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The kinetic study on lipase-catalyzed asymmetric alcoholysis of α-cyano-benzyl acetate in organic media

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Abstract

Optically active mandelonitrile was synthesized by the lipase-catalyzed asymmetric alcoholysis of α-cyano-benzyl acetate in organic media. Through screening, the preferable system for the asymmetric reaction were: lipase *Alcaligenes* sp., tetrahydrofuran and methanol. Effects of various parameters were studied to deduce the kinetics and mechanism of the reaction. The optimal temperature was 40 °C. The external diffusion limitation could be excluded by raising the rotation speed and the internal diffusion could be ignored. The experimental results indicated that the decomposition of the mandelonitrile was the main reason of the decrease of the yield and e.e. value and benzaldehyde could greatly inhibit the enzyme activity. The kinetics was found to obey the Ping-Pong bi-bi mechanism with substrate inhibition of methanol.
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Keywords: Lipase; Asymmetric alcoholysis; Organic media; α-Cyano-benzyl acetate; Kinetics

1. Introduction

Enantiopure cyanohydrin compounds are useful intermediates in organic synthesis and their derivatives, such as α-hydroxy acids, α-hydroxy ketones and aldehydes and β-hydroxy amines, are important for the production of pharmaceuticals and agrochemicals [1]. Optically active mandelonitrile was obtained by asymmetric addition of HCN to benzaldehyde under the catalysis of hydroxynitrile lyases [2]. Meanwhile, the enzymatic formation and esterification of mandelonitrile [3] and the combined reactions named one-pot reaction [4,5] were investigated.

However, these methods all have problems and cannot achieve both high yields and excellent e.e. values in reasonable time. Since the synthesis of α-cyano-benzyl acetate is not difficult and expensive, the method of lipase-catalyzed asymmetric alcoholysis of α-cyano-benzyl acetate in organic media (Scheme 1) was tried to obtain the satisfied product. Although both the separation of (R)-ester from (S)-cyanohydrin by solvent extraction [6] or distillation [7] and the racemization of (R)-ester [6] should be further studied, our research indeed contributed to the understanding of the kinetics and mechanism of the lipase-catalyzed reaction in organic media. In this paper, an effective system for the asymmetric reaction was screened and various parameters were studied to deduce the kinetics and mechanism of the reaction.

2. Experimental

2.1. Chemicals

Mandelonitrile and α-cyano-benzyl acetate were prepared in the conventional manner in our lab. Other materials and solvents were commercially available and were appropriately purified, if necessary.

2.2. Analytical method

The concentrations and e.e. values of the samples were analyzed using an Agilent 1100 Series HPLC instrument with a chiral HPLC column (SUMICHIRAL OA-4400) and Agilent ChemStation for LC.

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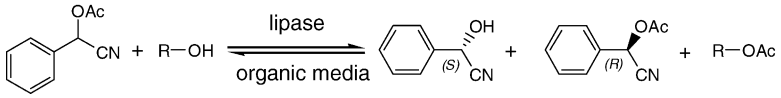


Scheme 1.

2.3. General methods for kinetic study

The reactions were carried out in a batch reactor (5 ml vial). The substrates were weighed accurately and the reaction was started by the addition of lipases. In the reaction course, the reactor was placed in a shaker in which the rotation speed could be controlled and temperature was kept at constant. A 5 μl of the well-stirred reaction mixture was taken at interval for analysis. Water activity of the solution was controlled at low level with two molecular sieves.

The reactions were generally performed in the following manner: 10 mg/ml lipase, 0.1 mol/l α-cyano-benzyl acetate, 0.1 mol/l methanol, tetrahydrofuran (THF) was used as organic media, 40 °C, 200 rpm. If necessary, the reaction conditions will be changed and the details will be explained in the text.

3. Results and discussion

3.1. Screening of lipase

Various lipases were tested. The results were listed in Table 1. Judging from these results, lipases no. 13 and 14 (both from *Alcaligenes* sp.) had both higher conversions and e.e. values. And the immobilized form lipase no. 14 was most suitable in terms of catalytic activity and enantioselectivity and was consequently used throughout further studies.

3.2. Choice of alcohols

Seven alcohols were compared in this reaction system (alcohol 100 mmol/l). Progress curves for the seven alcohols (methanol, ethanol, *n*-butanol, *sec*-butanol, *tert*-butanol, *n*-octanol, *n*-cetanol) were measured. The results were shown in Figs. 1 and 2 and Table 2. Judging from Fig. 1, the reaction courses of all the alcohols were similar [9]. Moreover, the data listed in Table 2 suggested quantitively that there were no remarkable difference between all the alcohols. Thus, methanol was chosen as the reactant for further studies. Fig. 2 showed that benzaldehyde concentration increased when the reaction proceeded and the e.e. value of the (S)-mandelonitrile decreased after 20 h in the reaction with *n*-octanol. From Table 2, although higher conversions were achieved at 44 h, the e.e. values decreased greatly after 18 h. This phenomena indicated that the decomposition of the mandelonitrile (Scheme 2), which caused the production of benzaldehyde and the racemization of the (S)-mandelonitrile, was the main reason of the decrease of the yield and e.e. value of products.

3.3. Choice of solvents

Enantioselectivity of enzyme-catalyzed reaction as well as the rate in non-aqueous media greatly depends on the solvents [10,11]. Eleven solvents were compared in this reaction system. Under the experimental condition (α-cyano-benzyl acetate 0.3 mol/l, alcohol 0.3 mol/l), the initial rates and con-

Table 1
The lipase sources and their conversions for alcoholysis of α-cyano-benzyl acetate and e.e. values of (S)-mandelonitrile at 24 h

Lipases no.	Sources	Suppliers	Conversions at 24 h (%)	e.e.%
1	*Chromobacterium viscosum*	Fluka	2.87	100
2	*Pseudomonas* sp.	Sigma	10.9	100
3	*Rhizopus niveus*	Fluka	5.15	59.4
4	*Aspergillus niger*	Fluka	6.22	76.3
5	*Aspergillus oryzae*	Fluka	8.09	55.4
6	*Candida antarctica*	Fluka	2.93	42.6
7	*Candida utilis*	Fluka	35.2	31.1
8	Hog pancreas	Fluka	15.3	88.4
9	*Mucor javanicus*	Fluka	57.5	74.4
10	*Rhizopus miehei*	Fluka	1.82	34.4
11	*Pencillium roqueforti*	Fluka	2.10	100
12	Lipase QL	Meito Sangyo	12.05	77.9
13	*Alcaligenes* sp.	Meito Sangyo	37.1	100
14	*Alcaligenes* sp.	Meito Sangyo	42.9	100
15	*Pseudomonas stutzeri*	Meito Sangyo	50.7	91.8
16	Lipoprime 50 TLs80000101	Novo Nordisk	5.43	75.6
17	Lipopan 50BGL A101009	Novo Nordisk	4.95	55.7
18	Lipolase ultra 507LE90310607	Novo Nordisk	2.69	100
19	Lipolase PPW3562	Novo Nordisk	3.98	61.8

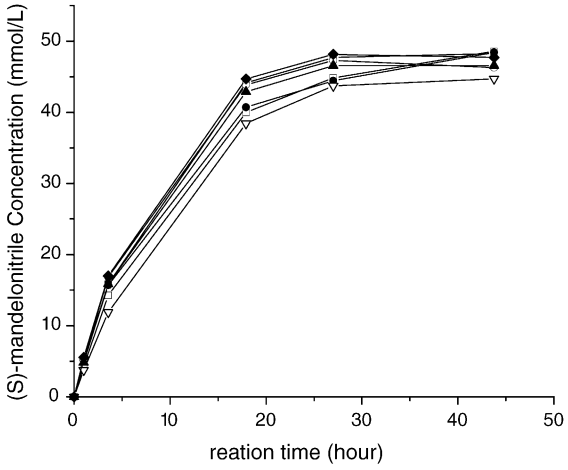


Fig. 1. Effect of different alcohols on the reaction: methanol (■); ethanol (□); *n*-butanol (●); *sec*-butanol (◯); *tert*-butanol (▲) *n*-octanol (▽); *n*-cetanol (♦).

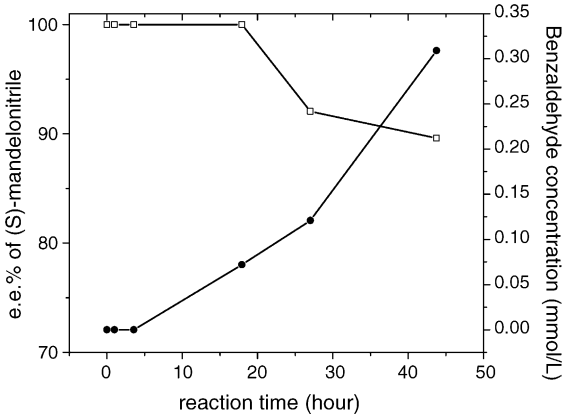
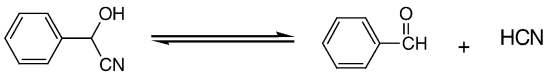


Fig. 2. Effect of decomposition of (S)-mandelonitrile on the reaction with *n*-octanol: e.e. % (□); benzaldehyde concentration (●).

versions and e.e. values at 40 h for the reaction at various solvents were measured. The results were shown in Table 3.

Although higher conversions were obtained with diethyl ether, diisopropyl ether, acetone, 1,4-dioxane and THF than other solvents, acetone, 1,4-dioxane and THF were more likely to get both high reaction rates and e.e. values. And tetrahydrofuran (THF) was the first choice for this lipase-catalyzed reaction because of its highest enantioselectivity.



Scheme 2.

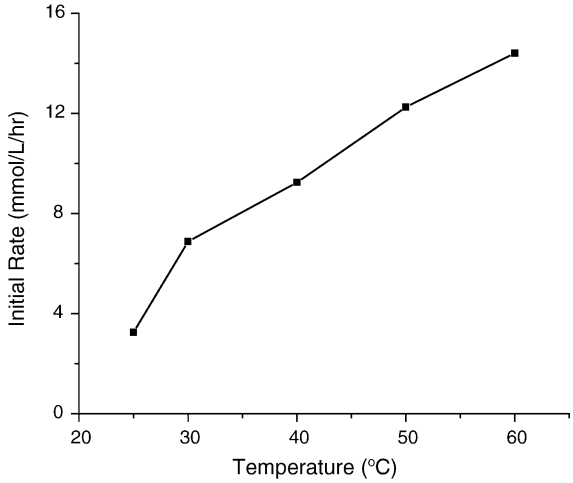


Fig. 3. Effect of temperature on the reaction initial rates.

3.4. Effect of temperature

The initial rates at different temperatures were shown in Fig. 3. The yields and e.e. values of (S)-mandelonitrile and the benzaldehyde concentrations at 40 h were shown in Fig. 4. Judging from Fig. 3, the initial rate increased when the reaction temperature was raised. From Fig. 4, benzaldehyde concentration increased and the e.e. value decreased while the reaction temperature raised. Consequently, the decomposition of the mandelonitrile (Scheme 2), which caused the production of benzaldehyde and the racemization of the (S)-mandelonitrile, were more intensive at high temperature. And the optimal temperature was 40 °C because of its highest yield and satisfied e.e. value of the product.

3.5. Effect of internal and external diffusion

The initial rate versus enzyme concentration curve was drawn in Fig. 5. The e.e. value always remained 100%. Fig. 5 showed that the curve was linear. This suggested that the internal diffusion limitation did not exist in this system [8].

Table 2
The initial rates and conversions and e.e. values at 44 h for the reaction with various alcohols

Alcohols	Initial rates (mmol/l h)	Conversions at 18 h (%)	e.e.% at 18 h	Conversions at 44 h (%)	e.e.% at 44 h
Methanol	7.57	39.0	100	47.5	93.4
Ethanol	7.50	42.8	100	48.2	92.3
n-Butanol	7.47	43.5	100	49.0	91.3
sec-Butanol	7.48	40.0	100	47.0	93.2
tert-Butanol	7.54	39.0	100	46.4	94.0
n-Octanol	7.45	43.8	100	48.9	89.6
n-Cetanol	7.48	43.1	100	48.6	91.3

Table 3
The initial rates and conversions and e.e. values at 40 h for the reaction at various solvents

Solvents	Initial rates (mmol/L h)	Conversions at 40 h (%)	e.e.% at 40 h
Benzene	12.8	23.2	60.5
Toluene	15.7	25.8	57.7
Ethyl benzene	15.3	26.7	69.8
Chloroform	5.8	23.1	62.2
Carbon tetrachloride	9.3	27.4	34.5
1,2-DichIoroethane	6.0	26.3	74.9
Acetone	6.4	33.2	93.8
Diethyl ether	25.4	42.7	77.4
Diisopropyl ether	21.4	48.0	65.8
1,4-Dioxane	10.3	45.0	92.9
Tetrahydrofuran	7.6	38.0	96.8

The effect of rotation speed on the reaction was shown in Fig. 6. The e.e. value always remained 100%. From Fig. 6, it can be realized that the reaction rate became greater with the rotation speed getting higher up to 200 rpm and was almost unchanged when the rotation speed was higher than 200 rpm. Thus, a conclusion would be reasonably drawn that the external diffusion can be excluded when the rotation speed is higher than 200 rpm.

3.6. Effect of benzaldehyde concentrations

Effect of benzaldehyde concentration on the initial rate was studied (Fig. 7). It was realized that the initial rate became lower when the benzaldehyde concentration got higher and accordingly benzaldehyde could greatly inhibit the enzyme activity.

3.7. Kinetics and mechanism

The double-reciprocal plots of initial rate versus ester concentration (Fig. 8) were measured. The plots in Fig. 8 at low methanol concentration tend to be parallel, whereas at high methanol concentration, the slopes of the lines appear to increase. This behaviour is typical of the so-called Ping-Pong bi-bi mechanism with methanol inhibition. The

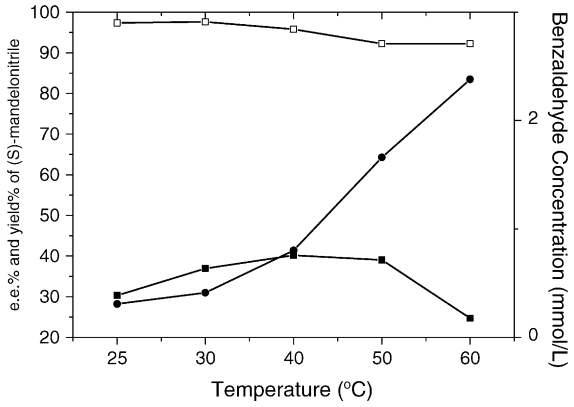


Fig. 4. Effect of temperature on the reaction at 40 h: yield % (■); e.e. % (□); benzaldehyde concentration (●).

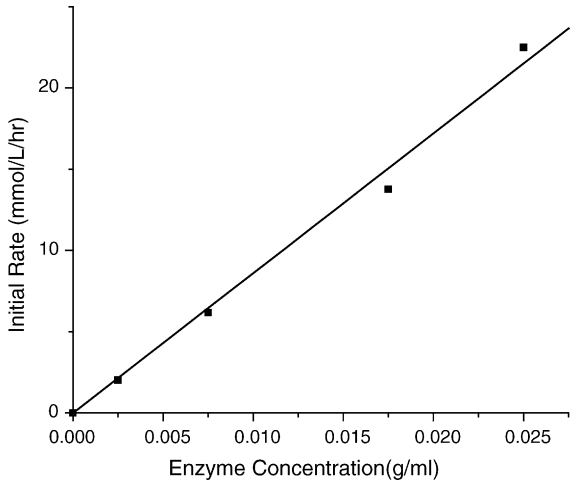


Fig. 5. Relationship between the initial rate and the enzyme concentration for the reaction.

reaction sequence may be given as follows (Scheme 3) – where A: methanol, EA: enzyme–methanol complex, E: free enzyme, EB: enzyme-α-cyano-benzyl acetate complex, F: enzyme–acyl complex, B: α-cyano-benzyl acetate, FA: binary complex of acyl enzyme and methanol, P: (S)-mandelonitrile and Q: methyl acetate.

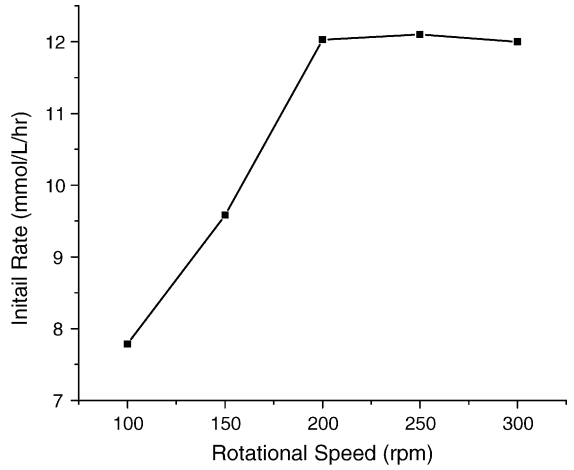


Fig. 6. Effect of rotation speed on the reaction initial rate.

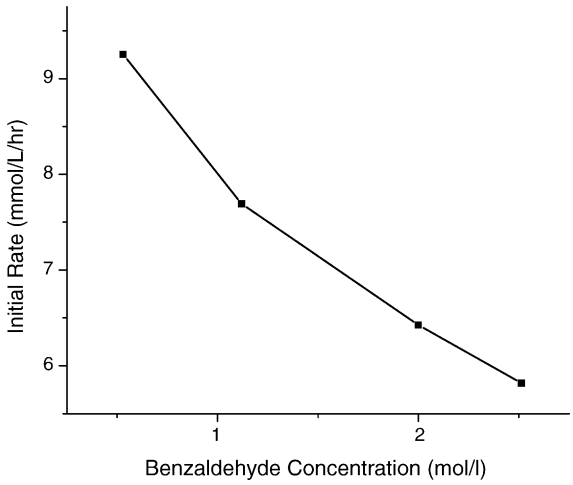


Fig. 7. Effect of benzaldehyde concentration on the initial rate.

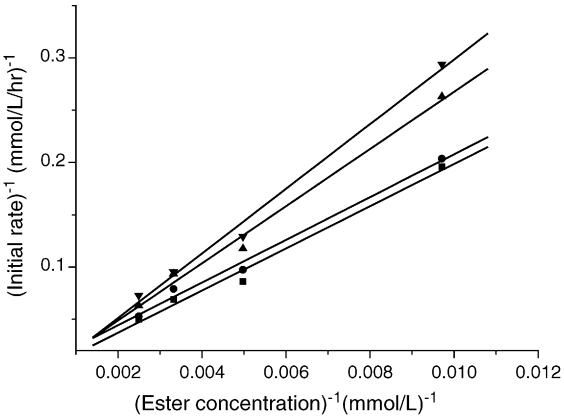


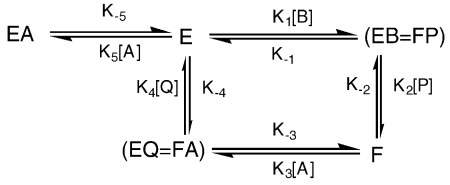
Fig. 8. . Double-reciprocal plots of initial rate versus ester concentration. The concentration of methanol: 40 mmol/l (■); 60 mmol/l (●); 200 mmol/l (▲); 300 mmol/l (▼).

The final initial rate equation is as follow:

$$v = \frac{v_m[A][B]}{[A][B] + K_A[B] + K_B(1 + [A]/K_{iA})[A]} \quad (1)$$

The data from initial rate measurements were used for the optimization of parameter. The result was given below (Table 4).

Fig. 9 shows the deviation of simulated and experimental values for four initial α-cyano-benzyl acetate concentrations, and it is found that the simulated values correspond to the experimental value quite well, and the R^2 value is 0.969.



Scheme 3.

Table 4
The parameter values and R^2 value of the simulated initial rate equation

Parameter	Value
V_m (mmol/L h)	169.11
K_A (mmol/L)	0.01
K_B (mmol/L)	1536.86
K_{iA} (mmol/L)	796.98
R^2	0.969

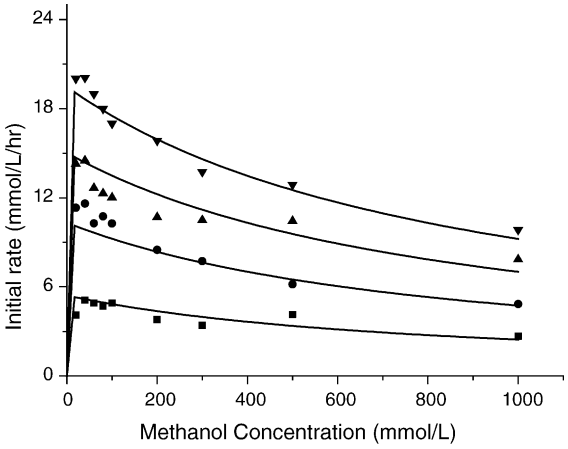


Fig. 9. Comparison of simulated values with the experimental data (the experimental values were scatters, the simulated values were lines). The concentration of cyano(phenyl)methyl acetate: 50 mmol/l (■); 100 mmol/l (●); 150 mmol/l (▲); 200 mmol/l (▼).

3.8. Effect of substrate concentrations

The effect of substrate concentration, which was equal both to the concentration of α-cyano-benzyl acetate and to the methanol concentration, was studied (Fig. 10).

When the concentrations of the two substrates were equal, the former initial rate equation with methanol inhibition (1)

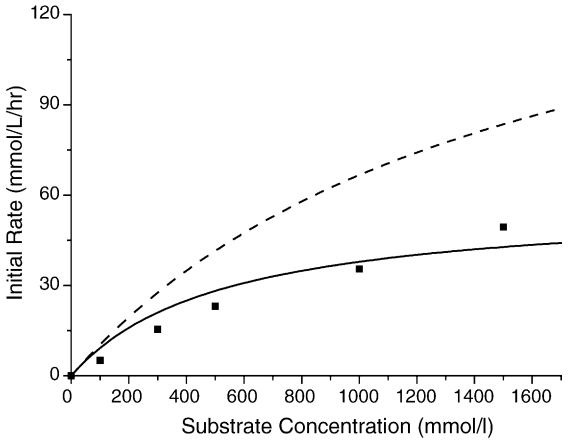


Fig. 10. Effect of substrate concentration on the initial rate (the experimental values were scatters, the simulated values with methanol inhibition were the solid line, the simulated values without inhibition were the dash line).

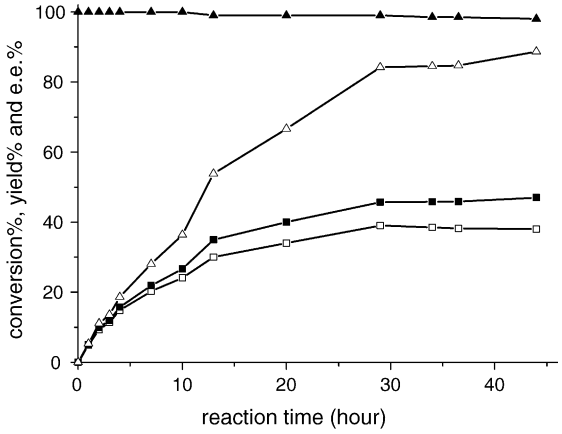


Fig. 11. Scale-up reaction (50 ml): α-cyano-benzyl acetate conversion% (■), mandelonitrile yield % (□), (S)-mandelonitrile e.e. % (▲),(R)-α-cyano-benzyl acetate e.e. % (△).

became:

$$v = \frac{v_m[A]}{K_A + K_B + (1 + K_B/K_{iA})[A]} \quad (2)$$

The parameter values were the same as Table 4. The simulated values were shown in Fig. 10 as the solid line. If there is no methanol inhibition, the former initial rate Eq. (2) became:

$$v = \frac{v_m[A]}{K_A + K_B + [A]} \quad (3)$$

The parameter values were the same as Table 4. The simulated values were shown in Fig. 10 as the dash line. The experimental values fitted the solid line quite well. Thus, the results indicated that the former kinetics and mechanism model was reasonable.

3.9. Scale-up reaction

The reaction in 50 ml scale was studied (Fig. 11) in the optimized reaction conditions (lipase *Alcaligenes* sp., THF and methanol, 40 °C, 200 rpm, substrate concentration 0.1 mol/l). The equilibrium was obtained after 30 h and 46% α-cyano-benzyl acetate conversion, 39% mandelonitrile yield, 99% (S)-mandelonitrile e.e. value and 84% (R)-α-cyano-benzyl acetate e.e. value were achieved at 30 h in 50 ml scale reaction.

4. Conclusions

The lipase-catalyzed production of (S)-mandelonitrile in organic solvent was studied. The preferable reaction system (lipase *Alcaligenes* sp., THF and methanol) and reaction condition (40 °C, 200 rpm) were determined. The decomposition of the mandelonitrile was the main reason of the decrease of the yield and e.e. value and benzaldehyde could greatly inhibit the enzyme activity. The kinetics was found to obey the Ping-Pong bi-bi mechanism with substrate inhibition of methanol and the simulated values correspond to the experimental value quite well. And 46% α-cyano-benzyl acetate conversion, 39% mandelonitrile yield, 99% (S)-mandelonitrile e.e. value and 84% (R)-α-cyano-benzyl acetate e.e. values were achieved at 30 h in 50 ml scale reaction.

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