oven at 70 °C for 48 h.

K. gibsonii SC0312 was stored in our laboratory and cultured in Luria-Bertani (LB) broth. The strain was first cultured at 37 °C and 180 rpm in LB broth overnight, and then 0.1 % of seed liquid was injected to a fresh LB broth to reproduce for 12–16 h. The cells were collected by centrifugation (6000 × *g*, 5 min) and washed twice with phosphate buffer (PB, 100 mM, pH 6.5) prior to their deployment for catalytic reaction.

2.2. General procedure for biocatalytic reduction of HAP

In a typical experiment, PB (100 mM, pH 7.5, 4 mL) containing 20 mM HAP, 30 mM glycerine, 30 mg/mL wet cells and a DES (4 %, v/v) was incubated at 35 °C and 180 rpm. Aliquots were withdrawn at regular intervals to monitor the initial reaction rate, enantiomeric excess (*ee*) and yield of (*R*)-PED. The initial reaction rate was based on the generated amount of PED after catalytic reaction for 30 min. The product yield was defined as the ratio of the generated amount of (*R*)-PED to the theoretical amount. The *ee* of (*R*)-PED was calculated based on the following equation.

$$ee = \frac{C_R - C_S}{C_R + C_S} \times 100\%$$

where C_S and C_R were the concentrations of (*S*)-PED and (*R*)-PED, respectively.

2.3. Cell metabolic activity in the system containing DESs

Metabolic activity of the cells was assessed by determining cell growth in LB broth containing the DESs. In brief, the cells were firstly incubated at 37 °C and 180 rpm in LB broth for 10–12 h, and then a seed sample with an inoculation amount of 0.1 % (v/v) was added to LB broth containing a DES, and the mixture was cultured at 37 °C and 180 rpm for 6 h. The biomass was determined at 600 nm by UV–vis spectrophotometry. The results were expressed in terms of relative biomass, with the biomass in the DESs-free LB broth being defined as 100 %.

Table 1
DESs and their characteristics.

Entry	DESs	Abbreviation of DESs	Mole ratio (HA:HD)[a]	Viscosity (Pa·s)[b]
1	choline chloride/urea	ChCl/U	1:2	0.214
2	choline chloride/ethanediol	ChCl/Et	1:2	0.025
3	choline chloride/glycerol	ChCl/Gly	1:2	0.177
4	choline chloride/triethylene glycol	ChCl/Teg	1:4	0.044
5	choline chloride/1,4-butanediol	ChCl/Bd	1:4	0.047

[a] HA: hydrogen-bond acceptor; HD: hydrogen-bond donor.
[b] These values were referenced by one of our publications [32].

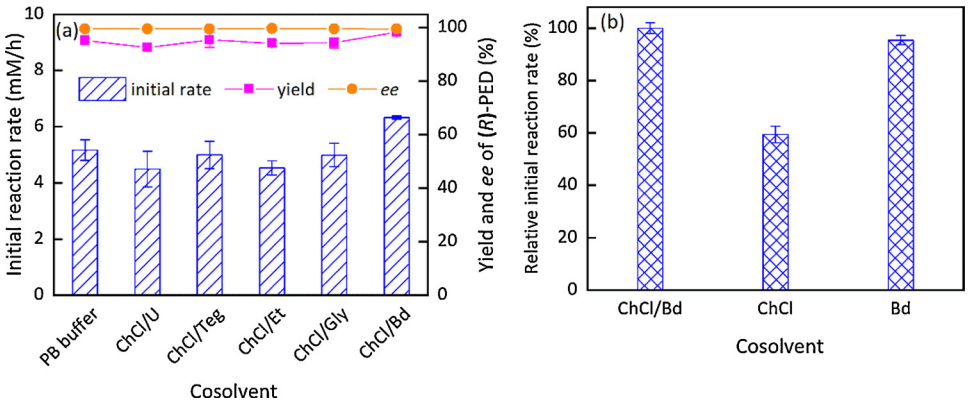

Fig. 1. The changes in the catalytic rate of *K. gibsonii* SC0312cell in the different reaction systems. Reaction conditions: 20 mM HAP, 30 mg/mL wet cell dosage, 35 °C and 180 rpm, 100 mM phosphate buffer, 2 % of DESs (v/v), reaction time 5 h.

2.4. Cell membrane permeability assay

The porous membrane of cell facilitates the leakage of intracellular nucleic acids and proteins. To assess cell membrane permeability, *K. gibsonii* SC0312 cells (OD_{600} = 0.2–0.3) were incubated in the saline solution containing a DES (2 %, v/v) at 35 °C and 180 rpm. The system without DESs was set as the control group. Samples were withdrawn at 0 h and 24 h to analyze the release of intracellular components by determining the cell-free supernatants at 260 nm and 280 nm. The final values are expressed in terms of the increase in absorbance from 0 h to 24 h.

The membrane integrity of the cells was also assayed by flow cytometry (FCM). Briefly, *K. gibsonii* SC0312 cells at 30 mg/mL were incubated for 12 h in the presence of the respective DES, and then diluted to 10^7 CFU/mL with saline solution. After incubating with 2.5 μg/mL propidium iodide (PI) at 4 °C in the dark for 15 min, membrane integrity of the strain suspension was measured using a Gallios flow cytometer (Beckman Coulter Inc., USA). The fluorescence emission was excited at 488 nm and recorded at 600–620 nm.

2.5. Assay of cell membrane fatty-acid composition

The effect of DESs on the cell membrane fatty acid composition was studied in PB (100 mM, pH 7.5) containing respective DESs (8 %, v/v) and 25 mg/mL wet cells. A DESs-free system was prepared as the control. Each system was incubated at 35 °C and 180 rpm for 12 h, and then the cells were collected to extract and analyze the fatty acid composition. Cell membrane fatty acids were extracted as described in a previous report [36] with some modifications. The collected cells were first mixed with 1.0 mL of saponification solution in a tube immersed in a boiling water bath for 30 min. The stock saponification solution consisted of distilled water (15.0 mL), methanol (15.0 mL), and NaOH (4.5 g). The mixture was then cooled to room temperature with cold water, whereupon 2.0 mL methylation solution was injected and the mixture was incubated at 80 °C for 10 min. The methylation solution consisted of methanol (27.5 mL) and 6.0 M aqueous HCl solvent (32.5 mL). Fatty acids in the above mixture were then extracted with 1.25 mL of extraction solution, which consisted of hexane and methyl *tert*-butyl ether (1:1, v/v). Finally, the extracted fatty acids were washed with 0.3 M aqueous NaOH solution (3 mL) and the organic phase was used to analyze the fatty acid composition.

The fatty acid methyl esters were determined by means of an Agilent 7890B gas chromatograph coupled to an Agilent 7000C GC/MS triple-quadrupole set-up operating in electron-ionization mode (Agilent, USA) and equipped with HP-5MS Ultra Inert capillary column (30 m × 0.25 mm ×0.25 μm; Agilent, USA). Ultra-pure helium was used as carrier gas at a constant flow rate of 1.0 mL/min. Finally, the fatty acids were identified by reference to the National Institute of Standards and Technology mass spectral library (2001).

2.6. Circular dichroism (CD) assay

ctDNA is often used to access the interaction with molecules. This study used ctDNA as a temple and accessed the effect of DESs on the structure of DNA by CD spectra assay. CD spectra of ctDNA in the absence and presence of the DESs were recorded using a Chirascan spectrometer (Applied Photophysics, UK). The concentrations of the DESs were set at 0 %, 2 %, 8 %, and 16 %, respectively, with a ctDNA concentration of 4 mg/mL. The observed CD spectra were corrected by the buffer signal (PB, 30 mM, pH 7.4).

2.7. Analytical methods

The yield and optical purity of (*R*)-PED produced from HAP catalyzed by *K. gibsonii* SC0312 were determined by means of reversed-phase high-performance liquid chromatography (HPLC) and normal-phase HPLC, respectively. A Waters HPLC set-up equipped with a UV detector and an XBridge C18 column (4.6 mm × 250 mm, 5 μm, Waters, USA) was applied to monitor changes in the concentrations of PED at 215 nm. The mobile phase consisted of water and acetonitrile (60:40, v/v) and was applied at a flow rate of 0.5 mL/min. The retention time of PED was at 6.5 min. For analysis of the product *ee* value, the sample was extracted and suitably diluted with ethyl acetate. The product *ee* was assayed by means of an Agilent HPLC 1100 equipped with a UV detector set at 215 nm using an OB-H analytical column (4.6 mm × 150 mm, 5 μm, Agilent, USA). The mobile phase comprised *n*-hexane/isopropanol (9:1, v/v) and was applied at a flow rate of 0.7 mL/min. The retention times of (*R*)-PED and (*S*)-PED were at 7.1 min and 8.7 min, respectively. The results were expressed as mean ± standard deviation and all experiments were performed at least in duplicate.

3. Results and discussion

3.1. Effects of various DESs on the reduction of HAP

Fig. 1 describes the effects of five DESs on the catalytic performance of *K. gibsonii* SC0312 for production of (*R*)-PED by asymmetric reduction of HAP. As depicted in Fig. 1a, 92.5–98.3 % of (*R*)-PED yields and more than 99 % of the product optical purities were obtained in various reaction systems. Amidst the reaction systems, ChCl/Bd-containing system showed the highest (*R*)-PED yield. As to the initial reaction rate, ChCl/Bd was capable of improving catalytic rate by 22 % compared with the control. However, the other four DESs (ChCl/U, ChCl/Et, ChCl/Teg, ChCl/Gly) had no such ability to enhance the catalytic rate. According to our previous study [37], the pH values of phosphate buffer

changed slightly when each examined DES above was added to the system at the concentration of 10 % (v/v). Thus, the catalytic activity of *K. gibsonii* SC0312 cells should not be obviously impacted due to trivial change of the pH in reaction system. Furthermore, the assay on the results of Table 1 and Fig. 1a showed the initial reaction rates had no clear correlations with the viscosities of the DESs. In terms of the stereoselectivity, Gotor-Fernández and Paul [38] reviewed many informed investigations that DESs enhanced the selectivity of various microbial cells and alcohol dehydrogenases for asymmetric reduction of prochiral ketones, and even led to inversion of stereoselectivity. Whereas some literatures described that the sterepreferences of whole cells [29] and alcohol dehydrogenases/carbonyl reductases [21] for asymmetric reduction of prochiral ketones were not visibly influenced by the DESs. The results shown in Table S1 indicate that the sterepreference of *K. gibsonii* SC0312 cells for the preparation of (*R*)-PED is not changed by the examined DESs during the biocatalytic processes. To better understand the delightful effects of ChCl/Bd, we compared the impacts of each component of ChCl/Bd on the initial rate of the reaction. The relative reaction rates of Bd-containing system and ChCl-containing system were 95.4 % and 59.5 % of that of the system containing ChCl/Bd (Fig. 1b), respectively, suggesting the stimulative impact on catalytic activity stemmed from ChCl/Bd. The results could provide a guidance for the application of ChCl/Bd in biocatalytic reactions.

3.2. Biocompatibility of DESs with K. gisbonii SC0312

The growth of the cells in LB broth containing DESs was used to characterize the change of cell metabolism. Fig. 2 demonstrates the influences of DESs on the growth of *K. gibsonii* SC0312 cells. When DESs concentration was at 2 %, the examined DESs except for ChCl/Bd contributed little to shift the biomass compared with the control. The biomass increased slightly following the addition of ChCl/Et, and decreased slightly after adding ChCl/U, ChCl/Teg and ChCl/Gly, respectively. However, a visibly higher biomass was achieved in the system containing 2 % ChCl/Bd in comparison to the control. The results manifest that ChCl/Bd and ChCl/Et have favorable biocompatibility to *K. gibsonii* SC0312. In terms of ChCl/Bd, the increase in the concentration would decrease the cell biomass, such as affording approximately 10 % biomass of the control at 20 % ChCl/Bd. The result indicates that a moderate amount of ChCl/Bd can accelerate the cells growth. Many reports have introduced none-aqueous media to enhance the catalytic efficiency of whole cells biocatalysts including organic solvents, ionic liquids and DESs [25,26,39]. In general, the enhancement in catalytic activity of whole cells was observed in the systems containing good biocompatible none-aqueous media. Our results from Figs. 1 and 2 are in parallel with their studies. These results suggest that the biocompatibility of the solvents is important for biocatalytic reaction of biocatalysts.

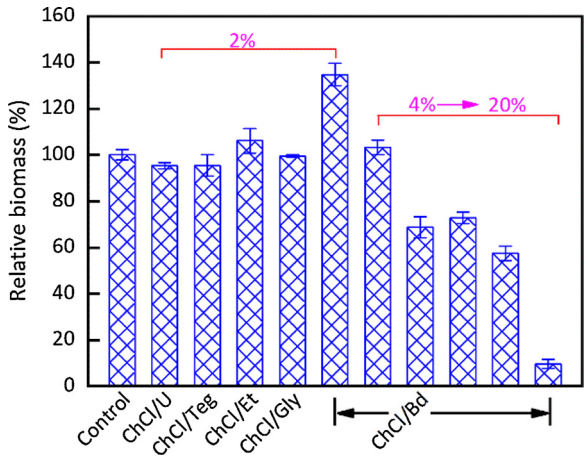

Fig. 2. The increase of cell biomass in the various DESs-containing system. Culture conditions: 37 °C, 6 h, 180 rpm, DESs concentration (ChCl/U, ChCl/Et, ChCl/Gly, ChCl/Teg) 2 %, and ChCl/Bd concentration: 2 %, 4 %, 8 %, 12 %, 16 %, 20 %.

3.3. The effects of DESs on the structure of DNA

The carrier of genetic information DNA is inextricably linked with the reproduction and function of cells. We here used ctDNA as a template and analyzed the impact of DESs thereon by CD spectroscopy. The impacts of the DESs on the structure of ctDNA were shown in Fig. 3. A positive band and a negative peak emerged at approximately 278 nm and 248 nm in the CD spectra of ctDNA, respectively, due to π-π base-stacking and helicity [33]. All examined DESs had no interference patterns at 220 − 320 nm, and B-conformation of ctDNA was not destructed by the DESs when the concentrations of the additives were from 0 % to 20 %. Some changes in the base-stacking and helicity of the DNA were observed after injection of the DESs. A high concentration of ChCl/U, such as 16 %, compressed the extended double-helix to a more compact form [40], presumably due to the presence of denaturant urea [32]. Adding ChCl/Et or ChCl/Gly rendered the positive band slightly red-shifted and less intense. The results suggest that structural changes of ctDNA ensue following the addition of ChCl/U, ChCl/Et, or ChCl/Gly. However, a slight impact on the ctDAN structure was observed when adding ChCl/Teg or ChCl/Bd at the examined concentrations. The change in the DNA structure may disturb gene expression as well as protein synthesis, showing difference in metabolic and catalytic activities. However, the correlation between structural change of DNA and catalytic properties remains unknown and requires in-depth study. Additionally, the change in the performances of some enzymes participating in duplication, transcription and translation of genes requires investigations.

3.4. Effects of DESs on the membrane permeability and fatty acid composition of the cells

The change in catalytic rate of the cells indicates that some interactions occur between *K. gibsonii* SC0312 and the DESs. The cell membrane is the first target of solvents [28], and the intracellular components (proteins and nucleic acids) release easily when it is damaged. Fig. 4a showed that higher absorbance values at 260 nm and 280 nm were observed in the presence of the DESs when compared with the control. The result suggests the DESs lead to more damaged *K. gibsonii* SC0312 cells within 12 h. In addition, the capacity of the DESs to expand the cell membrane was strengthened in the order of ChCl/Gly < ChCl/Teg < ChCl/Bd < ChCl/Et < ChCl/U. In terms of ChCl/Bd, the leakage of the intracellular material displayed an improvement with increased concentration, up to a maximum at 8 %, and then decreased.

The damaged and dead cells can be distinguished by flow cytometer using PI as fluorescein dye [41,42]. As shown in Fig. 4b, compared with the control, ChCl/Teg, ChCl/U and ChCl/Bd led to slight increase in the proportion of the damaged cells when the concentration of DESs was at 2 %. However, visible increases in the ratio of damaged cells were observed at the presence of the other two DESs, especially ChCl/Gly. The low ratio of damaged cells in the ChCl/Bd-containing system stood line with the bioconversion data, showing an enhancement in catalytic rate (Fig. 1). To better understand the effects of ChCl/Bd, the proportion of the damaged cells were measured with the increase of the DES. When the concentrations of ChCl/Bd varied from 2 % to 8 %, the ratio of damaged cells changed slightly. Thereafter, the proportion rapidly increased with the increment of ChCl/Bd level, up to the maximum at 16 %, then followed by a slight decrease. The results imply the membrane integrity is impacted by the level of ChCl/Bd, which is likely owing to the changed osmotic pressure in buffer.

Fig. 4 shows that the additives can enhance the membrane

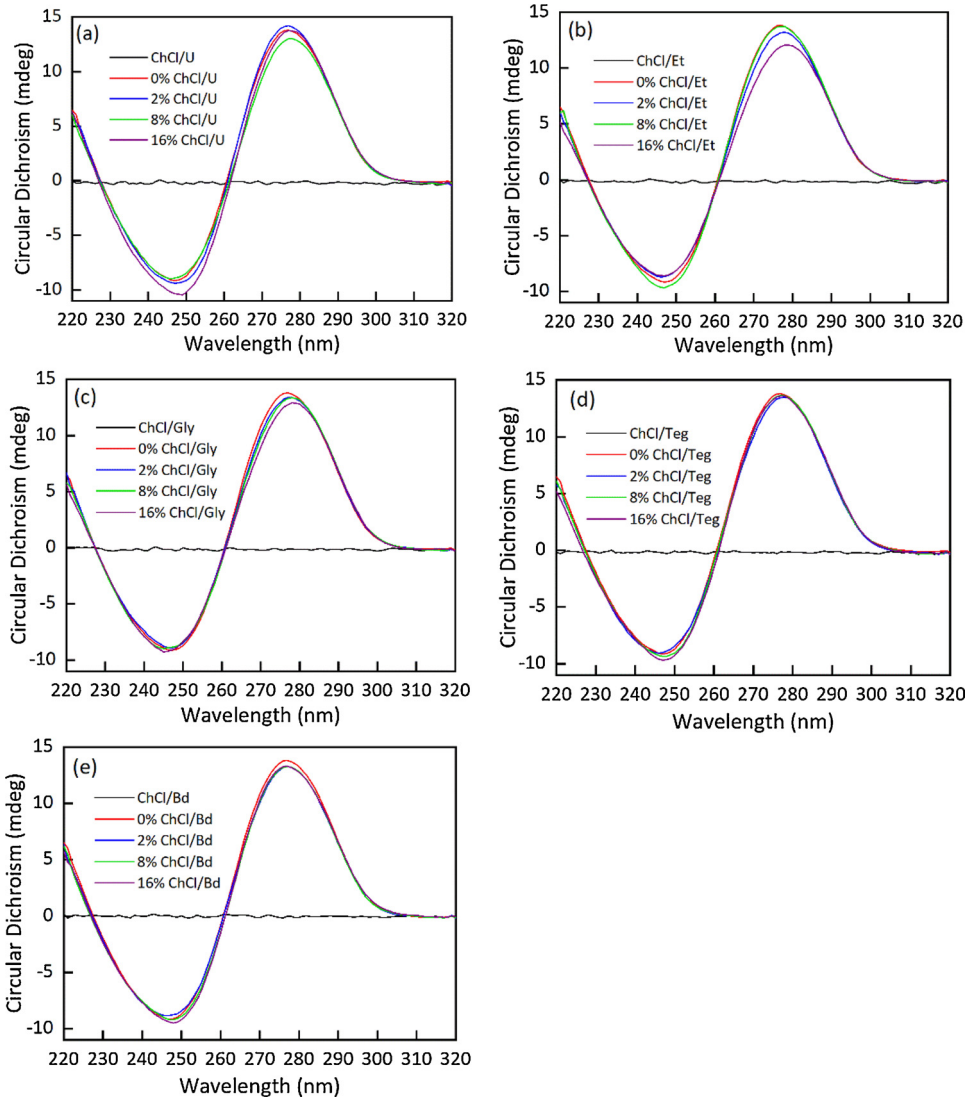

Fig. 3. CD spectra of ctDNA in the presence of DESs.

permeability of *K. gibsonii* SC0312, which is favorable not only in the contact between intracellular enzymes and the substrate but also in the easing of product inhibition so as to enhance catalytic efficiency. Ni et al. [43] has certified the catalytic activity of cells would be augmented by outer membrane mutation due to the enhancement of membrane permeability. A comparison of data between UV–vis spectrophotometry and flow cytometry shows some differences in the changing trend. For instance, ChCl/Gly led to a small leakage of intracellular materials, but resulted in a tremendous increase in the impaired degree of the cell membranes. We considered the higher viscosity of ChCl/Gly [35] to be an important factor for this marked discrepancy, which can restrict mass transfer. In the case of ChCl/Bd, the opposite change between UV–vis spectrophotometry and flow cytometry was seen with the increase of the additive, which again

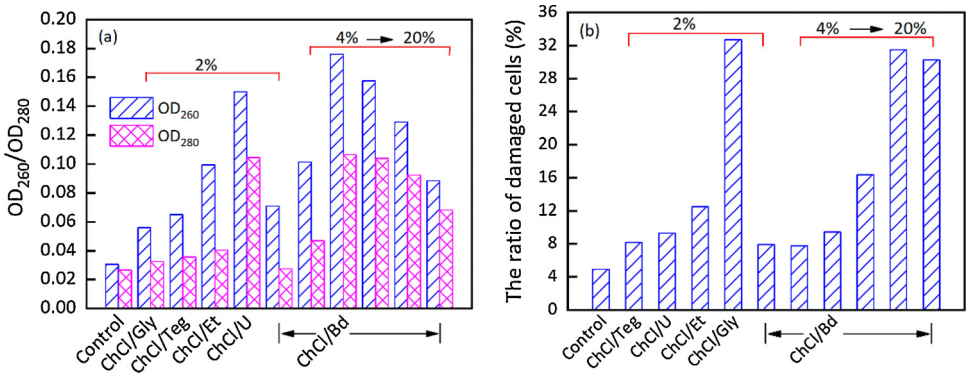

Fig. 4. The impacts of various DESs on the membrane integrity of *K. gibsonii* SC0312 by ultraviolet spectroscopy (a) and Flow cytometer (b). Incubation conditions: DESs concentration (ChCl/U, ChCl/Et, ChCl/Gly, ChCl/Teg) 2 %, and ChCl/Bd concentration: 2 %, 4 %, 8 %, 12 %, 16 %, 20 %.

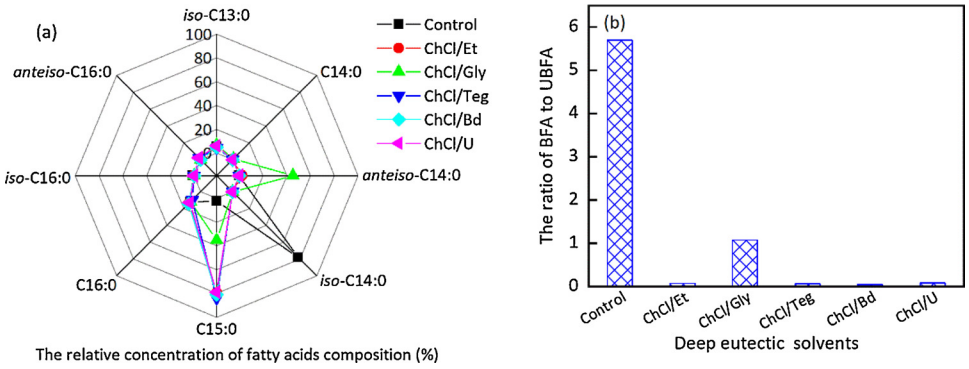

Fig. 5. The effects of DESs on the cell membrane fatty acid compositions (a)and the ratio of UBFA to BFA (b).

suggests that the viscosity increase may account for the discrepancy.

Membrane lipids are capable of changing in response to perturbations induced by external factors [44,45]. Relative levels of individual major fatty acids and the ratios of branched to unbranched fatty acids (BFA/UBFA) are shown in Fig. 5. The fatty acid *iso*-C14:0 accounted for 78.3 % of the total fatty acids of *K. gisbonii* C0312 suspended in the buffer. When the cells were exposed to the buffer systems containing the DESs, the relative level of *iso*-C14:0 would approach to 0 % as well as C15:0 increased strikingly, even reached to 78.8–83.8 % in the presence of the DESs except for ChCl/Gly (Fig. 5a). A significant improvement in the relative level of *ante*iso-C14:0 was also observed after injection of ChCl/Gly, reaching to 45.2 %. Furthermore, the additional DESs induced the content of branched-chain fatty acids to decrease, affording the ratio of branched-chain fatty acids to unbranched fatty acids from 5.7 % to 0.1–1.1 % (Fig. 5b). *K. gibsonii* SC0312 possesses a high level of branched-chain saturated fatty acids since it is a Gram-positive bacterium [46]. An increase in fatty acid chain length will change the membrane permeability [46] and improve the survival rate [47] in response to environmental stresses. In addition, the maintenance of an abundance of straight-chain fatty acids with respect to branched fatty acids in a cell membrane is viewed as an adaptive response to sublethal stresses, and straight-chain fatty acids in a cell membrane are beneficial for its stability [44,46]. The above results indicate that the cells will change fatty acid compositions in response to the addition of DESs.

3.5. Effects of main reaction conditions on the bioreduction of HAP in the ChCl/Bd-containing system

The impacts of key factors on the reduction of HAP by *K. gibsonii* SC0312 in the buffer system containing ChCl/Bd were studied (Fig. 6). Fig. 6a shows the influences of ChC/Bd concentration on the preparation of (*R*)-PED by *K. gibsonii* SC0312 cells. Highly optical purities of the product (> 99 %) were achieved as well as slight changes in the product yields were observed when the concentrations of ChCl/Bd varied from 2 % to 20 %. The initial reaction rates were slightly different when ChCl/Bd concentrations were no more than 16 %. As the concentration was at 20 %, a decline trend in the initial reaction rate was observed. The effects of reaction temperatures on the asymmetric reduction of HAP were shown in Fig. 6b. The reaction temperature had a substantial effect on the initial rate, but a slight impact on the yield of the product, except that a lower yield was obtained at 20 °C. In addition, excellent optical purities of the product were observed at the different reaction temperatures. Fig. 6c depicts the impacts of buffer pH on the production of (*R*)-PED. The optimal buffer pH for the catalytic rate was at 7.5 as well as the neutral or weakly alkaline environment were beneficial to achieve high reaction rates. Moreover, negligible variations in the yields and *ee* values of the product were observed within the pH range tested. Fig. 6d shows the effects of HAP concentrations on the reduction of HAP by *K. gibsnoii* SC0312 cells. Relatively high product yields were obtained at 30 − 40 mM HAP. Further increasing HAP concentration would lead to a certain amount of decline in the yield, for instance affording 80 % the product yield at 80 mM. The toxicity of HAP to the strain might give rise to the decrease of the product yield. [48]. Additionally, we studied the reduction of HAP for the fabrication of (*R*)-PED by *K. gibsonii* SC0312 in DESs-free reaction system (Fig. S1). A comparison of (*R*)-PED yield in reaction systems with ChCl/Bd and without the DES showed that the obviously higher product yields were achieved in the ChCl/Bd-containing system when HAP concentrations were at 70 mM and 80 mM, respectively. The results indicate ChCl/Bd can improve catalytic efficiency of *K. gibsonii* SC0312 for preparation of (*R*)-PED.

The increase in DESs concentration could be attributed to the improvement of hydrogen bond network, with a higher viscosity of buffer system. The increase in the viscosity of buffer system is able to increase the mass-transfer limitations, leading to a decline in catalytic activity. Furthermore, the additional amount of ChCl/Bd also impacted the osmotic pressure and polarity of reaction system, which were deemed as important factors to the catalytic properties of biocatalysts [49]. Additionally, the results from the cell biomass and the ratio of the damaged cells showed excessive ChCl/Bd could not benefit the whole cell catalytical reaction. The results in the reaction temperatures showed the desirable initial rates and yields of (*R*)-PED could be achieved at the temperature of 30 − 45 °C, suggesting that the cell biocatalyst may be employed in the reduction of HAP at room or higher temperature. Buffer pH is an important factor to the catalytic performances of the cells. The addition of DESs may make the cells more sensitive to environmental pH, due to the expansion of cell membrane. Additionally, the results indicate a neutral or alkalescent condition could be acceptable when considering appropriate range of buffer pH for the reduction of HAP by the functional enzymes from *K. gibsonii* SC0312. In comparison to previous works, using *K. gibsonii* SC0312 to prepare (*R*)-PED in ChCl/Bd-containing system presented some superiorities, such as high HAP concentration. *Saccharomyces cerevisiae* JUC15 produced (*R*)-PED by asymmetrically reducing of HAP at 8 g/L (58 mM) under optimum conditions, affording 92.4 % yield and > 99 % *ee* [50]. A cofactor self-sufficiently recombinant *Escherichia coli* BL21(DE3) by expression of glucose dehydrogenase and carbonyl reductase from *Candida parapsilosis* CCTCC M203011 was capable of asymmetrically reducing HAP to (*R*)-PED in 95.5 % yield at 6 g/L of substrate concentration (44 mM) [51]. In summary, an efficient reaction system containing ChCl/Bd for the production of (*R*)-PED from HAP catalyzed by *K. gibsonii* SC0312 has been constructed, giving the product in 80 % yield with > 99 % *ee* at a HAP concentration of 80 mM.

4. Conclusion

We have studied the effects of five DESs on the *K. gibsonii* SC0312 cells for the preparation of (*R*)-PED by asymmetric reduction of HAP. Of the additives, ChCl/Bd showed a pleasurable biocompatibility for the

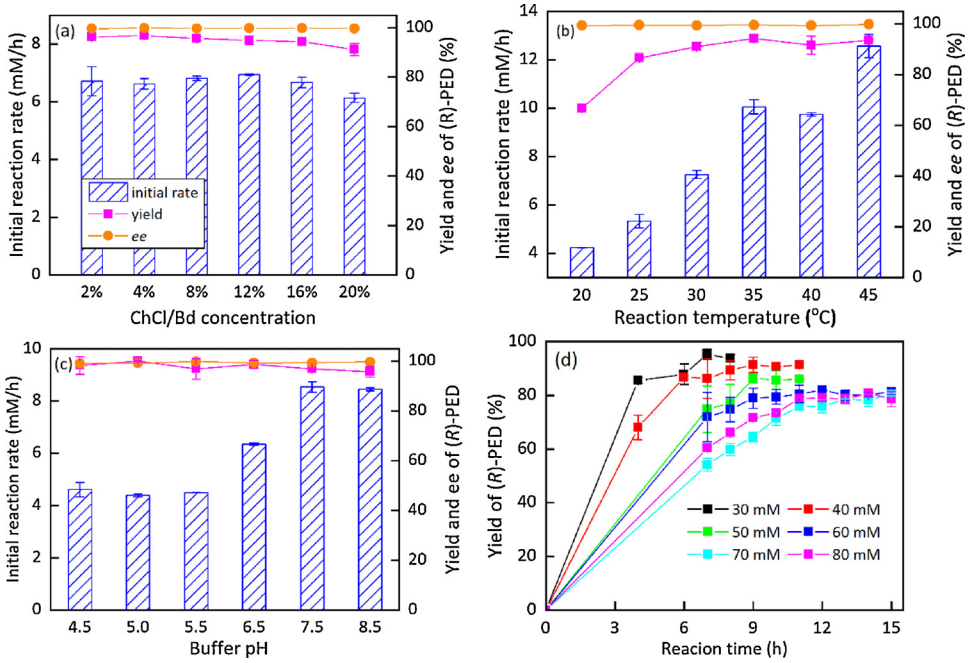

Fig. 6. The effects of several key factors on the catalytic properties of *K. gibsonii* SC0312. Reaction conditions: 4 % ChCl/Bd (b–d), 30 mg/mL wet cells, 180 rpm, 20 mM HAP (a–c), reaction time 5 h (a–c), temperature 35 °C (a, c, d).

cells, stimulating the cells growth. On basis of the results from UV–vis and FCM, all examined co-solvents were capable of enhancing the membrane permeability of *K. gibsonii* SC0312 cells. Further assay on the membrane fatty acids of the cells confirmed that the strain could change membrane components in response to the addition of the DESs. Finally, a highly efficient reduction of 80 mM HAP by *K. gibsonii* SC0312 in the ChCl/Bd-containing system was established, affording (*R*)-PED in 80 % yield and optical purity > 99 % at 30 mg/mL wet cells. Further work should focus on identifying the functional enzyme from *K. gibsonii* SC0312 and exploring the effects of DESs on the catalytic performance and structure of the related enzymes.

CRediT authorship contribution statement

Fei Peng: Data curation, Investigation, Writing - original draft, Writing - review & editing. **Qing-Sheng Chen:** Data curation, Investigation. **Fang-Zhou Li:** Writing - review & editing. **Xiao-Yang Ou:** Writing - review & editing. **Min-Hua Zong:** Methodology, Writing - review & editing. **Wen-Yong Lou:** Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mcat.2020.110773.

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