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Kinetic study of lipase catalyzed asymmetric transesterification of mandelonitrile in solvent-free system

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Abstract

Optically active mandelonitrile was obtained by the lipase-catalyzed asymmetric transesterification of mandelonitrile in solvent-free system. The lipase *Alcaligenes* sp. was screened to be used in this system. Effects of various parameters were studied to deduce the kinetics and mechanism of the reaction. The optimal reaction temperature was 40 °C. The external diffusion limitation could be reduced greatly by raising the rotation speed and the internal diffusion could be ignored. The experimental results indicated that benzaldehyde and benzoic acid could greatly inhibit the enzyme activity. Using King–Altman method, a kinetic model of the transesterification of mandelonitrile with vinyl acetate in solvent-free system without substrate and production inhibition was proposed based on ping-pong bi–bi mechanism. Using Matlab program to simulate the processes and six parameters are obtained. The simulated values can fit the experimental values quite well and the relative error of the model was 8.61%.

Keywords: Mandelonitrile; Lipase; Asymmetric transesterification; Solvent-free system; Kinetics

1. Introduction

The synthesis of optically pure cyanohydrins is an area of growing interest in synthetic chemistry. Cyanohydrins and their derivatives are important compounds for the production of pharmaceuticals and agrochemicals [1]. Mandelonitrile (MN) is one of the most important cyanohydrin and enantioselective synthesis of MN has attracted considerable attention recently. Optically active MN had been prepared by chemical methods using chiral catalyst [2,3]. Meanwhile, the enzymatic approaches have been investigated by hydroxynitrile lyase [4,5]. However, all these methods have problems and cannot achieve both high yields and excellent e.e. (enantiomeric excess) value. Although the method of lipase-catalyzed asymmetric alcoholysis of mandelonitrile acetates was developed [6], it is still defective, such as the long reaction time and needing a large volume of solvents. Thus, better processes need to be developed.

In recent years, the enzyme-catalyzed transesterification received extensive research and application. Especially, the lipase-catalyzed asymmetric transesterification already succeeded in resolving many chiral alcohols in organic solvents [7,8]. Application of vinyl or isopropenyl esters as the acylating agent for transesterification offers an effective solution to overcome equilibrium because the enol co-product can be irreversibly transformed into acetaldehyde or acetone immediately [9,10]. Meanwhile, a solvent-free system, which is a simple mixture of reactants, presents the major advantage of the absence of solvents which facilitates downstream processing. Moreover, the elimination of solvents from the production step offers significant cost saving and minimizes environment impact. Therefore, the method of lipase-catalyzed asymmetric transesterification of mandelonitrile (Scheme 1) in solvent-free system was tried to be employed as an effective means to obtain enantiopure mandelonitrile here. Until now, to the best of our knowledge, the kinetics of lipase-catalyzed asymmetric transesterification of mandelonitrile in solvent-free system has not been reported. In this paper, a highly effective lipase for the asymmetric reaction was screened first and various parameters were studied to deduce the kinetics of the reaction. The kinetic studies were conducted with vinyl acetate as the acyl donor.

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Nomenclature

A	substrate, vinyl acetate
B	substrate, (*S*)-mandelonitrile
E	enzyme
F	acyl-enzyme intermediate
k_i	rate constant ($i = \pm1, \pm2, \pm3, \pm4$)
K_x	parameters of kinetics modeling (x = A, B, AB, Q, BQ, BA, QA, BQA)
P	product, enol
Q	product, mandelonitrile acetate
S_0	substrate concentration
T	temperature
v	reaction rate
V_m	maximal rate of reaction
V_0	initiate rate

2. Materials and methods

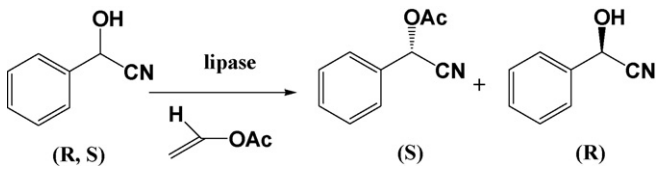
2.1. Chemicals

Mandelonitrile and mandelonitrile acetate were provided by J&K Chemical Ltd. Lipases which origins were *Rhizopus arrhizus*, *Candida lipolytica*, *Lipoprime* 50T, *Mucor javanicus*, *Rhizopus miehei*, *Pseudomonas stutzeri* and *Alcaligenes* sp. were used. Other chemicals and solvents were commercially available of analytical grade. High-performance liquid chromatography (HPLC) grade *n*-hexane, 1,2-dichloroethane and ethanol were supplied by Tedia.

2.2. Analytical method

Analysis of mandelonitrile, mandelonitrile acetate and benzaldehyde are performed by HPLC (Agilent 1100). A SUMICHIRAL OA-4400 column (4.6 mm × 250 mm) was used. Samples were diluted with ethanol if necessary. About 87% *n*-hexane, 10% 1, 2-dichloroethane and 3% ethanol were used as eluents and the flow rate was 1 mL/min. The wavelength of UV–vis detector was 254 nm. Retention times: benzaldehyde 4.3 min, mandelonitrile acetate 5.1 min, (*S*)-mandelonitrile 18.4 min, (*R*)-mandelonitrile 19.8 min. The e.e. value of mandelonitrile could be calculated from the HPLC data.

The e.e. value of mandelonitrile acetate was analyzed by Fuli 9790 GC instrument with a chiral column (10% permethylated β-cyclodextrin). The temperatures of column, injection and detection were set on 120, 230 and 230 °C, respectively. Retention times: (*R*)-mandelonitrile acetate 4.7 min, (*S*)-mandelonitrile acetate 5.2 min.



Scheme 1. Lipase-catalyzed asymmetric transesterification of mandelonitrile.

2.3. General methods for kinetic study

The reactions were carried out in a batch reactor (10 mL vial). The substrate was weighed accurately and the reaction was started with the addition of lipase. In the reaction course, the reactor was placed in a shaker in which the rotation speed could be controlled and the temperature was kept at constant. About 5 μL of the well-stirred reaction mixture was taken at intervals 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, 100 mmol/L mandelonitrile, and vinyl acetate was used as reactant and solvent, 40 °C, 200 rpm. The reaction conditions were changed and details are explained in the text.

3. Results and discussion

3.1. Selection of lipase

Some lipases were tested to examine their catalytic activity and selectivity first. The results were shown in Table 1. From these results, it seems that the immobilized form of *Alcaligenes* sp. lipase was the most suitable in terms of catalytic activity and enantioselectivity. So it was consequently used throughout further experiments.

3.2. Effect of temperature

The effect of temperature was studied in the range of 30–50 °C. The initial reaction rates at different temperature were shown in Fig. 1. The yield of mandelonitrile acetate and the concentration of benzaldehyde at 5 h are shown in Fig. 2. The initial rate increased when the reaction temperature was raised according to Fig. 1. From Fig. 2, the concentration benzaldehyde increased while the reaction temperature was raised. And

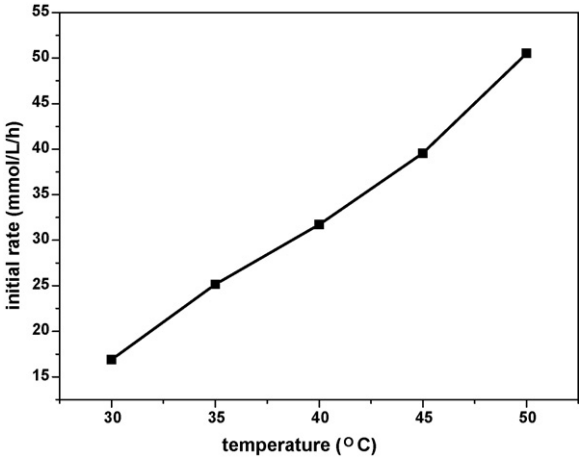


Fig. 1. Effect of temperature on initial rate.

Table 1
Lipase sources and the conversion of transesterification and e.e. values of (*S*)-mandelonitrile acetate at 5 h

Number	Sources	Company	State	Conversion (%)	e.e. (%)
1	*Rhizopus arrhizus*	Fluka	Free	79.4	71.9
2	*Candida lipolytica*	Fluka	Free	35.2	71.7
3	*Lipoprime* 50T	Novo Nordisk	Immobilized	26.1	>99
4	*Mucor javanicus*	Fluka	Free	8.2	87.1
5	*Rhizopus miehei*	Fluka	Free	30.0	62.4
6	*Pseudomonas stutzeri*	Prepared in our lab [11]	Free	42.3	63.8
7	*Alcaligenes* sp.	Meito Sangyo	Immobilized	47.9	>99

the yield of mandelonitrile acetate reached the maximum value at the middle temperature of 40 °C. The reason is that mandelonitrile will decompose and produced benzaldehyde and HCN more quickly at higher temperature. Consequently, the optimal temperature was 40 °C because of the highest yield. According to Arrhenius law, EA is calculated to be 43.08 kJ/mol from this figure.

3.3. Effect of speed of agitation

In the case of immobilized enzyme, the reactants have to diffuse from the bulk liquid to the external surface of the catalyst. External mass transfer can be minimized by carrying out the reaction at an optimum speed of agitation. The effect of speed of rotation was studied over the range of 90–240 rpm. The curve of the initial rate versus speed of rotation was shown in Fig. 3. It was found that the initial rate increased with speeds from 90 to 180 rpm and was almost unchanged when the rotation speed was higher than 180 rpm. Therefore, a conclusion that the effect external diffusion can be reduced greatly when the rotation speed is higher than 180 rpm would be reasonably drawn.

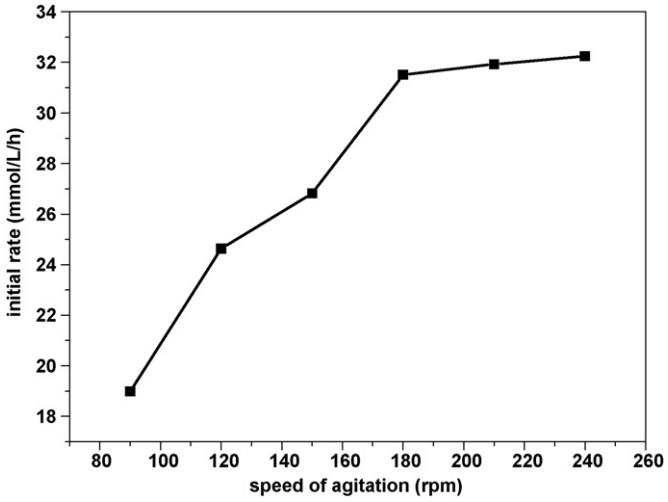


Fig. 3. Effect of rotation speed on initial rate.

3.4. Effect of enzyme concentration

The *Alcaligenes* lipase is an immobilized enzyme. In the case of immobilized preparations internal diffusion problems could occur in the case of porous supports where enzyme molecules could be attached on the surface of the support and in the inner part of the support when the loading of enzyme is big. Internal diffusion problems could therefore happen when the substrate could not reached the inner parts of the support. Effect of enzyme concentration was studied to reveal the effects of the internal diffusion limitation. The effect of enzyme concentration was shown in Fig. 4. It was found that the initial rate of the reaction increased linearly with an increase in enzyme concentration in reaction mixture, ranging from 5 to 25 mg/mL. This suggested

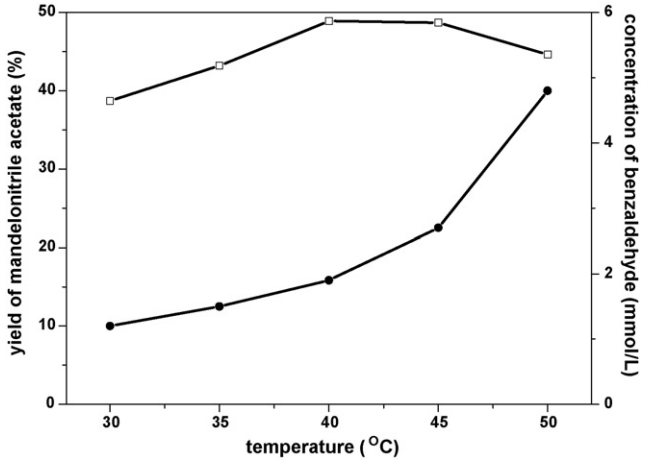


Fig. 2. Effect of temperature on the reaction at 5 h. (□) Yield of mandelonitrile acetate; (●) concentration of benzaldehyde).

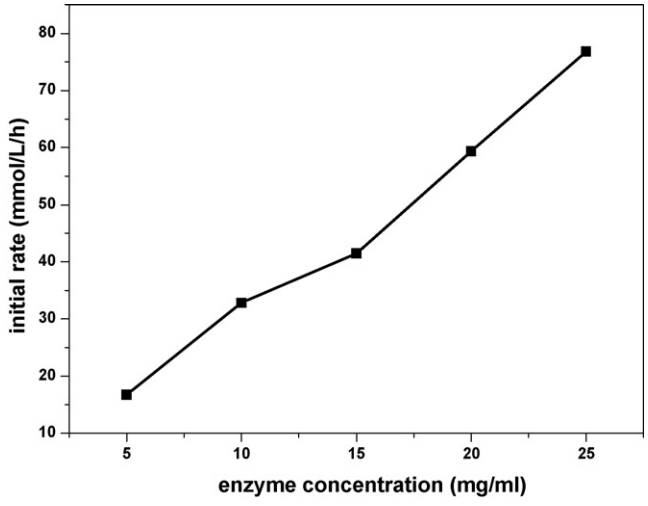


Fig. 4. Effect of enzyme concentration on initial rate.

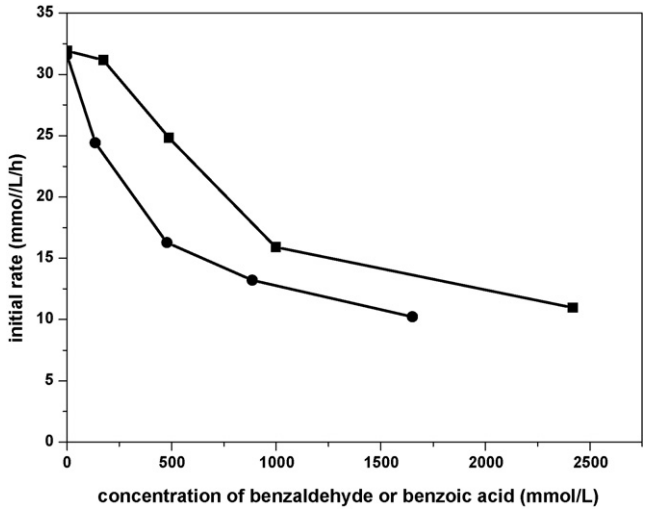


Fig. 5. Effect of the concentration of benzaldehyde and benzoic acid on initial rate. (■) Benzaldehyde; (●) benzoic acid.

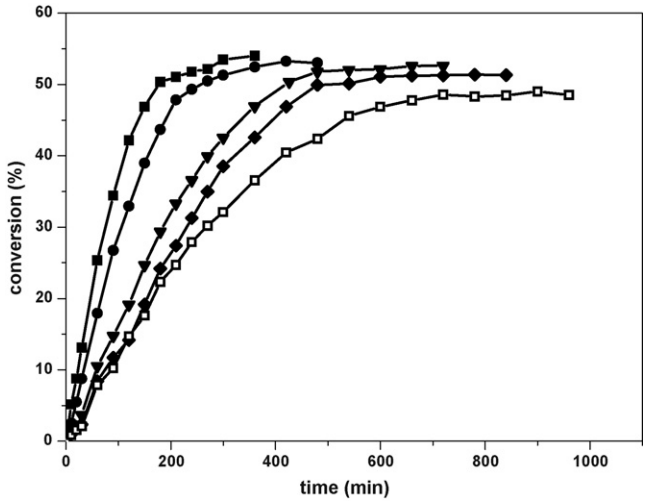


Fig. 6. The progress curve of different concentration of mandelonitrile: (■) 50 mmol/L, (●) 100 mmol/L, (▼) 200 mmol/L, (♦) 300 mmol/L, (□) 400 mmol/L.

that the internal diffusion limitation did not exist in the system [12]. The reason may be that the enzyme was just immobilized on the surface of the carrier.

3.5. Effect of benzaldehyde and benzoic acid

Mandelonitrile is very easy to decompose and produce benzaldehyde and HCN. And benzaledhyde is also easy to be oxidized and converted into benzoic acid. So the effects of benzaldehyde and benzoic acid were studied in Fig. 5. It was realized that the initial rate became lower when the concentration of benzaldehyde and benzoic acid got higher. Accordingly, benzaldehyde and benzoic acid could greatly inhibit the enzyme activity. The effect of benzoic acid was more remarkable to the effect of benzaldehyde. The results also suggested that mandelonitrile must carry on the purification to eliminate the influence of impurity before using. And the results verified that the higher temperature was unfavorable for carrying on the reaction, because mandelonitrile would convert into benzaldehyde at higher temperature to inhibit the reaction.

3.6. Effect of substrate concentration

The effect of concentration of mandelonitrile was studied over the range of 50–400 mmol/L by keeping the catalyst quantity constant. The concentration of vinyl acetate can be seen as constant because it was far excessive. Fig. 6 showed that each progress curve at different substrate concentration. From Fig. 6, the reaction rate was enhanced when the initial substrate concentration increased. This phenomenon is very common in an enzymatic reaction and it revealed that there is not serious substrate inhibition under above experiment conditions. The initial rate (V_0) of each progress was calculated and a $1/V_0$ versus $1/S_0$ (S_0: concentration of substrate) curve was drawn as shown in Fig. 7. From this figure, it can be found that $1/V_0$ is almost linearly with $1/S_0$. It confirmed the deduction that the substrate inhibition can be neglected.

3.7. Kinetic model based on initial rate measurements

Most of kinetic studies on lipase-catalyzed transesterification are described by a ping-pong bi–bi kinetic model [13–15]. During the course of experiment, the inhibition of products was tested. From the results, it was determined that there was no obvious inhibition of products. Therefore, the kinetics of lipase-catalyzed asymmetric transesterification of mandelonitrile suggests that the model is based on the bi–bi mechanism without inhibition by both substrates and products. The reaction sequence may be given as follows (Scheme 2).

In this model, vinyl acetate (A) binds first to the free enzyme (E) and forms a non-covalent enzyme-ester complex and this central complex upon isomerization forms the acyl-enzyme intermediate (F), with the release of the first product, enol (P). The second substrate, (*S*)-mandelonitrile (B), binds to the acyl-enzyme complex and forms another complex, which again undergoes isomerization to ester-enzyme complex, which finally breaks into the second product, mandelonitrile acetate (Q) and the free enzyme.

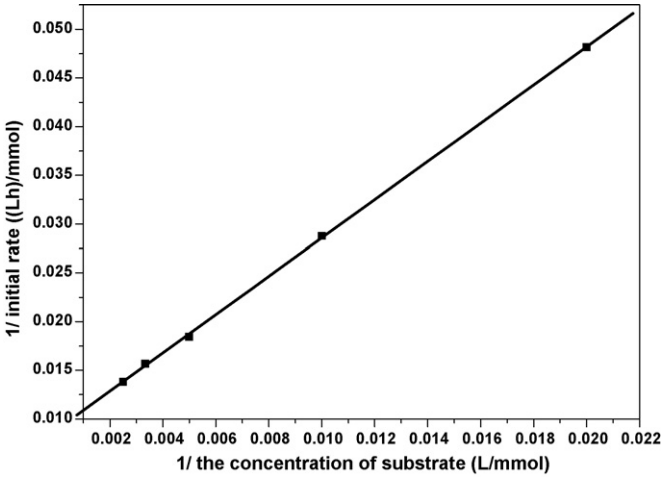
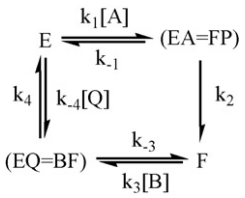


Fig. 7. Double-reciprocal plots of initial rate vs. concentration of mandelonitrile.



Scheme 2. King–Altman scheme of enzyme-catalyzed bi–bi transesterification. A, vinyl acetate; E, enzyme; (EA = FP), non-covalent complex; F, acyl-enzyme intermediate; P, enol; B, (*S*)-mandelonitrile; (EQ = BF), another complex; Q, (*S*)-mandelonitrile acetate.

The rate equation of the reaction was postulated by using King–Altman method [16]. The items that contain enzyme were [E], [EA = FP], [F], [EQ = BF], and the total of enzyme was

$$\Sigma = (k_{-3} + k_4)k_1k_2[\mathrm{A}] + (k_{-1} + k_2)k_3k_4[\mathrm{B}] + (k_2 + k_4)k_1k_3[\mathrm{A}][\mathrm{B}] + (k_{-1} + k_2)k_{-3}k_{-4}[\mathrm{Q}] + (k_{-1} + k_2)k_3k_{-4}[\mathrm{B}][\mathrm{Q}] \quad (1)$$

The reaction rate can be expressed as Eq. (2):

$$v = k_1[\mathrm{E}][\mathrm{A}] - k_{-1}[\mathrm{EA} = \mathrm{FP}] \quad (2)$$

because,

$$\frac{[\mathrm{E}]}{[\mathrm{E}]_t} = \frac{k_{-1}k_3k_4[\mathrm{B}] + k_2k_3k_4[\mathrm{B}]}{\Sigma} \quad (3)$$

$$\frac{[\mathrm{EA} = \mathrm{FP}]}{[\mathrm{E}]_t} = \frac{k_1k_3k_4[\mathrm{A}][\mathrm{B}]}{\Sigma} \quad (4)$$

Therefore,

$$v = \frac{k_1k_{-1}k_3k_4[\mathrm{A}][\mathrm{B}][\mathrm{E}]_t + k_1k_2k_3k_4[\mathrm{A}][\mathrm{B}][\mathrm{E}]_t - k_1k_{-1}k_3k_4[\mathrm{A}][\mathrm{B}][\mathrm{E}]_t}{\Sigma} = \frac{k_1k_2k_3k_4[\mathrm{A}][\mathrm{B}][\mathrm{E}]_t}{\Sigma} = \frac{k_1k_2k_3k_4[\mathrm{A}][\mathrm{B}][\mathrm{E}]_t}{K_{\mathrm{A}}[\mathrm{A}] + K_{\mathrm{B}}[\mathrm{B}] + K_{\mathrm{AB}}[\mathrm{A}][\mathrm{B}] + K_{\mathrm{Q}}[\mathrm{Q}] + K_{\mathrm{BQ}}[\mathrm{B}][\mathrm{Q}]} \quad (5)$$

where $K_{\mathrm{A}} = (k_{-3} + k_4)k_1k_2$, $K_{\mathrm{B}} = (k_{-1} + k_2)k_3k_4$; $K_{\mathrm{AB}} = (k_2 + k_4)k_1k_3$, $K_{\mathrm{Q}} = (k_{-1} + k_2)k_{-3}k_{-4}$; $K_{\mathrm{BQ}} = (k_{-1} + k_{-2})k_3k_{-4}$.

The reaction was carried out in non-solvent system, so the concentration of vinyl acetate was constant. The finally rate equation was as following:

$$v = \frac{V_{\mathrm{m}}[\mathrm{B}]}{K_{\mathrm{A}} + K_{\mathrm{BA}}[\mathrm{B}] + K_{\mathrm{AB}}[\mathrm{B}] + K_{\mathrm{QA}}[\mathrm{Q}] + K_{\mathrm{BQA}}[\mathrm{B}][\mathrm{Q}]} \quad (6)$$

where $V_{\mathrm{m}} = k_1k_2k_3k_4[\mathrm{E}]_t$; $K_{\mathrm{BA}} = K_{\mathrm{B}}/[\mathrm{A}]$; $K_{\mathrm{QA}} = K_{\mathrm{Q}}/[\mathrm{A}]$; $K_{\mathrm{BQA}} = K_{\mathrm{BQ}}/[\mathrm{A}]$.

Using Matlab program to simulate the processes and the six parameters are obtained. The results were given in Table 2. Fig. 8 shows the derivation of simulated curves and experimental values for different concentrations of mandelonitrile. It is found that the simulated values can fit the experimental values quite well. The relative error of this model was calculated to be 8.61%. Such error is acceptable for kinetic study in this system. Therefore, this model would be useful in the range of experimental substrate concentrations for this kind of transesterification catalyzed by immobilized lipase in solvent-free system.

Table 2
The parameter values of the simulated rate equation

Parameter	Value
V_{m} (mol/L min)	0.0060
K_{A} (mol/L)	0.4390
K_{AB}	12.9552
K_{BA}	0.3431
K_{QA}	0.3059
K_{BQA} (L/mol)	12.7496

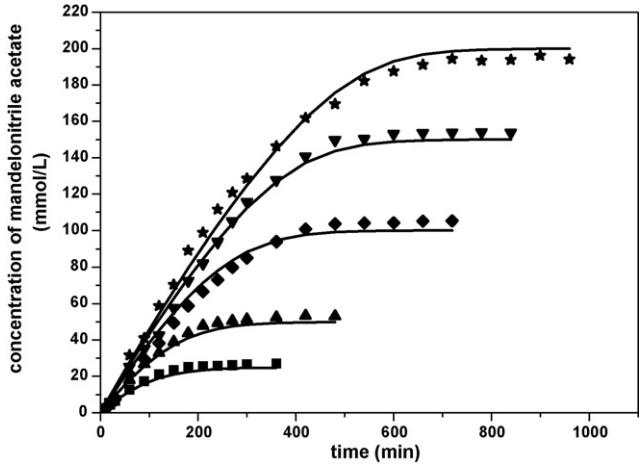


Fig. 8. Comparison of simulated values with the experimental data. The initial concentration of mandelonitrile: experimental: (■) 50 mmol/L, (▲) 100 mmol/L, (♦) 200 mmol/L, (▼) 300 mmol/L, (⋆) 400mmol/L; simulated: lines.

4. Conclusion

The lipase-catalyzed transesterification of mandelonitrile with vinyl acetate in solvent-free system was studied including the effect of various parameters. Immobilized lipase *Alcaligenes* sp. was found to be the most efficient catalyst. The reaction time is only 5 h. The external diffusion limitation could be reduced greatly by raising the rotation speed and the internal diffusion could be ignored. Benzaldehyde and benzoic acid could greatly inhibit the enzyme activity. By using King–Altman method, a kinetic model of the transesterification was proposed based on ping-pong bi–bi mechanism without inhibition of production and substrate. The simulated values can fit the experimental values quite well and the relative error of the model was 8.61%.

Acknowledgments

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