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Kinetic and Reactor Modelling of Lipases Catalyzed *(R,S)*-1-phenylethanol Resolution

[1]*Chua Lee-Suan,* [2]*Cheng Kian-Kai,* [2]*Lee Chew-Tin,* [1]*Mohamad-Roji Sarmidi,*
[1]*Ramlan Abdul-Aziz and* [3]*Tang Boon-Seng*

[1]Chemical Engineering Pilot Plant, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
[2]Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
[3]Department of R and D, ECO-IEE Pte. Ltd., 40 Tuas West Road, Singapore 638389

Abstract: This study was focused on the development of a kinetic model and a reactor model for the enzymatic resolution of *(R,S)*-1-phenylethanol. The reaction progress curves catalyzed by immobilized lipases, ChiroCLEC-PC in batch stirred tank reactor were used to develop the kinetic model. The resolution followed Ping-Pong Bi-Bi mechanism with the inhibition of lauric acid, *(R,S)*-1-phenylethanol and water. The validity of the model was verified by fitting it to another experimental data catalyzed by immobilized lipases, Chirazyme L2, c.-f., C3, lyo at the same reaction conditions. The rate equation was then applied for the development of reactor model in a recirculated packed bed reactor system. The overall effectiveness factor and Peclet number were used to determine the mass transfer and axial dispersion limitation in the reactor performance. The reactor model was verified by fitting it to the larger scale reactor data with the correlation coefficient value more than 0.99.

Key words: Immobilized lipases · Kinetic model · Reactor model · *(R,S)*-1-phenylethanol · Ping-pong Bi-Bi · Kinetic resolution

INTRODUCTION

A real manual experimentation in conjunction with a proper simulation program will provide better understanding about the reaction. This understanding is very important in developing a model. Having the knowledge on a reaction will be easier for the work of modelling, thereby increasing the confident level of the model accuracy. Moreover, a good qualitative understanding of the system will also simplify the optimization process. As a result, the model can be used for better experimental design.

However, the majority of bioreaction creates nonlinear data. The technique of nonlinear regression is used to fit the data into a model. The nonlinear regression will be able to find the best-fit values of the model parameters such as rate constants in enzymatic reaction. The linearizing methods such as Lineweaver-Burk plot for enzyme kinetic data, Scatchard plot for binding data and logarithmic plot for kinetic data are less accurate. This technique usually requires a large number of experimental data to satisfactory fit the model equation. Furthermore, the outcome is only valid within the narrow range of the evaluated data.

Recently, the mathematical modelling of bioprocess using nonlinear regression technique has become increasingly important among the scientific approaches. Mathematics based computer simulation is an essential part of research because of cost effectiveness and time saving features. The studies on modelling of enzymatic reactions are become crucial in the last few years [1]. However, there are very limited papers reported on the kinetic modelling of lipases catalyzed reactions, including esterification [1-4], transesterification [5-7] and hydrolysis [8-10].

In this study, the kinetic data used for the modelling of lipases catalyzed *(R,S)*-1-phenylethanol resolution was obtained from the experiments reported in previous paper

Corresponding Author: Chua Lee-Suan, Chemical Engineering Pilot Plant, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia, Tel: +60 19 7214378, E-mail: chualeesuan@yahoo.com.

[11]. The resolution of *(R,S)*-1-phenylethanol catalyzed by immobilized lipases (ChiraCLEC-PC) in batch stirred tank reactor was used to develop the mechanistic model. The developed model was then verified by fitting it to another set of experimental data catalysed by immobilized lipases (Chirazyme L2, c.-f., C3, lyo) from different source of microorganism. The kinetic data coupled with the computer modelling studies will be able to describe the interaction between the enzymes and substrate molecules.

Subsequently, an immobilized lipase recirculated packed bed reactor was used to carry out the similar resolution. This plug flow type of reactor is preferable for the resolution as it can readily be scaled up. The other advantages are the possibility of enzyme reusability, ease of reactor maintenance as well as operation. Furthermore, many researches repeatedly reported that an ideally mixed reactor is inferior for the kinetic resolution process [12-13]. However, there are two types of operation mode in packed bed reactor system. The recirculated mode was chosen rather than recycled mode because of the better performance of the former [14].

In ideal case, immobilized enzyme reactor accounts only the intrinsic kinetic of enzyme. However, the use of immobilized enzymes is usually accompanied by mass transfer resistance even in the laboratory scale reactor. Both axial and radial concentration gradients are also expected present in the reaction system. This is because substrate molecules were transported to the surface of enzyme particles and subsequently into their pores by molecular diffusion. This diffusional restriction will be of greater significant for a reactive system. As reported by Xiu *et al*. [15], the presence of intraparticle diffusion in the steoreselective reaction would lead to an apparent decrease in enantioselectivity. Therefore, the overall steady state conversion depends not only on the kinetics and the mass transfer characteristics, but also on the dispersion effects of the bioreactor [16]. The effects of these factors have been studied separately by several researchers [15, 17-19]. They concluded that the quantitative knowledge of these factors is important for efficient design of immobilized enzyme reactor.

In present study, a reactor model has been constructed using the rate equation developed from the kinetic model. Chirazyme L2, c.-f., C3, lyo was packed in the column as enzyme bed. The validity of the model was verified by fitting it to the experimental data from five-fold larger scale recirculated packed bed reactor catalyzed by the same enzyme. The model is very useful for the prediction of enzyme performance and further optimization.

Therefore, the objectives of this study were developed a kinetic model and a reactor model for the enzymatic resolution of *(R,S)*-1-phenylethanol. The strategies that used for model construction were explained and the models that proposed were further verified with satisfied results.

METHODOLOGY

Development of Kinetic Model: This reaction was a multisubstrate and multiproduct reaction. It behaved like a single substrate reaction obeying Michaelis-Menten equation because one of the substrates concentrations was varied, while the other was held constant.

The strategy used to carry out the kinetic modelling process is presented in Fig. 1. Overall, there are 6 major steps involved in developing a kinetic model for the reaction. First, a plausible reaction mechanism was determined from the experimental observation and theoretical understanding. Thereafter, the model equation was formulated from the reaction mechanism which was represented by a series of chemical equations. The model, nonlinear differential equations was solved numerically using Matlab 7.6. The Runge-Kutta 4,5 was used as the ordinary differential equation (ODE) solver, while nonlinear least squared method was used for the optimization of initial estimate values of elementary rate constants. The necessary information including the

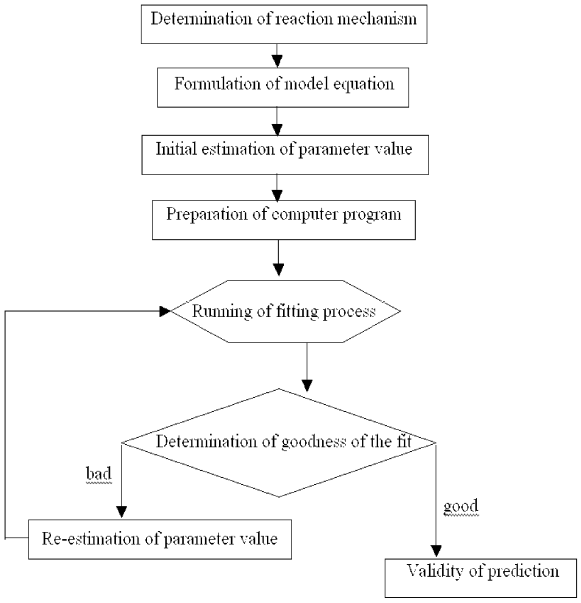

Fig. 1: Kinetic model development strategy

concentrations of substrates and enzymes were incorporated into the program preparation step. Once the program was constructed, the fitting process could be carried out. The goodness of the fit was determined by the values of sum of the squared residual and correlation coefficient. A few runs were carried out using the generated rate constant values. A good fit was obtained when the output of the model fits reasonably well both qualitatively and quantitatively. The validity of the kinetic model was verified by fitting it to another set of experimental data catalyzed by the same class of enzyme under the same reaction conditions from different source of microorganism.

Determination of Reaction Mechanism: The real enzymatic reaction mechanism is very complex and difficult to understand. This is mainly due to the interactive nature of biological processes. Nevertheless, most authors [20-22] agreed that the reaction occurs via the formation of an active complex between an acyl group of substrate and the active centre of lipases. The other phenomena such as inhibition and mass transfer effect also need to be considered in the enzymatic system. However, mass transfer limitation could be neglected in this study. This was because the reactions were carried out in a well-stirred tank reactor [11] and the particle size of the lipases was relatively small. Since high concentration of substrates was employed, it is sufficient to determine a plausible mechanism of the reaction including the reversibility and inhibition phenomena.

Trials and Errors Approach: The mechanism of Michaelis-Menten with different types of inhibition had been tested for initial trials in selecting a correct mechanism. The mechanisms included simple Michaelis-Menten, competitive, uncompetitive and noncompetitive inhibition. Neither of these mechanisms nor product inhibition could represent the reaction.

When Michaelis-Menten mechanism with the competitive inhibition of substrates and product was introduced, the goodness of the fit was significantly improved. The product inhibition refers to water inhibition only. The inhibition caused by *(R)*-ester was eliminated as this inhibition step could lead to a large deviation in the fit.

The reversibility of each inhibition step was also determined in order to improve the fitting results. The results indicated that there were two possible mechanisms that could represent the reaction mechanism. The first type was Michaelis-Menten with irreversible inhibition of racemic alcohol and lauric acid and reversible water inihibition. The other type of mechanism was Michaelis-Menten with irreversible racemic alcohol, lauric acid and water inhibition. The only difference between these two mechanisms was the reversibility of water inhibition step. The second type of mechanism was chosen for the modelling works because of the decrease of enzyme performance in the second run of experiment [23]. The decrease was assumed mainly due to the change of hydration level of the recycled enzymes [24].

Straathof's Approach: The strategy developed by Straathof [25] was used to validate the selected reaction mechanism. He reported that the strategy in combination with the experimental observation was enough and easier to determine the correct structure of the reaction mechanism.

The stoichiometry of the reaction is bi-bi. This is because both enzymes, ChiroCLEC-PC and Chirazyme L2, c.-f., C3, lyo used in this resolution belong to the enzyme classification of hydrolases (EC 3.1.1.3).

The order of reactants can only be determined under the steady state kinetics. It determines the sequence in which the substrates enter or the products leave the enzyme active site. This order determines which rate equation is valid for the reaction. The possible mechanisms followed by bi-bi reaction are ping-pong, sequential and random ordered ternary complex [13]. The ping-pong mechanism is usually followed by the reaction involving two substrates and two products [3]. Ping-Pong mechanisms are assumed to be typical of group transfer or substituted enzyme reactions [26]. The bi-bi reaction can also be either sequential ordered or random ordered depending on which substrates or products will enter the enzyme first. According to Straathof [25], the random mechanism seems to occur much less frequently than the ordered mechanism.

Based on the experimental observation [11], the use of *(R,S)*-1-phenylethanol and lauric acid in excess would lead to a decrease in enzyme performance. Water as by-product was the main factor in restricting the reaction towards completion. Therefore, a Ping-Pong Bi-Bi mechanism with the inhibition of *(R,S)*-1-phenylethanol, lauric acid and water was postulated. The mechanism presented in schematic diagram can be referred to Fig. 2.

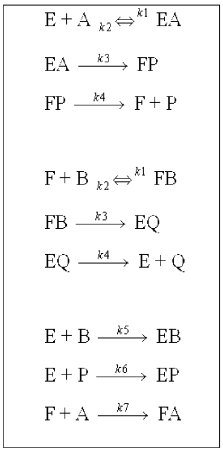

Fig. 2: Schematic diagram of Ping-Pong Bi-Bi mechanism

The mechanism consisted of two steps, namely reaction step and inhibition step. In the reaction step, the first product must leave before the second substrate binds to the active site. Lauric acid was the first substrate, which bound to the enzyme and followed by the release of water. After the first product, water was released, the second substrate, racemic alcohol bound to the enzyme complex before the chiral ester was synthesized. It is worthy to note that the substrates enter into the active site in the same order as the products leave [25].

The formation of nonproductive enzyme complex in the inhibition steps was irreversible. This is because the rate of reaction steps was much higher than the inhibition steps. As a consequence, the amount of nonproductive enzyme complex formed was relatively small and the reverse rate of inhibition steps was negligible.

Formulation of Kinetic Model Equation: After selecting the plausible reaction mechanism, a rate equation was formulated based on the reaction rates of experiments. The reaction rates could be analyzed by using either initial rate analysis or progress curve analysis under steady state condition. The initial rate analysis is easier and widely used when only a limited set of kinetic properties is required. The traditional Lineweaver-Burk plot uses the method of initial rate analysis. The progress curve analysis becomes attractive if the parameters such as reaction equilibrium, enzyme inactivation, product inhibition and substrate decomposition are required. A relatively modest set of progress curves may contain more information than a large set of initial reaction rates. Hence, the mechanistic study using progress curve analysis may reduce the experimental efforts.

By taking into account all the elementary steps, the mathematical kinetic model for the resolution of *(R,S)*-1-phenylethanol in batch stirred tank reactor was derived using mass action law. The principle of mass action law was used to convert the reaction mechanism into mathematical equations. These differential equations were derived using steady state assumption. The change of free enzyme, enzyme complex and enzyme intermediate concentration with time is equalled to zero as shown in Equations (1) to (8).

$$\frac{d[EA]}{dt} = k_1[E][A] - k_2[EA] - k_3[EA] = 0 \quad (1)$$

$$\frac{d[FP]}{dt} = k_3[EA] - k_4[FP] = 0 \quad (2)$$

$$\frac{d[FB]}{dt} = k_1[F][B] - k_2[FB] - k_3[FB] = 0 \quad (3)$$

$$\frac{d[EQ]}{dt} = k_3[FB] - k_4[EQ] = 0 \quad (4)$$

$$\frac{d[EB]}{dt} = k_5[E][B] = 0 \quad (5)$$

$$\frac{d[EP]}{dt} = k_6[E][P] = 0 \quad (6)$$

$$\frac{d[FA]}{dt} = k_7[F][A] = 0 \quad (7)$$

$$\frac{d[F]}{dt} = k_4[FP] - k_1[F][B] + k_2[FB] - k_7[F][A] = 0 \quad (8)$$

Therefore,

$$[EA] = \frac{k_1[E][A]}{k_2 + k_3}$$

$$[FP] = \frac{k_1 k_3[E][A]}{k_4(k_2 + k_3)}$$

$$[FB] = \frac{k_1[B]}{k_2 + k_3}\left(\frac{k_1 k_3[E][A]}{k_1 k_3[B] + k_2 k_7[A] + k_3 k_7[A]}\right)$$

$$[EQ] = \frac{k_1^2 k_3^2[E][A][B]}{k_4(k_2 + k_3)(k_1 k_3[B] + k_2 k_7[A] + k_3 k_7[A])}$$

$$[F] = \frac{k_1 k_3[E][A]}{k_1 k_3[B] + k_2 k_7[A] + k_3 k_7[A]}$$

As known, the initial concentration of enzyme is equal to the sum of enzyme in the intermediate forms as presented in Equation (9).

$$[E] = Eo - \{[EA] + [FP] + [EB] + [EQ] + [EB] + [EP] + [FA] + [F]\} \quad (9)$$

Finally, the rate of substrate depletion (Equations (10) and (11)) and product formation (Equations (12) and (13)) could be derived.

$$V_A = -\frac{d[A]}{dt} = -k_1[E][A] + k_2[EA] + k_7[F][A] \quad (10)$$

$$V_B = -\frac{d[B]}{dt} = -k_1[F][B] + k_2[FB] - k_5[E][B] \quad (11)$$

$$V_P = \frac{d[P]}{dt} = k_4[FP] - k_6[E][P] \quad (12)$$

$$V_Q = \frac{d[Q]}{dt} = k_4[EQ] \quad (13)$$

These equations were then incorporated into computer program in order to simultaneously fit multiple parameters of multiple equations on multiple curves. The program fitted simultaneously a number of elementary rate constants by the numerical integration of the batch differential equations. A series of conversion curves at fixed reaction conditions, such as temperature and solvent composition was used in the fitting process. This model is valid for the substrates concentration ranging from 25 - 250 mM with a unique set of kinetic constants.

Preparation of Program: Matlab 7.6 was used as a modelling tool to prepare the fitting program. The non-stiff medium order (4-5) ordinary differential equation (ODE) solver was used to numerically solve the differential equation model. The solvers repeatedly call the oDE file to evaluate the system of differential equation at various times. ODE45 is based on an explicit Runge-Kutta formula, which is used to generate a series of points. This numerical integration of the differential rate equations in combination with a nonlinear iteration fitting process is more applicable route, as the analytical integration is exceedingly complex [27].

Another function of the software is the nonlinear least square method from optimization toolboxes. This method was used to optimize the initial estimate values of elementary rate constants and to minimize the error between input and output. The sum of squared residuals was calculated with the assumption that there was no error on the time axis, but only on the concentration axis. The differences between the experimental data and the calculated data were squared and summed in the fitting procedure.

Designing Reactor Model Equation: A mathematical model has been developed for the immobilized lipase-catalyzed resolution in a recirculated packed bed reactor. The schematic diagram of the reactor is shown in Fig. 3. The concept of dispersion model was used to describe the non-ideal reactor in this study. The design equation of the reactor at steady state can be written as Equation (14) [28]. The kinetic resolution was assumed to be carried out under isothermal conditions, constant effective diffusivity of substrate inside the porous support and homogeneously distributed enzyme activity. It was also assumed no flow in radial direction in the evenly packed enzyme bed with optimized flow rate. Furthermore, the reaction rates are independent of lauric acid concentration as the acyl donor was employed in large excess.

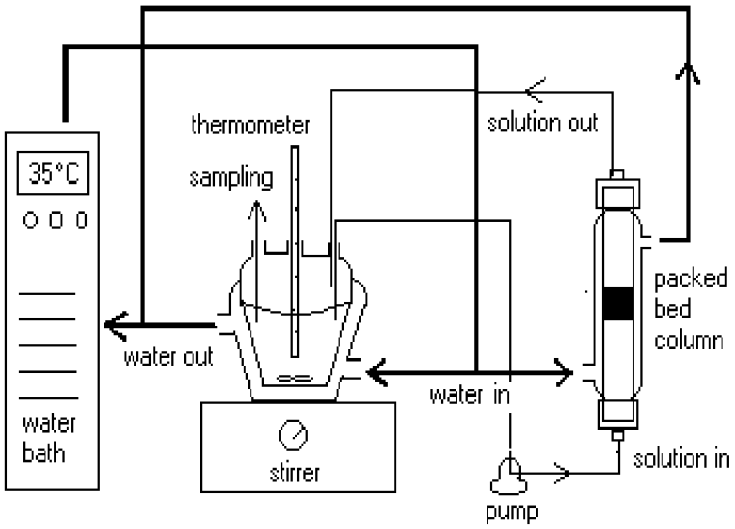

Fig. 3: Schematic diagram of recirculated packed bed reactor

$$D_e \frac{\partial^2 C}{\partial l^2} - \frac{\partial (U.C)}{\partial l} = \frac{\partial C}{\partial t} \quad (14)$$

Kobayashi and Moo-Young [29] were the first who applied the dispersion model to immobilized enzyme reactor. They studied the influence of dispersion and external mass transfer on the conversion of fixed bed reactor. Lortie and Pelletier [30] have shown that plug flow reactor model with external mass transfer resistance can represent adequately a fixed bed immobilized enzyme reactor. The mathematical model can be further expressed in the dimensionless variables as Equation (15). The dimensionless expressions for substrate concentration and reactor length are given by Equations (16) and (17). The concentration of substrate is expressed as fractional conversion (X) and the reactor length is converted into the length ratio of reactor (*Z*). The term of $\frac{dC}{dt}$ is replaced by the parameter of reaction rate (r) as shown in Equation (18).

$$\frac{D_e}{UL}\frac{d^2X}{dZ^2} - \frac{dX}{dZ} = -\frac{Lr}{UC_{Bo}} \quad (15)$$

$$X = \frac{C_{Bo} - C}{C_{Bo}} \quad (16)$$

$$Z = \frac{l}{L} \quad (17)$$

$$\frac{dC}{dt} = r \quad (18)$$

The quantity of $\frac{D_e}{UL}$ can be written into the form of Peclet number as Equation (19). It measures the ratio of transport rate by convection to the transport rate by diffusion or dispersion. If the value of Peclet number is infinity, the first term of Eq. (15) will be negligible. The equation reduced to the plug flow reactor equation. It is known that the value of Peclet number is approximately 10^6 for open tubes and 10^3 for packed bed [28].

$$P_e = \frac{UL}{D_e} \quad (19)$$

Equation (15) becomes Equation (20) after incorporating the overall effectiveness factor in the right hand term. The introduction of the overall effectiveness factor (Ω) in the equation is mainly due to the presence of mass transfer limitation in the reaction system.

$$\frac{1}{P_e}\frac{d^2X}{dZ^2} - \frac{dX}{dZ} = -\frac{\Omega Lr}{UC_{Bo}} \quad (20)$$

Therefore, this model equation evaluates the performance of two major components in recirculated packed bed reactor such as packed column and stirred tank vessel. The length of packed column determines the degree of conversion. This is because reaction took place in the column packed with enzyme particles. However, the reactions were affected by the problem of mass transfer limitation. The mixing effect in the stirred tank vessel is also another important factor have to be considered in designing model equation. The mixing effect of the reactor can be characterized by Peclet number. It measures the degree of dispersion. Hence, the model equation takes into account the effects of mass transfer and axial dispersion by evaluating the overall effectiveness factor and Peclet number. Data gathered (unpublished yet) during various experiments revealed that the effect of dispersion in radial direction was negligible for Re < 1. Danckwerts boundary condition for a closed-closed vessel system was assumed for this study. There was no dispersion either at the entrance or at the exit section. Numerically, the input to the reactor at time, t+1 was its output at time, t.

Determination of Reactor Design Parameters: The values of the reactor design parameters such as the total length of reactor and superficial velocity from the design equation can be determined using the Equations (21) and (23), respectively. The total length of reactor (L) can be determined by multiplying the number of passes (n) that gone through by the reacting fluid to the single unit of reactor length (L_{unit}). One single unit of reactor length can be determined from the value of bed height (8 mm). The internal diameter of the packed column was 16 mm. The optimal flow rate from previous studies was 21.1 ml/min and the total reaction time was 380 min [11]. Equation (22) was used to determine the number of passes.

$$L = nL_{unit} \quad (21)$$

$$n = \frac{TQ'}{V} \quad (22)$$

The superficial velocity can be determined by multiplying the voidage of enzyme bed (ϵ = 0.50) to the interstitial velocity of fluid (v) as Equation (23). Equation (24) was used to calculate the value of interstitial velocity.

$$U = v\epsilon \quad (23)$$

$$v = \frac{Q'}{A'\varepsilon} \quad (24)$$

The corresponding rate equation (r) from kinetic model was incorporated into the reactor design equation. Since the experimental data available for model fitting is the time course of substrate conversion, it is necessary to convert the reaction progress into the expression of dimensionless length ratio of reactor. The formulation for converting the reaction time into the length ratio of reactor is shown in Equation (25).

$$Z = \frac{Q't}{A'L} \quad (25)$$

RESULTS AND DISCUSSION

Interpretation of Kinetic Model: The kinetic data from the experiments of varying racemic alcohol concentrations catalyzed by ChiroCLEC-PC was used to fit the model. The data consisted of six full reaction progress curves. The alcohol concentration was varied from 25 - 250 mM at the fixed lauric acid concentration (150 mM). The result of the fit is presented in Fig. 4. From the qualitative point of view, the model fits the experimental data satisfactory.

The goodness of the fit was also evaluated using the dimensionless parameter of correlation coefficient. The model equation fitted the experimental data with the correlation coefficient values close to one, except for the curve at 25 mM of racemic alcohol (Table 1). The reason for the low value of correlation coefficient was attributed by the high reaction rate of resolution at the high ratio of enzyme to substrate (0.164). The resolution achieved conversion up to 20 - 30 per cent just after a few seconds of reaction time. The rapid reaction rate caused difficulty in manually sampling. Therefore, the lack of initial portion of experimental data resulted in a low value of correlation coefficient for this curve.

Table 1: Correlation coefficients of model fitting

	Lauric acid (150 mM)	
(R,S)-1-phenylethanol (mM)	ChiroCLEC-PC	Chirazyme L2, c.-f., C3, lyo
25	0.7428	0.2779
50	0.9409	0.8966
100	0.9743	0.9625
150	0.9903	0.9957
200	0.9867	0.9934
250	0.9908	0.9876

Since the number of elementary steps in the reaction mechanism was relatively large, an assumption was made in order to reduce the number of parameters that going to be optimized as minimum as possible. The steps of first substrate binds to the enzyme to produce first product were assumed to have the same constant values to the steps that second substrate binds to the enzyme complex to produce second product [11]. It is important because the precision of fitting decreases exponentially with an increase number of parameters. The absolute minimum

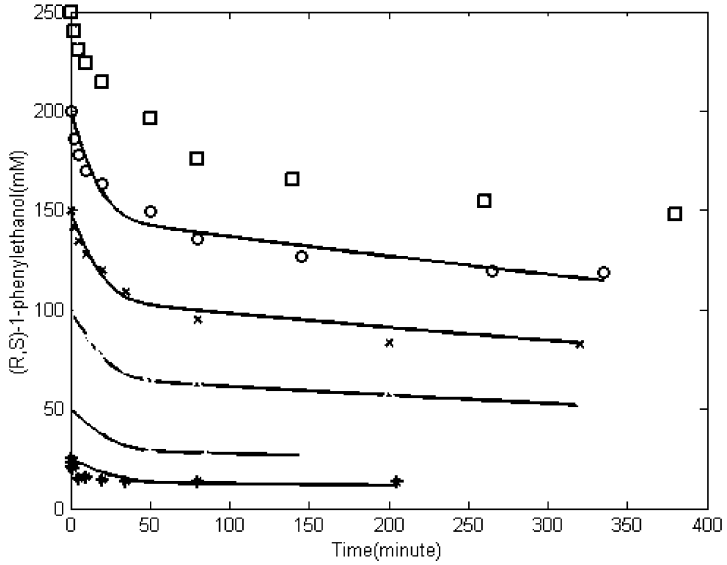

Fig. 4: ChiroCLEC-PC catalyzed resolutions at various initial concentrations of racemic alcohol. (Solid line, — : model result. Symbol, □, O, X, ◇, + and ♦ : experimental data at the initial concentration of (*R,S*)-1-phenylethanol, 25, 50, 100, 150, 200 and 250 mM)

Table 2: Elementary rate constants values

Elementary steps (unit)	Rate constants
k_1 ($mM^{-1}s^{-1}$)	0.2238
k_2 (s^{-1})	1.5594×10^{5}
k_3 (s^{-1})	1.9597×10^{5}
k_4 (s^{-1})	1.0367×10^{5}
k_5 ($mM^{-1}s^{-1}$)	1.1050×10^{-4}
k_6 ($mM^{-1}s^{-1}$)	4.4408×10^{-14}
k_7 ($mM^{-1}s^{-1}$)	0.5839

Table 3: Mean squared errors for Chirazyme L2, c.-f., C3, lyo catalyzed reactions

(R,S)-1-phenylethanol (mM)	Mean squared error (mM)
25	1.21
50	1.44
100	1.41
150	0.67
200	1.14
250	1.81

cannot be found easily when the number of parameters is large. The possibility leads to local minima in the parameter space is increasing as the rate equations is nonlinear. The program Encora 1.2 developed by Straathof [25] has also not been designed to work properly if the number of parameters to be optimized exceeds six.

For the initial estimation of the elementary rate constants, relatively large values were given for the reaction steps and small values were given for the inhibition steps. The fitting procedure was carried out starting from different initial estimate values until the predicted profile matched the experimental profiles. It is essential to have a good starting point in order to obtain the best fit curve. A good starting point can only be obtained from the qualitative understanding of the reaction. Hence, it is more beneficial by carrying out the experimental work before modelling.

The elementary rate constant values generated by the program are presented in Table 2. Comparison was made among the constants sharing with the same unit. The values of k2, k3 and k4 were not much different. Among them, k4 was the lowest value compared to k2 and k3. The conclusion could be drawn from these values was the reaction more favourable to reverse direction (k2>k4) compared to product formation step. This conclusion in line with the finding of experimental work that excessive lauric acid was needed to speed up the reaction. However, the use of lauric acid in excess inhibited the enzyme complex. It was found that the optimum concentration of lauric acid was 150 mM [11].

On the other hand, the degree of lauric acid inhibition was higher than the other inhibitors, such as *(R,S)*-1-phenylethanol (k7>k5) and water (k7>k6). Since k7 was greater than k5 and k6, lauric acid was more favourable to bind with complex enzyme in the inhibition step. However, the degree of inhibition due to racemic alcohol and water was not predominant in this study. It is noticed that the change in the k6 value has a negligible effect on the fit. This is because no significant amount of water was formed within the substrate concentration range from 25 to 250 mM during the reactions.

The mean squared errors of the progress curves were in the range of 0.84 to 1.50. These values were acceptable, since the number of data points used for modelling was only 9 to 11 in one single progress curve. The validity of the model was then verified by fitting the experimental data from Chirazyme L2, c.-f., C3, lyo catalyzed resolutions.

Validity of Kinetic Model: The acceptance level of the model was further enhanced when a good agreement between the predicted and the experimental data from Chirazyme L2, c.-f., C3, lyo catalyzed resolutions was achieved. From the quantitative point of view, the curves also achieved good correlation coefficient, except for the curve at the initial alcohol concentration of 25 mM (Table 1). The same reason given in the previous section was used to explain the observation.

Another quantitative parameter that used for evaluating the goodness of the fit result is the mean squared errors as presented in Table 3. The mean squared errors of the progress curves were ranging from 0.67 to 1.81. The simulated data fit well to the experimental data as the mean squared errors were less than 2. To further illustrate the goodness of the fit, the percent relative errors of the progress curves were determined. It was found that the percent relative errors of the progress curves at higher *(R,S)*-1-phenylethanol concentration are relatively low, less than 7 per cent. However, the progress curves at the low concentration of *(R,S)*-1-phenylethanol have slightly higher percent relative errors. This may be due to the rapid reaction rate at lower *(R,S)*-1-phenylethanol concentration for manual sampling. Overall, the experimental data fit very well to the simulated results.

Interpretation of Reactor Model: The developed reactor model fits well to the recirculated packed bed reactor for the *(R,S)*-1-phenylethanol resolution. Fig. 5 is the result of the fit. The goodness of the fit was evaluated by

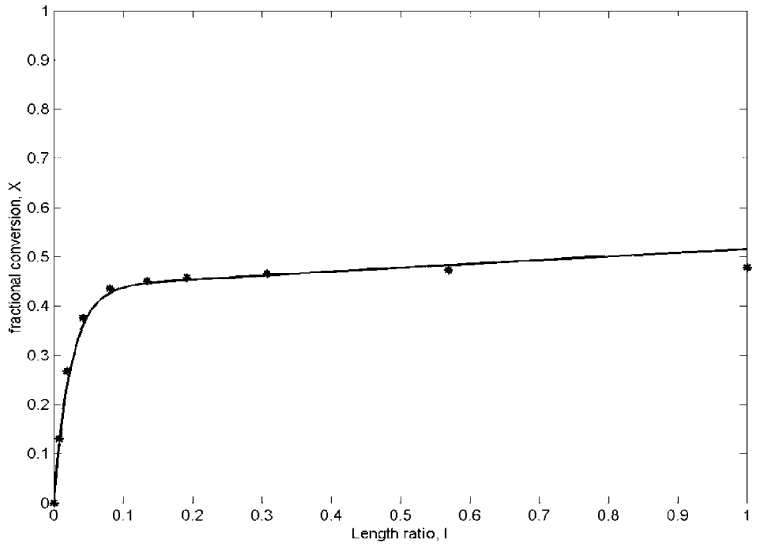

Fig. 5. Fitting result of data from small scale reactor (Solid line, — : model result. Symbol, *: experimental data)

determining the correlation coefficient value. This value can be achieved up to 0.9938 in this study. It reflects that 99.38 per cent of the experimental data fit to the simulated data.

The value of overall effectiveness factor is 0.48. It means that 48 per cent of the secondary alcohol was converted into fatty ester. As known, the racemic alcohol contains *(R)*- and *(S)*-enantiomers at equal molar ratio. The enzymes used for the resolution are highly selective towards *(R)*-enantiomer only. Hence, the obtained factor value is near to theoretical value, 50 per cent.

The Peclet number of this model was 40. The incorporation of recirculation mode in the packed bed reactor resulted a batch mode reactor system [11,31]. According to Xiu *et al*. [15], Peclet number should be controlled at more than 50 in order to minimise the external mass transfer and the axial dispersion limitations on the enzyme performance in fixed bed reactor. However, the enantioselectivity of the enzyme was not affected by the low Peclet number value in this study. The enzyme remained highly selective towards the (R)-enantiomer of the secondary alcohol.

The presence of external mass transfer limitation in the packed column was evaluated using Damkohler number, Da as presented in Equation (26). The maximum volumetric rate of reaction, V_{max} reported from previous kinetic studies was 0.106 m.mol/L/s. The mass transfer coefficient, K_L which was determined from Equation (27) was 2.657 x 10^{-8} m/s [32]. The effectiveness diffusivity, D_{eff} of *(R,S)*-1-phenylethanol was estimated from Wilke-Chang correlation to be 1.328 x 10^{-12} m^2/s [32]. It was found that the value of Da was less than unity, the mass transfer rate must be greater than the rate of reaction. The reaction solution was pumped through the packed enzyme at the optimal flow rate, 21.1 ml/min. This flow rate was assumed to preclude external mass transfer limitation.

$$Da = \frac{V_{max}}{K_L - C_{Bo}} \quad (26)$$

$$K_L = \frac{2D_{eff}}{d} \quad (27)$$

The existence of internal mass transfer limitation was investigated by using Thiele modulus as discussed in Chua and Sarmidi [11]. Based on Weisz's criteria, the internal mass transfer limitation was not significant because the observable modulus was less than 0.3 ([33]. The particle size of the enzymes was relatively small, thus did not create an obvious gradient of substrate concentration within the particles.

Besides that, Biot number was used to evaluate the absence of mass transfer limitation in the reactor system. This number measures the ratio of intraparticle diffusion resistance against the external mass transfer resistance. Its value became one after replacing the values of the diffusivity of substrate (D_{eff}) and the mass transfer coefficient (K_L) into Equation (28). The result indicated that the degree of intraparticle resistance equalled to the external mass transfer resistance. Since the intraparticle diffusion was negligible due to the relatively small enzyme particles, the external mass transfer problem was also not significant in the packed bed reactor.

$$Bi = \frac{K_L d}{2D_{eff}} \quad (28)$$

The observation was also interpreted from another quantitative point of view. Both the time constant for reaction, t_r (1132.6 s) and the time constant for diffusion, t_d (1.0 s) were calculated using Equation (29) and Equation (30), respectively [34]. Since $t_r \gg t_d$, the reactions were not controlled by mass transfer.

$$t_r = \frac{C_{Bo}}{\upsilon_{obs}} \quad (29)$$

$$t_d = \frac{D_{eff}}{K_L^{\ 2}} \quad (30)$$

In order to further confirm the absence of mass transfer limitation in the reaction system, two more parameters were compare; the reaction rate per unit interfacial area, r_r (Equation (31) and the estimated rate of substrate diffusion per unit interfacial area, r_d (Equation (32) [35]. Since the substrate diffusion rate per unit area (2.32 x 10^{-6} m.mol/L/s) was greater than the reaction rate per unit area (7.35 x 10^{-10} m.mol/L/s), mass transfer limitation did not influent the reaction rate catalyzed by the enzymes in this study. Therefore, the reactions were solely controlled by enzyme kinetic.

$$r_r = \frac{\Phi r_a}{a} \quad (31)$$

$$= \frac{r_a \upsilon_{obs}}{3}$$

$$r_d = K_L C_{Bo} \quad (32)$$

The validity of the proposed model was quantified by means of simulation experiments. The developed model was used to simulate the results from larger scale reactors. The output of the model indicated that the model fit to the experimental data satisfactory with the correlation coefficient value, 0.9901.

CONCLUSION

The resolution of *(R,S)*-1-phenylethanol followed Ping-Pong Bi-Bi mechanism with irreversible inhibition from *(R,S)*-1-phenylethanol, lauric acid and water. The small deviation between the experimental results and those calculated by the rate equation showed that a good agreement was achieved within the experimental range. Hence, the kinetic model is valid for subsequent reactor design and for simulation purposes. The developed reactor model can be used to predict the steady state performance of the recirculated packed bed reactor for lipased-catalyzed resolutions with high accuracy by coupling with both mass transfer and axial dispersion effect.

NOTATION

A	Lauric acid
B	*(R,S)*-1-phenylethanol
E	Enzyme
Eo	Initial concentration of enzyme
F	Enzyme complex
P	Water
Q	Chiral ester
EA	Enzyme intermediate
EB	Enzyme intermediate
FA	Enzyme intermediate
FB	Enzyme intermediate
EP	Enzyme intermediate
EQ	Enzyme intermediate
FP	Enzyme intermediate
k1-7	Elementary rate constants ($mM^{-1}s^{-1}$, s^{-1})
V_A	Rate of lauric acid depletion ($-mMs^{-1}$)
V_B	Rate of *(R,S)*-1-phenylethanol ($-mMs^{-1}$)
V_P	Rate of water formation (mMs^{-1})
V_Q	Rate of chiral ester formation (mMs^{-1})
D_a	Damkohler number
D_e	Effective dispersion coefficient (m^2s^{-1})
C	Substrate concentration in bulk flow (M)
L	Total length of reactor (m)
U	Superficial velocity (ms^{-1})
ν	Interstitial velocity of fluid (ms^{-1})
T	Total reaction time (second)
X	Fractional conversion
Z	Length ratio of reactor
r	Reaction rate (mMs^{-1})
r_a	Radius of enzyme particle (m)
r_r	Reaction rate per unit interfacial area ($m.Ms^{-1}$)
r_d	Substrate diffusion per unit interfacial area ($m.Ms^{-1}$)
C_{Bo}	Initial concentration of *(R,S)*-1-phenylethanol (M)
Ω	Overall effectiveness factor
V	Volume of packed column (litre)
V_{max}	Maximum volumetric rate of reaction (mMs^{-1})
ν_{obs}	Observed initial reaction rate (Ms^{-1})
Q'	Flow rate of solution (m^3L^{-1})
A'	Cross sectional area of packed bed (m^2)
Bi	Biot number
K_L	Mass transfer coefficient (ms^{-1})
D_{eff}	Diffusivity of *(R,S)*-1-phenylethanol (m^2s^{-1})
a	Interfacial area per volume of organic phase (m^2L^{-1})
d	Diameter of enzyme particle (m)
n	The number of passes
t_r	Time constant for reaction (s)
t_d	Time constant for diffusion (s)
Φ	Phase volume ratio

REFERENCES

1. Garcia, T., A. Coteron, M. Martinez and J. Aracil, 2000. Kinetic Model for the Esterification of Oleic Acid and Cetyl Alcohol using an Immobilized Lipase as Catalyst. Chemical Engineering Science, 55: 1411-1423.

2. Mukesh, D., S. Jadhav, A.A. Banerji, K. Thakkar and H.S. Bevinakatti, 1997. Lipase-catalyzed Esterification Reactions – Experimental and Modelling Studies. J. Chemical Technol. Biotechnol., 69: 179-186.
3. Paiva, A.L., D. van Rossum and X.F. Malcata, 2002. Kinetics of Lipase-mediated Synthesis of Butyl Butyrate in n-hexane. Biocatalysis and Biotransformation, 20: 43-51.
4. Znidarsic-Plazl, P. and I. Plazl, 2009. Modelling and Experimental Studies on Lipase-catalyzed Isomyl Acetate Synthesis in a Microreactor. Process Biochemistry, 44: 1115-1121.
5. Chulalaksananukul, W., J.S. Condoret and D. Combes, 1992. Kinetics of Geranyl Acetate Synthesis by Lipase Catalysed Transesterification in n-hexane. Enzyme and Microbial Technol., 14: 293-298.
6. Hita, E., A. Robles, B. Camacho, P.A. Gonzalez, L. Esteban, M.J. Jimenez, M.M. Munio and E. Molina, 2009. Production of Structured Triacylglycerols by Acidolysis Catalyzed by Lipases Immobilized in a Packed Bed Reactor. Biochemical Engineering J., 46: 257-264.
7. Mukesh, D., A.A Banerji, R. Newadkar and H.S. Bevinkatti, 1993. Mathematical Modelling of Enzymatic Butanolysis of Vegetable Oils. Biocatalysis, 8: 191-199.
8. Goswami, D., R. Sen, J.K. Basu and S. De, 2010. Surfactant Enhanced Ricinoleic Acid Production using *Candida rugosa* Lipase. Bioresource Technol., 101: 6-13.
9. Hermansyah, H., M. Kubo, N. Shibasaki-Kitakawa and T. Yonemoto, 2006. Mathematical Model for Stepwise Hydrolysis of Triolein using *Candida rugosa* Lipase in Biphasic Oil-Water System. Biochemical Engineering J., 31: 125-132.
10. Rice, K.E., J. Watkins and C.G. Hill, 1999. Hydrolysis of Menhaden Oil by a *Candida cylindracea* Lipase Immobilized in a Hollow Fiber Reactor. Biotechnol. Bioeng., 63: 33-45.
11. Chua, L.S. and M.R. Sarmidi, 2004. Immobilised Lipase-catalysed Resolution of *(R,S)*-1-phenylethanol in Recirculated Packed Bed Reactor. Journal of Molecular Catalysis B: Enzymatic, 28: 111-119.
12. Rakels, J.L.L., H.T. Paffen, A.J.J. Straathof and J.J. Heijnen, 1994. Comparison of Enzymatic Kinetic Resolution in a Batch Reactor and a CSTR. Enzyme and Microbial Technol., 16: 791-794.
13. Straathof, A.J.J., J.L.L. Rakels and J.J. Heijnen, 1992. Kinetics of the Enzymatic Resolution of Racemic Compounds in Bi-bi Reactions. Biocatalysis, 7: 13-27.
14. Wong, T.H., 2002. Modelling of a Recirculated Packed Bed Immobilized Enzyme Reactor. Master Degree Thesis. Malaysia: Universiti Teknologi Malaysia.
15. Xiu, G.H., L. Jiang and P. Li, 2001. Mass-Transfer Limitations for Immobilized Enzyme-catalyzed Kinetic Resolution of Racemate in a Fixed Bed Reactor. Biotechnology and Bioengineering, 74: 29-39.
16. Papathanasiou, T.D., N. Kalogerakis and L.A. Bechie, 1988. Dynamic Modelling of Mass Transfer Phenomena with Chemical Reaction in Immobilized-enzyme Bioreactors. Chemical Eng. Sci., 43: 1489-1498.
17. Indlekofer, M., F. Brotz, A. Bauer and M. Reuss, 1996. Stereoselective Bioconversions in Continuously Operated Fixed Bed Reactors: Modeling and Process Optimization. Biotechnol. Bioeng., 52: 459-471.
18. Matson, S.L. and J.L. Lopez, 1989. Multiphase Membrane Reactors for Enzymatic Resolution: Diffusional Effects on Stereoselectivity. In: Frontiers in Bioprocessing. CRC Press, Boca Raton, FL, pp: 391-403.
19. Xiu, G.H., L. Jiang and P. Li, 2000. Mass-transfer Limitations for Immobilized Enzyme-catalyzed Kinetic Resolution of Racemate in a Batch Reactor. Industrial and Engineering Chem. Res., 39: 4054-4062.
20. Guit, R.P.M., M. Kloosterman, G.W. Meindersma, M. Mayer and E.M. Meijer, 1991. Lipase Kinetics: Hydrolysis of Triacetin by Lipase from *Candida cynlindracea* in a Hollow-fiber Membrane Reactor. Biotechnol. Bioeng., 38: 727-732.
21. Lortie, R., M. Trani and F. Ergan, 1993. Kinetic Study of the Lipase-catalyzed Synthesis of Triolein. Biotechnol. Bioeng., 41: 1021-1026.
22. Shimada, Y., K. Maruyama, A. Sugihara, T. Baba, S. Komemushi, S. Moriyama and Y. Tominaga, 1998. Purification of Ethyl Docosahexanoate by Selective Alcoholysis of Fatty Acid Ethyl Esters with Immobilized *Rhizomucor miehei* Lipase. J. American Oil Chemists' Society, 75: 1565-1571.
23. Chua, L.S. and M.R. Sarmidi, 2006. Effect of Solvent and Initial Water Content on *(R,S)*-1-phenylethanol Resolution. Enzyme and Microbial Technol., 38: 551-556.
24. Khalaf, N., C.P. Govardham, J.J. Lalonde, R.A. Persichetti, Y.F. Wang and A.L. Margolin, 1996. Cross-linked Enzyme Crystals as Highly Active Catalysts in Organic Solvents. Journal of the American Chemical Society, 118: 5494-5495.

25. Straathof, A.J.J., 2001. Development of a Computer Program for Analysis of Enzyme Kinetics by Progress Curve Fitting. Journal of Molecular Catalysis B: Enzymatic, 11: 991-998.
26. Segel, I.H., 1993. Enzyme Kinetics-Behavior and Analysis of Rapid Equilibrium and Steady-state Enzyme Systems. Wiley, New York.
27. Rakels, J.L.L., B. Romein, A.J.J. Straathof and J.J. Heijnen, 1994. Kinetic Analysis of Enzymatic Chiral Resolution by Progress Curve Evaluation. Biotechnol. Bioeng., 43: 411-422.
28. Fogler, H.S., 1992. Elements of Chemical Reaction Engineering, second ed. Prentice Hall PTR, United States of America, pp: 765-773.
29. Kobayashi, T. and M. Moo-Young, 1971. Backmixing and Mass Transfer in the Design of Immobilized-enzyme Reactors. Biotechnol. Bioeng., 13: 893-910.
30. Lortie, R. and D. Pelletier, 1992. Comparison between Dispersion Model and Plug Flow Models for Fixed-bed Enzyme Reactors. American Institute of Chemical Engineers J., 38: 1477-1480.
31. Abu Reesh, I.M., 1997. Predicting the Performance of Immobilized Enzyme Reactors using Reversible Michaelis-Menten Kinetics. Bioprocess Engineering, 17: 131-137.
32. Geankoplis, C.J., 1993. Transport Processes and Unit Operations. 3rd ed. Prentice Hall PTR, New Jersey.
33. Doran, P.M., 1995. Bioprocess Engineering Principles. Academic Press, London.
34. Briggs, G.E. and J.B.S. Haldane, 1925. A Note on the Kinetics of Enzyme Action. Biochem. J., 19: 338-339.
35. Cardenas, F., M.S.D. Castro, J.M.S. Montero, J.V. Sinisterra, M. Valmaseda, S.W. Elson, E. Alvarez, 2001. Novel Microbial Lipase: Catalytic Activity in Reaction in Organic Media. Enzyme and Microbial Technology, 28: 145-154.