MODIFIED KINETIC MODELLING OF ISOAMYL ACETATE PRODUCTION

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2017

MODIFIED KINETIC MODELLING OF ISOAMYL ACETATE PRODUCTION

by

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Thesis submitted in partial fulfilment of the requirement

for the degree of Bachelor of Chemical Engineering

June 2017

ACKNOWLEDGEMENT

I would like to express my greatest gratitude to my thesis supervisor, Associate Professor Dr. Syamsul Rizal Abd Shukor, who has given me the opportunity to conduct this research. Despite his busy schedule, he reviewed my modelling progress at all the time, including providing suggestion on improvement in modelling and report writing. Without his support, this project would not have come this far.

I would like to thank Nurhazwani Yusoff Azudin for her help in explaining her experimental work in detailed including her observation and understanding in the experiment. I would like to thanks her again for providing me the experimental data, and guiding me on modelling and simulation, so that I able to do the modelling fitting in modelling. Without her help, this project may need more time to be completed.

Lastly, my deepest gratitude would go to my beloved family members who had given their full support as well as being concerned on my research project. Their spiritual support gave me motivation to strive for the completion of this research.

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LIST OF SYMBOLS

Symbol	Description	Unit
A	Frequency factor	-
[A]	Concentration of acetic anhydride	mol/L
[B]	Concentration of isoamyl alcohol	mol/L
Ea	Activation energy	J/mol
[E]	Concentration of free enzyme	mol/L
[EA]	Concentration of enzyme-acetic anhydride complex	mol/L
[EP]	Concentration of enzyme-acetic acid complex	mol/L
[EQ]	Concentration of enzyme-isoamyl acetate complex	mol/L
[E'A]	Concentration of inhibited enzyme-acetic anhydride complex	mol/L
[E'P]	Concentration of inhibited enzyme-acetic acid complex	mol/L
$[E_T]$	Total concentration of enzyme	mol/L
[F]	Concentration of enzyme-acyl complex	mol/L
[FB]	Concentration of enzyme-acyl-isoamyl alcohol complex	mol/L
[FP]	Concentration of enzyme-acyl-acetic acid complex	mol/L
[FQ]	Concentration of enzyme-acyl-isoamyl acetate complex	mol/L
[FR]	Concentration of enzyme-acyl-water complex	mol/L
k	Rate constant	$L^2/(mol^2\ min)$
K	Inhibition constant	L/mol
[P]	Concentration of acetic acid	mol/L
[Q]	Concentration of isoamyl acetate	mol/L
R	Gas constant	J/(mol. K)
[R]	Concentration of water	mol/L
T	Temperature	K
t	Time	s
Vmax	Maximum velocity	mol/(L. s)

LIST OF ABBREVIATIONS

CALB	*Candida Antartica Lipase B*
PSSH	Pseudo Steady State Hypothesis

PEMODELAN DIUBAHSUAI PROSES PENGHASILAN ISOAMIL ASETAT

ABSTRAK

Isoamil Asetat mempunyai pasaran yang besar. Model kinetik diperlukan untuk memahami hubungan antara parameter operasi untuk mencapai kadar pengeluaran dan hasilnya dengan tinggi, Pemodelan kinetik dikaji untuk menganggarkan profil kepekatan dari semasa ke semasa. Model kinetik tidak dapat menggambarkan profil disebabkan oleh perubahan dalam pH, andaian tidak sah dan perisian had. Profil kepekatan boleh diramal dengan baik apabila nisbah Ac/Al adalah rendah dan ia ralat kuasa dua adalah rendah. Oleh itu, model kinetik adalah hanya sah untuk keadaan / Al nisbah Ac rendah dan pada suhu optimum 30 - 40 ℃ . Keadaan optimum nisbah Ac/Al adalah 0,217 untuk mencapai hasilan 90%. masa optimum diambil adalah untuk 4 wt% , 8wt% dan 12% berat enzim adalah 30.6 min, 23.3 min dan 19.3 min.

MODIFIED KINETIC MODELLING OF ISOAMYL ACETATE PRODUCTION

ABSTRACT

Isoamyl acetate has high market demand. To achieve high production rate and yield, kinetic model is required in order to the understand relationship between operating parameters. The kinetic modelling is studied to estimate the concentration profile from time to time. The kinetic model developed in this work was not perfect due to the variation in pH, invalid assumption and software limitation. Concentration profile can be predicted well when Ac/Al ratio is low and it squared error is low. Therefore, the kinetic model was only valid for condition of low Ac/Al ratio and at optimum temperature 30 – 40 ℃. The optimum condition of Ac/Al ratio was 0.217 to achieve 90% yield. Optimum time taken for 4 wt%, 8wt% and 12 wt% enzyme were 30.6 min, 23.3 min and 19.3 min respectively.

CHAPTER ONE

INTRODUCTION

1.1 Esters Overview

Ester is an organic chemical with general chemical formula of RCOOR. There are a few ways to obtain ester, for example, it can be extracted from natural resources such as plant and fruits (Serra et al., 2005). Besides, it can be produced synthetically through esterification and transesterification reaction. Esterification is defined as ester formed by using carboxylic acid and alcohol as reactants while transesterification is defined as new ester formed by using ester and alcohol as reactants.

Simple esters normally have fruit-like flavor. Table 1.1 shows some of the esters and their corresponding flavors or fragrances. Ester flavors are important raw materials used in food, cosmetics and pharmaceutical industries (Berger, 2009). Esters are expensive if it is obtained through extraction method since the only small amount of ester is extracted which increase the processing cost (Serra et al., 2005). Therefore, catalytic esterification and transesterification reaction are recommended.

Table 1.1: Examples of esters and its corresponding flavors or fragrances.

Esters	Flavors / Fragrances	References
Benzyl acetate	Peach	Lampkowski et al., 2015
Butyl acetate	Apple	Martins et al., 2011
Butyl butyrate	Pineapple	Mendes et al., 2012
Isoamyl acetate	Banana	Torres et al., 2009
Octyl Acetate	Orange	Lampkowski et al., 2015
Propyl acetate	Pear	Lampkowski et al., 2015

There are two types of catalyst available for the production of ester which are inorganic catalyst such as concentrated sulphuric acid and biocatalyst such as

enzyme. Enzyme has more advantages compare to inorganic catalyst. Esters produced from enzyme convinces people to buy the product, since the product can be labeled “natural” (Romero et al., 2007). Besides, enzyme has high selectivity (Mateo et al., 2007). The only drawback for enzyme is the activity or rate of reaction will be affected at high temperature due to denaturation (Romero et al., 2005).

1.2 Production of Isoamyl Acetate

Isoamyl acetate is a colourless ester which has banana aroma (Torres et al., 2009). The chemical structure is shown in Figure 1.1.

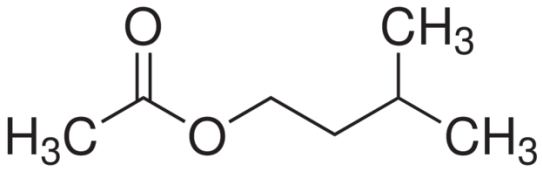


Figure 1.1: Chemical structure of isoamyl acetate.

Production method of isoamyl acetate through extraction from banana is low. 25.6 %w/w and 17.1 %w/w of isoamyl acetate was extracted respectively from normal state of matured banana and very late state of matured banana (Salmon et al., 1996). Isoamyl acetate extracted from banana is expensive for commercial used (Yang et al., 2010). This is because a lot of banana was required to produce low quantity of isoamyl acetate.

Conventional commercial method to produce isoamyl acetate through chemical catalytic esterification reaction between carboxylic acid and alcohol by using sulphuric acid causes some problems such as corrosion to the reactors and pipes due to strong sulphuric acid oxidize the surface of the reactors and pipes.

Furthermore, both reactants and products are in liquid form which is in homogeneous creates difficulty in product separation and catalyst recovery (Yang et al., 2010).

Biocatalyst catalytic esterification reaction to produce isoamyl acetate is sustainable because of often save raw materials, energy, chemicals due to high selectivity compared to conventional processes (Jegannathan and Nielsen, 2013). Synthesis of isoamyl acetate can be carried out in organic solvent and solvent free system (Rizzi et al., 1992). High conversion yield of production was achieved in solvent. However, there are many problems still exist in the separation, the toxicity and the inflammability of the organic solvents (Ghamgui et al., 2006). Since solvent used in enzymatic reaction causes a lot of problem to the society, so many researchers start to study solvent free system in enzymatic reaction.

1.3 Enzyme for production of isoamyl acetate

Enzyme are protein catalyst that can increase the rate of conversion of substrate into product. The same enzyme may not able to catalyse other reaction, this is because different enzymes have different structure and functional group of active site. *Candida Antarctica* is an enzyme which can used as biocatalyst in esterification of isoamyl acetate. Denaturation effect is small for *Candida Antarctica* if the operating temperature not beyond 65℃ (Romero et al., 2005). Therefore, it is high thermal stability enzyme (Salmon et al., 1996), hence, activity of enzyme can be maintained if disturbance such as low cooling effect surround the reactor and higher feed temperature temporary affect the reactor system. *Candida Antarctica* was used in this study because of high yield production of isoamyl acetate. Comparison between enzymes used for esterification of isoamyl acetate as shown in Table 1.2.

Table 1.2: Examples of enzymes and the yield of production.

Enzyme	Range of temperature (℃)	Optimum temperature (℃)	Yield (%)	Reference
Amycoltopsis mediterranei	30-50	50	59	Dheeman et al., 2011
Candida Antarctica	30-65	40	91	Romero et al., 2005
Mucor miehei	32-55	45	80	Razafindralambo et al., 1994
Staphylococcus simulans	30-45	37	64	Ghamgui et al., 2006

1.4 Problem Statements

Production of isoamyl acetate is high due high market demand. To have better quality of product, kinetic modelling need to be studied to find the micro kinetic constant in order to have better understanding the reaction rate.

To optimize the profit in industry, the parameters such as temperature, amount of enzyme, ratio of acid to alcohol, and time must be controlled well. Time is money as for the industry, therefore, normally higher temperature than room temperature is preferred as it (Romero et al., 2005) increases the rate of production. However, CALB will be denatured if the temperature is not controlled well. This will lead to the loss of enzyme, in other word, production cost will increase due to the need for purchasing new enzyme. Besides, ratio of acid to alcohol must be controlled as well to maximize the yield of production. High enzyme concentration increases the production rate, however, excess amount of immobilized CALB will cause inhibition of substrates toward enzyme which leads to lesser yield (Romero et al., 2005).

1.5 Research Objective

The objectives of this research are:

i. To develop mathematical models for the isoamyl acetate synthesis process catalyzed by immobilized lipase from *Candida Antarctica Lipase B* in a solvent-free system.

ii. To find the optimum operating parameters of the production of isoamyl acetate.

1.6 Scope of study

The process modelling of enzymatic synthesis of isoamyl acetate from acetic anhydride and isoamyl alcohol catalyzed by CALB in solvent-free system is studied. Model will be developed according to Ping-Pong Bi-Bi mechanism. The model is developed by assuming Pseudo Steady State Hypothesis.

Model fitting is carried out by using Microsoft Excel Solver Tool to find all the kinetic parameters. Next, the model developed will be used to find its optimum operating condition.

1.7 Thesis Organization

Chapter 1 covers the introductory part of the thesis. The research backgrounds of ester, production of isoamyl acetate and enzyme used. The problem statement, research objectives, scope of study and thesis organization are also explained in the later part of this chapter.

Chapter 2 presents a review on the parameter studies, binding site of enzyme and kinetic modelling of isoamyl acetate production.

Chapter 3 explains the methodology of the research. Kinetic equation was developed and methods to find the kinetic parameters and optimum operating conditions.

Chapter 4 describes about results and discussions. Error analysis of the modelling was described and optimum operating condition was obtained ad discussed.

Chapter 5 describes the conclusion and recommendations for future work.

CHAPTER TWO

LITERATURE REVIEW

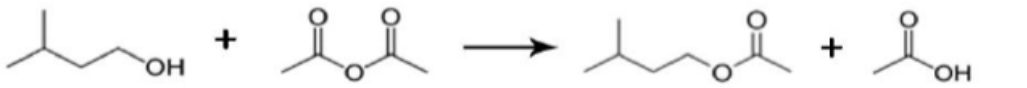
This chapter illustrates the research background of the present work. Enzymatic synthesis of isoamyl acetate are reviewed. The literatures reviewed parameters studies such as temperature, enzyme concentration, and molar ratio of acetic anhydride to isoamyl alcohol. This is then followed by enzyme kinetic of Ping Pong Bi Bi mechanism.

2.1 Enzymatic Synthesis of Isoamyl Acetate

In this study, esterification of acetic anhydride and isoamyl alcohol was catalysed by CALB. Acetic anhydride has two acyl groups. In the reaction, one of the acyl from acetic anhydride will bind with isoamyl alcohol to form acetic acid and isoamyl acetate. The esterification reaction is followed by acetic acid reacts with isoamy acetate to form isoamyl acetate. (Azudin et al., 2014)

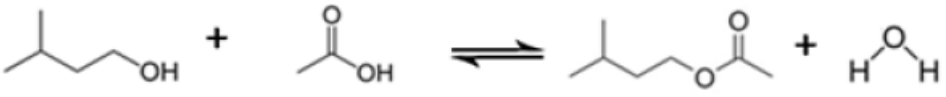
Reaction scheme are as below :

(a) Main reaction



(Isoamyl alcohol) (Acetic anhydride) (Isoamyl acetate) (Acetic acid)

(b) Secondary reaction



(isoamyl alcohol) (acetic acid) (isoamyl acetate) (water)

Acetic acid is a potent inhibitor of lipase activity causing dead-end inhibition (Segel, 1975). High yields of synthesis is difficult to be achieved for acetic acid ester due to lipase inactivation by acid (Langrand et al., 1988). Conversion of acetic acid was about 25% when it was used as acyl donor, whereas when acetic anhydride was used, the conversion achieved 97% (Hari Krishna et al., 2001). Hence, acetic anhydride is good for the synthesis of isoamyl acetate.

2.1.1 Mass Transfer

Reaction rate might be controlled by mass transfer limitation or reaction limitation. Mass transfer limitation may be solved by increasing the agitation speed (Romero et al., 2007). A number of experiments were carried out at various stirring rates (100–300 rpm), reaction rate did not improve by the increasing stirrer speed (Romero et al., 2007). Thus, the reaction is not influenced by mass transfer but controlled by enzyme kinetic.

2.2 Parameters Studies

2.2.1 Temperature

Temperature has a strong effect on enzyme configuration. This will affect its activity and stabilit(Illanes and Vera, 2014). There is a trade-off between activity and stability. When the temperature increase, rate of reaction will be increased, however, stability of enzyme reduced (Illanes and Vera, 2014). Arrhenius equation (Eqn 2.1) is used to describe kinetic constant at different temperature.

$$k = Ae^{-\frac{E_a}{RT}} \qquad \text{Eqn 2.1}$$

where k= rate constant

Ea=activation energy

R=gas constant

T= absolute temperature

2.2.2 Enzyme Loading

Enzyme is able to lower the activation energy of a reaction. As a result, the rate of reaction with enzyme is higher than that without enzyme, the reaction rate is usually 10^3 to 10^{17} times faster than uncatalyzed reaction (Fogler, 2014). Higher rate in esterification reaction is obtained at low enzyme concentration (Romero et al., 2005). Reaction rate is dependent on enzyme to substrate ratio. At low enzyme to substrates ratios, the substrate is in excess and so an increase on enzyme content enhances reaction rate. However, at high enzyme to substrate ratios, all the available substrate are forming the enzyme/substrate complex (Romero et al., 2005). Therefore, the excess enzyme molecules (active sites) cannot further improve reaction rate. Besides, initial reaction rate does not increase linearly with the enzyme added (Romero et al., 2005). High enzyme loading will cause inhibition too which reduce the yield (Yang et al., 2010).

2.2.3 Acid/Alcohol Molar Ratio

Production yield of isoamyl acetate through esterification of acetic anhydride and isoamyl alcohol is highly dependent on the reactants ratio. High alcohol concentration can slow down the reaction rate, as a result, production time become longer and hence increase the production cost (Chowdary et al., 2000). The optimum

condition of acid/alcohol molar ratio is 2:1 (Güvenç et al., 2002, Hari Krishna et al., 2001).

2.3 Deactivation of enzyme

Biocatalysts in reactors usually undergo irreversible conformational changes which is known as denaturation or deactivation. Deactivation normally causes an exponential decrease of activity with time. First-order reaction rate process can be used to describe the phenomena (J.Dunn et al., 2003).

$$r_d = -k_d E \qquad \text{Eqn 2.2}$$

For a batch reactor,

$$\frac{dE}{dt} = r_d \qquad \text{Eqn 2.3}$$

The integrated form can be written as below :

$$E = E_o \, e^{-k_d t} \qquad \text{Eqn 2.4}$$

where E_o = initial concentration of enzyme

2.4 Enzyme inhibition

Enzyme inhibition is defined as the reduction in reaction rate on enzyme activity by ligands (inhibitors) which bind to enzyme (Bisswanger, 2002). The inhibition effect can be reduced by manipulating the pH of the reaction medium or by the addition of high salt (Bisswanger, 2002, Romero et al., 2007). For example, the inhibition effect for the synthesis of isoamyl acetate by acylation of isoamyl alcohol with acetic anhydride using the lipase Novozym 435 in n-hexane is reduced

by adding Na2HPO4 at a concentration of 0.14 M to the reaction medium (Romero et al., 2005).

Reversible inhibition is defined as inhibitor bind reversely and can be released from the enzyme whereas irreversible inhibition is defined as inhibitor bind to the enzyme and form strong covalent which preventing the inhibitor detached from the enzyme (Bisswanger, 2002). Most of the enzyme inhibition is reversible (Bisswanger, 2002). Besides, enzyme and irreversible inhibitor can never achieve equilibrium, which mean all the inhibitors in the reaction medium will bind to the enzyme until either the enzyme becomes inactive or the inhibitor depletes (Stein, 2011).

Kinetic mechanism of an inhibitor does not necessarily indicate where the inhibitor binds (Stein, 2011). Usually for competitive inhibition, it is assumed that the inhibitor binds to the active site of the enzyme, however, inhibitor can bind to allosteric site of enzyme which induce the conformational change of enzyme that can close down the active site of the enzyme (Stein, 2011).

2.4.1 CALB inhibition during synthesis of isoamyl acetate

Enzyme inhibition can be caused by solvent, reactants or products formed after reaction. CALB inhibition caused by acetic anhydride and acetic acid (Romero et al., 2005). Acetic acid showed strong inhibition to the enzyme which cause the reaction rate to decrease when the concentration of acetic acid is low (0.1M) (Romero et al., 2007). No inhibition phenomenon was observed for both isoamyl alcohol and isoamyl acetate (Romero et al., 2007).

2.5 Ping Pong Bi Bi mechanism

In most enzyme reaction, two or more substrates may take part in the reaction (Bisswanger, 2002). Many enzyme have been found to catalysed reaction according to Ping Pong Bi Bi mechanism (Piszkiewicz, 1977). Ping Pong describes a mechanism in which one or more products must be released before all substrates can react. The reaction involved bimolecular in reactants and bimolecular in products, hence it is termed as Bi Bi (Piszkiewicz, 1977).

For Ping Pong Bi Bi mechanism, the first product after released from the enzyme, normally the reactive group in first substrate will be retained in enzyme to form an intermediary form. The reactive group is then transferred to the second substrate to form second product (Bisswanger, 2002). Figure 2.1 represents Ping Pong Bi Bi mechanism.

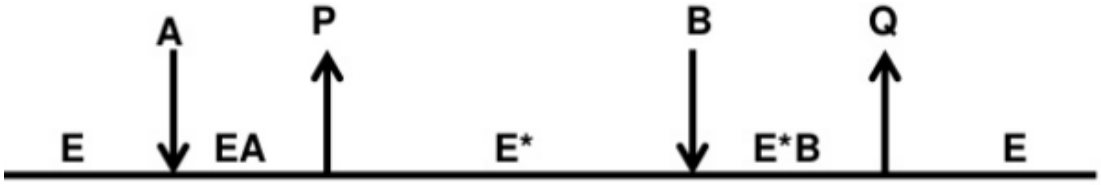


Figure 2.1: Ping Pong Bi Bi mechanism

Substrate binding is generally assumed that before an enzyme-catalyzed reaction take place, the substrates form complexes with the enzyme which exist in equilibrium with the free species (Piszkiewicz, 1977). Ping Pong Bi Bi is one of the ordered Bi Bi mechanism. Therefore, rules of substrates binding for Ping Pong Bi Bi is the first substrate binding before the second substrate bind to the enzyme.

The acyl donor first binds to the free enzyme forming a non-covalent anhydride enzyme complex which is converted to an enzyme-acyl intermediate with

the release of the first acetic acid product. Next, the second substrate, isoamyl alcohol will binds to the binary enzyme-acyl complex and form an acyl-alcohol complex. This complex is also isomerized by a unimolecular reaction to an enzyme-ester complex, which the second product, isoamyl acetate will be produced while the enzyme recovers its initial conformation (Romero et al., 2007).

2.6 Kinetic studies

2.6.1 Initial rate of reaction

Most of the kinetic modelling of enzymatic reaction are studied at initial condition. At initial condition, the effect of inhibition by product is small since the product formed is less. Therefore, there is no inhibition caused by product was assumed. Kinetic equation gets more complex, being necessary to include new parameters that correspond to inhibition constants (Romero et al., 2007).

In isoamyl acetate synthesis from acetic anhydride in the hexane solvent by using CALB as catalyst, only acetic anhydride acts as a lipase inhibitor, while isoamyl alcohol has no negative effect on the reaction rate which means it won't inhibit the enzyme (Romero et al., 2007). For the study of initial reaction rate, any effect of both products are not considered. Thus, the rate equation is shown following Eqn 2.5.

$$v = \frac{V_{max}[A][B]}{K_{mB}[A]\left(1+\frac{[A]}{K_{iA}}\right)+K_{mA}[B]+[A][B]} \qquad \text{Eqn 2.5}$$

where A = concentration of acetic anhydride

B = concentration of isoamyl alcohol

Vmax = maximum reaction rate

K_{mA} & K_{mB} = binding constants

K_{iA} = inhibition constant

2.6.2 Progress curve analysis

Kinetic mechanism of reaction is the main interest while kinetic parameter is the second interest in kinetic investigations on enzyme-catalyzed reactions, and therefore, progress curve analysis was introduced (Duggleby and Morrison, 1978). Progress curve analysis is used to study the reaction rate over the time but not just limit to initial reaction rate.

For single substrate, enzymatic reaction, Lambert W function is used to obtain explicit, closed-form solutions to differential rate expressions that describe the rate of reaction (Goudar et al., 2004). The explicit solution is shown in Eqn 2.6 :

$$x = \frac{S_o}{K_m} \exp\left(\frac{S_o - V_{max}t}{K_m}\right) \qquad \text{Eqn 2.6}$$

where x = concentration of substrate at time,t

So = initial concentration of reactant

.

This explicit solution simplified the nonlinear estimation kinetic parameters from the process curve data as conventional method used differential/integral rate equations (Goudar et al., 2004).

2.7 Numerical Methods

The finite difference method (FDM), a numerical modelling method based on the model equations, was used to solve the system of partial differential equations (Miložič et al., 2017). There is a trade-off of computational method between Euler method and Runge Kutta Fourth Order to solve the model by using regression. Less time consuming if Euler method is used but with lower accuracy compare to Runge Kutta (C.Chapra and P.Canale, 2010).

CHAPTER THREE
MATERIALS AND METHODS

In this chapter, the methodology of process modeling was described in Section 3.2. The final equations from the derivation of kinetic equations of Ping Pong Bi Bi was shown step by step with all the assumption made. Next, the final equations was used in the methodology of progress curve analysis by using Microsoft EXCEL Solver to find the optimum kinetic parameters was shown in the next section. Finally, Section 3.3 described the methodology to find the optimum condition such as ratio of acetic anhydride to isoamyl alcohol, temperature and enzyme loading were elaborated to maximize the production.

3.1 Experimental Procedure

Experimental data was obtained from Nurhazwani Yusoff Azudin. Isoamyl acetate synthesis was carried out without any organic solvent (solvent-free system) in 100ml stopped rubber Erlenmeyer flask with working volume of 15ml. Enzyme *Candida Antarctica Lipase B* was added into the reaction media containing a mixture of isoamyl alcohol and acetic anhydride at different temperature. 0.5ml of the reaction mixture is withdrawn from the reaction medium periodically and it was analysed by using gas chromatograph (Agilent Technologies 7820A) equipped with a hydrogen flame ionization detector and a SGE BP21 (FFAP) column (60m x 0.32mm x 0.25 um) to determine concentration of components in the mixture.

3.2 Methodology of Process Modelling

3.2.1 Derivation of kinetic equation

Ping-Pong Bi-Bi mechanism was used as the mechanism of the model. Fast reaction rate between the intermediate complexes was assumed. Therefore, the reaction happened in the synthesis of isoamyl acetate was assumed as shown as the equations below :

$$E + A \overset{k_1}{\rightarrow} F + P \qquad \text{Eqn 3.1}$$

$$F + B \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + Q \qquad \text{Eqn 3.2}$$

$$E + P \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} F + R \qquad \text{Eqn 3.3}$$

$$E + A \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} E'A \qquad \text{Eqn 3.4}$$

$$E + P \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} E'P \qquad \text{Eqn 3.5}$$

where E = Active site of CALB

F = CALB-acryl complex

A = Acetic anhydride

B = Isoamyl alcohol

P = Acetic acid

Q = Isoamyl acetate

R = Water

The rate of reaction that represent the reaction as follow :

$$\frac{d[E]}{dt} = -k_1[E][A] + k_2[F][B] - k_{-2}[E][Q] - k_3[E][P] + k_{-3}[F][R] - k_4[E][A] + k_{-4}[E'A] - k_5[E][P] + k_{-5}[E'P] \quad \text{Eqn 3.6}$$

$$\frac{d[F]}{dt} = k_1[E][A] - k_2[F][B] + k_{-2}[E][Q] + k_3[E][P] - k_{-3}[F][R] \quad \text{Eqn 3.7}$$

$$\frac{d[E'A]}{dt} = k_4[E][A] - k_{-4}[E'A] \quad \text{Eqn 3.8}$$

$$\frac{d[E'P]}{dt} = k_5[E][P] - k_{-5}[E'P] \quad \text{Eqn 3.9}$$

Pseudo steady state hypothesis was assumed which the complex in the reaction reached equilibrium quickly, and hence the rate of change in concentration of complexes to be zero.

The rate of reaction derived was shown as below:

$$\frac{d[A]}{dt} = \frac{-k_1[A][E_T]}{1+\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}+K_4[A]+K_5[P]} \quad \text{Eqn 3.10}$$

$$\frac{d[B]}{dt} = \frac{\left(-k_2[B]\left[\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}\right]+k_2[Q]\right)E_T}{1+\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}+K_4[A]+K_5[P]} \quad \text{Eqn 3.11}$$

$$\frac{d[P]}{dt} = \frac{\left(k_1[A]-k_3[P]+k_{-3}[R]\left[\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}\right]\right)E_T}{1+\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}+K_4[A]+K_5[P]} \quad \text{Eqn 3.12}$$

$$\frac{d[Q]}{dt} = \frac{\left(k_2[B]\left[\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}\right]-k_2[Q]\right)E_T}{1+\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}+K_4[A]+K_5[P]} \quad \text{Eqn 3.13}$$

$$\frac{d[R]}{dt} = \frac{\left(k_3[P] - k_{-3}[R]\left[\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}\right]\right)E_T}{1+\frac{k_1[A]+k_{-2}[Q]+k_3[P]}{k_2[B]+k_{-3}[R]}+K_4[A]+K_5[P]} \quad \text{Eqn 3.14}$$

3.2.2 Optimum kinetic parameter

Microsoft Excel Solver was used to find the optimum kinetic parameter.

Objective function of the Microsoft Excel Solver was minimize the squared error of all the experiment data.

$$Squared\ Error = \left(data_{model} - data_{experiment}\right)^2 \quad \text{Eqn 3.15}$$

Model data was predicted by using Euler method. The equations are shown as below:

$$[A]_{t+1} = [A]_t + \frac{d[A]}{dt} \times 0.01 \quad \text{Eqn 3.16}$$

$$[B]_{t+1} = [B]_t + \frac{d[B]}{dt} \times 0.01 \quad \text{Eqn 3.17}$$

$$[P]_{t+1} = [P]_t + \frac{d[P]}{dt} \times 0.01 \quad \text{Eqn 3.18}$$

$$[Q]_{t+1} = [Q]_t + \frac{d[Q]}{dt} \times 0.01 \quad \text{Eqn 3.19}$$

$$[R]_{t+1} = [R]_t + \frac{d[R]}{dt} \times 0.01 \quad \text{Eqn 3.20}$$

where 0.01 is the step time with the unit of minute

Kinetic parameters calculated may not accurate due to the enzyme complexes haven't reach equilibrium since the earlier assumption was PSSH. Therefore, enzyme complexes were assumed that achieved equilibrium after 10 minutes.

Since acetic anhydride contains two acyl group, both acetic acid and isoamyl acetate contain one acyl group, therefore initial concentration of acetic anhydride can be predicted as the formula below:

$$[A]_o = \frac{2\times[A]_t+[P]_t+[Q]_t}{N-1} \quad \text{Eqn 3.21}$$

where N = total number of period to take experiment data

Since there is no inhibition of isoamyl alcohol observed, therefore the initial concentration of isoamyl alcohol was predicted by the equation below:

$$[B]_o = [B]_{tf} + [Q]_{tf} \quad \text{Eqn 3.22}$$

where tf = time where last reading was taken

3.2 Optimization of kinetic model

Concentration of isoamyl alcohol were assign as 1 mol/L, 2 mol/L, 3 mol/L, 4 mol/L and 5 mol/L at each set of temperature and concentration. This is because the system is in solvent free which means there is a specific value for concentration of anhydride at specific concentration of B, however, the relationship cant well described by mass balance, so the optimum condition calculated is the mean value of results from concentration of isoamyl alcohol 1 mol/L, 2 mol/L, 3 mol/L, 4 mol/L and 5 mol/L

Acetic anhydride, acetic acid and isoamyl acetate consists of two, one and one acyl group respectively. The yield is defined as percentage or ratio of acetic anhydride was converted into isoamyl acetate. Therefore, the formula of yield of production of isoamyl acetate used in this study is

$$yield = \frac{concentration\ of\ isoamyl\ acetate\ at\ t{=}360min}{2{\times}concentration\ of\ acetic\ anhydride\ at\ t{=}0} \quad \text{Eqn 3.22}$$

The target yield in this study is set as 90% at time = 360 min.

The method used to predict the concentration of isoamyl acetate was euler method same as the methodology in section 3.2.2, so Eqn 16, Eqn 17, Eqn 18, Eqn 19 and Eqn 20 were used.

3.2.1 Optimization of temperature and concentration of CALB

Microsoft Excel Solver was used to find the optimum concentration of temperature and concentration of CALB. The objective function of Microsoft Excel Solver was maximum yield of isoamyl acetate.

3.2.2 Optimization of ratio of acetic anhydride to isoamyl acetate

Microsoft Excel Goal Seek was used instead of Microsoft Excel Solver because Microsoft Excel Solver tends to reach the objective function in the shortest time, as consequences, the concentration of acetic anhydride calculated was zero and led to error in calculation, besides, even constraints was set (i.e. concentration of acetic anhydride $\geq$ 0.0001 mol/L) , the smallest value of the constraints was be chosen and led to lower production.

The objective function of Microsoft Excel Goal Seek was set as 90 % yield production at time = 360 min. The manipulating number was concentration of acetic anhydride.

3.2.3 Optimization of time

The optimum time was obtained by searching time where the relative percentage difference between less than 0.01 %. The searching formula used in excel was shown in Figure 3.1. A28:A36028 was the table of calculation result by using Euler method. H22 was the cell of relative percentage difference. The cells in K28:K36028 is the calculation of percentage difference was shown in Eqn 3.23. The value of one in the MATCH function used in Figure 3.1 represented the optimum time was chosen when the relative percentage difference is equal or less than 0.01%.

Figure 3.1 Excel searching formula

```
=INDEX(A28:A36028,MATCH(H22,K28:K36028,1))
```

$$relative\ percentage\ difference \qquad \text{Eqn 3.23}$$

$$= \frac{[isoamyl\ acetate](t) - [isoamyl\ acetate](t-1)}{[isoamyl\ acetate](t-1)}$$

$$\times 100\%$$

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter presented the experimental results and discussion which consist of three main sections. The first section discussed the error caused by different types of mathematical models used. The second section illustrated model fitting and model validation. Finally, the optimization condition of production of isoamyl acetate was predicted by using the developed kinetic equations.

4.1 Error analysis on different mathematical kinetic model

Poor mathematical model unable to describe the progress curve in the production of isoamyl acetate. Mathematical model with low robustness can be considered as poor mathematical model since it is not valid for most of the cases, in other words, it cannot predict the progress of a reaction. Stiff kinetic equation is one of the problems that causes low robustness equation which will be further discussed in Section 4.1.1. Besides, Microsoft Excel Solver has its own limitation, so some of kinetic parameters calculated are not logic and it highly rely on initial guess.

4.1.1 Stiff kinetic equation

Stiff equation is a differential equation which causes unstable output when it is solved numerically. This problem can be solved if the step size is very small. However, the step size smaller than 0.01 min requires longer time to get the kinetic parameter, therefore, smaller step size was not applied in this modelling due to time constraint.

The reaction scheme and equations below were used in modelling in this section.

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EA \overset{k2}{\rightarrow} F + P \qquad \text{Eqn 4.1}$$

$$F + B \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} FB \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} E + Q \qquad \text{Eqn 4.2}$$

$$E + P \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} EP \underset{k_{-6}}{\overset{k_6}{\rightleftarrows}} F + R \qquad \text{Eqn 4.3}$$

$$E + A \underset{k_{-7}}{\overset{k_7}{\rightleftarrows}} E'A \qquad \text{Eqn 4.4}$$

$$E + P \underset{k_{-8}}{\overset{k_8}{\rightleftarrows}} E'P \qquad \text{Eqn 4.5}$$

$$\frac{d[E]}{dt} = -k_1[E][A] + k_{-1}[EA] + k_4[FB] - k_{-4}[E][Q] - k_5[E][P] + k_{-5}[EP] - k_7[E][A] + k_{-7}[E'A] - k_8[E][P] + k_{-8}[E'P] \qquad \text{Eqn 4.6}$$

$$\frac{d[EA]}{dt} = k_1[E][A] - k_{-1}[EA] - k_2[EA] \qquad \text{Eqn 4.7}$$

$$\frac{d[F]}{dt} = k_2[EA] - k_3[F][B] + k_{-3}[FB] + k_6[EP] - k_{-6}[F][R] \qquad \text{Eqn 4.8}$$

$$\frac{d[FB]}{dt} = k_3[F][B] - k_{-3}[FB] - k_4[FB] + k_{-4}[E][Q] \qquad \text{Eqn 4.9}$$

$$\frac{d[EP]}{dt} = k_5[E][P] - k_5[EP] - k_6[EP] + k_{-6}[F][R] \qquad \text{Eqn 4.10}$$

$$\frac{d[E'A]}{dt} = k_7[E][A] - k_{-7}[E'A] \qquad \text{Eqn 4.11}$$

$$\frac{d[E'P]}{dt} = k_8[E][P] - k_{-8}[E'P] \qquad \text{Eqn 4.12}$$

$$\frac{d[A]}{dt} = -k_1[E][A] + k_{-1}[EA] - k_7[E][A] + k_{-7}[E'A] \qquad \text{Eqn 4.13}$$