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Asymmetric reduction of 3-chloropropiophenone to (*S*)-3-chloro-1-phenylpropanol using immobilized *Saccharomyces cerevisiae* CGMCC 2266 cells

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ABSTRACT

(*S*)-3-Chloro-1-phenylpropanol is an important chiral precursor for numerous antidepressants such as tomoxetine. A high enantiomeric excess (e.e.) of (*S*)-3-chloro-1-phenylpropanol can be achieved by asymmetric reduction of 3-chloropropiophenone using *Saccharomyces cerevisiae* CGMCC 2266 cells immobilized in calcium alginate. Thermal pretreatment of the immobilized cells at 50 °C for 30 min resulted in high enantioselectivity (99% e.e.) and good percent conversion (80%). The effects of various conditions on the reduction reaction were investigated. The optimal conditions were found to be as follows: sodium alginate concentration, 2%; bead diameter, 2 mm; temperature, 30 °C; re-culture time, 24 h; and batch addition of the substrate. After reusing these three times, the immobilized cells retained approximately 60% of their original catalytic activity with their enantioselectivity intact.

1. Introduction

The search for novel methods to synthesize chiral drugs is a focus area in contemporary organic synthesis due to a worldwide increase in the demand for enantiomerically pure forms of chiral drugs. Enzymes can catalyze reactions with remarkable chemo-, regio-, and stereoselectivity under mild conditions of pH and temperature. Consequently, the number of biocatalysts used in the production of chiral compounds has rapidly increased during the last few decades [1].

Chiral alcohols with additional functional groups are very important intermediates in the synthesis of enantiomerically pure pharmaceuticals and other important chemicals [2]. For example, (*S*)-3-chloro-1-phenylpropanol and (*R*)-3-chloro-1-phenylpropanol are building blocks of (*S*)-fluoxetine (**3**), (*R*)-tomoxetine (**4**), and nisoxetine (**5**), which are prescription drugs used in the treatment of major depressive disorders [3–7]. Unlike chemical tricyclic antidepressants such as imipramine, (*R*)-tomoxetine and (*S*)-fluoxetine have been shown to specifically inhibit norepinephrine and selective serotonin uptake in humans at doses that are clinically well tolerated. These compounds are also reported to be relatively weak ligands for α-1, α-2, and β-adrenergic receptors [8,9]. These receptors are believed to be responsible for the undesirable side effects associated with antidepressants. Clearly, an enantioselective preparation of (*R*)-tomoxetine and (*S*)-fluoxetine would be desirable. Based on these observations, we initiated studies on the reduction of 3-chloropropiophenone (**1**) because (*S*)-3-chloro-1-phenylpropanol (**2**) is a simple precursor of popular serotonin/norepinephrine reuptake inhibitors [3]. The reduction of 3-chloropropiophenone (**1**) is an economical method for obtaining optically active (*S*)-3-chloro-1-phenylpropanol (**2**) because 3-chloropropiophenone is easily synthesized and cheap (Fig. 1).

The conversion of a ketone to its corresponding optically active alcohol is one of the most common redox reactions in organic chemistry. The vast majority of dehydrogenases and reductases used for ketone reduction and alcohol oxidation require nicotinamide cofactors such as NADH and NADPH [10]. In fermentative processes, internal cofactor regeneration occurs in whole cells; as a result, the addition of cheap glucose is sufficient to drive the reaction [1,11–13]. In comparison to the application of isolated enzymes,

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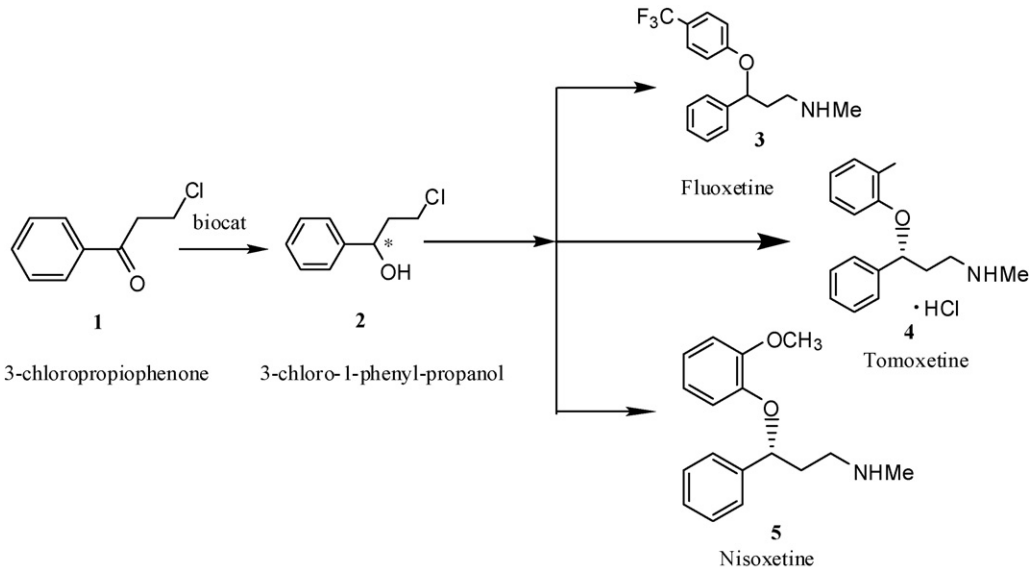

Fig. 1. Synthesis of *R*-(-)-tomoxetine, fluoxetine, and nisoxetine from 3-chloro-1-phenyl-propanol.

the use of whole cells has distinct characteristics. Whole cells contain enzymes that are generally more stable since they are present in their natural environment. There are reports on several carbonyl reductases that reduce ketones to optically active alcohols in microorganisms [14–16]. Michio and Yoshinori [17,18] reported the production of optically active (*S*)-3-chloro-1-phenylpropanol by several microorganisms. Although these microorganisms could produce optically active (*S*)-3-chloro-1-phenylpropanol of high enantiomeric purity, the conversion was very low. Bakers' yeast (*Saccharomyces cerevisiae*) is a popular biocatalyst in organic synthesis and has been shown to catalyze C–C bond formation and dissociation, oxidation, hydrolysis, and a variety of reduction reactions [19]. It is widely used for the asymmetric reduction of prochiral ketones because such reductions are easy to perform, and the cells are inexpensive and readily available [20]. In some cases, several reductases present in a single cell are involved in the reduction of a particular ketone. Due to the different stereospecificities of these enzymes, the desired alcohol has low optical purity [21,22]. Moreover, many of the *S. cerevisiae*-mediated methods used for the asymmetric reduction of ketones are unsuitable for the large-scale production of chiral alcohol. Some recent studies on the use of immobilized cells for the enantioselective reduction of ketones have attempted to overcome these drawbacks [23–25]. The use of immobilized microbial cells in organic synthesis is considered to be both technically and economically advantageous [26–28].

In this paper, we report the use of the *S. cerevisiae* CGMCC 2266 strain (accession number: CGMCC 2266, China General Microbiological Culture Collection Center (CGMCC)) as a novel biocatalyst for the production of (*S*)-3-chloro-1-phenylpropanol from the prochiral compound 3-chloropropiophenone. The biocatalyzed reduction reaction was carried out with thermally pretreated immobilized cells to achieve high enantioselectivity. We determined the effects of different immobilization methods, such as crosslinking with glutaraldehyde–gelatin and entrapment in an agar or Ca-alginate gel, on the reduction of 3-chloropropiophenone by immobilized *S. cerevisiae* CGMCC 2266 cells. We also investigated other factors that influenced the percent conversion and enantiomeric excess (e.e.) of 3-chloro-1-phenylpropanol.

2. Experimental

2.1. Chemical materials

3-Chloropropiophenone, (*R*)-3-chloro-1-phenylpropanol, and (*S*)-3-chloro-1-phenylpropanol were purchased from Aldrich Chemical Co., Inc. All other chemicals were of analytical grade and obtained commercially.

2.2. Strain and culture conditions

The microorganism used in this study was *S. cerevisiae* CGMCC 2266, which was obtained from Zhejiang University of Technology, Hangzhou, Zhejiang Province, PR China. The strain was maintained at 4 °C on MYPG solid medium. The composition of 1 L of MYPG medium was as follows: wort (10 g), yeast extract (3 g), peptone (5 g), glucose (10 g), and agar (20 g). The liquid medium for the growth of strains contained glucose (30 g/L), yeast extract (3 g/L), $(NH_4)_2SO_4$ (5 g/L), $MgSO_4{\cdot}7H_2O$ (0.5 g/L), $K_2HPO_3{\cdot}3H_2O$ (1 g/L), and KH_2PO_3 (1 g/L).

To prepare the seed culture, the strain was inoculated in sterilized liquid medium (100 mL) in a 500-mL shake flask that was shaken (160 rpm) at 30 °C for 24 h. The seed culture (10 mL) was added to the liquid medium (100 mL), which was shaken at 30 °C for one day. The cells were collected by centrifugation and washed with distilled water.

2.3. Immobilization of S. cerevisiae CGMCC 2266 cells

2.3.1. Immobilization in crosslinked glutaraldehyde–gelatin beads

The cells were immobilized in crosslinked glutaraldehyde–gelatin beads as follows. Gelatin (8 g) was dissolved in distilled water (36 mL) by warming to 60 °C. *S. cerevisiae* CGMCC 2266 cell suspension (10 mL) was mixed with the gelatin solution at 40 °C, and the gelatin concentration was adjusted to 10% (w/v). After the microbial cells were well dispersed, glutaraldehyde (25%, 1 mL) was added with stirring, and the solution was poured into a petri plate and allowed to dry overnight at room temperature. After sieving, immobilized microbial granules of particle size 3 mm were obtained.

2.3.2. Immobilization in agar

Agar (1 g) was dissolved in 20 mL of distilled water by heating to 100 °C and then sterilized by autoclaving (121 °C, 20 min). After cooling to 55 °C, 10 mL of *S. cerevisiae* CGMCC 2266 cell suspension was added and mixed. The mixture was immediately poured and cooled in a rectangular mold that was 5 mm deep; the resulting gel was then chopped into small cubes of size 3 mm. The cubes were washed with sterilized distilled water and wiped with sterilized absorbent paper.

2.3.3. Immobilization in Ca-alginate gel beads

S. cerevisiae CGMCC 2266 cells were entrapped in calcium alginate gel beads as follows. An *S. cerevisiae* CGMCC 2266 cell suspension (10 mL) was mixed with an equal volume (1:1, v/v) of sodium alginate solution (2%) and stirred for 5 min. The solution was placed in a cylindrical reservoir and dropped into a well-stirred sterile $CaCl_2$ solution (3.5%) using a syringe. The alginate drops solidified upon contact with $CaCl_2$ and formed beads that encapsulated the *S. cerevisiae* CGMCC 2266 cells. The beads were left to harden for 30 min at 37 °C and then washed with sterile saline solution to remove excess calcium ions and unencapsulated cells.

2.4. Re-culture of immobilized cells

The immobilized cells obtained above were suspended in the liquid culture media and re-cultured for a period of time. The re-cultured immobilized cells were used in the biotransformation trial.

2.5. Reduction of 3-chloropropiophenone

2.5.1. Reduction of 3-chloropropiophenone by resting cells

The culture conditions were the same as those described above. The cell culture broth (100 mL, 430 mg dry weight) was transferred to a Falcon tube and centrifuged for 15 min at 4000 rpm. The biomass was removed and washed three times using 20 mL phosphate buffer (50 mmol/L, pH 8.0), vortexed, and the cells were separated from the liquid. When the cells were free of culture media, they were resuspended in 200 mL of the same buffer. 3-Chloropropiophenone (0.1 g) was added to the reaction media. After incubation for a certain time period, the aqueous phase was extracted with ethyl acetate (25 mL ethyl acetate/100 mL cell culture medium) for 30 min. The organic phase was analyzed by gas chromatography (GC, HP6890) equipped with a flame ionization detector. An HP chiral 10% β-cyclodextrin (30 m × 0.32 mm × 0.25 μm) chromatography column was used.

2.5.2. Reduction of 3-chloropropiophenone by immobilized cells

The reduction was carried out by adding 0.1 g of 3-chloropropiophenone to the re-cultured immobilized cells in a 500-mL flask containing 100 mL Tris buffer (0.1 mol/L, pH 8.0). The reaction mixture was incubated in a rotary shaker (160 rpm) at 30 °C for a period of time. The immobilized cells were filtered, and the filtrate was extracted with ethyl acetate (25 mL). The e.e. and percent conversion were determined by GC as described above.

2.5.3. Reduction of 3-chloropropiophenone by thermal pretreatment of Ca-alginate-immobilized cells

The re-cultured Ca-alginate-immobilized cells were suspended in a 500-mL flask containing 100 mL of Tris buffer (0.1 mol/L, pH 8.0) and then heated at 50 °C for different time periods (5, 10, 15, 20, 25, 30, and 35 min). 3-Chloropropiophenone (0.1 g) was added, and the reaction mixture was incubated at 30 °C with shaking (160 rpm). After the designated time period, the immobilized cells were filtered, and the remaining filtrate was extracted with ethyl acetate (25 mL). The e.e. and percent conversions were determined by GC as described above.

3. Results and discussion

3.1. Reduction by resting cells versus that by immobilized cells

The asymmetric bioreduction of 3-chloropropiophenone was accomplished by both resting and immobilized cells. Table 1 shows that (*S*)-3-chloro-1-phenylpropanol can be obtained by reducing 3-chloropropiophenone with *S. cerevisiae* CGMCC 2266 cells. The e.e. of (*S*)-3-chloro-1-phenylpropanol was higher with the immobilized cells than with the resting cells. This suggests that of the two catalytic activities that convert 3-chloropropiophenone to the *R*- and *S*-enantiomers, the one responsible for the conversion to the *R*-enantiomer is inhibited to a greater extent by the immobilization technique. Comparison of the three immobilized methods used in our experiments showed that the best results were obtained with the cells immobilized in Ca-alginate gel beads. The e.e. and yield of 3-chloro-1-phenylpropanol from Ca-alginate-immobilized cells were 60% and 50%, respectively. The above results also confirmed the observation that cells entrapped in calcium alginate gels are advantageous in terms of simplicity of operation and nontoxicity [29].

3.2. Effect of thermal pretreatment of Ca-alginate-immobilized S. cerevisiae CGMCC 2266 cells on reduction

In order to obtain a higher e.e. of (*S*)-3-chloro-1-phenylpropanol, we heated the Ca-alginate-immobilized cells at 50 °C for different time periods prior to the reduction reaction. The results of these experiments are shown in Figs. 2 and 3. As the pretreatment time period increased, the e.e. of (*S*)-3-chloro-1-phenylpropanol increased; however, the conversion were lower than those of the cells prior to heating. With a preheating time of 30 min, (*S*)-3-chloro-1-phenylpropanol was obtained with 99% e.e. and 28% yield. The use of thermally pretreated Ca-alginate-immobilized cells was advantageous and led to increases in the e.e. of (*S*)-3-chloro-1-phenylpropanol. It could be assumed that the enzymes that convert 3-chloropropiophenone to (*R*)-3-chloro-1-phenylpropanol are more easily denatured by thermal pretreatment than those that

Table 1
Reduction by resting cells versus that by immobilized cells

	Percent conversion (%)	Enantiomeric excess (e.e. %)	Configuration
Resting cells	87	42	*S*
Immobilized in crosslinked glutaraldehyde–gelatin beads	42	56	*S*
Immobilized in agar	51	50	*S*
Immobilized in Ca-alginate gel beads	50	60	*S*

Reaction conditions: bio-transformation time, 72 h; substrate concentration, 1 g/L; pH, 8.0; temperature, 30 °C; rotary shaker velocity, 160 rpm; re-culture time, 24 h; and cell biomass, 43 g/L (cell dry weight/reaction mixture volume). Percent conversion (%) and enantiomeric excess (e.e. %) were determined by GC.

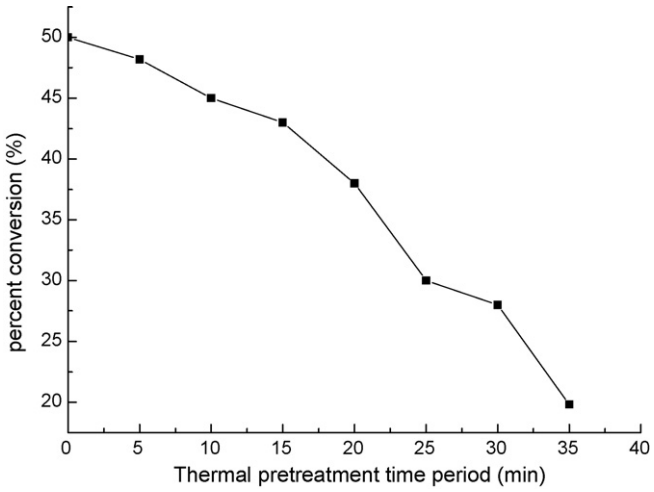

Fig. 2. Effect of the thermal pretreatment time period on percent conversion. Reaction conditions: bio-transformation time, 72 h; substrate concentration, 1 g/L; pH, 8.0; temperature, 30 °C; rotary shaker velocity, 160 rpm; re-culture time, 24 h; sodium alginate concentration, 2%; bead diameter, 2 mm; and cell biomass, 43 g/L. Percent conversion (%) was determined by GC.

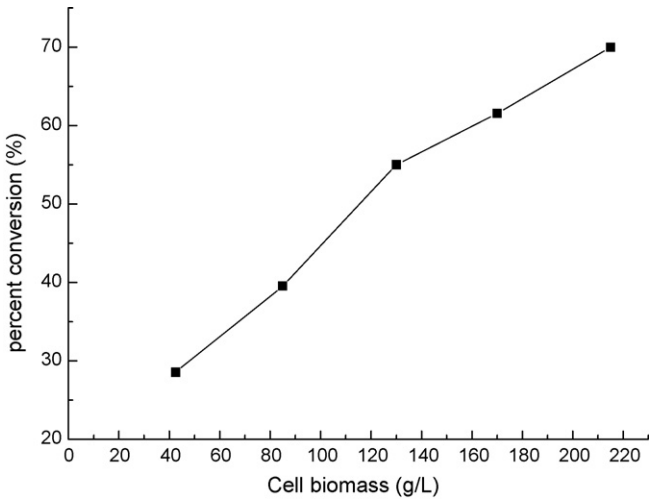

Fig. 4. Effect of the biomass of thermally pretreated Ca-alginate-immobilized *S. cerevisiae* CGMCC 2266 cells on reduction. Reaction conditions: bio-transformation time, 72 h; substrate concentration, 1 g/L; pH, 8.0; temperature, 30 °C; rotary shaker velocity, 160 rpm; re-culture time, 24 h; sodium alginate concentration, 2%; bead diameter, 2 mm; and thermal pretreatment time period, 30 min. Percent conversion (%) was determined by GC.

convert 3-chloropropiophenone to (*S*)-3-chloro-1-phenylpropanol. The results of our study indicated that the optimum preheating time was 30 min.

3.3. Effect of the biomass of thermally pretreated Ca-alginate-immobilized S. cerevisiae CGMCC 2266 cells on reduction

Fig. 4 shows the effect of the biomass of thermally pretreated Ca-alginate-immobilized cells on reduction percent conversion. The e.e. of (*S*)-3-chloro-1-phenylpropanol remained 99% at different biomasses. As the cell biomass increased, the percent conversion also increased. A percent conversion of 70% was obtained when the biomass was 215 g/L (cell dry weight/reaction mixture volume). A higher biomass was difficult to immobilize.

3.4. Effect of the sodium alginate concentration on reduction

Some capsule characteristics, such as its thickness or permeability to different substrates of the gel membrane, can be easily controlled by modulating the gelation conditions [30]. To study the effect of the sodium alginate concentration on the reduction reaction, various amounts of sodium alginate (1–5%, w/v) were added to the gel mixture. The gelation of the mixture was induced by a 3.5% (w/v) $CaCl_2$ solution. Fig. 5 shows the effect of the sodium alginate concentration on reduction. The results indicate that the maximum (*S*)-3-chloro-1-phenylpropanol percent conversion was obtained with 2% sodium alginate. After 54 h, 70% percent conversion and 99% e.e. were achieved under this condition. Beads produced with 1% sodium alginate are too soft, and the microbial cells may easily

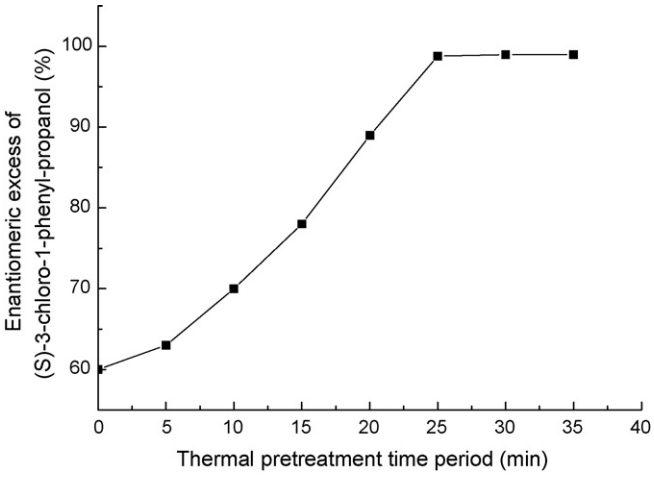

Fig. 3. Effect of the thermal pretreatment time period on the enantiomeric excess of (*S*)-3-chloro-1-phenylpropanol. Reaction conditions: bio-transformation time, 72 h; substrate concentration, 1 g/L; pH, 8.0; temperature, 30 °C; rotary shaker velocity, 160 rpm; re-culture time, 24 h; sodium alginate concentration, 2%; bead diameter, 2 mm; and cell biomass, 43 g/L. Enantiomeric excess (e.e. %) were determined by GC.

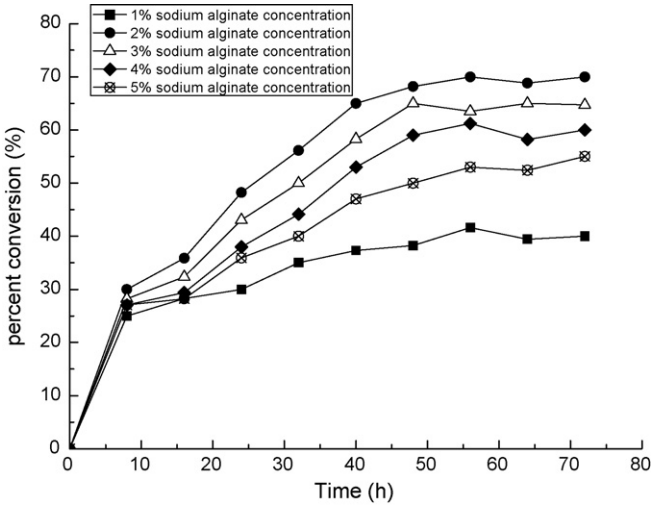

Fig. 5. Effect of the sodium alginate concentration on reduction. Reaction conditions: bio-transformation time, 72 h; substrate concentration, 1 g/L; pH, 8.0; temperature, 30 °C; rotary shaker velocity, 160 rpm; re-culture time, 24 h; bead diameter, 2 mm; cell biomass, 215 g/L; and thermal pretreatment time period, 30 min. Percent conversion (%) was determined by GC.

leak from these [31]. In contrast, if the sodium alginate concentration is increased beyond 2%, the number of biopolymer molecules per unit solution probably increase; as a result, the number of Ca^{2+} ion binding sites also increase. A more densely crosslinked gel structure is formed that leads to diffusion resistance of the substrates through the beads [32]. Using 2% sodium alginate, a higher (*S*)-3-chloro-1-phenylpropanol percent conversion was obtained. This was probably because the crosslinking of the alginate molecules was lower at this concentration; consequently, less densely packed three-dimensional lattices were formed from the outermost layer to the core of the drop. This would enable easy diffusion of substrates through the porous beads, leading to increased percent conversion.

3.5. Effect of the bead diameter on reduction

Fig. 6 shows the effect of four different bead diameters on the reduction reaction. A higher conversion was obtained with a smaller bead diameter. Good percent conversion (70%) and high e.e. (99%) were obtained when the diameter of the Ca-alginate bead containing immobilized *S. cerevisiae* CGMCC 2266 cells was approximately 2.0 mm. A lower conversion was observed when the bead diameter increased beyond 2.0 mm. Increase of the bead diameter to 4.0 mm resulted in a decrease in the percent conversion to 43%. This was probably because the smaller bead diameter has a higher surface/volume ratio. Therefore, the substrate can easily diffuse to the microbial cells when the bead diameter is small.

3.6. Effect of temperature on reduction

Temperature is an important factor that affects the reduction activity of the microorganism. The effect of temperature on reduction is shown in Fig. 7. The highest conversion was obtained at 30 °C. The e.e. was approximately 99% at four different temperatures in this study. Temperatures above and below 30 °C did not improve the percent conversion. The highest reduction activity of *S. cerevisiae* CGMCC 2266 cells was observed at 30 °C.

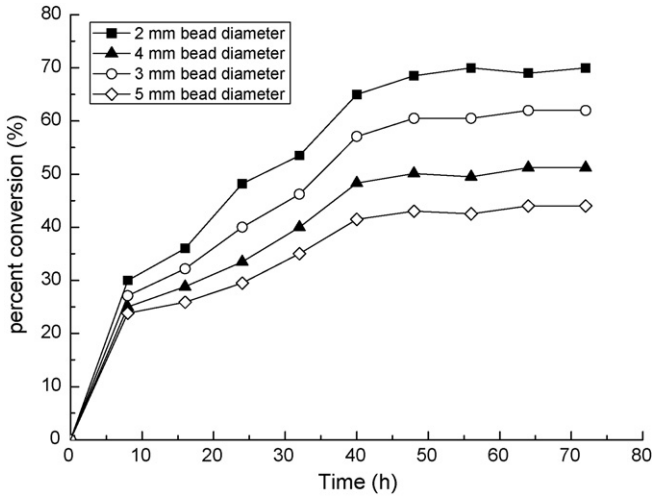

Fig. 6. Effect of the bead diameter on the reduction catalyzed by Ca-alginate-immobilized *S. cerevisiae* CGMCC 2266 cells. Reaction conditions: temperature, 30 °C; sodium alginate concentration, 2%; substrate concentration, 1 g/L; re-culture time, 24 h; pH, 8.0; rotary shaker velocity, 160 rpm; cell biomass, 215 g/L; and thermal pretreatment time period, 30 min. Percent conversion (%) was determined by GC.

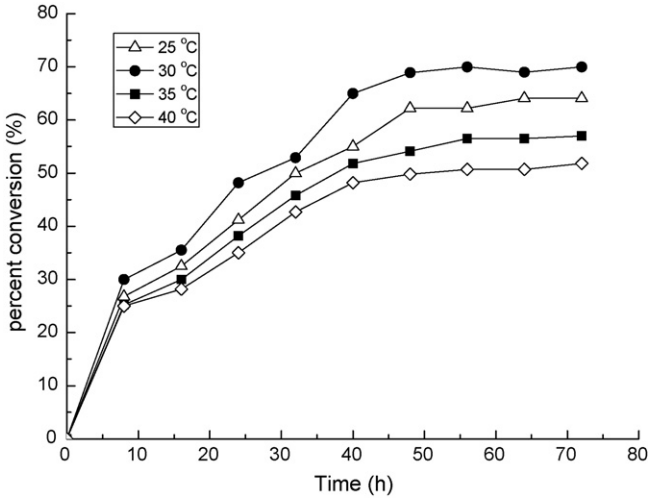

Fig. 7. Effect of temperature on reduction. Reaction conditions: sodium alginate concentration, 2%; substrate concentration, 1 g/L; bead diameter, 2 mm; re-culture time, 24 h; pH, 8.0; rotary shaker velocity, 160 rpm; cell biomass, 215 g/L; and thermal pretreatment time period, 30 min. Percent conversion (%) was determined by GC.

Table 2
Effect of the re-culture time of Ca-alginate-immobilized *S. cerevisiae* CGMCC 2266 cells on reduction

Re-culture time (h)	0	24	48	72
Percent conversion (%)	0	70	72	73
e.e. (%)	0	99	99	99

Reaction conditions: temperature, 30 °C; pH, 8.0; bead diameter, 2 mm; sodium alginate concentration, 2%; substrate concentration, 1 g/L; rotary shaker velocity, 160 rpm; cell biomass, 215 g/L; and thermal pretreatment time period, 30 min. Percent conversion (%) and enantiomeric excess (e.e. %) were determined by GC.

3.7. Effect of the re-culture time of Ca-alginate-immobilized *S. cerevisiae* CGMCC 2266 cells on reduction

The effect of the re-culture time of Ca-alginate-immobilized cells on reduction is shown in Table 2. Ca-alginate-immobilized cells that were not re-cultured had no reduction activity. At 24 h of re-culture, the percent conversion and e.e. of the product were 70% and 99%, respectively. After 24 h, the re-culture time had no effect on the e.e. of the product. Thus, the optimum re-culture time was 24 h.

3.8. Effect of batch addition of the substrate on reduction

Table 3 shows the effect of batch addition of the substrate on reduction. 3-Chloropropiophenone was added in three aliquots to the reaction medium over a period of 48 h; the total amount added was 100 mg. A percent conversion of 80% was obtained when 100 mg substrate was added by the abovementioned method. This represents an increase of 10% over the 70% percent conver-

Table 3
Effect of batch addition of the substrate on reduction

	First addition	Second addition	Third addition
Percent conversion (%)	91	86	80
e.e. (%)	99	99	99

Reaction conditions: temperature, 30 °C; bead diameter, 2 mm; pH, 8.0; re-culture time, 24 h; reaction time, 72 h; rotary shaker velocity, 160 rpm; sodium alginate concentration, 2%; cell biomass, 215 g/L; and thermal pretreatment time period, 30 min. Percent conversion (%) and enantiomeric excess (e.e. %) were determined by GC.

Table 4
Reuse of Ca-alginate-immobilized *S. cerevisiae* CGMCC 2266 cells in the reduction of 3-chloropropiophenone

	First	Second	Third	Fourth
Percent conversion (%)	70	66	50	42
e.e. (%)	99	99	99	99

Reaction conditions: temperature, 30 °C; bead diameter, 2 mm; pH, 8.0; re-culture time, 24 h; reaction time, 72 h; substrate concentration, 1 g/L; rotary shaker velocity, 160 rpm; sodium alginate concentration, 2%; cell biomass, 215 g/L; and thermal pretreatment time period, 30 min. Percent conversion (%) and enantiomeric excess (e.e. %) were determined by GC.

sion obtained when the substrate was added in a single step of 100 mg to the reaction medium. This indicates that a larger amount of substrate can inhibit the reduction activity of the immobilized cells. Thus, batch addition of the substrate is a valuable method for increasing the percent conversion, and it can relieve substrate inhibition in the reduction reaction.

3.9. Reuse of Ca-alginate-immobilized S. cerevisiae CGMCC 2266 cells

Although resting *S. cerevisiae* CGMCC 2266 cells cannot be reused, immobilized cells can be used for several reaction cycles, as shown in Table 4. The results of this study reinforced the convenience of using immobilized-stabilized whole cells for the reduction process. After reusing these three times, the immobilized cells retained approximately 60% of their original activity with their enantioselectivity intact. This shows that good percent conversion can be obtained by reusing Ca-alginate-immobilized *S. cerevisiae* CGMCC 2266 cells for the reduction of 3-chloropropiophenone.

4. Conclusions

In this study, we compared the bioreduction of 3-chloropropiophenone by resting cells and immobilized cells. Based on our results, we conclude that thermally pretreated Ca-alginate-immobilized *S. cerevisiae* CGMCC 2266 cells have the highest catalytic activity and stereoselectivity. Using thermally pretreated Ca-alginate-immobilized cells, (*S*)-3-chloro-1-phenylpropanol can be obtained with 80% percent conversion and 99% e.e. The highest reduction activity of the immobilized cells was observed under the following conditions; temperature, 30 °C; re-culture time, 24 h; sodium alginate concentration, 2%; and bead diameter, 2 mm. Batch addition of the substrate relieved enzyme inhibition, and this method resulted in good reduction percent conversion and low cell toxicity. Reuse of Ca-alginate-immobilized *S. cerevisiae* CGMCC 2266 cells can increase the reduction efficiency.

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References

[1] K. Goldberg, K. Schroer, S. Lütz, A. Liese, Appl. Microbiol. Biotechnol. 76 (2007) 249–255.
[2] A. Armstrong, D.R. Carbery, Annu. Rep. Prog. Chem. Sect. B Org. Chem. 101 (2005) 33–48.
[3] M. Srebanik, P.V. Ramachandran, H.C. Brown, J. Org. Chem. 53 (1988) 2916–2920.
[4] G. Fronza, C. Fuganti, P. Grasselli, et al., J. Org. Chem. 56 (1991) 6019–6023.
[5] A. Kumar, D.H. Ner, S.Y. Dike, Tetrahedron Lett. 32 (1991) 1901–1908.
[6] M.P. Schneider, U. Goergens, Tetrahedron: Asymmetry 3 (1992) 525–528.
[7] E.J. Corey, G. Reichard, Tetrahedron Lett. 30 (1989) 5207–5210.
[8] R.L. Zerbe, H. Rowe, G.G. Enas, D. Wong, N. Farid, L. Lemberger, J. Pharmacol. Exp. Ther. 232 (1985) 139–143.
[9] D.T. Wong, F.P. Bymaster, L.R. Reid, R.W. Fuller, K.W. Perry, Drug Dev. Res. 6 (1985) 397–403.
[10] K. Goldberg, K. Schroer, S. Lütz, A. Liese, Appl. Microbiol. Biotechnol. 76 (2007) 237–248.
[11] K. Nakamura, T. Matsuda, in: K. Drauz, H. Waldmann (Eds.), Enzyme catalysis in organic synthesis, vol. III, 2nd ed., Wiley-VCH Verlag GmbH, Weinheim, 2002, pp. 991–1047.
[12] K. Faber, Biotransformations in Organic Chemistry, 5th ed., Springer, Berlin, 2004.
[13] K. Nakamura, R. Yamanaka, T. Matsuda, T. Harada, Tetrahedron: Asymmetry 14 (2003) 2659–2681.
[14] J.D. Stewart, Curr. Opin. Biotech. 11 (2000) 363–368.
[15] H. Hioki, T. Hashimoto, M. Kodama, Tetrahedron: Asymmetry 11 (2000) 829–834.
[16] W. Kroutil, H. Mang, K. Edegger, K. Faber, Curr. Opin. Chem. Biol. 8 (2004) 120–126.
[17] M. Ito, Y. Kobayashi, JP Patent 04,234989 (1992).
[18] M. Ito, Y. Kobayashi, JP Patent 04,316489 (1992).
[19] R. Csuk, B.I. Glanzer, Chem. Rev. 91 (1991) 49–97.
[20] H. Engelking, R. Pfaller, G. Wich, D. Weuster-Botz, Enzyme Microb. Technol. 38 (2006) 536–544.
[21] S. Rodriguez, M. Kayser, J.D. Stewart, Org. Lett. 1 (1999) 1153–1155.
[22] I.A. Kaluzna, T. Matsuda, A.K. Sewell, J.D. Stewart, J. Am. Chem. Soc. 126 (2004) 12827–12832.
[23] R. Wendhausen Jr., P.J.S. Moran, I. Joekes, J.A.R. Rodrigues, J. Mol. Catal. B Enzyme 5 (1998) 69–73.
[24] E.M. Buque, I. Chin-Joe, A.J.J. Straathof, J.A. Jongejan, J.J. Heijnen, Enzyme Microb. Technol. 31 (2002) 656–664.
[25] H.M.S. Milagre, C.D.F. Milagre, P.J.S. Moran, M.H.A. Santana, J.A.R. Rodrigues, Org. Process Res. Dev. 10 (2006) 611–617.
[26] K.P. Santosh, C. Anju, Tetrahedron: Asymmetry 16 (2005) 2790–2798.
[27] H.M.S. Milagre, C.D.F. Milagre, P.J.S. Morana, M.H.A. Santana, J.A.R. Rodrigues, Enzyme Microb. Technol. 37 (2005) 121–125.
[28] Y. Chen, J.H. Xu, J. Pan, Y. Xu, J.B. Shi, J. Mol. Catal. B Enzyme 30 (2004) 203–208.
[29] J.C. Rossi-Alva, M.H.M. Rocha-Leao, Biotechnol. Appl. Biochem. 38 (2003) 43–51.
[30] A. Blandino, M. Macías, D. Cantero, Enzyme Microb. Technol. 27 (2002) 319–324.
[31] P. Boyaval, J. Goulet, Enzyme Microb. Technol. 10 (1988) 725–728.
[32] A. Blandino, M. Macías, D. Cantero, J. Biosci. Bioeng. 88 (1999) 686–689.