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The kinetic study on lipase-catalyzed transesterification of α-cyano-3-phenoxybenzyl alcohol in organic media

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Abstract

Optically active form of α-cyano-3-phenoxybenzyl alcohol (CPBA), building block of pyrethroid insecticides, was synthesized as its acetate by the combination of anion-exchange resin-catalyzed transcyanation between *m*-phenoxybenzaldehyde and acetone cyanohydrin, and lipase-catalyzed enantioselective transesterification of the resulting cyanohydrin with vinyl acetate. Through a screening of enzymes, *Alcaligenes* sp. lipase showed the highest activity and the conversion exceeded 50%. Effects of solvents and temperatures on this reaction were studied. Among four kinds of solvent, diisopropyl ether was a best choice. The optimal temperature was 50 °C. Although the external diffusion limitation could be excluded by raising the rotational speed, internal diffusion could not be ignored, since the enzyme was an immobilized one on the particles with a considerably large diameter. The e.e. values of CPBA ester were measured by polarimeter and NMR.

Keywords: Lipase; Transesterification; Organic media; α-Cyano-3-phenoxybenzyl alcohol

1. Introduction

Cyanohydrin compounds are useful synthetic intermediates in organic synthesis. They can be converted to α-hydroxy carboxylic acids, α-hydroxy esters, α-hydroxy ketones, and β-hydroxy amines, which are important starting materials for the synthesis of pharmaceuticals and alcohols in organic media [1]. Optically active cyanohydrin esters can be obtained with enzymatic transesterification of racemic cyanohydrins in organic media [2]. Lipases were widely used in organic media. Lipases from different kinds of sources vary greatly in catalytic ability for catalyzing transesterification in organic media. Inagaki et al. [3] first proposed a one-pot method to synthesize α-cyano-3-phenoxybenzyl alcohol (CPBA) acetate. This is an approach to the synthesis of (*S*)-CPBA ester through the in situ racemization of corresponding cyanohydrin which is formed from *m*-phenoxybenzaldehyde and acetone cyanohydrin by the combination of chemical and biochemical catalysts of anion-exchange resin and lipase. By using enol esters such as vinyl acetate, the reaction became irreversible and the yield of the resolution tended to 100%. Furthermore, the racemization of product was greatly reduced when the hydroxyl group of cyanohydrin was protected by acylation. They used lipase from *Pseudomonas cepacia* as a catalyst for the transesterification. It cost 2.9 days to get a conversion of 88%. Therefore, a more effective lipase

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Nomenclature

c	the weight in gram of the chiral substance in 100 ml solution (g/100 ml)
C_p	concentration of product (mol/l)
E_0	initial enzyme concentration (mol/l)
h	hour
k	rate constant (l/(mol s))
L	the length of the tube of polarimeter (dm)
pK_a	dissociation equilibrium constant of acids (mol/l)
R	gas constant (8.314 J/(mol K))
S_0	initial substrate concentration (mol/l)
V_m	maximal rate (mol/(l s))
V_0	initial rate (mol/(l s))
x	the content of (*S*)-CPBA acetate in sample (%)

Greek letters

α	the rotation measured at 20 °C
$[\alpha]_D^{20}$	specific rotation at 20 °C

should be screened. Mitsuda et al. [4] studied some factors affecting the hydrolysis of CPBA ester such as temperature and pH.

Our group [5] compared the differences of the effect of different kinds of lipases, acylating agents (vinyl acetate and isopropenyl acetate) and resins (D301 and D296) on this reaction. The whole reaction is depicted as the following scheme (Scheme 1; in which R is *m*-phenoxybenzyl).

But, the conversions of the reaction were too low (the highest was 17%) though the reaction time was as long as 24 h. In this paper, a more effective lipase catalyzing this reaction was screened. The effects of solvents, reaction temperatures, substrate concentrations, and internal and external diffusion limitations were also studied.

2. Experimental

2.1. Chemicals

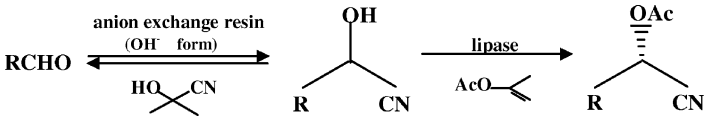
Vinyl acetate, diisopropyl ether, isooctane, cyclohexane, *n*-hexadecane, CPBA were all obtained from commercial suppliers at analytical pure grade. CPBA acetate was prepared in the conventional manner.

2.2. Instruments

The name, type and suppliers of the instruments are given in Table 1.

2.3. The method to keep the water content stable

The lyophilized powder was put into a container, which contained a saturated LiCl solution. The container was kept in a refrigerator at 4 °C so that the



Scheme 1.

Table 1
The name, type and suppliers of the instruments used

Name	Type	Supplier
Isothermal shaker	THZ-82A	Shanghai Yuejing Medical Instrument Factory
Electronic balance	MD200-3	Shanghai Balance Factory
Gas chromatograph	GC-9790	Zhejiang Wenlin Analytical Instrument Factory
Chromatographic work station	N2000	Institute of Intelligence and Information Engineering, Zhejiang University
NMR spectrometer	DMX500	BRUKER SPECTROSPIN Corporation
Automatic polarimeter	WZZ-2	Shanghai Physical Optics Instrument Factory

relative moisture was constant (11%) for at least 48 h before the use. The reproducibility in regard to the reaction rate of lipase-catalyzed reaction was very high, so long as the experiments followed this procedure [6].

2.4. Analytical method

The samples were analyzed with gas chromatograph, using a capillary of 30 m long, FID detector with temperature of 280 °C and nitrogen as the carrier gas. The temperature for the oven is programmed from 80 to 200 °C with rate of 20 °C/min and from 200 to 270 °C with rate of 10 °C/min. The injector temperature is 320 °C. The quantitative method is internal standard method and the internal standard is hexadecane. The calibration coefficient of product CPBA acetate was defined to be 2.634.

2.5. Measurement of e.e. value of CPBA acetate by NMR method

A sample (ca. 20 mg) was dissolved in a $CDCl_3$-TMS (0.03%) solution (total 0.5 ml), and $Eu(hfc)_3$ (ca. 50 mg) was added. Two signals appeared, δ 2.31 for (*R*)-isomer, and 2.27 for (*S*)-isomer. Comparison of the integration of these two signals brought about the precise determination of the e.e.

2.6. General methods for kinetic study

The reaction was carried out in a batch reactor (10 ml-vial). The substrates were weighed accurately and the reaction started by the addition of lipases. In the reaction course, the reactor was placed in a shaker in which the rotational speed could be controlled and temperature was kept at constant. A 0.05 μl of the well-stirred reaction mixture was taken at interval. The variation of CPBA acetate concentrations were measured to study the reaction nature. Water activity of the solution was controlled to 0.01 with definite amount of molecular sieve [7].

3. Results and discussion

3.1. Screening of lipases

Lipase nos. 1–15 were chosen. The reactions were performed in the following manner; lipase 50 mg, vinyl acetate 2 ml, CPBA 165 mmol/l, hexadecane 100 mg, 45 °C, 220 rpm. The progress curves were

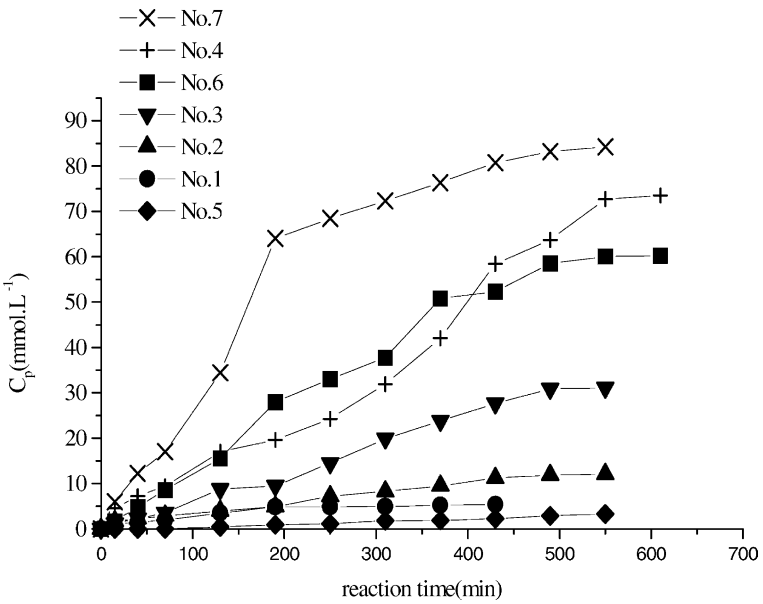


Fig. 1. Screening of lipases at 45 °C, 220 rpm, $[CPBA]_0 = 165$ mmol/l.

Table 2
The lipase sources and their initial reaction rates and conversions for synthesis of CPBA ester

Lipases	Sources and/or suppliers	Initial rates (mmol/(l min))	Conversions at 550 min (%)
No. 1	From *Aspergillus* sp. and donated by Prof. Kou Xiufeng[a]	0.069	3.42
No. 2	From *Candida rugosa* and supplied by Sigma (Cat. no: L1754)	0.112	7.31
No. 3	Lipase QL supplied by Meito Sangyo	0.079	18.84
No. 4	Lipase TL supplied by Meito Sangyo	0.302	44.09
No. 5	From Porcine pancreatic and supplied by Sigma (Cat. no: L3126)	0.0034	1.99
No. 6	Lipase UL supplied by Meito Sangyo	0.113	36.39
No. 7	Lipase PL immobilized and supplied by Meito Sangyo	0.401	51.06

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shown in Fig. 1 as well as the conversions at 550 min (Table 2). Judging from these results, immobilized form of *Alcaligenes* lipase (no. 7) was most suitable in terms of catalytic activity and was used throughout the further studies.

3.2. Choice of solvents

Enantioselectivity of enzyme-catalyzed reaction as well as the rate in non-aqueous media greatly depends on the solvents [8,9]. Four solvents were compared in this reaction system. Under the experimental condition (45 °C, 220 rpm, initial CPBA concentration 165 mmol/l), progress curves for the four solvents (vinyl acetate, diisopropyl ether, isooctane, cyclohexane) were measured. The results were shown in Fig. 2.

Judging from Fig. 2, diisopropyl ether was shown to be the first choice to get more product during the shortest time, and vinyl acetate can make do with the second best. Isooctane would be the third choice and cyclohexane should not be considered anytime.

3.3. Effect of temperature

It is widely believed that enzymes must exhibit their highest stereoselectivity at low temperatures [10]. This belief has been supported by some experimental observations. The reactions were performed in the

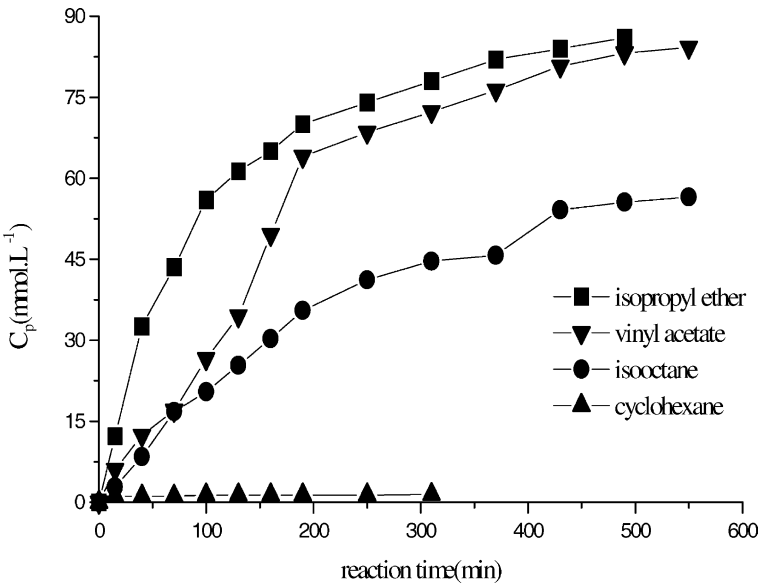

Fig. 2. Comparison of solvents on the reaction at 45 °C, 220 rpm, $[CPBA]_0 = 165$ mmol/l, no. 7 lipase 0.025 g/ml.

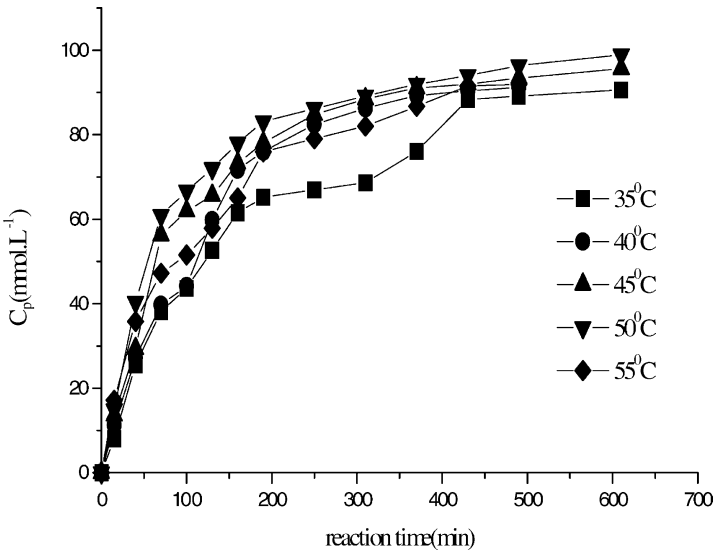

Fig. 3. Effect of temperature on the reaction at 220 rpm, $[CPBA]_0 = 170$ mmol/l, no. 7 lipase 0.025 g/ml.

following manner; 220 rpm, initial CPBA concentration 170 mmol/l, no. 7 lipase 25 mg/ml, vinyl acetate as acyl donor and solvent. The progress curves of the reaction at different temperatures was shown in Fig. 3 as well as the initial rates and conversions (Table 3).

Judging from Fig. 3 and Table 3, one will realize that 50 °C is the optimal temperature though the initial rate at this temperature is little lower than that at 55 °C. The enantioselectivity of the lipase decreased while the reaction temperature was raised.

3.4. Effect of internal and external diffusion

Due to the insolubility of enzyme in organic media, external diffusion limitation must exist in this reaction system. In contrast, the *Alcaligenes* lipase (no. 7) is an immobilized enzyme, the substrates must diffuse from the surface of the enzyme to the inner parts of the enzyme and it can then be transformed to products. Therefore, internal diffusional limitation may exist. Effects of rotational speed and enzyme concentration on the reaction when the substrate concentration is high enough were studied to reveal the effects of the external and internal diffusion limitations. The effect of rotational speed on the reaction was shown in Fig. 4 and Table 4.

From Fig. 4, one can realize that the reaction rate becomes greater with the rotational speed getting higher up to 220 rpm and be almost unchanged when the rotational speed is higher than 220 rpm. Thus, a conclusion that the external diffusion can be excluded when

Table 3
The initial rates and conversions at 490 min for the reaction at various temperatures

	T (°C)				
	35	40	45	50	55
V_m ($\times 10^5$ mol/(l s))	1.02	1.25	1.511	1.677	1.908
Conversions at 490 min (%)	52.5	53.7	55.0	56.7	54.1
e.e.%	88.2[a]	82.5[a]	65.0[a]	50.0[a]	40.2[a], 41.3[b]

[a] e.e. value was measured by polarimeter.
[b] e.e. value was measured by NMR method.

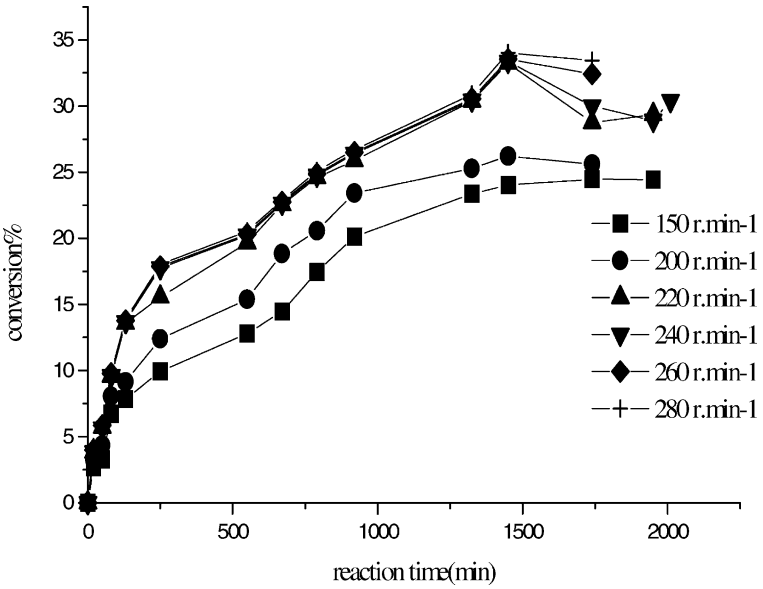

Fig. 4. Effect of rotational speed on the reaction at 45 °C, no. 7 lipase 0.01 g/ml, $[CPBA]_0 = 313$ mmol/l, e.e.% = 77.5.

Table 4
Effect of rotational speed on the initial rates of the transesterification reaction

Rotational speed (rpm)	155	200	220	240	260	280
Initial rate (mmol/(l min))	0.376	0.449	0.531	0.538	0.539	0.538

Reaction conditions: 45 °C, $S_0 = 313$ mmol/l, no. 7 lipase 0.01 g/ml.

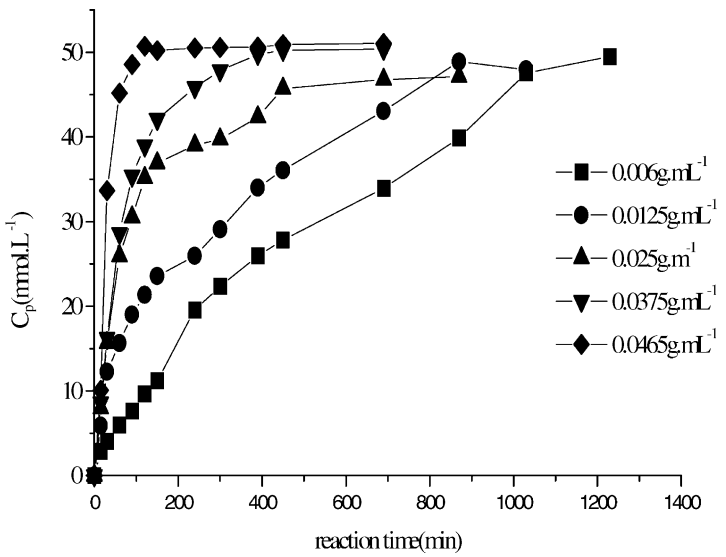

Fig. 5. Effect of enzyme concentration on the transesterification reaction at 45 °C, 220 rpm, $[CPBA]_0 = 102$ mmol/l.

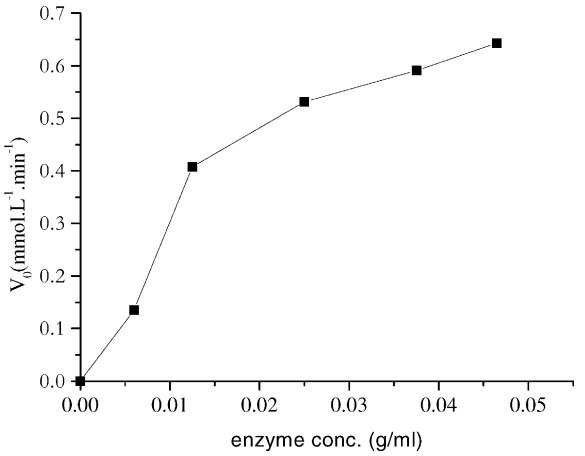

Fig. 6. Relationship between V_0 and E_0 for the transesterification reaction at 45 °C, 220 rpm, $[CPBA]_0 = 102$ mmol/l, no. 7 lipase.

the rotational speed is higher than 220 rpm would be reasonably drawn.

Under the experimental condition (45 °C, $S_0 = 102$ mmol/l, 220 rpm), the progress curves of the reaction in different enzyme concentrations were measured (Fig. 5). The reaction rate becomes greater as the enzyme concentration gets higher (Fig. 5). The initial rates of each progress curve were calculated and a V_0 versus E_0 curve was drawn (Fig. 6).

Fig. 6 showed that V_0 is not linearly with E_0. This suggested that the internal diffusion limitation existed in this system [11].

To verify the internal diffusion effect, different kinds of enzyme with different particle diameter were used as catalysts to catalyze the reaction and the progress curves were measured (Fig. 7). The reaction condition was 45 °C, 220 rpm, no. 7 lipase 0.025 g/ml. From Fig. 7, one can find that the reaction rate becomes greater as the particle diameter of the enzyme diminishes. This hinted that the internal diffusion limitation can not be ignored.

3.5. Effect of substrate concentrations on the transesterification reaction

Under the reaction condition (220 rpm, 45 °C, no. 7 lipase 25 mg/ml, vinyl acetate as acyl donor and solvent), the effect of substrate concentration on the transesterification has been studied. Fig. 8 shows each progress curve at different substrate concentrations.

From Fig. 8, one will realize that the reaction rate becomes higher when the initial substrate concentration gets higher.

The initial rates of each progress curve were calculated and a V_0 versus S_0 curve was drawn as shown in Fig. 9. From this figure, one can find that V_0 is almost linearly with S_0.

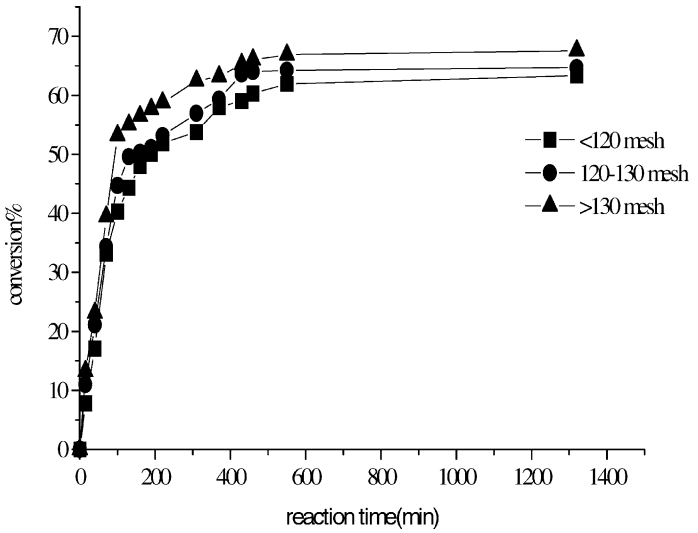

Fig. 7. Effect of lipase particle diameter on the transesterification reaction at 45 °C, 220 rpm, $[CPBA]_0 = 160$ mmol/l, no. 7 lipase 0.025 g/ml.

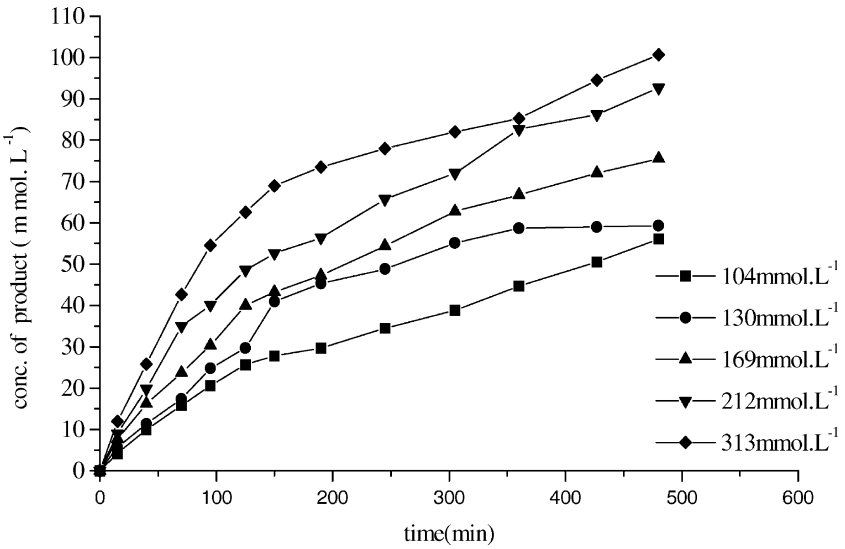

Fig. 8. Effect of the substrate concentration on the reaction at 45 °C, 220 rpm, no. 7 lipase 0.025 g/ml.

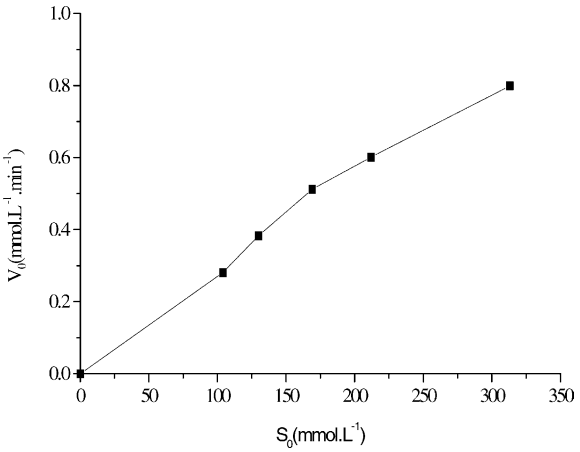

Fig. 9. Relationship between V_0 and S_0 for the reaction at 45 °C, 220 rpm, no. 7 lipase 0.025 g/ml.

4. Conclusions

The enzyme-catalyzed production of CPBA acetate in organic solvent was studied. From the screening of the enzyme, immobilized form of lipase with origin of *Alcaligenes* sp. showed the highest activity. The efficiency was much more improved than that had previously been achieved. Effects of solvents and temperatures on this reaction were studied, and the use of diisopropyl ether at 50 °C was the best choice. External diffusion limitation could be excluded by raising the rotational speed. In contrast, internal diffusion could not be ignored, since the enzyme was an immobilized one on the particle, whose diameter was not small enough. The rate was substantially accelerated with the increase of the substrate concentration.

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References

[1] A.-M. Klibanov, Asymmetric transformation catalyzed by enzymes in organic solvents, Acc. Chem. Res. 23 (1990) 114–120.
[2] Y.-F. Wang, S.-T. Chen, K.K.-C. Liu, C.-H. Wong, Lipase-catalyzed irreversible transesterification using enol

esters: resolution of cyanohydrins and synthesis of ethyl(*R*)-2-hydroxy-4-phenylbutyrate and (*S*)-propranolol, Tetrahedr. Lett. 30 (1989) 1917–1920.

[3] M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, Lipase-catalyzed kinetic resolution with in situ racemization: one-pot synthesis of optically active cyanohydrin acetates from aldehydes, J. Am. Chem. Soc. 113 (1991) 9360–9361;
M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, One-pot synthesis of optically active cyanohydrin acetates from aldehydes via lipase-catalyzed kinetic resolution coupled with in situ formation and racemization of canohydrins, J. Org. Chem. 57 (1992) 5643–5649.

[4] S. Mitsuda, H. Yamamoto, T. Umemura, Enantioselective hydrolysis of α-cyano-3-phenoxybenzyl acetate with arthrobacter lipase, Agric. Biol. Chem. 54 (1990) 2907–2912.

[5] Y. Zhu, L.R. Yang, Z.Q. Zhu, S.J. Yao, P.L. Cen, Lipase-catalyzed enantioselective transesterification of cyanohydrins for the synthesis of (*S*)-α-cyano-3-phenoxybenzyl acetate, Ann. New York Acad. Sci. 864 (1999) 646–648.

[6] P.J. Halling, Thermodynamic predictions for biocatalysis in non-conventional media: theory, tests, and recommendations for experimental design and analysis, Enzyme Microb. Technol. 6 (1994) 178–206.

[7] R.H. Valivety, P.J. Halling, A.R. Macrae, Reaction rate with suspended lipase catalyst shows similar dependence on water activity in different organic solvents, Biochim. Biophys. Acta 1118 (1992) 218–222.

[8] G. Carrea, S. Riva, Properties and synthetic applications of enzymes in organic solvents, Angew Chem. Int. Ed. 39 (2000) 2226–2254.

[9] A.M. Klibanov, Improving enzymes by using them in organic solvent, Nature 409 (2001) 241–246.

[10] R.S. Phillips, Temperature modulation of the stereochemistry of enzymatic catalysis: prospects for exploitation, TIBTECH 14 (1996) 13–16.

[11] B.A. Bedell, V.V. Mozhaev, D.S. Clark, J.S. Dordick, Testing for diffusion limitations in salt-activated enzyme catalysts operating in organic solvents, Biotech. Bioeng. 58 (1998) 654–657.