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Process Modelling of Isoamyl Acetate Synthesis Catalysed by Lipase from *Candida antarctica* in a Solvent-free System

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Abstract: *The objective of this work is to propose a reaction mechanism and develop a model for the synthesis of isoamyl acetate from isoamyl alcohol and acetate anhydride by using lipase from Candida antarctica in a solvent-free system. Ping-Pong Bi-Bi reaction mechanism with acetic anhydride and acetic acid inhibition is found to be the mechanism which is able to describe this reaction. Unknown parameters of the model developed are obtained by minimising deviation of model prediction from experimental result and it is performed by the Solver Tool in Microsoft Excel. The model prediction is found to match the experimental data quite well (average percentage error = 8.08%).*

Keywords: Isoamyl acetate, ester, process modelling, Ping-Pong Bi-Bi, solvent-free system

1. INTRODUCTION

Ester is a class of chemical compounds which contains carbonyl group (–COO–) as the functional group. Many short-chain esters have distinctive fruit-like odours, and many esters exist naturally in the essential oils of plants.[1] Generally, different esters are responsible for different specific fruity fragrances. However, very often one single compound plays a leading role. Due to its unique characteristic, esters are widely used in flavour, fragrance, food, cosmetic and pharmaceutical industries.[2]

Isoamyl acetate is an ester which is a clear, colourless liquid with a banana-like odour. It appears as one of the most important esters in food industries, with a production capacity of 74,000 kg per year.[3] Due to its characteristic fruity odour, isoamyl acetate is used widely as a component in perfumes and flavouring. Apart from that, isoamyl acetate possesses high solvency property and is used as solvents for paints, coatings, adhesives, cellulose, plastics, fats and wood stains.[4]

Generally, there are three ways to produce ester: (i) extraction from plant materials; (ii) chemical synthesis; and (iii) biological synthesis. However,

extraction from plant materials is not practical for ester production as it is too expensive, which is caused by the bulky property of plant materials. Hence, tremendous amount of processing work is required to produce only minor quality of ester.[5,6]

The production of esters through chemical synthesis is much cheaper compared to plant extraction. However, acid must be employed to catalyse the reaction and hence the product will be greatly polluted.[7] With the increasing orientation toward 'natural' production, biotechnology has become more important in the recent years.[8] By applying biotechnology, the ester production is catalysed by enzyme under milder conditions. Esters produced by this manner will obtain the 'natural' label.[7] Biological processes for ester productions are widely employed in the last decade because they are more environmental friendly. The enzyme can also be reused so that less waste will be discarded.[2]

In enzymatic esterification, lipase can be free or immobilised. However, immobilised lipase is preferred because unit operation required to separate lipase from product stream can be omitted. Furthermore, less amount of enzyme will be needed since immobilised enzyme can retain high catalytic activity over a long time.[9] Based on the review from several literatures, it can be observed that *Candida antarctica* has the best and most stable catalytic performance since it always give high maximum ester conversion.[2,3,5–13]

Although high ester conversion can be achieved in organic solvents such as n-hexane, toxicity can be a problem in most applications.[14] Furthermore, some of the solvents are too expensive to be utilised in a large-scale ester production.[6] Therefore, solvent-free system has been explored for enzymatic synthesis of isoamyl acetate. Solvent-free system facilitates downstream processing as less components exist in the reaction mixture at the end of the reaction and helps in reducing operating cost and reduce environmental impacts.

In isoamyl acetate synthesis process, acetic acid and acetic anhydride are the most common acyl donors. Isoamyl acetate conversion is found to be drastically reduced as the acetic acid concentration increases.[9] This is because acetic acid may be bound to the enzyme intermediate which causes dead-end inhibition and dissolves in the microaqueous layer that surrounds the enzyme, causing protein denaturation. Conversely, as acyl donor in esterification process, acetate anhydride is more superior compared to acetic acid.

Based on the justifications listed above, enzymatic synthesis of isoamyl acetate from isoamyl alcohol and acetic anhydride catalysed by lipase from *Candida antarctica* in solvent-free system is studied. A theoretical model is

developed according to Ping-Pong Bi-Bi mechanism and it is further discussed later.

2. EXPERIMENTAL

2.1 Chemicals

The chemicals used in this study are analytical grades. The purity, usage and supplier for all chemicals used in this study are listed in Table 1.

Table 1: List of materials/chemicals used.

Materials/Chemicals	Purity (%)	Usage	Supplier
Isoamyl alcohol	99.8	Production medium	Merck Co.
Acetic Anhydride	98	Production medium	ACROS Organics
Acetic acid	100	GC standard	Merck Co.
Isoamyl acetate	100	GC standard	Merck Co.
Candida antarctica immobilized lipase	N/A	Production medium	Sigma-Aldrich
n-Hexane	99.8	GC standard	Merck Co.

2.2 Equipment

The enzymatic synthesis of isoamyl acetate from acetic anhydride ad isoamyl alcohol is carried out at lab scale. Throughout the experiment, the synthesis of isoamyl acetate is carried out by using 100 ml Erlenmeyer flasks with stopped rubber which are then placed in incubator shaker. The incubator shaker is used to maintain the mixing rate and control the temperature. Next, Flame Ionization Detector Gas Chromatography (GC-FID) is used to analyse the concentration of all compounds in the samples which are taken out from the reaction mixture in certain period of time. Table 2 shows the list of equipment used in this study.

Table 2: List of equipment used.

Equipment	Usage	Brand
Mini incubator shaker	Production	Benchmark (Incu-shaker mini)
Gas chromatograph	Analysis	Agilent Technologies (7820A GC system)

2.3 Synthesis Procedure

Isoamyl acetate synthesis is carried out without any organic solvent (solvent-free system) in 100 ml stopped rubber Erlenmeyer flask with a working volume of 15 ml. An appropriate amount of enzyme is added into the reaction media which contains a mixture of isoamyl alcohol and acetic anhydride at a variety of temperatures. The reaction mixture is then incubated in an incubator shaker (Benchmark) at 150 rpm for 360 min. Samples are taken at different time intervals and analysed with GC-FID for isoamyl acetate content.

2.4 Analysis

After esterification reaction is done, 0.5 ml of the reaction mixture is withdrawn from the reaction medium at certain time intervals. Samples are analysed by gas chromatograph (Agilent Technologies 7820A). Quantification of data is done by calibration with standard samples. Compounds which have different chemical and physical properties are separated at a different retention time, while the amount or concentration of the compound in the sample is represented by the peak area.

2.5 Determination of Compound Concentration

The concentration of isoamyl acetate is determined by gas chromatography analysis. Before processing the samples of product from the mixture, a standard analysis of the compound in the samples is carried out. Pure materials are used and diluted with n-hexane to achieve certain concentration of the compound. Then it is analysed with gas chromatography to get standard calibration curve for each compound.

3. MODELLING

3.1 Derivation of Rate Equations

Since mathematical model plays a vital role in process control and optimisation, a set of rate equations for esterification is desired to be derived. In the following derivation, Ping-Pong Bi-Bi mechanism is assumed since it has been proven to describe plenty of enzymatic esterification quite well.[9] Most of the models of initial esterification rate developed by using this mechanism produce satisfactory result, hence, this mechanism is used to further develop the model such that the concentration profile of all substrates and products can be predicted based on initial condition. The esterification process of acetic anhydride and isoamyl alcohol is occurring via two reactions as shown below:[2]

Reaction 1 (main reaction):

$$\underset{(A)}{\text{Acetic Anhydride}} + \underset{(B)}{\text{Isoamyl Acetate}} \rightarrow \underset{(P)}{\text{Acetic Acid}} + \underset{(Q)}{\text{Isoamyl Acetate}}$$

Reaction 2 (secondary reaction):

$$\underset{(P)}{\text{Acetic Acid}} + \underset{(B)}{\text{Isoamyl Alcohol}} \rightarrow \underset{(R)}{\text{Water}} + \underset{(Q)}{\text{Isoamyl Acetate}}$$

Assumption is made that both Reaction 1 and Reaction 2 stated above follow Ping-Pong Bi-Bi mechanism. Both Reaction 1 and Reaction 2 are illustrated in Figure 1(a) and (b) respectively.

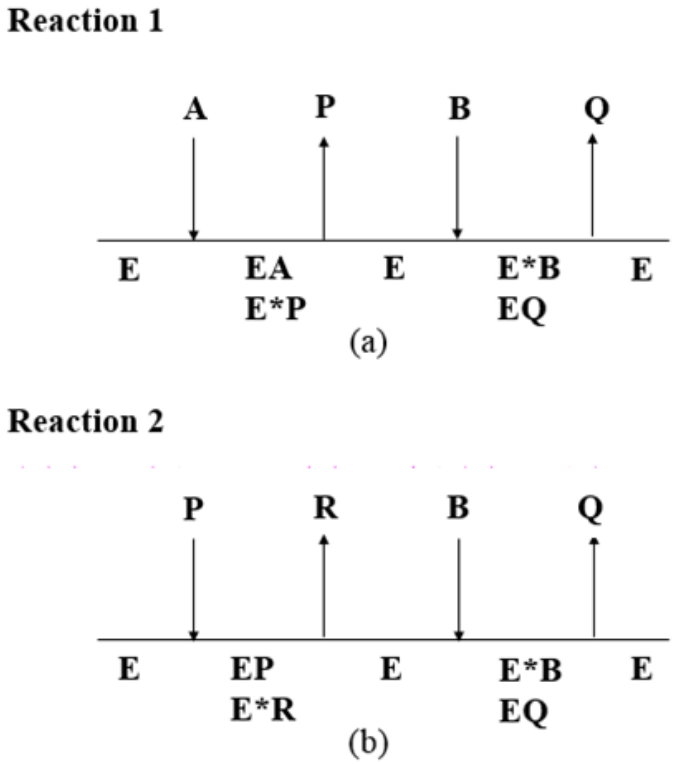

Figure 1: Illustration of the esterification process between acetic anhydride and isoamyl alcohol to produce isoamyl acetate, with (a) main reaction, and (b) secondary reaction.

From Figure 1, the reaction mechanism can be described by Equations (1) to (3).

$$E + A \overset{k_1}{\Leftrightarrow} EA \overset{k_2}{\rightarrow} E^* + P \quad (1)$$

$$E^* + B \overset{k_3}{\Leftrightarrow} E^*B \overset{k_4}{\rightarrow} E + Q \quad (2)$$

$$E + P \overset{k_5}{\Leftrightarrow} EP \overset{k_6}{\rightarrow} E^* + R \quad (3)$$

For Reaction 1, acetic anhydride (A) binds first to the free enzyme (E) forming non-covalent enzyme-anhydride complex (EA) which is transformed by a unimolecular isomerisation reaction to an enzyme-acyl intermediate (E*) with the release of the first product, acetic acid (P). In the second step, the second substrate, isoamyl alcohol (B), binds to the binary enzyme-acyl complex (E*) to form a ternary complex enzyme-acyl-alcohol (E*B). This complex will be isomerised by a unimolecular reaction to an enzyme-ester complex (EQ), which results in the release of the second product, isoamyl acetate (Q), while the enzyme recovers its initial conformation (E).

For Reaction 2, the product from Reaction 1, acetic acid (P) serves as the substrate in this reaction since it binds with free enzyme (E) to form enzyme-acid complex (EP) which is isomerised unimolecularly into enzyme-acyl intermediate (E*) with the release of water (R). Next, the enzyme-acyl complex follows the same route with Reaction 1 as it binds with isoamyl alcohol (B) to form ternary complex enzyme-acyl-alcohol (E*B) which is further transformed into isoamyl acetate (Q) and enzyme in initial form (E).

It has been well known that subtract inhibition is playing an important role in esterification process. In addition to the reactions shown in Equations 1 to 3, acetic anhydride (A) and acetic acid (P) may combine with the other site of free enzyme to form enzyme-anhydride (EA_i) and enzyme-acid (EP_i) complex which is unable to further undergo isomerization to form enzyme-acyl complex. On the other hands, isoamyl alcohol does not involve in the enzyme inhibition. The enzyme inhibition reactions are shown in Equations 4 and 5.[16]

$$E + A \overset{k_7}{\Leftrightarrow} EA_i \quad (4)$$

$$E + P \overset{k_8}{\Leftrightarrow} EP_i \quad (5)$$

According to Pseudo Steady State Hypothesis (PSSH), the net rate of reaction intermediate is equal to zero as it is highly energetic, disappears virtually as fast as it forms, and present in very low concentration.[17] During the esterification process, E, EA, E*, E*B EP, EA_i and EP_i are behave as reactive intermediate. Hence, their rate of formation is equal to zero, which are shown in Equations 6–12.

$$\frac{d[E]}{dt} = -k_1[E][A] + k_{-1}[EA] - k_5[E][P] + k_{-5}[EP] + k_4[E^*B] - k_7[E][A] + k_{-7}[EA_i] - k_8[E][P] + k_{-8}[EP_i] = 0 \quad (6)$$

$$\frac{d[EA]}{dt} = k_1[E][A] - k_{-1}[EA] - k_2[EA] = 0 \quad (7)$$

$$\frac{d[E^*B]}{dt} = k_3[E^*][B] - k_{-3}[E^*B] - k_4[E^*B] = 0 \quad (8)$$

$$\frac{d[EP]}{dt} = k_5[E][P] - k_{-5}[EP] - k_6[EP] = 0 \quad (9)$$

$$\frac{d[E^*]}{dt} = k_2[EA] + k_6[EP] - k_3[E^*][B] + k_{-3}[E^*B] = 0 \quad (10)$$

$$\frac{d[EA_i]}{dt} = k_7[E][A] - k_{-7}[EA_i] = 0 \quad (11)$$

$$\frac{d[EP_i]}{dt} = k_8[E][P] - k_{-8}[EP_i] = 0 \quad (12)$$

By assuming enzyme is conserved throughout the reaction,

$$[E_T] = [E] + [EA] + [EP] + [EA_i] + [EP_i] + [E^*] + [E^*B] \quad (13)$$

Rearranging Equations 6–13, the rate of reaction of ester, acetic acid, acetic anhydride and isoamyl acetate can be obtained.

$$\frac{d[Q]}{dt} = k_4[E^*B] = \frac{N_{Q_1}[A][B] + N_{Q_2}[P][B]}{D_{Q_1}[A] + D_{Q_2}\left(1 + \frac{[A]}{K_{iA}} + \frac{[P]}{K_{iP}}\right)[B] + D_{Q_3}[P] + D_{Q_4}[A][B] + D_{Q_5}[P][B]} \times [E_T] \quad (14)$$

$$\frac{d[A]}{dt} = k_{-1}[EA] - (k_1 + k_7)[E][A] + k_{-7}[EA_i] = -\frac{N_{A_1}[A]^2[B] + N_{A_2}[A][P][B]}{\left\{\begin{array}{c} D_{A_1}[A]^2 + D_{A_2}[P]^2 + D_{A_3}\left(1 + \frac{[A]}{K_{iA}} + \frac{[P]}{K_{iP}}\right)[A][B] + D_{A_4}\left(1 + \frac{[A]}{K_{iA}} + \frac{[P]}{K_{iP}}\right)[P][B] + \\ D_{A_5}[A][P] + D_{A_6}[A]^2[B] + D_{A_7}[P]^2[B] + D_{A_8}[A][P][B] \end{array}\right\}} \times [E_T] \quad (15)$$

$$\frac{d[P]}{dt} = k_2[EA] - (k_5 + k_8)[E][P] + k_{-5}[EP] + k_{-8}[EP_i]$$

$$= \frac{N_{P_1}[A]^2[B] + N_{P_2}[A][P][B]}{\left\{\begin{array}{c} D_{P_1}[A]^2 + D_{P_2}[P]^2 + D_{P_3}\left(1 + \frac{[A]}{K_{iA}} + \frac{[P]}{K_{iP}}\right)[A][B] + D_{P_4}\left(1 + \frac{[A]}{K_{iA}} + \frac{[P]}{K_{iP}}\right)[P][B] + \\ D_{P_5}[A][P] + D_{P_6}[A]^2[B] + D_{P_7}[P]^2[B] + D_{P_8}[A][P][B] \end{array}\right\}} \times [E_T] \quad (16)$$

$$\frac{d[B]}{dt} = -\frac{d[Q]}{dt} = -\frac{N_{Q_1}[A][B] + N_{Q_2}[P][B]}{D_{Q_1}[A] + D_{Q_2}\left(1 + \frac{[A]}{K_{iA}} + \frac{[P]}{K_{iP}}\right)[B] + D_{Q_3}[P] + D_{Q_4}[A][B] + D_{Q_5}[P][B]} \times [E_T] \quad (17)$$

N_{ij} and D_{ij} are multiples of various rate constant at the expression is shown in Table 3.

Table 3: Expression of N_{ij} and D_{ij} from Equations 14 to 17.

$N_{Q1} = k_1k_2k_3k_4(k_{-5} + k_6)$	$N_{Q2} = k_3k_5k_6(k_{-1} + k_2)$
$D_{Q1} = k_1k_2(k_{-3} + k_4)(k_{-5} + k_6)$	$D_{Q2} = k_3k_4(k_{-1} + k_2)(k_{-5} + k_6)$
$D_{Q3} = k_5k_6(k_{-1} + k_2)(k_{-3} + k_4)$	
$D_{Q4} = k_1k_2k_3(k_{-5} + k_6) + k_1k_3k_4(k_{-5} + k_6)$	
$D_{Q5} = k_3k_4k_5(k_{-1} + k_2) + k_3k_5k_6(k_{-1} + k_2)$	
$N_{A1}/N_{p1} = k_1^2k_2^2k_3k_4(k_{-5} + k_6)^2$	$N_{A2} = k_1k_2k_3k_4k_5k_6(k_{-1})(k_{-5} + k_6)$
$N_{p2} = -k_3k_4k_5^2k_6^2(k_{-1} + k_2)^2$	
$D_{A1}/D_{p1} = k_1^2k_2^2(k_{-3} + k_4)(k_{-5} + k_6)^2$	$D_{A2}/D_{p2} = k_5^2k_6^2(k_{-1} + k_2)^2(k_{-3} + k_4)$
$D_{A3}/D_{p3} = k_1k_2k_3k_4(k_{-1} + k_2)(k_{-5} + k_6)^2$	
$D_{A4}/D_{p4} = k_3k_4k_5k_6(k_{-1} + k_2)^2(k_{-5} + k_6)$	
$D_{A5}/D_{p5} = 2k_1k_2k_5k_6(k_{-1} + k_2)(k_{-3} + k_4)(k_{-5} + k_6)$	
$D_{A6}/D_{p6} = k_1^2k_2k_3(k_2 + k_4)(k_{-5} + k_6)^2$	$D_{A7}/D_{p7} = k_3k_5^2k_6(k_4 + k_6)(k_{-1} + k_2)^2$
$D_{A8}/D_{p8} = k_1k_2k_5(k_{-1} + k_2)(k_{-5} + k_6)\{k_2(k_4 + k_6) + k_6(k_2 + k_4)\}$	

According to Arrhenius equation, rate constant varies exponentially with temperature which is shown in Equation 18.[18]

$$k = Ae^{-\frac{E}{RT}} \quad (18)$$

where A = frequency factor
E = activation energy
R = gas constant
T = temperature

Since parameters N_{ij} or D_{ij} in Equations 14–17 are multiples or quotients of various rate constants,

$$D_{ij} = \prod_{i=1}^{n} k_i = \prod_{i=1}^{n} A_i e^{-\frac{E_i}{RT}} = \prod_{i=1}^{n} A_i \times e^{-\frac{\sum_{i=1}^{n} E_i}{RT}} = A_T e^{-\frac{E_T}{RT}} \quad (19)$$

where $A_T = \prod_{i=1}^{n} A_i$
$E_T = \sum_{i=1}^{n} E_i$

From Equation 19, it can be observed that all parameters N_{ij} or D_{ij} in Equations 14–17 are following Arrhenius equation as A_T and E_T are constant for a particular parameter.

3.2 Model Fitting

Microsoft Excel is used to fit the models and evaluate unknown parameters of Equations 13–17. Solver Tool is employed to minimise the deviation of model prediction from experimental result by evaluating the values of unknown parameters. This tool is chosen to fit the model because it is the powerful tool that is able to present the way of problem solving in a transparent and systematic approach. In addition, it is easier to set up and solve the problem by using Microsoft Excel spreadsheet.

Equations 10–12 cannot be solved analytically due to their complication. The complication is mainly due to the high degree of non-linearity of the differential equations. Since analytical approach is not practical to solve the problem, numerical method is employed in model fitting. Euler method is used in the present work, which is given by Equation 20, is a first-order numerical procedure for solving ordinary differential equations with a given initial value.[19]

$$y_{n+1} = y_n + hf(t_n, y_n) \quad (20)$$

where y_{n+1} = $(n+1)$th value of y
y_n = nth value of y
h = step size

$f(t_n, y_n)$ = gradient of the curve point *n*

Euler method is chosen due to its simple algorithm and it is able to give high degree of accuracy when step size is small.

The model fitting is based on 14 runs of experimental data in different set of operating conditions (temperature, Ac/Al ratio and concentration of enzyme). Solver Tool in Microsoft Excel is used to find value of all parameters so that the deviation of model prediction from experimental result is minimised. The parameters to be evaluated are frequency factor, A and activation energy, E for each parameter (N_{ij} and D_{ij}). The best solution for the model parameters is listed in Table 4.

Table 4: Solution of parameters for the model.

Parameter	A	E (kJ mol^{-1})	Parameter	A	E (kJ mol^{-1})
N_{A1}/N_{p1}	2.568×10^5	1.850×10^4	D_{A8}/D_{p8}	1.037×10^{15}	8.430×10^4
N_{A2}	3.505×10^{11}	5.555×10^4	N_{B1}	8.228×10^{10}	4.707×10^4
N_{p2}	7.148×10^{15}	8.932×10^4	N_{B2}	2.828×10^{15}	8.691×10^4
D_{A1}/D_{p1}	8.404×10^5	1.647×10^4	D_{B1}	9.956×10^{14}	8.419×10^4
D_{A2}/D_{p2}	8.013×10^{17}	8.027×10^4	D_{B2}	2.726×10^9	3.839×10^4
D_{A3}/D_{p3}	1.272×10^{15}	8.483×10^4	D_{B3}	1.894×10^{15}	6.161×10^4
D_{A4}/D_{p4}	1.103×10^{15}	8.446×10^4	D_{B4}	9.865×10^{14}	8.417×10^4
D_{A5}/D_{p5}	1.929×10^{15}	8.591×10^4	D_{B5}	9.860×10^{14}	8.417×10^4
D_{A6}/D_{p6}	1.236×10^{15}	8.475×10^4	K_{iA}	1.027×10^{16}	6.601×10^4
D_{A7}/D_{p7}	1.030×10^{15}	8.428×10^4	K_{iP}	9.696×10^{15}	9.012×10^4

By using this solution, the average percentage error for model prediction and actual value is given by 8.08%. The model prediction and experimental result for arbitrary run of experiment is shown in Figure 2. From the figure, it can be observed that the model developed is able to predict the trend of the experimental result and match the experimental data quite well.

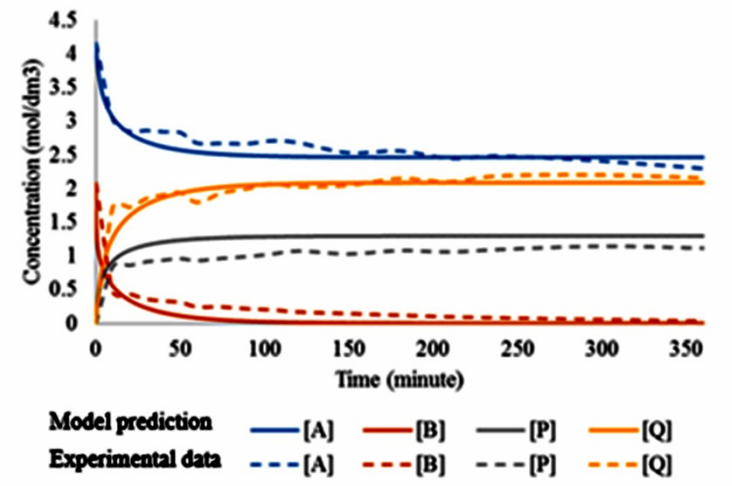

Figure 2: Comparison between model prediction and experimental data for arbitrary run of experiment.

Nevertheless, the model cannot fit the experimental data perfectly. The deviation of model prediction from experimental data occurs due to several reasons. First, the data obtained from experiment may not represent the true value because the sample is not quenched (stop the reaction) when it is taken out from the reaction mixture for composition analysis. The reaction will proceed when the sample is brought to gas chromatograph (GC) in order to determine its composition. Generally, there is a time delay of 5–10 min and the experimental data may not represent the exact value of the particular period.

Second, the model may not be the best model to describe the esterification process as enzyme deactivation is not considered during the derivation of the mathematical model. In contrast, enzyme deactivation is an important factor that affects the reaction rate throughout the reaction.[20] Third, the parameter values in Table 4 may not the best solution to the model as it is there are a lot of solution to this problem depending on the initial guess. There is possibility of the existence of solution for the unknown model parameters which give is able to give prediction that can match the experimental findings better.

4. CONCLUSION

The production of isoamyl acetate through esterification of acetate anhydride and isoamyl alcohol catalysed by immobilised lipase from *Candida antarctica* in a solvent-free system has been studied and investigated. During the

development of the model, Ping-Pong Bi-Bi mechanism is assumed throughout the reaction. By using PSSH, a set of differential equations is obtained and it is able to be solved by numerical method to predict the concentration profile for each substrate, if initial value is given, throughout the reaction. Experimental data is used to fit the model (by evaluating the model parameters) and the best solution obtained is found to match the experimental data quite well, with the average percentage error of 8.08%. The model can be used to predict the concentration profile of each substrate under different temperature, enzyme concentration and initial condition.

5. ACKNOWLEDGEMENT

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