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Asymmetric Bioreduction of Ethyl (S)-4-chloro-3-hydroxy Butanoate Using Dried Baker's Yeast in Aqueous/Ionic Liquid Biphasic System

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A process of highly stereoselective reduction of ethyl 4-chloro-3-oxobutanoate to ethyl (S)-4-chloro-3-hydroxy butanoate with Baker's yeast was established. Compared with the aqueous/organic solvent biphasic system, the aqueous/ionic liquid biphasic system could eliminate high toxicity of organic solvent and make the convenience of product separation. Baker's yeast showed the best catalytic activity and enantioselectivity in the aqueous/[Bmim]PF_6 biphasic system among the biphasic systems. Under the optimum conditions: [Bmim]PF_6 180 g/L, dried baker's yeast 60 g/L, glucose 0.8 mol/L, ethyl 4-chloro-3-oxobutanoate 0.08 mol/L, temperature 30 °C, pH 7.0, 24 h and shaking speed 180 rpm, the yield and the e.e. value of (S)-4-chloro-3-hydroxy butanoate reached 92.6 % and 95.4 %, respectively.

Keywords: Baker's yeast, Ethyl 4-chloro-3-hydroxybutanoate, Aqueous-ionic liquid biphasic system.

INTRODUCTION

Optically active ethyl 4-chloro-3-hydroxy butanoate is one of key chiral building blocks applied to the synthesis of pharmaceuticals[1]. Particularly, (S)-ethyl-4-chloro-3-hydroxybutanoate [(S)-CHBE] reduced by ethyl 4-chloro-3-oxobutanoate (COBE) is a significant chiral intermediate in the synthesis of Slagenins B and C[2], 1,4-dihydropyridine type β-blockers and HMG-CoA reductase inhibitors that lower the cholesterol level in human blood[3]. It can be obtained by the asymmetric reduction of the prochiral ketone[4]. And 4-chloro-3-oxobutanoate ethyl as a prochirality to prepare (S)-ethyl-4-chloro-3-hydroxybutanoate [(S)-CHBE] is quite suitable because COBE is easily synthesized and low cost.

It is well known that biological catalyst method with its incomparable advantage is one of the preferred methods for the synthesis of optically active compounds, which contain a whole cell system and an isolated enzyme[5,6]. Therefore, several preparing methods for optically active ethyl 4-chloro-3-hydroxybutanoate have been reported. Hunt *et al.*[7] obtained (S)- and (R)-CHBE by using glucose, glycerol as a co-substrate with effective production by *Zygosaccharomyces rouxii* (yield 78 %, 98 % ee) and *Pichia capsulate* (yield 73 %, 61 % ee). Wu *et al.*[8] achieved the yield of ethyl (S)-4-chloro-3-hydroxybutanoate 93.9 % and the e.e. value of (S)-CHBE reached 91.4 % by Baker's yeast in the water/isooctane biphasic system.

Asymmetric reduction of 4-chloro-3-oxobutanoate ethyl (COBE) to (S)-CHBE by Baker's yeast has several attractive characteristics, containing low cost, mild reaction conditions, high yield and remarkable enantioselectivity[9,10]. It is known that a very important factor for Baker's yeast biocatalysis is water, but water with a disadvantage of a low solubility of organic substrates and products. Kataoka and Shimizu[11,12] considered an aqueous/organic solvent biphasic system reaction for biocatalysis as appropriate, because the substrate (COBE) is unstable in an aqueous solvent. Nevertheless, there are still drawbacks to some extent. In the wake of the reduction, product inhibition and toxicity of the organic solvent seem to cause a drastic decrease till a total cessation in the reaction rate.

However, room temperature ionic liquids (ILs) as potential green solvents are recently emerging as desirable substituents for toxic, volatile and flammable organic solvents, which are primary cause of environmental pollution[13]. Thus the aqueous/ionic liquids solvent biphasic system may be a proverbial alternative to solve the problem currently.

In this work, Baker's yeast was applied to catalyze COBE to (S)-CHBE instead of an aqueous/organic solvent two-phase system as substrate reservoir aqueous/ionic liquids(ILs) biphasic system which had been investigated as possible replacements for volatile organic solvents. The effects of several parameters on the asymmetric reduction were tested. Furthermore, it showed the best catalytic activity, a convenient

product separation and enantioselectivity in the aqueous/[Bmim]PF_6 biphasic system.

EXPERIMENTAL

Baker's yeast was kindly provided by meishan-mali yeast Co. Ltd. (Guangdong, China). Ethyl 4-chloro-3-oxobutanoate (ECOB) (> 98 %, analytical grade) were purchased from Leqi Chemical Co. Ltd. (Shangdong, China). Enantiopure standards, (R)-(96 %) and (S)-ECHB (97 %) were procured from Sigma-Aldrich, Inc., (Steinheim, Germany). Ionic liquids were made in the laboratory. All other chemicals used in the work were analytical pure and were used without further purification.

Preparation of ionic liquid: The SO_3H-functionalized ionic liquid was prepared in laboratory according to the procedure outlined in the literature[14,15]. The ionic liquids [Bmim][BF_4]/[Bmim][PF_6] were prepared in the laboratory following the procedure outlined in literature[16]. In a typical ionic liquid preparation procedure, N-methyl imidazole (0.1 mol) and 1-chlorobutane (0.12 mol) were added into a 50 mL stainless steel autoclave. N_2 was charged several times to replace air and the final pressure of N_2 was 0.7 MPa. The synthesis reaction was carried out at 393 K for 12 h. After reaction, the production was washed three times with ethyl acetate and yellow viscous liquid 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) was obtained. [Bmim]Cl (0.1 mol), stoichiometric amount of $NaBF_4$ or $NaPF_6$ (0.1 mol) and acetone (100 mL) were then added to the reactor. The mixture was then stirred at 363 K for 4 h to obtain the ionic liquid. The catalyst was abbreviated as [Bmim][BF_4] or [Bmim][PF_6].

Characterization of ionic liquids: The ionic liquids(ILs) were characterized by FT-IR absorption spectrometer(Nicolet 380) and ^1H and ^{13}C NMR spectroscopy (Bruker AV500 spectrometer). The spectrum data showed the structures were consistent with those reported in literature[1,17,18]. The spectrum data of [Bmim][BF_4]/[Bmim][PF_6] were listed as follows:

[Bmim][BF_4]: IR (KBr,ν_{max}, cm^{-1}): ν = 3186, 3173, 3146, 3127, 2968, 2961, 2952, 2878, 2874, 1573, 1572, 1467, 1466, 1170, 1169, 830; ^1H NMR (500 MHz, DMSO-d_6), δ 0.90 (t, 3H), 1.26 (m, 2H), 1.77 (m, 2H), 3.86 (s, 3H), 4.19 (t, 2H), 7.73 (s, 1H), 7.80 (s, 1H), 9.21 (s, 1H) ppm; ^{13}C NMR (500 MHz, DMSO-d_6), δ 13.37, 19.62, 32.48, 36.53, 49.72, 122.98, 124.34, 137.44 ppm.

[Bmim][BF_6]: IR (KBr,ν_{max}, cm^{-1}): ν = 3186, 3173, 3146, 3127, 2968, 2961, 2952, 2878, 2874, 1573, 1572, 1467, 1466, 1170, 1169, 1030; ^1H NMR (500 MHz, DMSO-d_6), d 0.91 (t, 3H), 1.27 (m, 2H), 1.77 (m, 2H), 3.84 (s, 3H), 4.16 (t, 2H), 7.68 (s, 1H), 7.75 (s, 1H), 9.08 (s, 1H) ppm; ^{13}C NMR (500 MHz, DMSO-d_6), δ 13.29, 19.58, 32.31, 36.20, 49.84, 123.00, 124.38, 137.02 ppm.

Bioreduction assay: The reduction of ECOB in an aqueous/ionic liquid biphasic system was conducted in a 50 mL shaking flask. A certain amount of dried cells and the ionic liquid were mixed in phosphate buffer (50 mL, 0.05 mol/L, pH 7) with a certain amount of glucose. After being pre-incubated with rotary shaking at 30 ºC and 180 rpm for 15 min, 0.1 mol/L ECOB was added to the medium and the incubation was continued. A fixed amount of medium was sampled and centrifuged (8000 rpm, 10 min) at 4 ºC to remove the cells. The supernatant was extracted by ethyl acetate and mixed with octane as the internal standard for GC determination.

Analytical methods: The concentrations of COBE and CHBE and enantiomeric excess (e.e.) of (S)-CHBE were determined by GC (Aglient GC_6890, USA) equipped with a chiral column (CP-Chirasil Dex CB, 0.32 mm diameter, 25 m length, Varian USA). For the determination of e.e., the CHBE should be derivated. The ethyl acetate layer was dried by the rotating evaporation and the remainder reacted with acetic anhydride containing excessive amount of pyridine catalyzed by DMAP at 100 ºC for 20 min.

RESULTS AND DISCUSSION

Effect of different aqueous/ionic liquid biphasic system: The ionic liquid played a significant role in a high product yield and enantioselectivity of the cells, if it had a low toxicity to the microbe[19]. Therefore, the selection of the ionic liquid was important in an aqueous/ionic liquid biphasic system. Four ionic liquids ([HSO_3-*p*TEA][HSO_4], [HSO_3-pmim][HSO_4], [Bmim][BF_4] and [Bmim][PF_6]) were tested for their influence on the asymmetric reduction of ECOB by Baker's yeast and the results were shown in Fig. 1. It was noted that aqueous/[Bmim][PF_6] and aqueous/[Bmim][BF_4] biphasic system had a higher product yield and enantioselec-tivity than aqueous/[HSO_3-*p*TEA][HSO_4] and aqueous/[HSO_3-pmim][HSO_4] biphasic system. Because the [Bmim][BF_4] and [Bmim][PF_6] are neutral ionic liquids, whereas the other two are acidic, which would inhibit the activity of reductase in baker's yeast with a lower product yield and the e.e. (S). In the presence of the [Bmim][BF_4] and [Bmim][PF_6], the latter with a property of slightly soluble in water had a remarkable increase in the products and e.e. (S) compared with aqueous/[Bmim][BF_4] of water-soluble (v/v, 0.13 %[20]). [Bmim][PF_6] is slightly soluble in water as medium which is more suitable for biological catalytic reaction of dual phase or multiphase system.

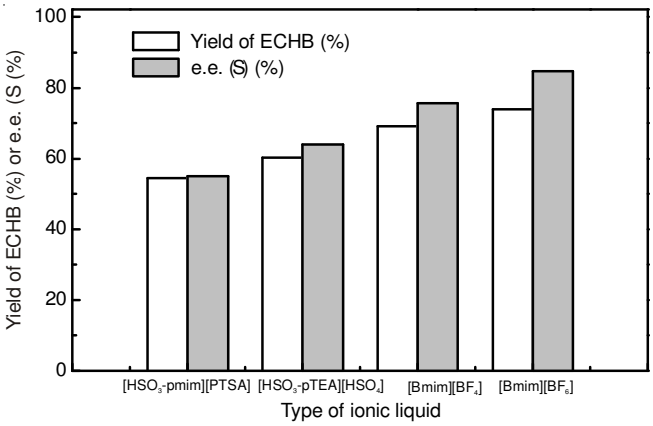

Fig. 1. Selection of the type of aqueous/ionic liquid biphasic system

Effect of phase ratio aqueous/[Bmim][PF_6] biphasic system on the reduction: As biotransformation in an aqueous/ionic liquid biphasic system, the volumetric phase ratio has an influence on the reaction rate, the product yield and e.e.(S)[21]. The effect of phase ratio on the reaction rate, the product yield and e.e.(S) were shown in Fig. 2. 87.2 % of the product yield

and 90.4 % of e.e. (S) were found to be maximum at [Bmim][PF_6] concentration of 180 g/L. Decrease in the e.e. (S) and yield of the asymmetric reduction was observed when the dosage of [Bmim][PF_6] was more than 180 g/L, indicating the increase of viscosity of aqueous/[Bmim][PF_6] biphasic system with the increase of [Bmim][PF_6][22], which seriously blocked the mass transfer of the system. Thus, the dosage of [Bmim][PF_6] 180 g/L was considered to be the optimum condition to obtain a high product yield and an excellent enantioselectivity.

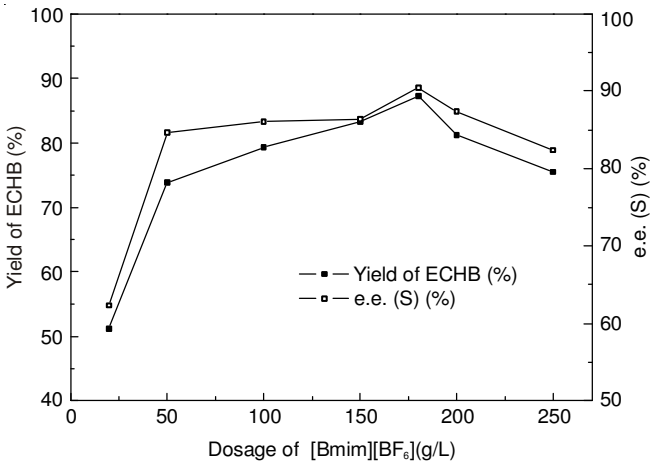

Fig. 2. Effect of the dosage of ionic liquid in aqueous/[Bmim][PF_6] biphasic system

Effect of dried yeast cell concentration on the reduction in aqueous/[Bmim][PF_6] biphasic system: The influence of dried yeast cell concentration on the asymmetric reduction of COBE revealed that the rate of asymmetric reduction increased with addition of the yeast cell concentration. The reaction carried out at cell concentration of 60 g/L recorded the maximum yield (90.9 %) and e.e.(S) (93.3 %) of asymmetric reduction (Fig. 3). Then, the product yield of CHBE decreased sharply, while the e.e.(S) kept constant with increasing dried yeast cell concentration. At the beginning of the reaction, the oxidoreductase participating in the reduction increased with increasing the cell concentration, which had a contribution to the asymmetric reaction. When cell concen tration increased to a certain extent, fixed glucose consumption were not enough

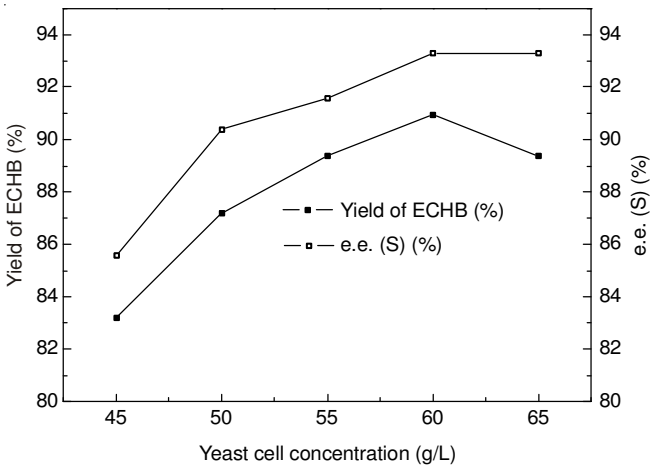

Fig. 3. Effect of the amount of yeast cells in water/[Bmim][PF_6] biphasic system

to meet the nutrient demand of too much yeast cells, which resulted in growing competition of yeast cells and the decrease of enzyme activity. Therefore, it was an advisable choice that the Baker's yeast concentration was 60 g/L.

Effect of the amount of glucose as co-substrate on the reduction in aqueous/[Bmim][PF_6] biphasic system: It is well known that glucose played an important role in the reaction medium for the regeneration of cofactors in yeast-mediated asymmetric reduction[23]. The effect of glucose concentration was shown in Fig. 4, maximum product yield 92.6 % and e.e.(S) 95.4 % was obtained when the amount of glucose was 0.8 mol/L. However, when the amount of glucose exceeded 0.8 mol/L, the product yield decreased and the e.e.(S) kept constant. It revealed that high glucose concentration could inhibit the activity of alcohol dehydrogenase. Therefore, the glucose concentration 0.8 mol/L was a good choice.

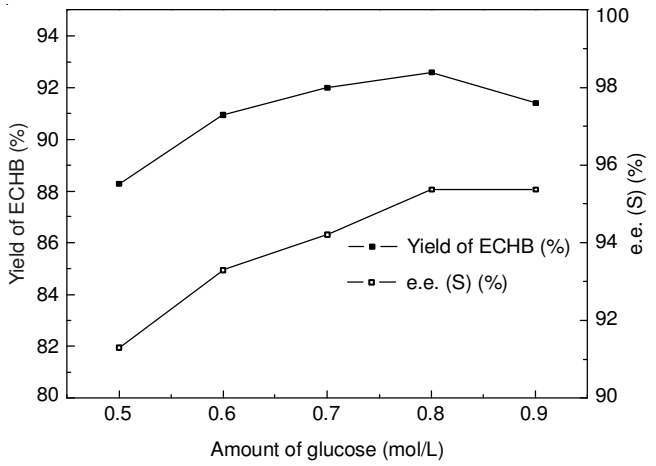

Fig. 4. Effect of the amount of glucose on the reduction of ECOB in water/[Bmim][PF_6] biphasic system

Conclusion

[HSO_3-pmim][HSO_4], [HSO_3-*p*TEA][HSO_4], [Bmim][BF_4] and [Bmim][PF_6] were synthesized and the reduction of Baker's yeast in four biphasic systems has been studied. It showed that the aqueous/[Bmim][PF_6] biphasic system was a better medium for the reduction of ECHB, in which Baker's yeast could keep high viability and possess a satisfactory catalytic activity and enantioselectivity. The optimum [Bmim][BF_6] concentration, substrate concentration, the amount of dried yeast cells and glucose concentration were 180 g/L, 0.1 mol/L, 50 g/L and 60 g/L, respectively, under which 93.9 % of molar conversion and 95.4 % e.e. of the optical purity of the product were acquired. So it was a potential biocatalytic process with broad application prospects.

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