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ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 54 (2008) 122–129
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Efficient synthesis of enantiopure (*S*)-4-(trimethylsilyl)-3-butyn-2-ol via asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one with immobilized *Candida parapsilosis* CCTCC M203011 cells

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Received 9 November 2007; received in revised form 19 December 2007; accepted 19 December 2007
Available online 31 December 2007

Abstract

In this paper, we show the substrate 4-(trimethylsilyl)-3-butyn-2-one is unstable, and can be easily cleaved into a carbonyl alkyne and trimethylhydroxysilane in aqueous buffer with pH above 6.0. However, this problem could be effectively solved by lowering the buffer pH. Meanwhile, the efficient synthesis of enantiopure (*S*)-4-(trimethylsilyl)-3-butyn-2-ol, a key intermediate for preparing a 5-lipoxygenase inhibitor, has been successfully conducted through the asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one with immobilized *Candida parapsilosis* CCTCC M203011 cells. For optimization of the reaction, various influential variables, such as buffer pH, co-substrate concentration, reaction temperature and substrate concentration, were systematically examined. All the factors mentioned above had effect on the reaction to some extent. The optimal buffer pH, co-substrate concentration, reaction temperature and substrate concentration were 5.0, 65.3 mM, 30 °C and 3.0 mM, respectively, under which the maximum yield and product *e.e.* were as high as 81.3% and >99.9% after a reaction time of 1 h, which are much higher than the corresponding values previously reported.

Keywords: Biocatalytic synthesis; Asymmetric reduction; *Candida parapsilosis* CCTCC M203011; 4-(Trimethylsilyl)-3-butyn-2-one

1. Introduction

Organosilicon compounds are non-natural compounds with carbon–silicon bonds which endow them with some unique chemical and physical characteristics compared to conventional organic compounds. Enantiomerically pure organosilicon compounds not only play an important part in asymmetric synthesis and functional materials, but also many of them are bioactive [1,2] and can be applied as silicon-containing drugs, such as Zifrosilone [3], Cisobitan [4] and TAC-101{4-[3,5-bis(trimethylsilyl)benzamido]benzoic acid} [5], with greater pharmaceutical activity, higher selectivity and lower toxicity than their carbon counterparts, resulting from the particular properties of the silicon atom, such as its larger atomic radius and smaller electronegativity compared to the carbon atom. Thus, the replacement of certain specific carbon atoms in drugs by silicon seems to be a useful and efficient strategy in drug design, and should be regarded as a complementary tool in the development of new drugs [6]. As a result, the preparation of organosilicon compounds, particularly enantiopure silicon-containing compounds is becoming more and more important. In recent years, many investigations on enzymatic and microbial synthesis of silicon-containing amino acids and peptides [1,7], alcohols [8–11] and cyanohydrins [12–14] have been carried out because of their great importance in the fundamental study of enzymology and the industrial production of useful organosilicon compounds. In particular, the biocatalytic asymmetric reduction of silicon-containing ketones to enantiopure organosilyl alcohols (the silicon counterparts of chiral, enantiopure alcohols, which are key synthons for a large number of pharmaceuticals) [15–20] has been the goal of extensive studies where both isolated dehydrogenases and whole cells have been employed as the biocatalysts. For example, horse liver alcohol dehydrogenase [21], *Rhodotorula* sp. AS2.2241 cells [11] and *Saccharomyces cerevisiae* cells [22] were successfully used as biocatalysts in our previous work for the synthesis of enantiomerically pure (*S*)-1-trimethylsilylethanol. In comparison with isolated enzymes, whole microbial cells are preferred

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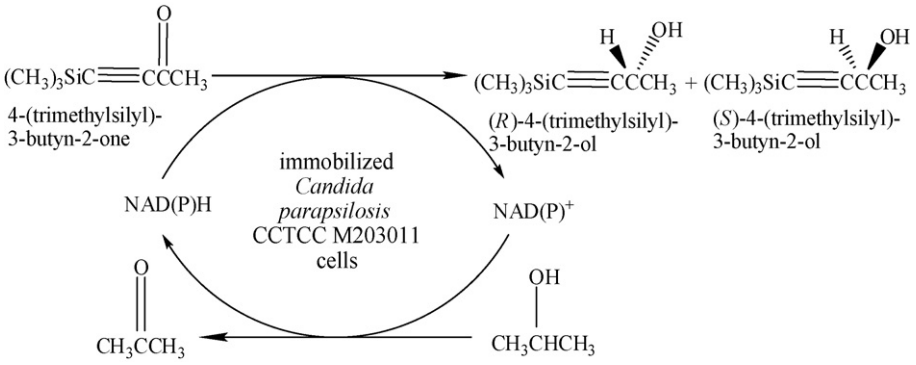
doi:10.1016/j.molcatb.2007.12.018



Scheme 1. The asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one catalyzed by immobilized *C. parapsilosis* CCTCC M203011 cells.

to avoid the need for enzyme purification and addition or complicated extra regeneration of the coenzyme. Among various microorganisms, the tremendous potential of the yeast cell as a practical biocatalyst has been well recognized owing to its high bioavailability, ease of use, low environmental pollution, cost-effectiveness, high efficiency and mild reaction conditions [23].

In the present paper, we, for the first time, describe the efficient synthesis of enantiopure (*S*)-4-(trimethylsilyl)-3-butyn-2-ol, which is a crucial intermediate for the synthesis of 5-lipoxygenase inhibitors [24] through asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one with immobilized *Candida parapsilosis* CCTCC M203011 cells (Scheme 1), which is a newly isolated yeast strain and is capable of catalyzing asymmetric reductions of prochiral ketones with high enantioselectivity [25]. 2-Propanol here acts as the co-substrate for coenzyme regeneration. 4-(Trimethylsilyl)-3-butyn-2-one is reduced to enantiopure (*S*)-4-(trimethylsilyl)-3-butyn-2-ol while converting NAD(P)H to $NAD(P)^+$. 2-Propanol is simultaneously oxidized to acetone, regenerating NAD(P)H from $NAD(P)^+$. To the best of our knowledge, only one attempt has been made so far to carry out the biocatalytic asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one to (*S*)-4-(trimethylsilyl)-3-butyn-2-ol, where the isolated carbonyl reductase was employed as the biocatalyst with disappointingly low yield (78%) and product *e.e*. (57%) [26].

2. Experimental

2.1. Biological and chemical materials

C. parapsilosis CCTCC M203011 was kindly provided by Professor Xu Yan, Key Laboratory of Industrial Biotechnology, Ministry of Education and School of Biotechnology, Southern Yangtze University (China). *Rhodotorula* sp. AS2.2241 was kindly supplied by Professor Xu Jian-He, the State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology (China). Other strains (*Candida tropicalis*, *S. cerevisiae*, *Trigonopsis variabilis*, *Lactobacillus brevis*, *Bacterium anthracoides*, *Geotrichum candidum*) used in this work were from the collection of the Division of Industrial Microbiology, College of Biological Sciences & Biotechnology, South China University of Technology (China).

4-(Trimethylsilyl)-3-butyn-2-one (97% purity), 4-(trimethylsilyl)-3-butyn-2-ol (97% purity) and *n*-decane (>99% purity) were purchased from Sigma–Aldrich (USA). All other chemicals were from commercial sources and were of analytical grade.

2.2. Cultivation of C. parapsilosis CCTCC M203011 cells

C. parapsilosis CCTCC M203011 cells were cultivated in medium containing 4% (w/v) glucose, 0.3% (w/v) yeast extract, and 10% (v/v) mineral solution (pH 7.0). The mineral solution consists of 13% (w/v) $(NH_4)_2HPO_4$, 7% (w/v) KH_2PO_4, 0.8% (w/v) $MgSO_4{\cdot}7H_2O$, and 0.1% (w/v) NaCl. A pre-culture was prepared by inoculation of 100 mL of the complex medium with fresh cells from an agar plate culture. Incubation was performed in a 500 mL Erlenmeyer flask, which was shaken at 150 r m^{-1} and 30 °C. After 24 h incubation the cells were in the exponential growth phase and were harvested by centrifugation (3500 r m^{-1}, 15 min), washed twice with distilled water, and separated from the aqueous medium by centrifugation to give a cell wet mass (cwm) of 12–15 g per 500 mL batch. The wet cells were immobilized on calcium alginate as described below.

2.3. Immobilization of C. parapsilosis CCTCC M203011 cells

A homogenous cell/sodium alginate suspension was prepared at 25 °C by adding 30 g of a suspension containing fresh cells (15 g wet cells in 15 mL water) into 60 mL of a homogeneous aqueous sodium alginate solution (2%, w/v), which was prepared by dissolving sodium alginate in deionized water, heating and stirring vigorously. The suspension was added dropwise by means of an injector to a gently stirred $CaCl_2$ solution (2%, w/v), where the calcium alginate pearls with a load of 31% (w/w) of *C. parapsilosis* CCTCC M203011 cells (based on cwm) precipitated. The pinhole size of injector and the drop rate were adjusted in such a way that the diameter of the pearls was around 1 mm. The pearls were kept in the $CaCl_2$ solution for another 1 h at 25 °C and collected by filtration, washed with water and resuspended in an aqueous solution of 20% (w/v) glucose, 0.9%

(w/v) NaCl and 0.05% (w/v) $CaCl_2$. They were stored at 4 °C for later use.

2.4. General procedure for asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one with immobilized C. parapsilosis CCTCC M203011 cells

In a typical experiment, 4.0 mL of TEA–HCl buffer (100 mM) containing 0.15 g mL^{-1} immobilized *C. parapsilosis* CCTCC M203011 cells and a fixed quantity of co-substrate was added to a 20-mL Erlenmeyer flask capped with a septum, and was pre-incubated in a water-bath shaker at 180 r m^{-1} and an appropriate temperature for 15 min. Then, the reaction was initiated by adding a fixed amount of 4-(trimethylsilyl)-3-butyn-2-one to the mixture. Aliquots (20 μL) were withdrawn at specified time intervals from the mixture system. The product and residual substrate were extracted with *n*-hexane (100 μL) containing 5.1 mM *n*-decane (internal standard) prior to GC analysis. Details about substrate concentration, co-substrate concentration, buffer pH and reaction temperature are specified for each case.

2.5. GC analysis

The reaction mixtures were analyzed by a Shimadzu GC2010 model with a flame ionization detector and a chiral column (20% permethylated β-cyclodextrin 30 m × 0.25 mm × 0.25 μm) from Hewlett Packard (USA). The split ratio was 100:1. The injector and the detector were both kept at 250 °C. The column temperature was held at 85 °C constant for 13 min. The carrier gas was nitrogen and its flow rate in the column was 1.2 mL min^{-1}. The retention-times for 4-(trimethylsilyl)-3-butyn-2-one, *n*-decane and 4-(trimethylsilyl)-3-butyn-2-ol were 5.1, 5.7, and 10.5 min, respectively.

The $[\alpha]_D^{20}$ of the reaction product, which was measured by a PerkinElmer 241 polarimeter, was compared with the $[\alpha]_D^{20}$ (−22.3°, *c* = 2.55 g/100 mL, $CHCl_3$) of enantiomerically pure (*S*)-4-(trimethylsilyl)-3-butyn-2-ol and the product was confirmed to be (*S*)-4-(trimethylsilyl)-3-butyn-2-ol as indicated by the optical rotation. The product *e.e.* was determined after the esterification of 4-(trimethylsilyl)-3-butyn-2-ol with acetic anhydride. Under the GC analysis described above, the retention-times for (*S*)-1-(trimethylsilyl)-3-acetyl-1-butyn and (*R*)-1-(trimethylsilyl)-3-acetyl-1-butyn were 11.7 and 12.0 min, respectively.

Quantitative data were obtained after integration on a Shimadzu GC integrator. An internal standard method was used for the calculations. The average error for this determination was less than 1.0%. All reported data are averages of experiments performed at least in duplicate.

2.6. GCMS analysis of trimethylhydroxysilane

GCMS: Agilent 6890 series GC system fitted with a Agilent 5975 mass selective detector (EI, 70 eV) and a DB-FFAP column (30 m × 250 μm), [T_{GC} (injector) = 250 °C, time programme (oven): $T_{0\,min}$ = 50 °C, $T_{8\,min}$ = 170 °C (15 °C/min)]; R_t = 4.8 min: *m/z* (%) = 75(100) [$M^+ - CH_3$], 60(1) [$M^+ - CH_3$–CH_3], 45(12) [$M^+ - CH_3$–CH_3–CH_3].

3. Results and discussion

3.1. Strains screening

Various microorganisms, including *C. parapsilosis*, *S. cerevisiae*, *L. brevis*, *G. candidum*, etc., have been found to possess an excellent ability to synthesize enantiomerically pure alcohols via asymmetric reduction of the corresponding prochiral ketones [27,28]. Therefore, 8 potential strains, including yeast (*C. parapsilosis* CCTCC M203011, *Rhodotorula* sp. AS2.2241, *C. tropicalis*, *S. cerevisiae*, *T. variabilis*), bacteria (*L. brevis*, *B. anthracoides*) and mold (*G. candidum*), were initially screened for promising strains for the preparation of enantiopure 4-(trimethylsilyl)-3-butyn-2-ol via asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one in triethanolamine (TEA)–HCl buffer (100 mM, pH 7.0). As shown in Table 1, only 3 strains, *C. parapsilosis* CCTCC M203011, *Rhodotorula* sp. AS2.2241 and *G. candidum*, were found to be capable of producing 4-(trimethylsilyl)-3-butyn-2-ol, among which *C. parapsilosis* CCTCC M203011 showed to be the best one with respect

Table 1
The screening of strains used for the reduction of 4-(trimethylsilyl)-3-butyn-2-one at pH 7.0

Strains	Conversion (%)	Yield (%)[a]	*e.e.* (%)[b]	Configuration[c]
Candida parapsilosis CCTCC M203011	>99.9	55.7	>99.9	*S*
Rhodotorula sp. AS2.2241	>99.9	13.8	>99.9	*S*
Candida tropicalis	>99.9	n.d.[d]	n.d.	n.d.
Saccharomyces cerevisiae	>99.9	n.d.	n.d.	n.d.
Trigonopsis variabilis	>99.9	n.d.	n.d.	n.d.
Lactobacillus brevis	>99.9	n.d.	n.d.	n.d.
Bacterium anthracoides	>99.9	n.d.	n.d.	n.d.
Geotrichum candidum	>99.9	4.7	>99.9	*S*

Reaction conditions: 4 mL TEA–HCl buffer (100 mM, pH 7.0); 3 mM 4-(trimethylsilyl)-3-butyn-2-one; 0.15 g mL^{-1} immobilized cells; 130.6 mM 2-propanol; 30 °C; 180 r min^{-1}.

[a] The maximum product yield of GC analysis.

[b] The product *e.e.*

[c] The configuration was determined by the optical rotation.

[d] Not determined.

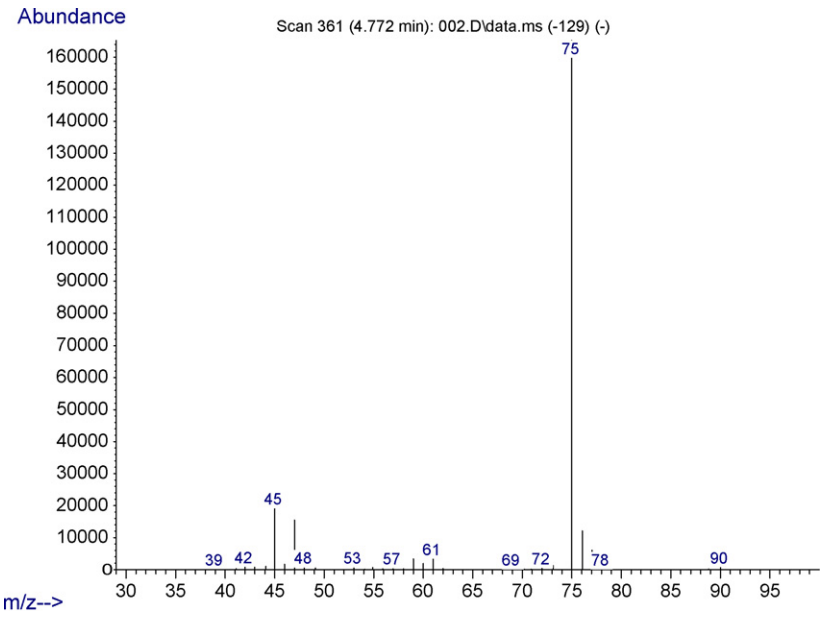


Fig. 1. Mass spectrum of the compound from 4-(trimethylsilyl)-3-butyn-2-one decomposition.

to yield and product *e.e*. For the tested microorganisms, only (*S*)-4-(trimethylsilyl)-3-butyn-2-ol could be produced, and no microorganisms were found to produce the (*R*)-isomer.

Surprisingly, from the data summarized in Table 1, whichever strain was employed as biocatalyst for the reaction, the substrate conversion reached above 99.9% after reaction for 1 h, but only with the three strains described above (*C. parapsilosis* CCTCC M203011, *Rhodotorula* sp. AS2.2241 and *G. candidum*) could the desired product 4-(trimethylsilyl)-3-butyn-2-ol be detected. Moreover, it was obviously observed that the achieved product yield was much lower than the substrate conversion, which is in agreement with the previous report on these strains [26]. It is speculated that the unexpected phenomenon may be attributable to one or more of the following: (1) the product 4-(trimethylsilyl)-3-butyn-2-ol may be unstable in the reaction system or partially decomposed; (2) the desired product may be metabolized by the strain; (3) the desired product may be adhered on the immobilization carrier calcium alginate or stay inside the cells; (4) the substrate may be decomposed via side reactions.

For a better understanding of the phenomenon, a series of experiments were performed. Initially, 4-(trimethylsilyl)-3-butyn-2-ol (3 mM) was added into the TEA–HCl buffer (100 mM, pH 7.0) system. After incubation for 24 h both in the absence and in the presence of the cells, no loss of 4-(trimethylsilyl)-3-butyn-2-ol was observed by GC analysis, clearly demonstrating that the desired product 4-(trimethylsilyl)-3-butyn-2-ol is quite stable in the reaction system and cannot be metabolized by the cells. Additionally, after the completion of the reaction under the conditions shown in Table 1, the immobilized cells were collected by centrifugation and washed with *n*-hexane. Then, the *n*-hexane was subjected to GC analysis and no product 4-(trimethylsilyl)-3-butyn-2-ol was found. Furthermore, the collected immobilized cells were dissolved in 0.1 M trisodium citrate to release the cells from the carrier calcium alginate, and subsequently the product was extracted with *n*-hexane from trisodium citrate solution after breaking up the cells with ultrasonic treatment. However, based on GC analysis of the extract, the desired product 4-(trimethylsilyl)-3-butyn-2-ol was not detected. Thus, it can be concluded that 4-(trimethylsilyl)-3-butyn-2-ol is not adhered on the immobilization carrier and dose not stay inside the cells. To prove whether there exists a non-biocatalytic side reaction in the reaction system, we repeated the experiment shown in Table 1 without the addition of the cells. Amazingly, it was found that the substrate concentration decreased sharply with reaction time, and 3 mM of substrate nearly disappeared within 1 h. This result indicated that the substrate 4-(trimethylsilyl)-3-butyn-2-one was extremely unstable and decomposed into the undesired by-products in the TEA–HCl buffer (100 mM, pH 7.0), which can well account for the unexpected phenomenon described above.

To gain a deeper insight into the substrate decomposition, it is essential to pay more attention to substrate structure. The substrate 4-(trimethylsilyl)-3-butyn-2-one consists of a trimethylsilyl (TMS) moiety and a carbonyl alkyne moiety. To the best of our knowledge, TMS carbonyl alkynes are quite susceptible to cleavage of the silicon–carbon bond under alkaline or neutral conditions. We presume that the mechanism of TMS carbonyl alkyne cleavage under alkaline or neutral conditions is probably by nucleophilic addition of the base to silicon to form a hypervalent silicate (5 bonds, anion localized on Si) which then is protonated at the silicon–carbonyl alkyne bond by solvent to give the carbonyl alkyne and the trimethylhydroxysilane. To confirm the hypothesis, we used *n*-hexane to extract the by-products of substrate cleavage in the absence of the cells from the non-biocatalytic reaction mixture, and then the extract was subjected to GCMS analysis. A clear peak which appeared at the retention time of 4.78 min on GC was observed, implying that a by-product was formed during the non-biocatalytic side reaction. The mass spectrum of the by-product exhibited a molecular

Table 2
The screening of strains used for the reduction of 4-(trimethylsilyl)-3-butyn-2-one between pH 3.0 and 6.0

Strains	pH 3.0			pH 4.0			pH 5.0			pH 6.0		
	Yield (%)[a]	*e.e.* (%)[b]	Configuration[c]	Yield (%)	*e.e.* (%)	Configuration	Yield (%)	*e.e.* (%)	Configuration	Yield (%)	*e.e.* (%)	Configuration
C. parapsilosis CCTCC M203011	52.6	>99.9	*S*	63.7	>99.9	*S*	74.1	>99.9	*S*	58.6	>99.9	*S*
Rhodotorula sp. AS2.2241	28.9	>99.9	*S*	40.8	>99.9	*S*	44.5	>99.9	*S*	43.2	>99.9	*S*
C. tropicalis	n.d.[d]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S. cerevisiae	n.d.	n.d.	n.d.	1.9	>99.9	*S*	2.7	>99.9	*S*	2.6	>99.9	*S*
T. variabilis	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L. brevis	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B. anthracoides	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
G. candidum	6.2	>99.9	*S*	7.1	>99.9	*S*	7.5	>99.9	*S*	6.4	>99.9	*S*

Reaction conditions: 4 mL TEA–HCl buffer (100 mM); 3 mM 4-(trimethylsilyl)-3-butyn-2-one; 0.15 g mL^{-1} immobilized cells; 130.6 mM 2-propanol; 30 °C; 180 r min^{-1}.
[a] The maximum product yield of GC analysis.
[b] The product *e.e.*
[c] The configuration was determined by the optical rotation.
[d] Not determined.

ion (M^+) at *m/e* 90 and its fragment pattern well explained the structure of trimethylhydroxysilane (Fig. 1). The GCMS result well supported our hypothesis described above. Certainly, the detailed mechanism of the cleavage of TMS–carbonyl alkyne bond still needs deeper investigation.

For efficient synthesis of the product, it is of considerable importance to improve the stability of the substrate in the reaction system and to effectively prevent the substrate from being cleaved. In the course of our ongoing investigation, it was found that buffer pH could clearly affect the substrate stability in TEA–HCl buffer. The experiment, where 3 mM 4-(trimethylsilyl)-3-butyn-2-one was incubated in TEA–HCl buffer systems with different pH values at 30 °C for 1 h, showed that the substrate was much more stable under acidic conditions than alkaline or neutral conditions. When the buffer pH was above 7.0, the substrate was completely cleaved into trimethylhydroxysilane. However, when the buffer pH was adjusted to the range between 3.0 and 6.0 where the cells still displayed reduction activity, the substrate concentration slightly decreased with incubation time and no notable cleavage of substrate was observed. Consequently, the experiment for the screening of strains was repeated within the pH range between 3.0 and 6.0. As can be seen in Table 2, *C. parapsilosis* CCTCC M203011, *Rhodotorula* sp. AS2.2241 and *G. candidum* showed better ability to reduce 4-(trimethylsilyl)-3-butyn-2-one. *S. cerevisiae* had low reduction activity towards 4-(trimethylsilyl)-3-butyn-2-one. However, four other strains (*C. tropicalis*, *T. variabilis*, *L. brevis*, *B. anthracoides*) still could not catalyze the reduction of 4-(trimethylsilyl)-3-butyn-2-one. Of the 8 tested strains, *C. parapsilosis* CCTCC M203011 proved to be the best catalyst for the bioreduction in terms of yield and product *e.e*. Therefore, the asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one with immobilized *C. parapsilosis* CCTCC M203011 was subsequently carried out with the buffer pH ranging from 3.0 to 6.0 in order to effectively protect the substrate from cleavage.

3.2. Optimization of the reaction conditions

For further improved results with respect to the initial reaction rate, the yield and the product *e.e*., it is of great interest to optimize the reaction conditions. Accordingly, a systematic study was made of the effects of several crucial variables, such as buffer pH, co-substrate concentration, reaction temperature and substrate concentration on the reaction.

3.2.1. Buffer pH

Buffer pH influences not only enzymatic selectivity and activity, but also the regeneration of the coenzyme present in the microbial cells, which in turn affects the reaction rate [29]. As can be recognized, variation in buffer pH will alter the ionic state of substrate and enzymes involved in this reaction, especially when the reaction was catalyzed by several isozymes with different enantioselectivity at different pHs, thus leading to changes in yield and *e.e*. value. As a result, there exists an optimum pH at which the desired reductases are most active, and the unde-

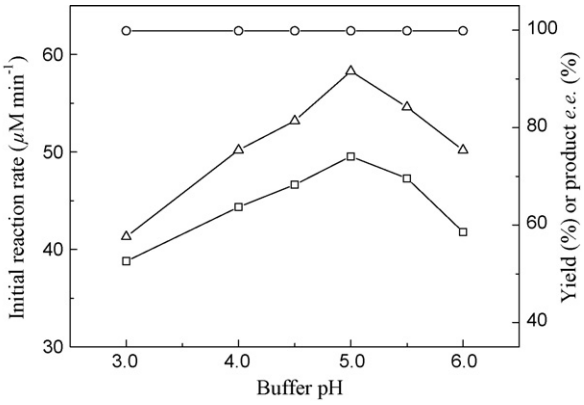


Fig. 2. Effect of buffer pH on the reaction {reaction conditions: 4 mL TEA–HCl buffer (100 mM); 3 mM 4-(trimethylsilyl)-3-butyn-2-one; 0.15 g mL^{-1} immobilized *Candida parapsilosis* CCTCC M203011 cells; 130.6 mM 2-propanol; 30 °C; 180 r min^{-1}}. Symbols: (△) the initial reaction rate; (□) the maximum product yield of GC analysis; (◯) the product *e.e*. All products have the (*S*) configuration.

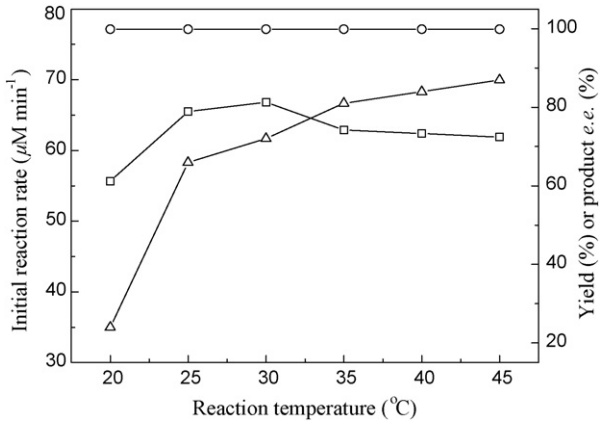


Fig. 3. Effect of temperature on the reaction {reaction conditions: 4 mL TEA–HCl buffer (100 mM, pH 5.0); 3 mM 4-(trimethylsilyl)-3-butyn-2-one; 0.15 g mL^{-1} immobilized *C. parapsilosis* CCTCC M203011 cells; 65.3 mM 2-propanol; 30 °C; 180 r min^{-1}}. Symbols: (△) the initial reaction rate; (□) the maximum product yield of GC analysis; (◯) the product *e.e*. All products have the (*S*) configuration.

sired isoenzymes show the lowest activity or even no activity [30]. Fig. 2 illustrates the significant effect of buffer pH on the reaction. Both the initial reaction rate and the yield increased with the rise of buffer pH from 3.0 to 5.0. However, further increase in buffer pH resulted in a sharp decline in the reaction rate and the product yield. Within the assayed buffer pH range between 3.0 and 6.0, the product *e.e*. was consistently above 99.9%, indicating that there was no problem with activity of undesired isoenzymes within this range of pH. Obviously, the optimal buffer pH for the reaction is 5.0.

3.2.2. Concentration of co-substrate

It is well known that the oxidoreduction reaction could proceed smoothly with whole cells because of the regeneration system within the biocatalyst, which avoids extra addition of the coenzyme [31]. However, there is only a small amount of coenzyme in the cells, and consequently the co-substrate is necessary for recycling the coenzyme. 2-Propanol has been found to be a suitable co-substrate for coenzyme regeneration [32]. So here 2-propanol was used as co-substrate and the effect of its concentration on the reaction was examined. As evident from the data summarized in Table 3, the initial reaction rate and the product yield went up with increasing 2-propanol concentration up to 65.3 mM, beyond which further rise in 2-propanol concentration led to a decline in the reaction rate and the yield, possibly owing to the detrimental effect of excess alcohol on the cells. The concentration of 2-propanol showed little influence on the product *e.e*., which kept above 99.9% within the range examined. Thus, the optimum concentration of co-substrate for the reaction was thought to be 65.3 mM.

3.2.3. Temperature

Fig. 3 depicts the great impact of reaction temperature on the bioreduction. Within the examined temperature range between 20 and 45 °C, the reaction accelerated markedly with the increase of reaction temperature, while the product *e.e*. showed little variation. When the temperature was below 30 °C, higher product yield was achieved along with the rise of reaction temperature. Further increase in temperature led to a notable drop in the yield, which could be attributed to the partial inactivation of the cells at a higher temperature. Taking into account the initial reaction rate, the yield and the product *e.e*., 30 °C was selected as the optimum reaction temperature for the reaction.

3.2.4. Substrate concentration

As can be seen in Table 4, the reaction markedly accelerated and the maximum yield showed no appreciable decrease when

Table 3
Effect of co-substrate concentration on the reaction

Concentration of 2-propanol (mM)	Initial reaction rate (μM min^{-1})	Yield (%)[a]	*e.e*. (%)[b]
21.8	31.7	50.8	>99.9
43.6	51.7	70.6	>99.9
65.3	63.3	81.3	>99.9
87.2	58.3	76.4	>99.9
130.6	56.7	74.1	>99.9
195.9	43.0	64.4	>99.9

Reaction conditions: 4 mL TEA–HCl buffer (100 mM, pH 5.0); 3 mM 4-(trimethylsilyl)-3-butyn-2-one; 0.15 g mL^{-1} immobilized *C. parapsilosis* CCTCC M203011 cells; 30 °C; 180 r min^{-1}.

[a] The maximum yield of GC analysis.

[b] The product *e.e*. All products have the (*S*) configuration.

Table 4
Effect of substrate concentration on the reaction

Substrate concentration (mM)	Initial reaction rate (μM min^{-1})	Yield (%)[a]	*e.e.* (%)[b]
1.5	45.0	81.7	>99.9
3.0	61.7	81.3	>99.9
4.5	59.2	68.8	>99.9
6.0	57.3	48.3	>99.9
12.0	55.0	33.6	>99.9
24.0	50.0	11.5	>99.9

Reaction conditions: 4 mL TEA–HCl buffer (100 mM, pH 5.0); a fixed concentration of 4-(trimethylsilyl)-3-butyn-2-one; 0.15 g mL^{-1} immobilized *C. parapsilosis* CCTCC M203011 cells; 65.3 mM 2-propanol; 30 °C; 180 r min^{-1}.
[a] The maximum yield of GC analysis.
[b] The product *e.e*. All products have the (*S*) configuration.

substrate concentration increased from 1.5 to 3.0 mM, beyond which further increase in substrate concentration gave rise to a clear drop in the initial reaction rate and the maximum yield. The results clearly show that there exists a pronounced substrate inhibition for the reaction performed in aqueous buffer system even if the substrate concentration is low. Throughout the range of substrate concentration, the product *e.e*. showed no variation and kept above 99.9%. On the other hand, the product inhibition also existed in aqueous buffer system. From the data listed in Table 5 it was clear that both the initial reaction rate and the maximum yield decreased sharply with increasing concentration of the product originally added into the reaction system. Accordingly, the substrate and product inhibition limited the increase of substrate concentration of the reaction conducted in aqueous buffer system. Taking into consideration the initial reaction rate, the maximum yield and the product *e.e*., the suitable substrate concentration for the bioreduction carried out in the aqueous buffer system was 3.0 mM.

After a reaction time of 1 h under the optimum conditions described above, the asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one with immobilized *C. parapsilosis* CCTCC M203011 cells efficiently produced enantiopure (*S*)-4-(trimethylsilyl)-3-butyn-2-ol with a yield of 81.3% and an *e.e*. value of >99.9%, which were much higher than those previously reported [26].

Table 5
Effect of 4-(trimethylsilyl)-3-butyn-2-ol addition on the reaction

Addition of 4-(trimethylsilyl)-3-butyn-2-ol (mM)	Initial reaction rate (μM min^{-1})	Yield (%)[a]	*e.e.* (%)[b]
0	61.7	81.3	>99.9
3.0	41.7	41.9	>99.9
6.0	16.7	11.6	>99.9

Reaction conditions: 4 mL TEA–HCl buffer (100 mM, pH 5.0); 3 mM 4-(trimethylsilyl)-3-butyn-2-one; a fixed concentration of 4-(trimethylsilyl)-3-butyn-2-ol; 0.15 g mL^{-1} immobilized *C. parapsilosis* CCTCC M203011 cells; 65.3 mM 2-propanol; 30 °C; 180 r min^{-1}.
[a] The maximum yield of GC analysis.
[b] The product *e.e*. All products have the (*S*) configuration.

4. Conclusions

In conclusion, it has been clearly shown that the cleavage of the substrate 4-(trimethylsilyl)-3-butyn-2-one readily takes place under neutral or alkaline conditions and can be effectively prevented by adjusting the buffer pH to the acidic range between 3.0 and 6.0. The synthesis of enantiopure (*S*)-4-(trimethylsilyl)-3-butyn-2-ol can be successfully conducted through the asymmetric reduction of 4-(trimethylsilyl)-3-butyn-2-one with immobilized *C. parapsilosis* CCTCC M203011 cells. However, the low substrate concentration (3 mM) in aqueous buffer system could severely limit the application of the biocatalytic process in industrial production. Attempt should be made to eliminate the substrate and product inhibition. Biocompatible ionic liquid or organic solvent-based biphasic systems might be good alternatives to the monophasic aqueous system.

Acknowledgements

We thank the National Natural Science Foundation of China (Grant No. 20602013) for financial support. We are also grateful to Professor Xu Yan (Southern Yangtze University, China) and Professor Xu Jian-He (East China University of Science and Technology, China) for the generous gift of the yeast strains *Candida parapsilosis* CCTCC M203011 and *Rhodotorula* sp. AS2.2241 used in this work.

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