PROCESS MODELLING AND CONTROL OF ISOAMYL ACETATE SYNTHESIS IN MICROREACTOR USING MATLAB SIMULINK

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2018

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by

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Thesis submitted is partial fulfilment of the requirement for the degree of Bachelor of Chemical Engineering

June 2018

ACKNOWLEGEMENT

I would like to express my deep and sincere gratitude to my thesis supervisor, Associate Professor Dr.Syamsul Rizal Abd Shukor, for giving me the opportunity to do this research and providing me the invaluable guidance with his knowledge and expertise in this research field. Despite of his busy schedule, he reviewed my simulation progress, gave his valuable suggestions and made sure I was on the right track of this research. I am extremely grateful for his patience, kindness and encouragement throughout my final year. Without his support, this project would not come to this far.

Apart from that, I would like to thank Leong Sim Siong for his generous help and willingness to spend the time to explain his modelling work and research in detailed to me. I would like to thank Nurhazwani Yusoff Azudin for providing me the experimental data so that I able to validate my simulation model with the experimental work. Without the helps from Sim Siong and Nurhazwani, it is almost impossible for me to carry out the model development and complete this dissertation.

I am extending my gratitude to the Dean, Prof Azlina Harun @Kamaruddin, all the lecturers and staffs of School of Chemical Engineering, USM for equipping me with the knowledge of chemical engineering and the precious soft skills in these four years.

Finally, my thanks go to my family for their love and care and the people who supported me to complete the research work directly or indirectly.

Elysia Chiang Chern Yuen

June 2018

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LIST OF SYMBOLS

Symbol	Description	Unit
A	Frequency factor	-
[A]	Concentration of acetic anhydride	mol L^{-1}
e	Mass fraction of enzyme	-
E	Activation energy	J/mol
[E]	Active enzyme concentration	mol L^{-1}
[EA]	Concentration of enzyme-anhydride complex	mol L^{-1}
[EP]	Concentration of enzyme-acid complex	mol L^{-1}
[E*]	Concentration of enzyme-acyl intermediate	mol L^{-1}
[E*B]	Concentration of enzyme-acyl-alcohol ternary complex	mol L^{-1}
$[EA_i]$	Concentration of inhibited enzyme-anhydride complex	mol L^{-1}
$[EP_i]$	Concentration of inhibited enzyme-acid complex	mol L^{-1}
$[E_T]$	Total enzyme concentration	mol L^{-1}
h	Step size	Min
K_{iA}	Inhibition constant for acetic anhydride (A)	mol L^{-1}
K_{iP}	Inhibition constant for acetic acid (P)	mol L^{-1}
k	Rate constant	s^{-1}
k_d	Denaturation constant	mol L^{-1}
[P]	Concentration of acetic acid	mol L^{-1}
[Q]	Concentration of isoamyl acetate	mol L^{-1}
r	Ac/Al ratio	-
r_0	Initial reaction rate	mol L^{-1} min^{-1}
R	Gas constant	J mol^{-1} K^{-1}
T	Temperature	K
t	Time	S
y	Ester yield	-
K_c	Process gain	-
τ_I	Process time constant	-
τ_D	Process delay	-

LIST OF ABBREVIATION

CALB	Candida Antarctica Lipase B
GC	Gas Chromatograph
PSSH	Pseudo Steady State Hypothesis
PID	Proportional-Integrative-Derivative Controller
PI	Proportional-Integrative Controller
P	Proportional Controller
PRC	Process Reaction Curve
ZN	Ziegler-Nichols tuning method
CC	Cohen-Coon tuning method

PROSES PEMODELAN DAN KAWALAN ATAS PENGELUARAN ISOAMEL ACETAT YANG DIMANGKIN OLEH SHANTHI DARI CANDIDA ANTARTIKA DALAM MICKOREAKTOR DENGAN MENGGUNAKAN MATLAB SIMULINK

ABSTRAK

Isoamyl asetat adalah ester yang mempunyai perisa pisang. Oleh sebab permintaan bagi industri perisa makanan dan perisa pisang telah meningkat kebelakangan ini, esterifikasi enzimatik telah didapati sebagai alternative yang berlabel semula-jadi untuk menghasilkan isoamil asetat. Proses berterusan berdasarkan teknologi mikroreaktor telah diberi tumpuan dalam proses biokimia kerana teknologi ini merupakan proses yang kurang membazir berbanding dengan kaedah tradisional. Objektif kajian ini adalah untuk mencadangkan strategi kawalan untuk mendapatkan hasil ester yang paling baik dengan menggunakan ujian langkah, kaedah Ziegler Nichols dan Cohen-Coon. Sebelum proses kawalan dilakukan, model proses dari skrip MATLAB diterjemahkan ke dalam blok Simulink. Simulasi dijalankan dalam MATLAB Simulink dan model yang dicadangkan telah disahkan dengan kerja eksperimen dan keputusan simulasi yang sebelumnya menggunakan EXCEL Solver. PID dengan nilai P, I dan D sebanyak 6.8, 0.56 dan 0.14 masing-masing telah didapati sebagai pengawal terbaik dari segi kriteria prestasi seperti overshoot, masa naik, masa puncak pertama, masa penyelesaian, nisbah keruntuhan dan lengkung tindak balas.

PROCESS MONITORING AND CONTROL OF ISOAMYL ACETATE SYNTHESIS CATALYZED BY LIPASE FROM CANDIDA ANTARCTICA IN MICROREACTOR USING MATLAB SIMULINK

ABSTRACT

Isoamyl acetate is an ester that exhibits banana-like flavour. As demand for food flavour industry and banana flavouring have risen this recent years, enzymatic esterification has been found to be a natural-labelled alternative to produce isoamyl acetate. Continuous process based on microreactor technology has been given focus in biochemical process as they offer a less wasteful process compared to traditional methods. The objective of this study is to develop a control strategy to optimize the ester yield by using step test, Ziegler Nichols and Cohen-Coon methods. Before process control was carried out, the process model from MATLAB script was translated into Simulink blocks. Simulation was carried out in MATLAB Simulink and the proposed model was validated with experimental work and previous simulation result using EXCEL Solver. PID with P, I and D values of 6.8, 0.56 and 0.14 respectively were found to be the best controller in term of performance criteria such as overshoot, rise time, time to first peak, settling time, decay ratio and response curve.

CHAPTER ONE

INTRODUCTION

1.1 Importance of Ester

Ester is an organic compound that produced from the reaction between alcohols and either organic or inorganic acids. The term ‘ester’ was first introduced by a German chemist Leopold Gmelin in the first half of the 19th century. Ester is mostly derived from carboxylic acid. Carboxylic acid esters of low molecular weight are colourless, volatile liquids with pleasant aroma, slightly soluble in water. They are mainly exploited in the industries of synthetic flavours, perfumes and cosmetics (Romero et al. (2007). Esters that are used for the fragrance and flavour of flowers and fruits; for instance, isoamyl acetate is found in banana, methyl salicylate in wintergreen, and ethyl butyrate in pineapple. Besides, the volatile esters, which act as solvent, serve the purpose in lacquers, paints and varnishes industries are commercially produced. Some examples of esters corresponding to their flavours and fragrances are shown in Table 1-1.

Table 1-1: Esters with their corresponding flavours and fragrances

Esters	Flavours or Fragrances
Bornyl acetate	Pine
Butyl acetate	Apple
Butyl butyrate	Pineapple
Ethyl butyrate	Pineapple
Ethyl cinnamate	Cinnamon
Isoamyl acetate	Banana
Methyl trans-cinnamate	Strawberry
Octyl acetate	Oranges
Propyl acetate	Pears

The rising demand for esters in industries gives rise to the development of technologies to yield esters. As esters occur naturally in plants and flowers, it is possible to extract esters from plant materials. Nevertheless, it is impractical for commercial exploitation as extraction is too costly (Romero et al., 2005b) and also demands exceeds supply (Güvenç et al., 2002). Therefore, chemical synthesis of esters is developed as it is much cheaper than plant extractions. However, the use of acid in catalytic reaction pollutes the products and requires a series of separation and purification processes that cost a lot (Ghamgui et al., 2006).

Application of biological processes using enzyme has become more crucial as it gains the label of ‘natural’ in esters production. Although it is more expensive than chemical synthesis, they are widely utilized in last decades as they are more environmental friendly since inorganic acids, which catalysing the chemical reaction, can be avoided. In addition, enzyme can be reused and thus less waste is generated (Romero et al., 2007).

1.2 Synthesis of Isoamyl Acetate

Isoamyl acetate, also known as isopentyl acetate, is a colourless ester that is slightly soluble in water yet exhibits high solubility in most organic solvent. The chemical structure is shown in Figure 1-1.

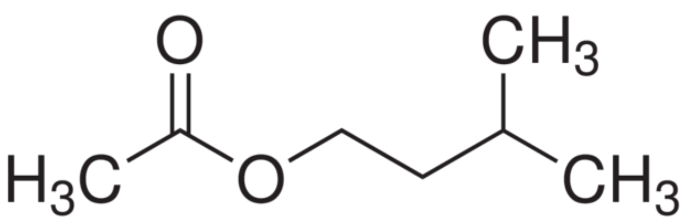


Figure 1-1: Chemical structure of isoamyl acetate

It has a strong banana aroma (Torres et al., 2009) and thus is important in food industry. In USA, it has about 74 tonnes of annual demand (Hari Krishna et al., 2000). (van der Merwe and van Wyk, 1981) stated that isoamyl acetate is one of the most remarkable esters to the aroma profile of white wines.

Traditional methods in isoamyl acetate production are through chemical synthesis and extraction from natural sources, for instance, normal and very late state of matured banana (Salmon et al., 1996). Chemical synthesis such as chemical catalytic esterification reaction between carboxylic acid and alcohol by using sulphuric acid is found to have some problems in corrosion to the reactors and pipes due to the strong oxidation of sulphuric acid to the surface of the reactors and pipes, and difficulty in separation and catalyst recovery due to the homogenous liquid phase of reactants and products. On the other hand, flavour extraction from natural sources is costly and not economical beneficial to the commercial use. This happens is due to the fact that a large quantity of banana is required to produce a small quantity of isoamyl acetate.

As a result, enzyme catalytic synthesis has been developed and attracted attention from researches as it catalyses a wide range of esterification and hydrolysis reactions at low temperature, provide high specificity towards the ester bond, eliminating the undesired side reaction and by-products. In food, cosmetic and pharmaceutical industry, the mean of using enzymatic production is highly recommended as the products are usually labelled as ‘natural’. Besides, the enzyme, specifically immobilized lipase is more favourable for industrial purposes as it can be easily separated from the reaction mixture and regenerated (Garcia et al., 1996), only performed at mild operation conditions and resulting in high purity of product (Žnidaršič-Plazl and Plazl, 2009) . Thus, a huge material cost can be saved.

In the past, esterification between isoamyl alcohol and acetic acid is used to synthesis isoamyl acetate. Acetic acid, acts as acyl donor, however, is found to give a low conversion rate and ester yield due to lipase inactivation by acid (Langrand et al., 1988).

1.3 Microreactor

Micro-structured devices have exhibited a number of benefits in chemical processes (Liu et al, 2016), for instance, high surface-area-to-volume ratio, associated with great heat and mass transfer, simpler control of process parameters and a recent concept of production by numbering-up instead of scale-up for the expansion of production capacity (Žnidaršič-Plazl and Plazl, 2009).

As the devices are in microscale, they greatly reduce the consumption of chemicals and the cost. An easier control of the process parameters essentially temperature, residence time and flow rate possess an attractive way for enzyme-catalysed production of fragrance esters in organic solvents (Žnidaršič-Plazl and Plazl, 2009).

Enzymatic microreactors have the potential to be introduced to industrial-scale synthesis as they can be easily assimilated into systems which operating in the external numbering-up mode. Due to the repetition of the fluidic path while the transport properties and hydrodynamics are preserved, this approach of scaling-up reactions provides good adjustability and control over the process. In addition, when there is any enzyme in microreactor is found to be less active, it can be easily substituted with new enzyme, with minimal effects on the whole system performance (Urban et al., 2006).

1.3 Research Gap

The previous research works are mainly done in batch process and focusing on the reaction and separation parts. To produce isoamyl acetate in microreactor, reaction models need to be converted from batch to continuous process so that a complete workable connection can be developed. By using simulation, we can imitate the future complete system into a simulation environment.

1.4 Problem Statements

In order to fulfil the high market demand and achieve maximum profit, optimum conditions of isoamyl acetate production such as temperature, ratio of acid to alcohol, amount of enzyme and duration of time need to be maintained and controlled. Among these four parameters, ratio of acid to alcohol was founded to cause the greatest effect to the isoamyl acetate production.

Miniaturization is accompanied by fast system dynamics and higher sensitivity to disturbances, which require faster measurement and actuation dynamics along with quick controller calculations. This seriously limits the size and complexity of models that can be used for control. A simple feedback control strategy is required to be developed as it is important to be an initiative step to develop a complete control system in industry.

1.5 Research Objectives

The objectives of this research are:

i. To translate MATLAB script into Simulink model.
ii. To validate the proposed simulation model with experimental data and previous simulation result using EXCEL Solver.
iii. To develop a simple control strategy to optimize yield of isoamyl acetate production.

1.6 Scope of Study

In this study, the process synthesis of isoamyl acetate enzymatic from acetic anhydride and isoamyl alcohol catalysed by *Candida Antarctica Lipase B* (CALB) is studied.

The equation models and experimental work which considered batch process were conducted by Leong (2014) and Nurhazwani (2015) are utilized in this simulation and control study. Simulation data is compared with the previous research works. Due to the time constraint, I utilized the validated model in Simulink model to further develop a simple control strategy by using step-test method as basic, Ziegler-Nichols and Cohen-Coon as the tuning method.

The best controller is chosen based on the best performance criteria in term of overshoot, rise time, time to first peak, settling time, decay ratio and response curve.

1.7 Thesis Organization

This thesis is divided into five chapters as follow:

Chapter 1 comprises the introductory part of the thesis. The research backgrounds of isoamyl acetate synthesis, advantages and challenges of microreactor are introduced. The problem statement, research objectives, scope of study and thesis organization are also disclosed in the later part of this chapter.

Chapter 2 presents a review on the research of isoamyl acetate production in respect to importance of food flavour industry, process simulation in food industry, process intensification, process control in food industry, enzymatic synthesis of isoamyl acetate such as acyl donor, enzyme, solvent free system and parameter studies.

Chapter 3 outlines the methodology of the research.

Chapter 4 depicts and interprets the results of the simulation model for the esterification. The comparison of different types of controller and selection of the best controller strategy are explained thoroughly.

Chapter 5 presents a summary of the research conducted. Some recommendations are suggested for future research.

CHAPTER TWO

LITERATURE REVIEW

In this chapter, background research of the present work will be described.

2.1 Importance of Food Flavour Industry and Its Global Market Presence

Global flavours industry can be categorised as highly technical, specialized and innovative industry due to its highly competitive and concentrated characteristics if compared to other products in food and beverage market. This can be observed by the high market value of global flavour at 12,474 million in 2016 and is expected to achieve 18,126 million by 2023 by a CAGR of 5.5% from 2017 to 2023 (Tandon, 2017).

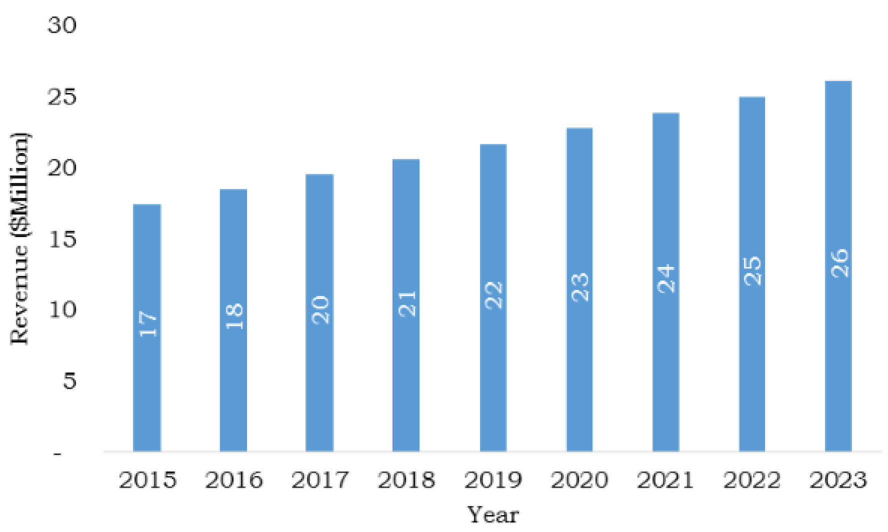


Figure 2-1: Morocco Flavours (Food and Beverages) Market, 2015-2023 (Tandon, 2017)

There are several driving factors of flavours industry in global, such as the growth in food and beverage industry, technological innovations in flavour manufacturing procedures, and rise in health and wellness trend. The demand for recognizable and authentic flavours is rising in various end user industries that are requesting high-quality product. The flavours (food and beverages) market is segmented based on type, end user and geography, For the basis of type, it is classified into natural and artificial flavours. While for end user basis, it is divided into beverages, dairy and frozen products, bakery and confectionery, savoury and snacks, as well as animal and pet food. The key segments of flavours market are summarised in Table 2-1.

Table 2-1: Key segments of flavours market (Tandon, 2017)

Type	**End User**	**Geography**	
• Natural • Artificial	• Beverages – Hot Drinks – Soft Drinks – Alcoholic Drinks • Dairy & Frozen Products – Dairy Products – Meat • Bakery & Confectionery – Bakery – Chocolate – Confectionery – Ice Cream • Savoury & Snacks – Savoury – Snacks • Animal & Pet Food – Animal Feed – Pet Food	• Asia-Pacific – China – Japan – India – Australia – South Korea – Rest of Asia-Pacific • North America – U.S. – Canada – Mexico • Western Europe – UK – Germany – France – The Netherlands – Spain – Belgium – Rest of Western Europe • Eastern Europe – Russia – Poland – Czech Republic – Rest of Eastern Europe	• Latin America – Brazil – Argentina – Colombia – Chile – Rest of Latin America • Middle East – Turkey – Israel – Iran – Saudi Arabia – Rest of Middle East • Africa – Morocco – South Africa – Nigeria – Rest of Africa

Due to the rising demand in flavour industry, applications of advanced technologies provide innovative and novel tastes in food. However, the chemical utilized flavours are less desirable and are believed to cause numerous problems for instance dizziness, nervous system depression, allergies, fatigues, headaches, brain damage, nausea and seizures. Thus, biotechnology is emerging as a competitive alternative in flavouring production.

As isoamyl acetate production by Candida Antarctica catalyst is a bio-processes, it has high potential to be industrially useful and could offer an alternative way to obtain natural banana flavour (Torres et al., 2010). Thus, it is a plausible mean to meet the global banana flour market demand that is anticipated to witness a sluggish growth of CAGR of around 3.8% from 2017-2027 (Analysts, 2017).

2.2 Process Simulation in Food Industry

Simulation is the utilization of mathematical model or models to describe the relationship between parameters that can be observed in a specific system. It is useful for the development of a new process or the alteration of an existing one. It can be very cost-effective in comparison to carrying out full-scale trials in the pilot plant. Calculations based on different conditions can be carried out rapidly on a computer yielding the results in a very short time. Full-scale experiments can thus be rationalised with considerable financial and time savings. If a production plant is to be made more efficient, with greater productivity and enhanced quality assurance, greater knowledge of the process allied with data on the raw material and product is needed. The development of model systems is also crucial. Increased knowledge of the physical process can save money and lead to better quality in the end-product (Skjiildebrand, 1993).

There was a project namely BATCHES from Batch Process Technologies has been successfully applied to the simulation of a brewhouse operation in a Belgium brewery. BATCHES has the ability to simulate both batch and semi-continuous processes in the biochemical, food and pharmaceutical industries. It allows the user to evaluate alternative system configurations and operating procedures, identify bottlenecks, assess the requirements for capacity expansion, and evaluate scheduling strategies (McGrath et al., 1998).

Another simulation was done on the performance of baking ovens using a computational fluid dynamics (CFD) model to quantify heat transfer to, and mass transfer from , the product surface within an oven. CFD is a simulation tool to solve the fluid flow and heat transfer problem by using Navier-Stokes transport equations, describing the conservation of mass, momentum and energy. It is also utilized in application of chillers and retail display cabinets where air distribution and temperature patterns are important factor. Besides, the use of CFD models for the flow of Newtonian fluids such as glucose in pipelines and non-Newtonian foods such as ketchup in a various continuous-flow processing equipment (Scott and Richardson, 1997).

In short, simulation is one of the important element in food industry to ensure quality consistency, cost savings and act as a tool to monitor the important parameter that beyond human limit.

2.3 Process Intensification

Process intensification is one of the most ancient and outstanding development methods to competitive advantage (Daoutidis et al., 2016). It is a technological advancement which leads to a smaller, safer, cleaner and energy efficient process (Reay et al., 2013).

Process intensification based on micro-devices is an innovative approach to minimize the costs of capital and energy as well as to reduce the sizes of chemical plant and equipment which give benefit to the environment. Besides, the micro-scales improve the safety factors since it reduces the exposure to toxic or hazardous materials and the enclosed nature of microreactors limit the probability of runaway reaction. Due to the small quantity of chemical is needed and acquisition of high heat and mass transfer rates, micro-scale system is capable with the reactions with highly flammable, toxic and explosive reactants as these reactants are required to eliminate by-products and to obtain maximum conversion and energy utilization. As the surface-to-volume ratio in microchannels is high, heat transfer is efficient and reaction temperatures in microreactors can be regulated by highly effective heat removal and applications. With the benefits of reduced transport limitations, giving nearly gradientless conditions, microreactors are desirable for determination of reaction kinetics and implementation of more precisely controlled reaction conditions if compared to conventional macroscale reactors. This gives rise to reaction with higher selectivity and yield of desired products (Pohar, 2009) .

Design has become one of the research interests in process intensification. As it involves combination of multiple operations, the issues of unique dynamics and control are raised. To be more specific, it brings about the increased interactions and nonlinear dynamics and in turn restricts the performance of traditional linear decentralized controllers (Baldea, 2015) and sometimes compromises process safety (Luyben and Hendershot, 2004). As a result, several control studies on specific intensified processes using traditional as well as advanced control systems such as decentralised PID control, linear and nonlinear model-based controls have been carried out. To achieve practical throughput, high quantity of parallel micro-units (stacks) is needed. As such, it is challenging to monitor and control the individual units. (Daoutidis et al., 2016)

2.4 Process Control in Food Industry and Its Applications

In 1994, there was a survey carried out by Leatherhead Food Research Association, suggested that food industry had been surprisingly slow to take up many recent technologies. As such, many food industries were characterised as the traditional 'art' food production. To transit from 'art' to 'science', any effective control system must be applied to reduce this intuitive element via appropriate control feedback and sensory inputs. Although we believe that there is no system for the foreseeable future will be able to entirely replace the experienced operator, an appropriate support mechanism is developed to assist both the skilled operator and supervisory personnel to effectively carry out their production or processing duties as well as free the operator from many mundane tasks so that they can concentrate their time and effort on more important tasks (McGrath et al., 1998).

Apart from that, automatic process control is desired to ensure food security and product consistency as the deviation of controlled variables from the desired values can be persistently monitored, adjusted and minimized to enhance the process operations from time to time (Huang, 2013). A major benefit of successful automation and process control is the ability to produce a range of components in large quantities that are consistent from one batch to batch (McGrath et al., 1998).

Data processing systems are capable of dealing with the variability and indeterminacy of food products. This brings a beginning impact on food process automation. Fuzzy logic systems and neural networks are the typical emerging technologies. These technologies are filtering into commercially available packages as add-on ‘toolboxes’ for standard programming environments such as MATLAB Math Works Inc.) and LabVIEW (National Instruments) (McGrath et al., 1998).

Proportional-integral-derivative (PID) control has been widely used in food industry. To enhance the control system performance, model-based control has been developed in food industry (Huang, 2013).

According to the survey by Bauer and Craig (2008), food processing was one of the industries utilized Advanced Process Control (APC). There are several advanced control schemes have been designed and applied on high-order non-linear dynamics by researchers (Huang, 2013). Belt weigh feeder is one of the food industrial applications utilizes advanced controller to precisely control the speed of motor or belt for accurate weighing purpose. A PI Fuzzy Logic with cascaded adaptive controller (Advanced BWF controller) was designed where the gains of PI controller obtained using Fuzzy Logics are further adapted. After that, the implementation of PI Fuzzy Logic Control along with adaptive gain compensation in plant Distributed (Digital) Control System (DCS) to improve the response of machine of all control parameters and control accuracy (Mahajan et al., 2018) . Besides advanced controller, conventional PI method has been proved to be successful in drum dryer application for the food industry to control the moisture content.

Table 2-2: Controllers used by different food industries.

Industry	**Controller**	**References**
Food industry	model-based control	(Huang, 2013)
Food industry	PID controller	(Huang, 2013)
Belt weigh feeder in food industry	Advanced BWF controller	(Mahajan et al., 2018)
Drum dryer in Food Industry	PI controller	(Rodriguez et al., 1996)

2.4.1 PI Controller

Based on research from Bhilai (2014), PI controller is most widely adopted in industrial application due to its cheaper cost, simple construction and design. However, there are some disadvantages, for instance, failure in controlling object with highly nonlinear and uncertain. Introducing integral mode will reduce the speed of the response and overall stability of the system as well. Besides, PI controller with no derivative action is unable to predict the error in the future. Nevertheless, in many cases when response time is not an issue, PI controllers are more preferred by industries. A control without D mode is used when

a) Fast response of the system is not required.

b) Large disturbances and noise are present during operation of the process.

c) There is only one energy storage in process (capacitive or inductive).

d) There are large transport delays in the system.

The controller output in this case is

$$u\,(t) = K_p\,.\,e\,(t) + K_i \int e\,(t\,)\,dt \qquad (2.1)$$

PI controller an integral error compensation scheme, the output response depends in some manner upon the integral of the actuating signal. This type of compensation is introduced by using a controller which produces an output signal consisting of two terms, one proportional to the actuating signal and the other proportional to its integral. Such a controller is called proportional plus integral controller or PI controller (Rao and Mishra, 2014).

2.4.2 PID Controller

Many industrial controllers employ a proportional, integral plus differential PID regulator arrangement that can be utilized to optimize a specific control system. PID controller or its advanced version is widely used in industry. The main types of PID controller include parallel controller, serial controller, and mixed controller. PID controller exhibits many essential dynamics: fast reaction on change of the controller input (D mode), increase in control signal to lead error towards zero (I mode) and suitable action inside control error area to eliminate oscillations (P mode). Derivative mode improves stability of the system and enables increase in gain K and decrease in integral time constant τ_i, which increases speed of the controller response. PID controllers are the most often used controllers in the process industry. The majority of control systems in the world are operated PID controllers. It has been reported that 98% of the control loops in the pulp and paper industries are controlled by single-input single output PI controllers and that in process control applications, more than 95% of the controllers are of the PID type controller. PID controller combines the advantage of proportional, derivative and integral control action (Rao, 2014).

PID controller is one of the classic-loop feedback control schemes. It is developed based on the study of second-order linear systems. To perform a desired feedback closed-loop system, PID controller can be added at the input of the system as shown in Figure 2-2.

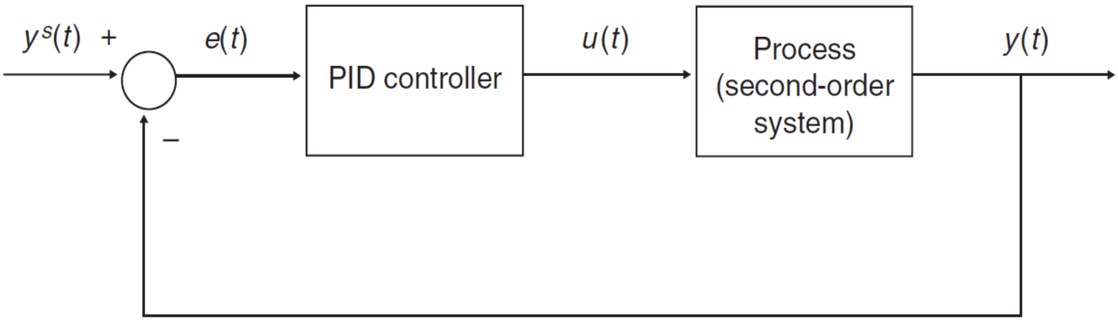


Figure 2-2: A feedback closed-loop control scheme with PID controller

In other way, A PID controller can be expressed by the equation as follow:

$$u(t) = K_p e\ (t) + K_i \int_0^t e(\tau)\ d\tau + K_d \frac{d}{dt} e(t) \qquad (2.2)$$

where

$u(t)$ = output of the PID controller and input to the process at time instant, t

$e\ (t)$ = y^s (t) − y(t) is the difference between the system output y(t) and the desired value y^s(t) at time instant, t

K_p = proportional gain constant

K_i = integral gain constant

K_d = derivative gain constant

2.5 Enzymatic Synthesis of Isoamyl Acetate

CALB is used to catalyse esterification of acetic anhydride and isoamyl alcohol. Chemical structure of acetic anhydride which comprises two acyl groups, -COOR is shown in Figure 2-3.

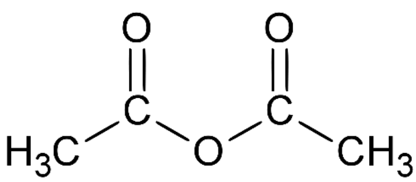
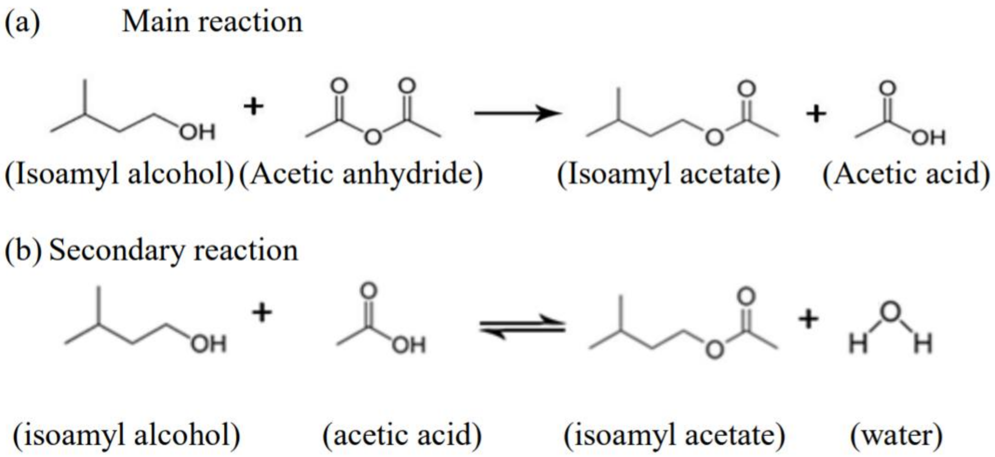


Figure 2-3: Chemical structure of acetic anhydride

There are two consecutive reactions happen in this esterification. First reaction takes place when one of the acyl group from acetic anhydride bind with isoamyl alcohol, discharge one H^+ to form acetic acid with one acyl group and isoamyl acetate (the first desired product). The second reaction is followed by reaction between acetic acid and isoamyl alcohol to form isoamyl acetate (the second desired product) and water (Nurhazwani et al., 2015).

Reaction schemes are as follow:



2.5.1 Acyl Donor

Acetic acid is a potent inhibitor of lipase activity causing dead-end inhibition (Segel, 1975), causes a deactivation of lipase due to acidification of enzyme aqueous environment. This inhibitory effect can be reduced by lower inlet concentrations of reactants, specifically acetic acid or longer residence times (Žnidaršič-Plazl and Plazl, 2009). Research from Langrand et al. (1988) depicted that high yield of esters was difficult to be synthesized due to lipase inactivation by acid (Langrand et al., 1988). Figure 2-4 showed that acetic acid resulted in only 25% conversion while acetic anhydride which contains higher acyl donor enhanced to a high 97% conversion (Hari Krishna et al., 2001).

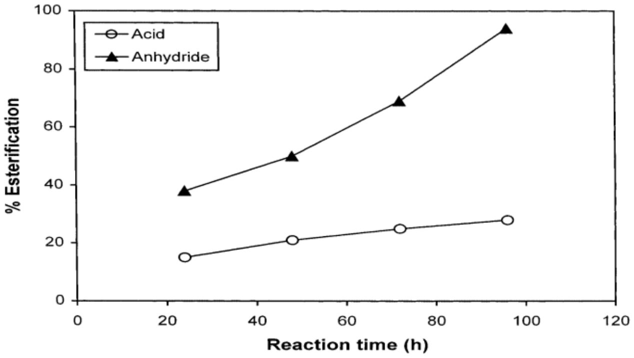


Figure 2-4: Effect of nature of acyl donor on the synthesis of isoamyl acetate by using 0.25 M substrate (equimolar acid/anhydride and alcohol), 10 g l−1 enzyme, *n*-heptane, 40°C and 150 rpm. (Hari Krishna et al., 2001)

As such, acetic anhydride is chosen over acetic acid as reactant in isoamyl acetate esterification.

Table 2-3: Different acyl donors used in isoamyl acetate synthesis

Acyl donors	**Acyl Groups**	**Maximum Conversion**	**References**
Acetic acid	1	25%	Hari Krishna, 2001
Acetic anhydride	2	75%	Hari Krishna, 2001

2.5.2 Enzyme

Enzymes are large protein molecules with specific three-dimensional structures. Every enzyme catalyses a specific reaction, or specific type of reaction, that involves specific substrate(s). Utilization of enzyme in food production and processing has a long tradition as it related to the characteristics of enzyme such as biocompatible nature, selective property and ability to operate under mild conditions. In addition, strict regulations, public perception and market trends further stimulate the implementation of innovative and enhanced processes to the goods. Consequently, research and development on enzyme related technology has becoming one of the promising ways to encounter the situations. Food production for instance the making of beer, bread, cheese or wine, are some examples where enzymes are widely used for years (Fernandes and Carvalho, 2017).

In esterification of enzymatic reactions, lipase can either be in free or immobilized state. Immobilized enzymes are used in this study as they are easy recovered, reused, possesses greater stability and allow continuous operations (Hari Krishna et al., 2001). (Ghamgui et al., 2006) reported that there is no significant decrease in synthesis activity of isoamyl acetate after four cycles of use and conserving maximum conversion of 64% until the synthesis activity is retained about 49% after seven cycles of use that may be caused by reduction in the effective biocatalyst loading or denaturation of enzyme after many cycles of use.

A summary of maximum ester yield by various lipases is shown in Table 2-4. It can be observed that *Candida Antarctica* shows the characteristics of most stable and best catalytic performance as it gives the highest conversion. Hence, immobilized enzyme from *Candida antarctica*, Novozyme 435 supplied by Sigma Aldrich has been chosen in this study.

Table 2-4: Lipase origins and corresponding maximum conversion based on previous researches.

Lipase Origins	Maximum Conversion, % (Based on acyl donor)	References
Candida antarctica	>90	Vija et al., 1997
	95.5	Gubicza et al., 2000
	80	Güvenç et al., 2002
	100	Romero et al., 2005a
	96	Romero et al., 2005b
	100	Fehér et al., 2008
	~99%	Wolfson et al., 2010
Rhizomucor miehei	>90%	Krishna et al., 2001
	~40%	Romero et al., 2005b
Mucor miehei	>80%	
	96.4	Hari Krishna et al., 2000
Staphylococcus simulans	64	Ghamgui et al., 2006
Thermomyces lanuginosus	~42	Romero et al., 2005b

2.5.3 Solvent-Free System

There were several types of solvent used by the researchers (Güvenç, 2002, Yahya, 1998). Lipase catalysed esterification using organic solvent has lately received the greater consideration compared to traditional chemical synthesis methods especially in natural flavour and fragrance industries. However, despite the high conversion is achieved using organic solvent, there are some shortcomings in separation, toxicity, and the flammability of the organic solvents in many applications (Güvenç et al., 2002). Besides, some organic solvents are too expensive to achieve profitable commercial scale-up (Yahya et al., 1998). Therefore, solvent-free system is used in this study as it capable to overcome those problems.

The main benefits of solvent-free system are including the absence of solvents facilitates downstream processing, since fewer components would be present in the reaction mixture at the end of reaction; the exclusion of solvents promotes great cost savings and reduces environmental impacts; high substrate concentrations can be carried out so that high product concentration can be obtained (Güvenç et al., 2002).

2.6 Parameters Studies

2.6.1 Molar Ratio of Alcohol to Acid

Study of the initial molar ratio of alcohol to acyl donor is important to optimize the ester yield. Hari Krishna et al. (2001) recorded that a maximum yield of isoamyl acetate (91% in 24 hours) is obtained when alcohol to acid molar ratio was 2:1 due to the competitive nature of alcohol and acid binding. When the ratio is higher that 2:1, an increase in alcohol binding slightly inhibites the equilibrium concentration of the bound acid because of the increased binding of alcohol thereby reducing the rate of esterification; nevertheless, acid shows stronger inhibitory effect compared to alcohol.

This similar result was showed by Güvenç et al. (2002) in the study of isoamyl acetate synthesis in solvent-free system. Ghamgui et al. (2006) obtained the maximum conversion of acid at alcohol/acid molar ratio of 1:2 in the synthesis of isoamyl acetate catalysed by immobilized lipase from *S. simulans* in solvent-free system.

Romero et al. (2007) carried a study of isoamyl acetate synthesis catalysed by Novozym 435 in n-hexane with acetic anhydride as the acyl donor. He recorded a decrease on reaction rate with increased ratio of acid to alcohol of 0.1:1 to 3:1. Oppositely, the activity percentage is increasing with ratio of alcohol to acid. No alcohol inhibition is observed (Romero et al., 2007).

Table 2-5: Al/Ac ratio used by researches to study the optimum ratio for isoamyl acetate synthesis

Al/Ac ratio	**Solvent**	**References**
2:1	Organic Solvent	(Hari Krishna et al., 2001)
1:2	Solvent-free system	(Güvenç et al., 2002)
1:0.1-1:3	n-hexane	(Romero et al., 2007)

2.6.2 Temperature

In lipase-catalysed reactions, temperature greatly affects both initial rate of the reaction and enzyme stability. Generally, when temperature increases, rate of reaction is increased and enzyme stability is reduced. (Mahapatra et al., 2009)

Ghamgui et al. (2006) reported that enzyme source, the type of immobilization and the nature of the substrate determine the optimum temperature of enzymatic reaction. While in the study of isoamyl acetate synthesis in a solvent-free systems, both reactions using Novozym 435 and Lipozyme RM1M showed the initial reaction rate increases with temperature from 30^oC to 50^oC (Güvenç et al., 2002). Žnidaršič-Plazl and Plazl

(2009) addressed that temperature above 45^oC is less favourable in lipase-catalysed isoamyl acetate synthesis in a microreactor due to thermal inactivation and temperature at 60^oC showed a denaturation of *candida antarctica* lipase B. (Yong and Al-Duri, 1996) also suggested that temperature above 45^oC will thermally denature almost all enzymes as it causes the tertiary structure of enzyme to be disrupted.

However, enzyme denaturation will be less significant at lower temperature. In this case, reaction rate changes according to Arrhenius equation,

$$k = Ae^{\frac{-E_a}{RT}} \tag{2.3}$$

where k = rate constant

A = frequency factor

E_a = activation energy

R = gas constant

T = temperature in Kelvin

From Arrhenius Equation 2.1, temperature increases will bring positive effect on the kinetic constant as defined by transition state theory (Romero et al., 2005a).

2.6.3 Enzyme Loading

Enzyme is used to speed up reaction by providing an alternative reaction pathway of lower activation energy. It is one of the important economic costs in enzymatic reactions. Therefore, conduct a high rate of esterification at low enzyme concentration is always the aim (Romero et al., 2005b). In Michaelis-Menten systems, reaction rate is influenced by enzyme to substrate ratio. When enzyme to substrate ratio is low, substrate is in excess and thus an increase in enzyme loading improves reaction rate; while at high