Process Biochemistry 45 (2010) 1445–1449

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

A new approach for the immobilization of permeabilized brewer's yeast cells in a modified composite polyvinyl alcohol lens-shaped capsule containing montmorillonite and dimethyldioctadecylammonium bromide for use as a biocatalyst

Geng-Hao Gong[a], Yi Hou[a], Quan Zhao[a], Ming-An Yu[a,*], Fei Liao[b], Lan Jiang[c], Xiao-Lan Yang[a]

[a] College of Pharmaceutical Science, Chongqing Medical University, #1, Medical College Road, Chongqing 400016, China
[b] Chongqing Key Laboratory of Biochemistry and Molecular Pharmacology, Chongqing Medical University, Chongqing 400016, China
[c] College of Environmental and Biological Engineering, Chongqing University of Technology and Business, Chongqing 400067, China

ARTICLE INFO

Article history:
Received 25 October 2009
Received in revised form 16 May 2010
Accepted 17 May 2010

Keywords:
Permeabilized brewer's yeast
Immobilization
Modified composite PVA
Diffusion capability
Ethyl (*S*)-4-chloro-3-hydroxybutanoate
Biocatalyst

ABSTRACT

Permeabilized brewer's yeast cells were immobilized in modified composite polyvinyl alcohol lens-shaped capsules containing montmorillonite (MONT) and dimethyldioctadecylammonium bromide (DDAB). The special properties of the capsules were then investigated. The results showed that the diffusion capability of hydrophobic molecules from bulk solution into the capsules was obviously improved by the introduction of 0.5% MONT and 0.05% DDAB into the 15% PVA matrix. More importantly, when ethyl 4-chloro-3-oxobutanoate (COBE) was reduced with the capsules as the biocatalyst, the yield and enantiomeric excess (ee) of the product reached 88% and 99.1%, respectively, within 8 h, which are significantly higher totals than those mediated by the unmodified PVA capsules under the same conditions. The intracellular alcohol dehydrogenase (ADH) in the capsules retained over 81% of its original activity after 1 month of storage and 88% of its initial activity after 20 cycles of reaction.

1. Introduction

Nowadays, biocatalysts are indispensable tools for the production of chiral alcohols whether they are whole cells [1], permeabilized cells [2–5] or isolated enzymes [6]. When compared with whole cells, permeabilized cells allow for the use of an externally added cofactor for efficient catalysis, cofactor recycling, easy substrate access and easy product release [3]. When compared with the isolated enzyme, permeabilized cells are cheap and easily available in large amounts. However, when the permeabilized cells were reused under the same conditions, the intracellular enzyme activities in permeabilized cells and the production yield of the bioreduction process significantly decreased when compared with the previous batch [3]. Therefore, the use of immobilization techniques to produce a new biocatalyst with good enzyme stability, higher operational stability and storage stability is very necessary. However, mass transfer is one of the major factors affecting the activity and effectiveness of immobilized cells because substrates and products have to be transported through the external boundary layers (external mass transfer) and within the matrix (internal mass transfer) [7].

Recently, attempts have been made to solve this problem by the introduction of new techniques that are able to adjust the size (microbeads) and shape (lenticular shape) of PVA hydrogels [8–11]. Although this method has proven successful, the PVA hydrogel is highly hydrophilic due to the abundance of hydroxyl pendant groups on its chains and is thus likely to limit the mass transfer of some organic compounds from the reaction mixture into the hydrogel layer. The main cause of diffusion limitations may be due to the fact that organic compounds contain hydrophobic groups, which are either totally insoluble or very sparsely soluble in the water-soluble gel matrix. To overcome this limit, two substances, montmorillonite (MONT) and dimethyldioctadecylammonium bromide (DDAB), a cationic lipid [12], were introduced into the PVA matrix.

MONT is one of the widely used silicate minerals for the preparation of polymer/mineral composites. MONT belongs to a class of natural bentonite clay and has some reactive hydroxyl groups on its surface [13]. During the last decade, polymer/clay nanocom-

Abbreviations: PVA, polyvinyl alcohol; MONT, montmorillonite; DDAB, dimethyldioctadecylammonium bromide; ADH, alcohol dehydrogenase; ee, enantiomeric excess; COBE, ethyl 4-chloro-3-oxobutanoate; (*S*)-CHBE, ethyl (*S*)-4-chloro-3-hydroxybutanoate; (*R*)-CHBE, ethyl (*R*)-4-chloro-3-hydroxybutanoate; NAD, nicotinamide adenine dinucleotide; CTAB, cetyltrimethylammonium bromide.

* Corresponding author. Tel.: +86 23 68485077; fax: +86 23 68485161.
E-mail address: minganyu666@yahoo.com.cn (M.-A. Yu).

1359-5113/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.procbio.2010.05.021

posites have attracted a lot of attention because they frequently exhibit unexpected properties. Polymer-layered silicate nanocomposites, which are representative of these materials, consist of 1-nm thick aluminosilicate layers dispersed in a polymer matrix. These nanometric fillers can greatly improve the thermomechanical and barrier properties of polymers with low clay content in comparison with unfilled polymers or more conventional microcomposites [14,15]. It has been found that when the silicate layers are exfoliated (i.e., individually dispersed) in the polymer matrix, maximum enhancement of the properties is attained [16]. Moreover, surfactants can also bind to the clay mineral, and the surface of the clay mineral becomes more hydrophobic [13,17].

In this paper, we first report the effects of the addition of MONT and DDAB into the PVA matrix on the immobilization of permeabilized brewer's yeast cells, including changes in the intracellular ADH activity and in the diffusion capacity of hydrophilic and hydrophobic molecules. Reusability and storage stability of the immobilized cells and the bioreduction of ethyl 4-chloro-3-oxobutanoate (COBE) to ethyl (*S*)-4-chloro-3-hydroxybutanoate ((*S*)-CHBE) by the immobilized cells were also investigated in a batch reaction before and after the addition of MONT and DDAB into the PVA matrix.

2. Materials and methods

2.1. Chemicals

Polyvinyl alcohol with an average degree of polymerization of 1799 ± 50 was purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Montmorillonite was supplied by Fenghong Clay Chemicals Co. Ltd. (Zhejiang, China). Lactose and L-glycine were purchased from Beijing Dingguo Biotechnology Co. Ltd. (Beijing, China). Dimethyldioctadecylammonium bromide, NAD^+, and ethyl 4-chloro-3-hydroxybutanoate [(*R*)- and (*S*)-enantiomers] were purchased from Sigma–Aldrich (St. Louis, MO, USA). Separated fresh brewer's yeast cells (a strain of *Saccharomyces cerevisiae*) were obtained from Chong Qing Beer Group Ltd. (Chongqing, China). All other chemicals used were of reagent grade except where noted.

2.2. Permeabilization of fresh brewer's yeast cells

Fresh brewer's yeast cells were separated by centrifugation from fresh brewer's yeast slurry, which was a by-product from the brewery with a solid content of about 20% and received after beer production from the Chongqing Beer Group Co. Ltd. (Chongqing, China). Fresh brewer's yeast cells were harvested by centrifugation at 2016 × *g* for 10 min. The yeast pellet was again diluted 2–3 times with 0–2 °C saline, screened on a sieve shaker equipped with a 100 mesh screen and centrifuged at 5600 × *g* for 20 min to obtain the separated fresh brewer's yeast cells. Dead and live cells were then examined according to our previous report [4]. The percentage of live cells is represented as the average of these three determinations.

When the total number of live cells reached 95%, the cells were permeabilized with cetyltrimethylammonium bromide (CTAB) according to the previously described method [18]. About 2 g of wet yeast cells was uniformly suspended in 10 ml of permeabilization solution containing 0.2% CTAB (w/v) in 0.1 M sodium phosphate buffer, pH 7.5. The yeast cells were kept in this solution for 15 min at 24 °C with intermittent shaking. The cells were then separated by centrifugation at 5600 × *g* for 10 min. The yeast pellet was washed twice with the above-mentioned sodium phosphate buffer. After centrifuging and washing, the permeabilized brewer's yeast cells were stored at 4 °C for further use.

2.3. Preparation of modified composite PVA gel matrix

PVA (15 g) and MONT (0.5 g) were added to water (100 ml) with stirring, and the mixture was heated to 95 °C for 3 h until the PVA particles were completely dissolved. The suspension was then equally divided into five aliquots (20 ml each), each of which was mixed with a certain amount of DDAB (0.05%, 0.1%, 0.2%, 0.3% and 0.5%, w/v) with mild stirring for 5 min. After homogenization for 100 min at 80 °C, the temperature of these modified composite PVA gel matrices was allowed to decrease to 25 °C for further use.

2.4. Immobilization of permeabilized brewer's yeast cells into modified composite PVA lens-shaped capsules

The immobilized cells were prepared as described previously with some modifications [11]. Briefly, about 2 g of permeabilized brewer's yeast cells (wet weight) were added to 10 ml of modified composite PVA gel matrix. The resulting mixture was dropped on a smooth polystyrene plate using a 9-gauge needle syringe (the distance between the needle and the plate was 2 cm) and the plate was immersed in a 2% (w/v) boric acid solution for 20 min. The plate was then removed from the solution and left at room temperature for 90 min to allow the gelation process to be completed. The new modified composite PVA lens-shaped capsules loaded with permeabilized brewer's yeast cells were transferred to 100 mM sodium sulfate for re-swelling. After 12 h, this solution was replaced with 50 mM Na_2HPO_4/KH_2PO_4 buffer, pH 7.5. The capsules were stored in this buffer at 4 °C. Test groups A, B, C, D and E are the new modified composite PVA lens-shaped capsules loaded with permeabilized brewer's yeast cells and contain different amounts of DDAB (0.05%, 0.1%, 0.2%, 0.3% and 0.5% (w/v), respectively). The capsules of control group F were prepared following the method described above except that the modified composite PVA gel matrix was replaced with PVA alone as the immobilization matrix for cells.

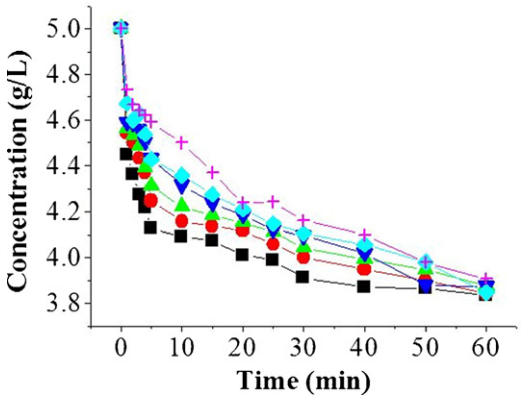

Fig. 1. Remaining lactose in an aqueous solution after lactose from bulk solution diffuses into the PVA capsules. Unmodified PVA capsules (■). Modified composite PVA capsules containing DDAB: 0.05% (w/v) (●); 0.1% (w/v) (▲); 0.2% (w/v) (▼); 0.3% (w/v) (◆); 0.5% (w/v) (+).

2.5. Diffusion capabilities of hydrophilic and hydrophobic molecules through the lens capsule

The diffusion capabilities of hydrophilic (lactose and L-glycine) and hydrophobic (benzyl benzoate) molecules from bulk solution into capsule A, B, C, D, E or F were measured in a conical flask, which was placed in an orbital water bath shaker at 30 °C and shaken at 125 rpm. A 50 ml aqueous solution of 0.5% (w/v) lactose or 0.02% (w/v) L-glycine or an isopropanol solution (isopropanol:water = 1:3) of 0.4% (w/v) benzyl benzoate was added to the flask and a definite amount of capsule A, B, C, D, E or F was added to the solution. The solute concentration in the solution would decrease over time because the solute would diffuse from the solution into the capsules. By measuring the change in concentration over time, the diffusion capacity can be determined. During the initial 5 min, samples from the bulk solutions were taken every 1 min. The time intervals were subsequently increased to 5 min and 10 min between 5–30 min and 30–60 min, respectively. After enough time, the concentration in the bulk solution and the concentration inside capsule A, B, C, D, E or F would be close to equilibrium. All the experiments were carried out in triplicate and the mean values were shown in the corresponding Figs. 1–3.

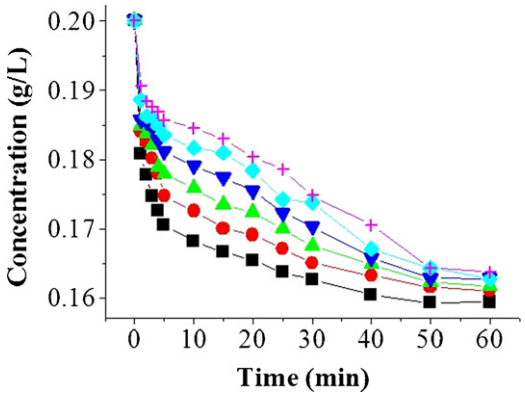

Fig. 2. Remaining L-glycine in an aqueous solution after L-glycine from bulk solution diffuses into the PVA capsules. Unmodified PVA capsules (■). Modified composite PVA capsules containing DDAB: 0.05% (w/v) (●); 0.1% (w/v) (▲); 0.2% (w/v) (▼); 0.3% (w/v) (◆); 0.5% (w/v) (+).

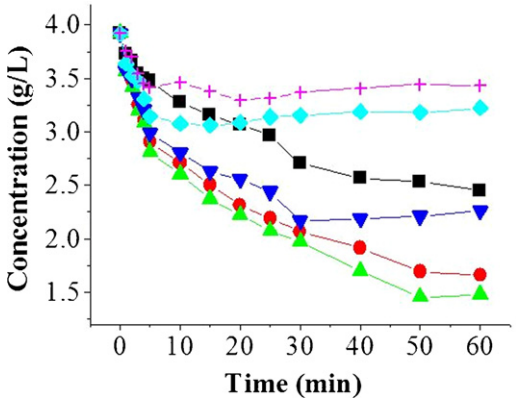

Fig. 3. Remaining benzyl benzoate in a water/isopropanol system after benzyl benzoate from bulk solution diffuses into the PVA capsules. Unmodified PVA capsules (■). Modified composite PVA capsules containing DDAB: 0.05% (w/v) (●); 0.1% (w/v) (▲); 0.2% (w/v) (▼); 0.3% (w/v) (◆); 0.5% (w/v) (+).

2.6. Effects of pH and temperature on intracellular ADH activity

To determine the effect of the change in pH on the ADH activity of free or immobilized cells, the free or immobilized cells were uniformly suspended in 0.1 M sodium phosphate buffer (pH 5.0–10.0) and incubated at 30 °C for 30 min. The effect of temperature on the ADH activity of free or immobilized cells was determined by varying the incubation temperature of the suspension from 10 °C to 50 °C at pH 7.5 for 30 min. The ADH activity of each sample was determined at least three times. The relative ADH activity was calculated as a percentage of its maximum activity.

2.7. Bioconversion of COBE to (S)-CHBE by capsule-immobilized cells

An appropriate amount of capsule-immobilized cells with modified composite PVA or unmodified PVA was added to reaction flasks containing 30 ml of 0.1 M phosphate buffer, pH 7.5; $MgCl_2$ and NAD^+ were added to final concentrations of 15 mM, and 0.1 M, respectively. Next, 2.5 ml of 2-propanol was added, followed by dilution with redistilled water to the final volume of 37.5 ml. The reaction was started by 12.5 ml of COBE/*n*-butyl acetate (COBE:*n*-butyl acetate = 1:3 v/v), which was continuously fed into the stirred suspension at a rate of 5 drops/min. Flasks were incubated in a 30 °C water bath for 8 h with continuous shaking at 125 rpm. The concentration of COBE, ethyl (*R*)-4-chloro-3-hydroxybutanoate ((*R*)-CHBE)) and (*S*)-CHBE were measured by gas chromatography (GC) at 1-h intervals as described in Section 2.10.3.

2.8. Reusability

To characterize the reusability of the immobilized cells, appropriate amounts of the capsule-immobilized cells with modified composite PVA or free cells were added to the reaction system as described above. The biocatalyst was harvested after the reaction and washed with 50 mM phosphate buffer, pH 7.5; this process was repeated 20 times. After each cycle, the ADH activities of the immobilized and free cells were determined. The residual ADH activity (relative activity) was calculated as a percentage of its initial activity.

2.9. Storage stability

The capsule-immobilized cells with modified composite PVA and free cells were stored in 50 mM Na_2HPO_4/KH_2PO_4 buffer, pH 7.5 at 4 °C. The storage stability was evaluated by determining the ADH activity at regular time intervals up to 1 month. The residual ADH activity (relative activity) was calculated as a percentage of its initial activity.

2.10. Analytical methods

2.10.1. Concentration analysis of hydrophilic and hydrophobic molecules

The concentration of lactose was determined by the colorimetric method (UV-7504, XinMao) of dinitrosalicylic acid (DNS) [19]. The absorbance was measured at 515 nm after cooling to room temperature. The concentration of L-glycine was measured by the ninhydrin method [20] using UV at 570 nm. The concentration of benzyl benzoate, which was diluted in 95% ethanol, was tested by UV at 260 nm. All data were presented as the mean values of triplicate experiments.

2.10.2. Enzyme assay

The following method was developed to prepare samples for the determination of the intracellular levels of ADH activity in the immobilized yeast cells. Approximately 100 mg of immobilized cell capsules were thoroughly chopped with a sharp razor blade in a Petri dish containing 5 ml of 0.1 M sodium phosphate buffer, pH 7.5. Subsequently, the suspension of immobilized cells was homogenized by grinding slightly with a mortar and pestle. Finally, the suspension of immobilized cells (the capsule concentration: 0.02 g/ml) was obtained (Sample 1). As a comparison group, free yeast cells were suspended in 0.1 M sodium phosphate buffer, pH 7.5 at a cell concentration of 0.2 g/ml (Sample 2). The ADH activity of the two groups of cells was determined using a previously described method [21]. Generally, 1.5 ml of 0.1 M sodium pyrophosphate buffer, 0.5 ml of 2 M ethanol and 1 ml of 20 mM NAD^+ solution were mixed in a test tube and then added to Sample 1 (0.1 ml) or Sample 2 (0.1 ml). The ADH activity was determined by measuring the steady state increase in the rate of absorbance change at 340 nm that results from the reduction of NAD^+. One unit of ADH activity was defined as the amount of enzyme required to reduce 1 μmol of NAD^+ per min at 25 °C and pH 7.5.

2.10.3. Analysis of reactant and products

At the end of the reaction, experimental procedures were carried out as previously described with some modifications [4]. Briefly, the capsule-immobilized cells separated from the reaction mixture described above were washed with deionized water. The remaining reaction mixture was centrifuged at 4160 × *g* for 15 min at 4 °C. The combined supernatant and filtrate were extracted twice with ethyl acetate. The combined organic layer was washed first with saturated sodium bicarbonate and then with saturated brine, dried over anhydrous magnesium sulfate and filtered. The concentrations of COBE, (*R*)-CHBE and (*S*)-CHBE were determined on a Varian 3800 GC equipped with a FID detector (Varian, Darmstadt, Germany). A Chiraldex-GTA capillary column was used (20 m, 0.25-mm i.d.; Advanced Separation Technologies Inc. (Astec), Whippany, New Jersey, United States). Acetophenone was used as an internal standard.

3. Results and discussion

3.1. Diffusion capabilities of hydrophilic and hydrophobic molecules through the lens capsule

The diffusion capabilities of different solutes from bulk solution into the PVA capsule-immobilized cells are all shown in Figs. 1–3. These solutes of low molecular weight are either hydrophilic or hydrophobic and include lactose (Fig. 1), L-glycine (Fig. 2), and benzyl benzoate (Fig. 3). All data were measured at least three times and the average values are shown in the figures.

First, we looked at the diffusion capabilities of hydrophilic molecules in the aqueous system. As shown in Figs. 1 and 2, during the initial 5 min the diffusion of lactose and L-glycine from bulk solution into the unmodified PVA capsules was the fastest and diffusion into the modified composite (containing 0.5% (w/v) DDAB) PVA capsules was the slowest. Moreover, there is a drastic and orderly reduction in the rate of penetration of the test molecules with increasing concentrations of DDAB. This decreasing trend gradually slowed over time and was close to equilibrium after 60 min. It can be observed that when compared with the unmodified PVA capsules, the modified composite PVA capsules were hydrophobic in the aqueous system. In water–isopropanol systems, the MONT–DDAB fillers can greatly improve the diffusion capability of benzyl benzoate from bulk solution into PVA capsules with low DDAB content (0.05% and 0.1%, w/v) when compared with unfilled PVA capsules. Surprisingly, the diffusion capability was sharply decreased when the DDAB content was increased up to 0.3% (w/v) and beyond (Fig. 3). The combination of these results indicates that small amounts of cationic surfactants bind to the clay mineral. The interface of the clay mineral becomes more hydrophobic and binding of the PVA to the clay mineral is strengthened. The materials become amphiphilic as a result of their architecture, i.e., the chemical constitution resulting from the balance between hydrophilic and hydrophobic segments or groups in the MONT and DDAB modified composite PVA capsules, which may provide a more conducive environment for the desired reaction. With the increasing concentration of the surfactant in the modified composite PVA capsules, the surfactant molecules bind to PVA and PVA becomes hydrophilic. These results are in strong agreement with previous results [13,17]. Therefore, in a water organic two-phase system (water–isopropanol system), the modified composite PVA capsules

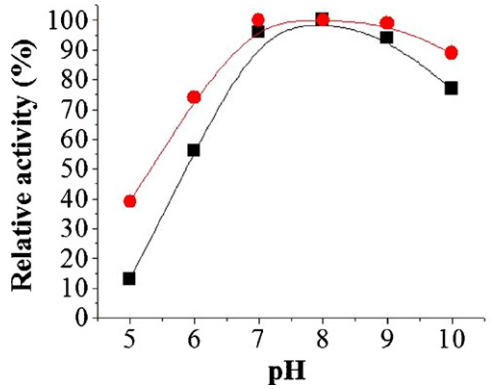

Fig. 4. Effect of pH on the relative ADH activity of free (■) and immobilized (●) cells.

containing 0.05% (w/v) DDAB are the most appropriate choice for the diffusion of hydrophilic and hydrophobic molecules.

3.2. Effects of pH and temperature on intracellular ADH activity

The results on the effect of pH on the ADH activity of free and immobilized cells are given in Fig. 4. The relative ADH activities of both cells showed obvious differences in pH dependence. The maximum ADH activity of free cells was observed at pH 8.0 and of immobilized cells was observed at pH 7.0–8.0. Furthermore, the retained relative ADH activity of the immobilized cells was higher both at lower and higher pH in comparison to that of the free cells. The results indicate that immobilization methods preserved the intracellular ADH activity over a wider pH range. The results on the effect of temperature on the ADH activity of free and immobilized cells are shown in Fig. 5. The free and immobilized cells exhibited similar temperature profiles and the maximum ADH activity of both cells was observed at 30 °C. At higher temperatures, however, immobilized cells were more active than the free cells. The immobilized cells showed essentially the same ADH activities over the range of 30–40 °C and started to sharply decline at 45 °C.

3.3. Bioconversion of COBE to (S)-CHBE with capsule-immobilized cells as a biocatalyst

The bioreduction of COBE to give the corresponding product (*S*)-CHBE is a very interesting reaction because the product is known to be a key intermediate in the synthesis of inhibitors of HMG-CoA reductase [22]. As shown in Fig. 6, when using unmodified

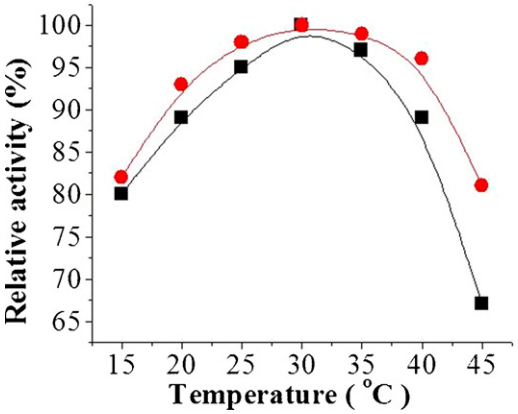

Fig. 5. Effect of temperature on the relative ADH activity of free (■) and immobilized (●) cells.

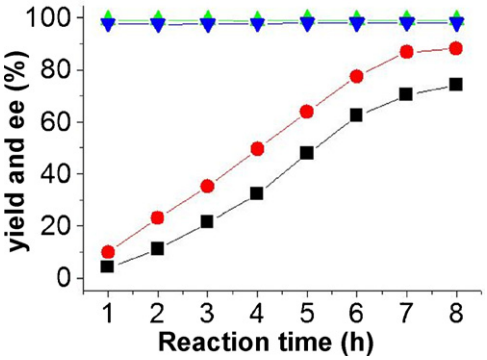

Fig. 6. Reaction time course of the bioconversion of COBE to (*S*)-CHBE by immobilized cells. The ee (▲) and yield (●) of modified composite PVA capsules and the ee (▼) and yield (■) of unmodified PVA capsules.

PVA capsules or modified composite PVA capsules as the biocatalyst in an organic solvent–water biphasic reaction system at pH 7.5 and 30 °C with continuous shaking at 125 rpm, the latter gave much higher yield of (*S*)-CHBE than former after reaction for 8 h (88% versus 74%). Additionally, the product ee was higher when the reaction was performed with modified composite PVA capsules (99.1% versus 98.2%). These results suggests faster diffusion of substrate from bulk solution into the modified composite PVA capsules and more interaction with the intracellular ADH in the immobilized cells compared to that in unmodified PVA capsules. This finding is consistent with the results shown in Figs. 1–3.

3.4. Reusability

The stability of a catalyst is an important feature for its potential application in industry. As shown in Fig. 7, the immobilized cells retained over 88% of their initial ADH activity after 20 cycles. However, the ADH activity of free cells decreased significantly and there was almost no activity after six cycles. This result is similar to the data in the literature [3]. The results indicated that the immobilized cells in the modified composite PVA capsules displayed good reusability.

3.5. Storage stability

Storage stability of a catalyst is also very important for industrial applications. Time courses of the retained activities of immobilized and free cells after a long period of storage at 4 °C are presented in Fig. 8. The experimental results indicate that the ADH activity of immobilized cells decreased more slowly than that of free

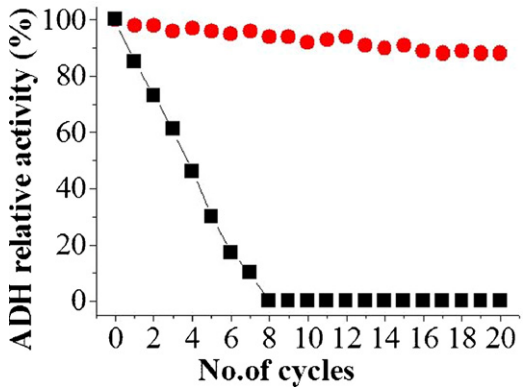

Fig. 7. Effect of reusability on the relative ADH activity of free (■) and immobilized (●) cells.

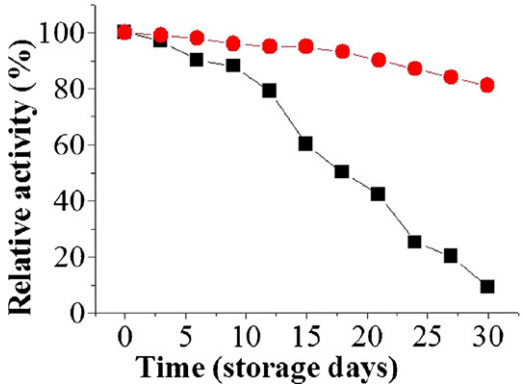

Fig. 8. Effect of storage time on the relative ADH activity of free (■) and immobilized (●) cells.

cells. After 13 days, the ADH activity of free cells decreased significantly and there was almost no activity after 30 days. This result is the same as that obtained in our previous report [5]. However, the ADH activity of immobilized cells retained 81% of its original activity after 30 days, which may be a potential benefit if used commercially. The results indicated that the immobilized cells demonstrated good storage stability.

4. Conclusion

A new approach for the immobilization of permeabilized brewer's yeast cells in modified composite PVA capsules was presented. An attractive feature of this method is that it is readily available and very easy to handle. More importantly, it showed significant advantages over the use of unmodified PVA gel with regard to the diffusion capabilities of hydrophobic molecules. Therefore, it may provide a more conducive environment for the desired reaction. Additionally, the permeabilized brewer's yeast cells immobilized in new modified composite PVA capsules not only exhibited good storage stability and reusability, but also could be successfully employed for the bioreduction of COBE to (*S*)-CHBE with high optical purity. The combination of these results indicates that the immobilization of permeabilized brewer's yeast cells into modified composite PVA capsules would probably be a more promising strategy for the synthesis of (*S*)-configured chiral alcohols.

Acknowledgements

This work was supported by the special funding of three items of expenditure in the Science and Technology Department of the Central District in Chongqing. We also thank the Chongqing Medical University for partial financial support of this work.

References

[1] Goldberg K, Schroer K, Lütz S, Liese A. Biocatalytic ketone reduction—a powerful tool for the production of chiral alcohols—part II: whole-cell reductions. Appl Microbiol Biotechnol 2007;76:249–55.
[2] Zhang J, Witholt B, Li Z. Efficient NADPH recycling in enantioselective bioreduction of a ketone with permeabilized cells of a microorganism containing a ketoreductase and a glucose 6-phosphate dehydrogenase. Adv Synth Catal 2006;348:429–33.
[3] Zhang J, Witholt B, Li Z. Coupling of permeabilized microorganisms for efficient enantioselective reduction of ketone with cofactor recycling. Chem Commun 2006;4:398–400.
[4] Yu MA, Wei YM, Jiang L, Zhu XB, Qi W. Bioconversion of ethyl 4-chloro-3-oxobutanoate by permeabilized fresh brewer's yeast cells in the presence of allyl bromide. J Ind Microbiol Biotechnol 2007;34:151–6.
[5] Yu MA, Hou Y, Gong GH, Zhao Q, Zhu XB, Jiang L, et al. Effects of industrial storage on the bioreduction capacity of brewer's yeast. J Ind Microbiol Biotechnol 2009;36:157–62.
[6] Goldberg K, Schroer K, Lütz S, Liese A. Biocatalytic ketone reduction—a powerful tool for the production of chiral alcohols—part I: processes with isolated enzymes. Appl Microbiol Biotechnol 2007;76:237–48.
[7] Ha J, Engler CR, Lee SJ. Determination of diffusion coefficients and diffusion characteristics for chlorferon and diethylthiophosphate in Ca-alginate gel beads. Biotechnol Bioeng 2008;100:698–706.
[8] Jekel M, Buhr A, Willke T, Vorlop KD. Immobilization of biocatalysts in LentiKats®. Chem Eng Technol 1998;21:275–8.
[9] Parascandola P, Branduardi P, De Alteriis E. PVA-gel (Lentikats®) as an effective matrix for yeast strain immobilization aimed at heterologous protein production. Enzyme Microb Technol 2006;38:184–9.
[10] Bezbradica D, Obradovic B, Leskosek-Cukalovic I, Bugarski B, Nedovic V. Immobilization of yeast cells in PVA particles for beer fermentation. Proc Biochem 2007;42:1348–51.
[11] Kubáč D, Čejková A, Masák J, Jirků V, Lemaire M, Galliene E, et al. Biotransformation of nitriles by Rhodococcus equi A4 immobilized in LentiKats®. J Mol Catal B: Enzym 2006;39:59–61.
[12] Li PC, Li D, Zhang LX, Li GP, Wang EK. Cationic lipid bilayer coated gold nanoparticles-mediated transfection of mammalian cells. Biomaterial 2008;29:3617–24.
[13] Liu J, Hoffmann H. Hydrogels in aqueous phases of polyvinyl alcohol (PVA), surfactants and clay minerals. Colloid Polym Sci 2004;283:24–32.
[14] Kokabi M, Sirousazar M, Hassan ZM. PVA–clay nanocomposite hydrogels for wound dressing. Eur Polym J 2007;43:773–81.
[15] Harrane A, Belbachir M. Synthesis of biodegradable polycaprolactone/montmorillonite nanocomposites by direct in situ polymerization catalysed by exchanged clay. Macromol Symp 2007;247:379–84.
[16] Solar L, Nohales A, Muñoz-Espí R, López D, Gómez CM. Viscoelastic behavior of epoxy prepolymer/organophilic montmorillonite dispersions. J Polym Sci Part B: Polym Phys 2008;46:1837–44.
[17] Williams-Daryn S, Thomas RK, Castro MA, Becerro A. The structures of complexes of a vermiculite intercalated by cationic surfactants, a mixture of cationic surfactants, and a mixture of cationic and nonionic surfactants. J Colloid Interface Sci 2002;256:314–24.
[18] Gowda LR, Bachhawat N, Bhat SG. Permeabilization of Bakers' yeast by cetyltrimethylammonium bromide for intracellular enzyme catalysis. Enzyme Microbiol Technol 1991;13:154–7.
[19] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for the determination of sugars and related substances. Anal Chem 1956;28:350–6.
[20] Starcher B. A ninhydrin-based assay to quantitate the total protein content of tissue samples. Anal Biochem 2001;292:125–9.
[21] Vallee BL, Hoch FL, Zinc A. A component of yeast alcohol dehydrogenase. Proc Natl Acad Sci USA 1955;41:327–38.
[22] Karanewsky DS, Badia MC, Ciosek Jr CP, Robl JA, Sofia MJ, Simpkins LM, et al. Phosphorus-containing inhibitors of HMG-CoA reductase I 4-[(2-arylethyl) hydroxyphosphinyl]-3-hydroxybutanoic acids: a new class of cell selective inhibitors of cholesterol biosynthesis. J Med Chem 1990;33:2952–6.