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BIODIESEL PRODUCTION FROM OLEIC ACID USING IMMOBILIZED LIPASE

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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สาวิตรี มุลาลี : กระบวนการผลิตไบโอดีเซลจากกรดโอเลอิกโดยใช้เอนไซม์ไลเปสที่ถูกตรึง (BIODIESEL PRODUCTION FROM OLEIC ACID USING IMMOBILIZED LIPASE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. เหมือนเดือน พิศาลพงศ์, หน้า.

ไบโอดีเซลเป็นเชื้อเพลิงที่เป็นมิตรกับสิ่งแวดล้อม ในงานวิจัยนี้ไบโอดีเซลผลิตขึ้นจากปฏิกิริยาเอสเทอริฟิเคชั่นจากกรดโอเลอิกกับแอลกอฮอล์สายสั้น (เมทานอล, เอทานอล, โพรพานอล, และบิวทานอล) โดยใช้ โนโวไซม์ 435 เป็นตัวเร่งปฏิกิริยา ซึ่งเริ่มจากการทดลองแบบกะและทำการศึกษาที่สภาวะ: อุณหภูมิ 45 องศาเซลเซียส, อัตราส่วนโดยโมลของแอลกอฮอล์กับกรดโอเลอิกคือ 2 ต่อ 1, โนโวไซม์ 435 5% โดยน้ำหนักเทียบกับกรดไขมัน, ความเร็วในการเขย่าเท่ากับ 250 รอบต่อนาที และเวลาในการทำปฏิกิริยา 8 ชั่วโมง พบว่าโนโวไซม์ 435 ให้ผลที่ดีที่สุดในปฏิกิริยาเอสเทอริฟิเคชันที่ใช้เมทานอล (ค่าการเปลี่ยนแปลงของกรดโอเลอิกเท่ากับ 94.82%) และพบว่าค่าคงที่ทางจลนพลศาสตร์ (k) ของปฏิกิริยาเอสเทอริฟิเคชั่นจากกรดโอเลอิกกับเมทานอล, เอทานอล, โพรพานอล, และบิวทานอล ที่อุณหภูมิ 45 องศาเซลเซียส มีค่าเท่ากับ 0.78, 0.52, 0.69 และ 0.17 ลูกบาศก์เมตรต่อเวลาต่อกิโลโมล ตามลำดับ และค่าพลังงานกระตุ้นในช่วงของอุณหภูมิ 45 ถึง 50 องศาเซลเซียสของปฏิกิริยาเอสเทอริฟิเคชันที่ใช้เมทานอลและเอทานอล เท่ากับ 4.7 และ 39.1 กิโลจูลต่อโมล ตามลำดับ ผลของอุณหภูมิต่อการเสื่อมสภาพของเอนไซม์เห็นได้ชัดจากปฏิกิริยาที่ใช้กรดโอเลอิกและเอทานอลที่ 50 องศาเซลเซียส อย่างไรก็ตาม โนโวไซม์ 435 สามารถนำกลับมาใช้ใหม่ได้ 13 รอบโดยที่ค่าการเปลี่ยนแปลงของกรดโอเลอิกยังมากกว่า 90% แต่เมื่อนำเอทานอลความเข้มข้น 96% และ 95% มาใช้ในปฏิกิริยา พบว่าความสามารถในการนำกลับมาใช้ลดลงเหลือ 10 รอบและ 8 รอบ ตามลำดับ จากภาพของกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดพบว่าการเปลี่ยนแปลงของพื้นผิวของตัวเร่งปฏิกิริยากับระดับการเสื่อมสภาพของตัวเร่งปฏิกิริยามีความสัมพันธ์กัน นอกจากนี้ในงานวิจัยนี้ยังได้พัฒนากระบวนการผลิตไบโอดีเซลจากปฏิกิริยาเอสเทอริฟิเคชันจากแบบกะเป็นแบบต่อเนื่อง โดยขั้นแรกได้ศึกษาสภาวะที่เหมาะสมโดยทำการทดลองโดยใช้ระบบหมุนวนในถังปฏิกรณ์ขยายเบดแบบถังเดี่ยว พบว่าได้ค่าสภาวะที่เหมาะสมที่ อัตราส่วนโดยโมลของแอลกอฮอล์กับกรดโอเลอิกคือ 2 ต่อ 1, อุณหภูมิ 45 องศาเซลเซียส, ความเร็วรอบในการหมุนที่ 600 รอบต่อนาที, อัตราการป้อนสารตั้งต้นขาเข้า 5 มิลลิลิตรต่อนาที, อัตราส่วนระหว่างปริมาตรของเบดและปริมาตรของตัวเร่งปฏิกิริยาที่ 2 ต่อ 1, โนโวไซม์ 435 10% โดยน้ำหนักเมื่อเทียบกับกรดไขมันและเวลาในการทำปฏิกิริยา 5 ชั่วโมง พบว่าโนโวไซม์ 435 สามารถนำกลับมาใช้ใหม่ได้ 22 ครั้งซึ่งให้ค่าการเปลี่ยนแปลงของกรดไขมันมากกว่า 90% งานวิจัยนี้ยังได้พัฒนาต่อไปเป็นกระบวนการเอสเทอริฟิเคชันแบบต่อเนื่องในถังปฏิกรณ์ขยายเบดที่ต่อกันแบบอนุกรมจำนวน 4 ถังปฏิกรณ์ พบว่าค่าร้อยละการได้ผลิตภัณฑ์ของไบโอดีเซลจากปฏิกิริยาเอสเทอริฟิเคชันที่ใช้กรดโอเลอิกและกรดไขมันปาล์มเท่ากับ 93.46% และ 88.50% ตามลำดับ ซึ่งทำให้คำนวณหาค่าอัตราการผลิตได้เท่ากับ 5.24 และ 4.68 กรัมของไบโอดีเซลต่อชั่วโมงต่อกรัมของโนโวไซม์ 435 ตามลำดับ

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต .....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2557

5471466221 : MAJOR CHEMICAL ENGINEERING

KEYWORDS: BIODIESEL / OLEIC / NOVOZYM / REUSAB / EXPANDED BED REACTOR

SAWITTREE MULALEE: BIODIESEL PRODUCTION FROM OLEIC ACID USING IMMOBILIZED LIPASE. ADVISOR: ASSOC. PROF. MUENDUEN PHISALAPHONG, Ph.D., pp.

In this work, biodiesel was produced from esterification of oleic acid and short chain alcohols (methanol, ethanol, propanol, and butanol) catalyzed by Novozym 435 in a batch system at conditions: 45°C, oleic to alcohol molar ratio of 1:2, Novozym 435 loading at 5% (w/w of oleic acid), 250 rpm and 8 h of reaction time. Novozym 435 exhibited the best catalytic activity in the production of methyl oleate (FFA conversion of 94.82%). At 45°C, the rate constants (k values) for the production of methyl oleate, ethyl oleate, propyl oleate, and butyl oleate by Novozym 435 were 0.78, 0.52, 0.69 and 0.17 $m^3 \cdot h^{-1} \cdot kmol^{-1}$, respectively. The activation energies for the production of methyl oleate and ethyl oleate over the temperature range of 40 °C to 55 °C were 4.7 and 39.1 kJ/mol, respectively. The effect of thermal deactivation on the reusability of Novozym 435 in the esterification of oleic acid with ethanol at 50°C was greater than that with methanol. Novozym 435 could be reused in the production of methyl oleate and ethyl oleate for 13 cycles with FFA conversions of > 90%. When 96.0% ethanol and 95.0% ethanol were used, the numbers of Novozym 435 reuse cycles were not greater than 10 cycles and 8 cycles, respectively. The effective development of esterification from FFAs (oleic acids and PFAD) and methanol catalyzed by Novozym 435 was studied. The optimal operating condition was obtained in the single expanded bed circulation reactor at; FFA to methanol molar ratio of 1:2, 45°C, rotation speed of 600 rpm, feed volumetric flow rate of 5 mL/min, the bed to catalyst volumetric ratio of 2:1, Novozym 435 of 10% w/w of FFA and 5h. Novozym 435 could be reused 22 cycles with FFA conversion>90%. The continuous process in four expanded bed reactors in series was also investigated. FAME yields of esterification from FFAs (oleic acid and PFAD) and methanol were 93.46% and 88.50%, respectively. The productivity of biodiesel production using oleic acid and PFAD were 5.24 and 4.68 g FAME•h^{-1}•g $enzyme^{-1}$, respectively.

Department: Chemical Engineering Student's Signature .....

Field of Study: Chemical Engineering Advisor's Signature .....

Academic Year: 2014

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Finally, I would like to express my heartfelt thanks to my parents and my family for their blessing, support and encouragement as well as my friends in the Department of Chemical Engineering, Chulalongkorn University for the completion of my study.

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CHAPTER I

INTRODUCTION

1.1 Motivation

Currently, concerning on the increasing of energy demand, continuous global warming effects, declining petroleum reserves, and rising petroleum price have raised the need to search for alternative renewable fuels. Therefore, the development of biodiesel as an alternative fuel to supplement or replace petro-diesel is receiving a great attention among researchers and policy makers for its numerous advantages such as renewability, biodegradability and lower gaseous emission profile [1]. In Thailand, government aims to reduce reliance on imported energy and promote domestic renewable energy programs, which could utilize domestic resources and create new economic activities.

Biodiesel is an environmentally compatible product that can be produced from renewable resources, such as vegetable oils, free fatty acids (FFAs), animal fats [2, 3] or waste cooking oil (WCO) [4, 5] with short-chain alcohols, such as methanol, ethanol, propanol and butanol [6]. The catalysts used in the production of biodiesel

can be classified as alkaline catalysts (NaOH or KOH), acid catalysts (H_2SO_4) [7] and biocatalysts (lipase enzymes).

Lipase can selectively and effectively catalyze both transesterification and esterification reactions with low energy consumption at mild operating temperatures of less than 50°C [5, 8, 9]. Moreover, this process is "greener" than chemical processes [10]. Because the use of enzymatic catalysts does not form soaps, it can esterify both FFA and TAG in one step without the need of a subsequent washing step [11]. Since there is very low discharge of chemicals and wastewater, the enzymatic process is considered to be a clean and environment friendly technique. However, the high cost of enzymatic catalysts is considered to be the limiting factor for their commercialization. Therefore, immobilization techniques are used to enhance the potential for industrial-scale enzymatic processes. Immobilization allows for the easy recovery of enzymatic catalysts by filtration and for reuse of the catalyst several times without significant losses in activity or stability [12].

Immobilized lipase from *Candida antarctica* lipase B (Novozym 435) is an attractive enzymatic catalyst for the production of biodiesel from many types of oil-containing seed plants in Thailand, such as palm (Elaeis guineensis), physic nut (Jatropha curcas), papaya (Carica papaya) and rambutan (Nephelium lappaceum) [13]. The operating conditions, such as the temperature, initial molar ratio of FFA to alcohol, mixing rate and enzyme concentration, have important roles in the

enzymatic conversion of FFAs. The optimal FFA to alcohol molar ratio can reduce the effect of alcohols on enzyme deactivation. A mild operating temperature could reduce energy consumption and could prolong the lipase life cycle. The influences of the operating conditions and the external mass transfer limitations on the synthesis of fatty acid esters using Novozyme 435 were investigated [14]. It was reported that the external mass transfer limitation could be minimized by using a mixing rate of 300 rpm. The production of butyl ester from crude high oleic sunflower oil was catalyzed by Novozym 435 in a packed-bed reactor, and the results indicated that Novozym 435 could be reused for more than 50 days without a significant loss in activity [15]. Influence of alcohol structure on the enzymatic activity was reported. It was shown that yields of greater than 90% were obtained from the esterification of normal-, di-, and sec-butanol. However, when Novozym 435 was used in the esterification of adipic acid with tert-butanol, the final yield was only 39% [16].

The first part of this work was the study of esterification of oleic acid, a major component of various oils, such as palm oil, rapeseed oil and used frying oil, with short-chain alcohols catalyzed by the immobilized lipase, Novozym 435 in a batch system. The optimal operating conditions such as the temperature, molar ratio of FFA to alcohol, mixing rate and enzyme concentration were investigated. The effect

of operating conditions on the kinetic parameters (k, E_a) and Novozym 435 stability and reusability were also focused.

On the second part, a continuous system of biodiesel production from oleic acid and short-chain alcohols catalyzed by Novozym 435 at the optimal operating condition obtaining from the batch experiment was developed for improve biodiesel productivity. The effects of operating factors such as a feed volumetric flow rate, a catalyst to bed volumetric ratio and the Novozym 435 loading on FFA conversion were investigated.

Moreover, for further development of "green technology" of a commercial biodiesel production from low-cost feedstocks, the esterification of palm fatty acid distillate (PFAD), a non-edible byproduct from palm oil refining and short-chain alcohol catalyzed by Novozym 435 under the optimal conditions in a continuous system in was demonstrated.

1.2 Objectives

1. To investigate the optimal operating conditions of the esterification of oleic acid and short-chain alcohols (methanol, ethanol, 1-propanol, 1-butanol) catalyzed by Novozym 435.

2. To study the effects of temperature and type of alcohol on the kinetic parameters (k, E_a) and the reusability of Novozym 435 in the esterification of oleic acid and short-chain alcohols.
3. To study the effect of initial water content in ethanol on the FFA conversion and the reusability of Novozym 435.
4. To develop an effective continuous process for the esterification of oleic acid and methanol catalyzed by immobilized lipase, Novozym 435.
5. To examine the use of a low cost feed stock, palm fatty acid distillate (PFAD) for biodiesel production, in the continuous system of esterification with methanol catalyzed by Novozym 435.

1.3 Scope of works

1.3.1 Batch Process

i. The operating conditions were; temperature of 45-50°C, FFA:alcohol molar ratio of 1:2, shaking rate of 250 rpm, and enzyme (Novozym 435) loading of 5% w/w of FFA.

ii. The effect of operating temperature during 40 and 55°C on kinetic parameters (k, E_a) of esterification of oleic acid with short-chain alcohols (methanol, ethanol) catalyzed by Novozym 435 was

investigated and compared with those of the esterification with 1-propanol and 1-butanol.

iii. The effect of operating temperature (45°C and 50°C) on the catalytic activity and reusability of Novozym 435 in the esterification of oleic acid and short-chain alcohols was studied.

iv. The effect of initial water content on the catalytic activity and reusability of Novozym 435 in the esterification of oleic acid and ethanol (ethanol 99.9%, 96.0%, and 95.0% v/v) was studied.

1.3.2 Continuous Process

1.3.2.1 A single expanded bed circulation reactor

i. The single expanded bed reactor was developed for esterification from oleic acid and methanol catalyzed by Novozym 435 in a circulation process at the optimal operating condition: 45°C and oleic acid to alcohol molar ratio of 1:2 and rotation speed of No.5 (~ 600 rpm, calculated based on IKAMAG manual).

ii. The optimal operating conditions were investigated as follows;

    a. Catalyst to bed volume ratio; 1:1 and 1:2

b. Novozym 435 loading; 5% and 10% (by weight of oleic acid)

c. Circulation flow rate of feed; 4, 5, and 6 ml/min

iii. The stability and catalytic activity of Novozym 435 of the esterification from oleic acid and methanol in a circulation process was investigated.

iv. The application of palm fatty acid distillate (PFAD) was examined in the esterification of PFAD with methanol in the single expanded bed circulation reactor.

1.3.2.2 Multiple expanded bed reactors in series

I. The single expanded bed circulation reactor was developed to the multiple expanded bed reactors in series for the esterification of oleic acid and methanol catalyzed by Novozym 435. The optimal number of expanded bed reactors was also investigated.

II. The use of palm fatty acid distillate (PFAD) was examined for the biodiesel production in the continuous system by the multiple expanded bed reactors in series catalyzed by Novozym 435.

1.4 Expected Benefit

The expected benefits of this study are;

1. The understanding of esterification of oleic acid and short-chain alcohol catalyzed by Novozym 435.
2. The development of an effective continuous process for biodiesel production from of oleic acid and short-chain alcohols catalyzed by Novozym 435.
3. The information for biodiesel production from low-cost renewable feedstock, such as palm fatty acid distillate (PFAD) and short-chain alcohols catalyzed by Novozym 435.

CHAPTER II

THEORY

2.1 Biodiesel

Biodiesel, an alternative and attractive diesel fuel, is produced from transesterification or esterification process of vegetable oils or animal fats with the addition of alcohol. It is also known as fatty acid alkyl ester. Biodiesel is quite similar to petroleum-derived diesel in its main characteristics such as cetane number, energy content, viscosity and phase changes [17]. Biodiesel contains no petroleum products, but it is compatible with conventional diesel and can be blended in any proportion with fossil-based diesel to create a stable biodiesel blend. Therefore, biodiesel has become one of the most common biofuels in the world [18].

The feedstock of biodiesel of the world depends on availability of each location. Normally, the typical feedstocks for biodiesel production are vegetable oil such as rapeseed oil, canola oil, soybean oil, sunflower oil and palm oil. Moreover, the animal fats such as tallow and poultry and waste oil such as waste cooking oil are also attractive sources of raw materials [18].

There are many advantages of biodiesel over normal diesel such as higher cetane number, higher flash point, and better lubrication. Table 1 shows the biodiesel fuel properties produced from different feedstocks and compared to normal diesel fuel.

Table 1 Some properties of diesel and biodiesel produced from different feedstocks [18].

Fuel	Kin. Viscosity (mm^2/s, at 40 °C)	Density (g/cm^3, at 21°C)	Cetane number	Flash point (°C)	Cloud point (°C)	Pour point (°C)
Diesel	2.0-4.5	0.820-0.860	51.0	55	-18	-25
Soybean ME	4.08	0.884	50.9	131	-0.5	-4
Rapeseed ME	4.83	0.882	52.9	155	-4	-10.8
Palm ME	4.71	0.864	57.3	135	16	12
Sunflower ME	4.60	0.880	49.0	183	1	-7
Jatropha ME	4.4	0.875	57.1	163	4	-
Tallow ME	5.00	0.877	58.8	150	12	9
Soapstock ME	4.30	0.855	51.3	169	6	-

Note: ME = methyl ester

2.2 Biodiesel process

2.2.1 Transesterification reaction

Transesterification reaction is chemical reaction between alcohol and triglyceride such as vegetable oils or animal fats. This reaction produces ester compounds, which can be used to replace the petroleum based diesel fuel. The by-product in this reaction producing is glycerol, which can be used as a substrate in chemical and cosmetic industrials. The transesterification reaction from triglyceride and alcohol has shown in Figure 1.;

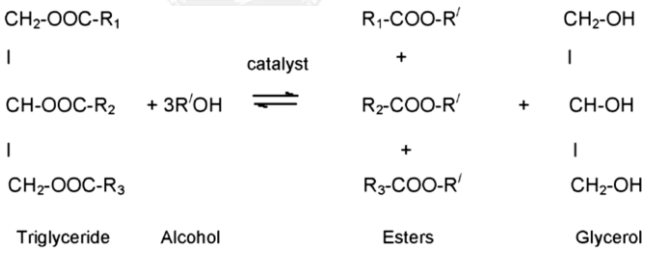


Figure 1 Transesterification reaction of triglyceride with alcohol [19].

2.2.2 Esterification reaction

Esterification reaction is a chemical reaction between carboxylic acids and alcohols deriving the ester compounds and water as a final product. Esterification is a reversible reaction. In order to increase the conversion yields of fatty acid alkyl esters, alcohol should be excess feed in the process, or water should be removed from the product. The esterification reaction from free fatty acid (FFA) and alcohol has shown in Figure 2.;

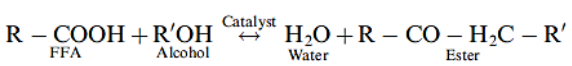


Figure 2 Esterification reaction [20].

Although transesterification may give high productivity of biodiesel, the reaction has several drawbacks such as energy intensive and difficult to recovery glycerol. On the other hand, no removal of glycerol is required under biodiesel synthesis by esterification of free fatty acids.

2.3 Alcohol

Alcohol is an alternative transportation fuel since it has properties, which would allow its use in existing engines with minor hardware modifications. Alcohols have higher octane number than gasoline. A fuel with a higher octane number can endure higher compression ratios before engine starts knocking, thus giving engine an ability to deliver more power efficiently and economically. Alcohol burns cleaner than regular gasoline and produce lesser carbon monoxide, HC and oxides of nitrogen [21, 22]. Alcohol has higher heat of vaporization; therefore, it reduces the peak temperature inside the combustion chamber leading to lower NOx emissions and increased engine power.

Methanol (CH_3OH) is a simple compound. It melts at – 97.7°C and boils at 65°C [23]. Methanol is a completely miscible with water and organic solvents. Moreover, it has a high octane and is easy to transport so it is widely used in chemical production such as biodiesel, plastics, adhesives and paints. It does not contain sulfur or complex organic compounds. The organic emissions (ozone precursors) from methanol combustion will have lower reactivity than gasoline fuels hence lower ozone forming potential. If pure methanol is used then the emission of benzene and PAHs is very low [24]. Moreover, methanol gives higher engine efficiency and is less flammable than gasoline.

Ethanol (C_2H_5OH) is a second number of the alcohol. Absolute ethanol is an ethanol which is completely free of water. It melts at - 117.3°C and boils at 78.5°C [23] . Ethanol is similar to methanol, but it is considerably cleaner, less toxic and less corrosive. It gives greater engine efficiency. Ethanol is grain alcohol and can be produced both a petrochemical and biological processes. In petrochemical process, ethanol is produced through the hydration of ethylene. It can be produced by fermenting sugars with yeast in biological processes. Ethanol is miscible with water. It is difficult to separate from water because it has an azeotrope point that is at 95% ethanol and 5% water. Therefore, ethanol 95% is usually used in industrial because the price of ethanol 95% is lower than absolute ethanol (99.9%). The physical properties of alcohols in comparison to CNG, DME and petroleum fuels are given in Table 2.

Table 2 Comparison of various properties of primary alcohols with gasoline and diesel [25].

Fuel	Methane	Methanol	Dimethyl Ether	Ethanol	Gasoline	Diesel
Formula	CH_4	CH_3OH	CH_3OCH_3	CH_3CH_2OH	C_7H_{16}	$C_{14}H_{30}$
MW (g/mol)	16.04	32.04	46.07	46.07	100.2	198.4
Density (g/cm^3)	0.000072	0.792	0.661	0.785	0.737	0.856
Normal boiling point (^oC)	-162	64	-24.9	78	38-204	125-400
LHV (kJ/cm^3)	0.0346	15.82	18.92	21.09	32.05	35.66
LHV (kJ/g)	47.79	19.99	28.62	26.87	43.47	41.66
Exergy (MJ/l)	0.037	17.8	20.63	23.1	32.84	33.32
Exergy (MJ/Kg)	51.76	22.36	30.75	29.4	47.46	46.94
Carbon Content (wt %)	74	37.5	52.2	52.2	85.5	87
Sulfur content (ppm)	7-25	0	0	0	200	250

Transesterification process normally utilizes methanol or ethanol and vegetable oils as the process inputs. This route of utilizing alcohol as a diesel engine fuel is definitely a superior route as the toxic emissions (aldehydes) are drastically reduced. The problem of corrosion of various engine parts utilizing alcohol as fuel is also solved by way of transesterification.

For biodiesel production, methanol is the alcohol most frequently used for triglyceride transesterification due to its low cost. Nevertheless, biodiesel obtained by ethanolysis is interesting since ethanol is renewable and can be produced from the fermentation of many agricultural products. Moreover, propanol

or butanol can also be used in this process. These two alcohols can also use to promote a better miscibility between the alcohol and the oil phases [26].

2.3 Fatty Acid [18]

The physical and chemical fuel properties of biodiesel basically depend on the fatty acids distribution of the triglyceride used in the production. The chemical structures of common fatty acids are shown is Table 3. and the fatty acid distributions of some feedstocks commonly used in biodiesel production are shown in Table 4. and Figure 3., respectively.

Table 3 The chemical structures of common fatty acids [18].

Fatty acid	Chemical structure
Lauric (12:0)	$CH_3(CH_2)_{10}COOH$
Myristic (14:0)	$CH_3(CH_2)_{12}COOH$
Palmitic (16:0)	$CH_3(CH_2)_{14}COOH$
Stearic (18:0)	$CH_3(CH_2)_{16}COOH$
Oleic (18:1)	$CH_3(CH_2)_7CH=CH(CH_2)_7(COOH)$
Linoleic (18:2)	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
Linolenic (18:3)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$
Arachidic (20:0)	$CH_3(CH_2)_{18}COOH$
Behenic (22:0)	$CH_3(CH_2)_{20}COOH$
Erucic (22:1)	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$

Table 4 The fatty acid distributions of some biodiesel feedstocks [18].

Feedstock	Fatty acids (% w/w)						
	12:0	**14:0**	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**
Sunflower	-	-	6.08	3.26	16.93	73.73	-
Rapeseed	-	-	3.49	0.85	64.40	22.30	8.23
Soybean	-	-	10.58	4.76	22.52	52.34	8.19
Palm	-	1	42.8	4.5	40.5	10.1	0.2
Peanut	-	0.3	12.3	4.6	53.6	29	0.1
Coconut	46.5	19.2	9.8	3	6.9	2.2	-
Soybean soapstock	-	-	17.2	4.4	15.7	55.6	7.1
Used frying oil	-	-	12	-	53	33	1
Tallow	-	3-6	24-32	20-25	37-43	2-3	-
Lard	-	1-2	28-30	12-18	4-50	7-13	-

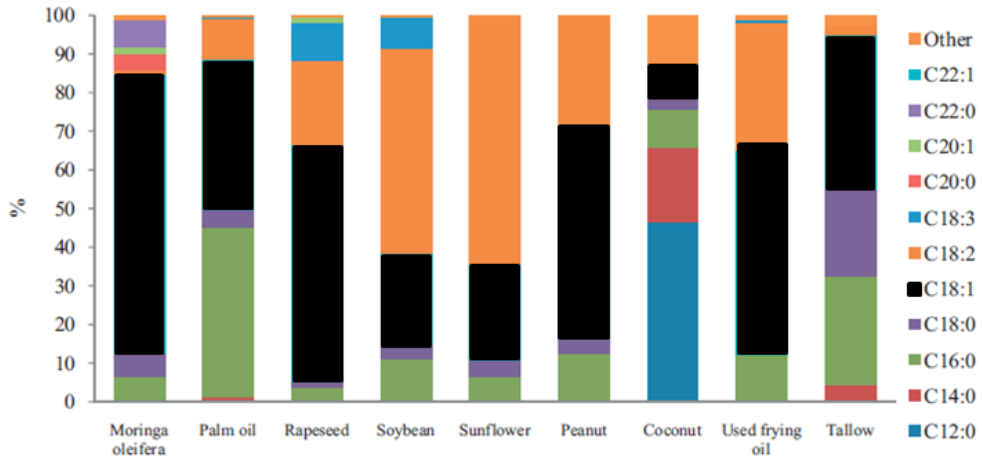


Figure 3 Fatty acid profile of some biodiesel feedstocks [27].

The study of Marty et al., (1999) informed that oleic acid (C18:1) which shows the highest composition of each feedstock (Figure 4) is more stable to thermo-oxidation than other fatty acids, since its structure has only one unsaturated bond.[28]

2.4 Palm fatty acid distillate (PFAD)

Palm oil is usually refined in several sections including degumming, neutralization, bleaching, dewaxing and deodorization [29]. The refining processes remove phospholipids, free fatty acid, pigment and other impurities in the oil. Refining methods for the vegetable oil refinery are two methods including physical refining and chemical refining [30] as shown in Figure 4.

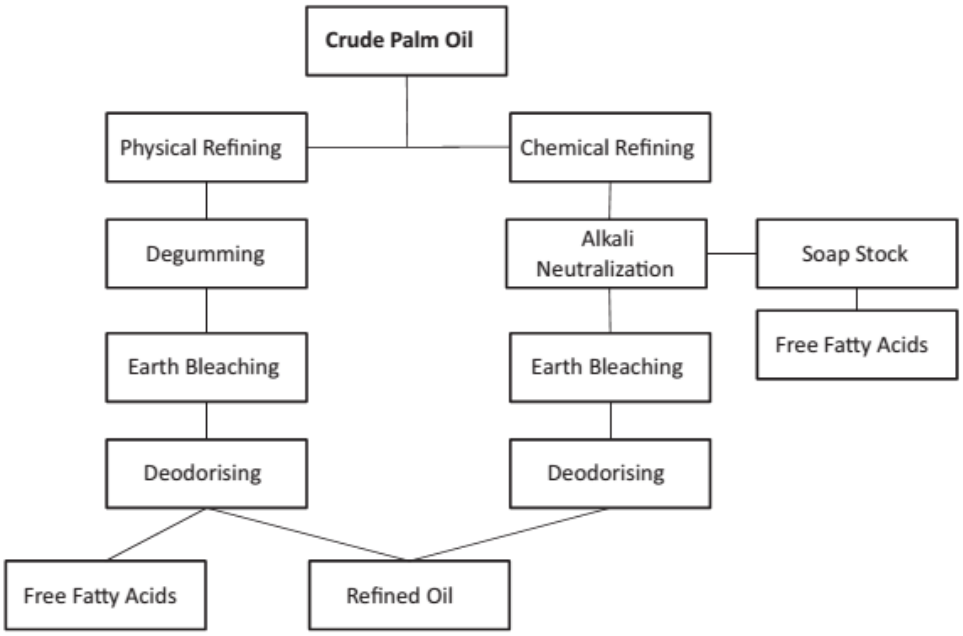


Figure 4 Palm oil refining process [31]

The refining process removes free fatty acids, phosphatides, odoriferous matter, water and impurities from the crude palm oil to produce high quality edible oil that meets industry standards. To achieve this chemical or physical objective, refining can be used as shown in Figure 4 and in both cases free fatty acids are obtained as a by-product of the refining processes.

The high cost of biodiesel is mainly due to its being produced from high quality virgin oil with low content of free fatty acid. A way of reducing biodiesel cost is to use less expensive feedstock containing high FFA, recycled or waste oil and products of refining vegetable oils [32]. Therefore, the use of PFAD for biodiesel

production is another alternative and interesting substrate to substitute the use of virgin oil [2].

2.5 Lipase Catalyst and its immobilization

Lipases are widely employed to catalyze hydrolysis, alcoholysis, esterification and transesterification of carboxylic esters. Lipases have excellent catalytic activity and stability in non-aqueous media, which facilitate the esterification and transesterification processes during biodiesel production. Immobilized lipase or immobilized enzyme are defined as "enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities which can be used repeatedly and continuously" [33]. There are several methods for lipase immobilization, including adsorption, covalent bonding, entrapment, encapsulation, and cross-linking. These immobilization methods have been employed to improve lipase stability for biodiesel production in recent years, and this is discussed in the following sections.

Adsorption

Adsorption is the attachment of lipase on the surface of the carrier by weak forces, such as van der Walls, hydrophobic interactions or dispersion forces [33]. Adsorption can be prepared under mild conditions without major activity loss and

the associated process is relatively easy and low cost. Moreover, the carrier can easily be recovered for repeated immobilization. With these advantages, adsorption is still the most widely employed method for lipase immobilization.

In general, all the biodiesel yields using the adsorption technique are higher than 80% with vegetable oil or waste cooking oil as feedstock. There are two kinds of lipase used most frequently, especially for large scale industrialization. One is the *Candida antarctica* [34, 35] lipase immobilized on acrylic resin, which is known by its commercial name of Novozym 435.

Novozym 435 can catalyze vegetable oil and cooking oil with yield higher than 90% [36]. The other is the Candida sp. 99–125 lipase immobilized on cheap textile membrane [37]. This immobilized lipase textile can catalyze lard, waste oil and various vegetable oil with yield higher than 87%. A comparison between these two lipases is summarized in Table 5.

Table 5 Comparisons of immobilized Candida antarctica and Candida sp. 99–125 [27].

Lipases	Carrier used	Substrate	Organic solvent	Effect of water on yield	Yield (%)	Stability	Cost
Candida antarctica	Acrylic resin	Vegetable oil, waste cooking oil	Hydrophobic solvents, solvent free, t-butanol	No water added	>90	500 h	High
Candida sp. 99-125	Textile membrane	Lard, waste oil, salad oil	Hydrophobic solvents, solvent free	10% wt to the oil	>87	210h	Low

From Table 5, *Candida Antarctica*, immobilized lipases, shows the higher yield, higher stability. However, the higher cost is still its bottleneck. Therefore, the reusability of this enzymatic catalyst is another important issue to concern.

Although adsorption has its special commercial advantages for its high activity toward biodiesel production at low cost, the lipase maybe stripped off from the carrier because of the weak adhesion forces between the enzyme and support. The immobilized lipase may not be stable enough to prevent lipase from desorption during the catalytic process. Thus, the optimum shaking rate or ration of speed in the reaction is another important factor for the stability and reusability of immobilize enzymatic catalyst [33].

2.6 Kinetic of reaction

2.6.1 The reaction rate constant

In the chemical reaction, one of the reactants that is disappearing as a result of the reaction is the basic of calculation a species A. Usually chosen the limiting reactant is basic for calculation. The rate of a reaction can be expressed in terms of the concentrations of the various species.

For a general reaction rate form: A + B $\longrightarrow$ C + D, where substance A and B are reacting to produce C + D.

$$\text{Rate} = k(T)\,[A]^{m}\,[B]^{n} \qquad (1)$$

In this equation, k is the rate constant for the reaction; [A], [B] is the concentration of A, B; m is the order of the reaction with respect to A and n is the order of the reaction with respect to B

2.6.2 The Arrhenius equation

Quantitatively the relationship between the temperature term and the rate of reaction term is determined by the Arrhenius Equation.

$$k(T) = Ae^{-Ea/RT} \quad (2)$$

Where, A = pre-exponential factor or frequency factor

Ea = activation energy, J/mol or cal/mol

R = gas constant = 8.314 J/mol.K = 1.987 cal/mol.K

T = absolute temperature, K

The activation energy is determined experimentally by carrying out the reaction at several different temperatures. After taking the natural logarithm of the Arrhenius equation, the modified equation is usually of the form:

$$\ln k = \ln A - E_a/RT \quad (3)$$

The activation energy can be determined from a straight line whose slope from a plot of ln k versus 1/T.

CHAPTER III

LITERATURE REVIEWS

There are many researchers studied the development of biodiesel production in both a batch and continuous process. For biodiesel production, various types of fatty acid, alcohol, and lipase catalyst were also evaluated.

3.1 Enzymatic biodiesel production in a Batch process

Chulalaksananukul et al., (1990) studied the kinetic of the esterification of oleic acid by ethanol catalyzed by immobilized lipase of *Mucor miehei* or Lipozyme in n-hexane as a solvent [38]. It was found that this reaction was followed with a Ping-Pong Bi Bi mechanism which indicated the inhibition of the excess of ethanol in the process. The mechanism of this reaction has shown below;

$$\frac{v}{v_{max}} = \frac{[Ol][Eth]}{k_{m(Ol)}[Eth]\{1+[Eth]/K_i\}+K_{m(Eth)}[Ol]+[Ol][Eth]} \quad (4)$$

Where; V_{max}= maximum rate of reaction

$[Ol]$= initial oleic acid concentration

$[Eth]$ = initial ethanol concentration

$K_{m(Ol)}$ and $K_{m(Eth)}$ = Michalis constant of oleic acid and ethanol, respectively

K_i = inhibitor constant of ethanol

Moreover, this study indicated that the external mass transfer limitation is not significant when the rotating speed is suitably high at 300 rpm [38]. However, the effect of internal diffusion was not studied in this research.

Trubiano et al., (2007) investigated the enzymatic esterification of fatty acid (mainly oleic acid) and ethanol catalyzed by Novozym 435 at solvent free conditions [14]. They studied several factors of the operating condition such as temperature, initial molar ratio of acid to alcohol, initial water percentage and enzyme percentage which affected to the equilibrium conversion and the initial reaction rate. Finally, they found that the optimal condition of this reaction was at 65^{o}C, using an enzyme amount of 1-5% by weight of acid, in the molar ratio of 1:1. The highest conversion of ethyloleate of 80-90% was obtained. However, they suggested that the high operating temperature might destroy the enzyme activity. Moreover, they also notified that the external mass transfer limitation was not a significant influence on the reaction rate [14].

Marchetti and Errazu., (2008) studied the influence of alcohol's carbon chain length and the presence of water on the esterification reaction of oleic acid with different heterogeneous catalyst and alcohols as shown in Table 6;

Table 6 The chemical substance for esterification of oleic acid with different heterogeneous catalyst and alcohols [6].

aFree Fatty Acid (FFA)	Alcohol	Catalyst	Condition
1. Oleic Acid	1.Ethanol anhydrous 2. Ethanol 96° 3. 1-Propanol 4. 2-Propanol 5. 1-Butanol	1. Solid Resin (Dowex Monosphere 550A) 2. Enzyme 2.1 Lipozyme CALB 2.2 Lipozyme T.L 100L 3. Zeolite 3.1 NaY 3.2 VOx over USY	1. Molar ratio of Alcohol/FFA= 6.13:1 2. Temp = 45°C 3. Catalyst weight of 3% w/w

The highest FFA conversion of the esterification from oleic acid and ethanol was obtained compared to that of propanol and butanol. Moreover, FFA conversion of FFA and 1-propanol was higher than that with of 2-propanol in the study of the effect of OH group location on propylester production. Furthermore, enzyme Lipozyme CALB showed the highest activity and final FFA conversion of esterification compared with other catalyst (98% of conversion). Additionally, the presence of water in the reaction had a negative effect on final FFA conversion of the esterification catalyzed by enzymatic catalyst [6].

Tan et al., (2010) pretreated the Candida sp. 99-125 lipase immobilized on textile membrane with several methods to improve its activity and methanol tolerance for biodiesel production. The lipase was treated with various types of solution as n-propyl alcohol, n-butanol, isopropyl alcohol, tert-butanol, isobutyl alcohol, 1 mM salt solutions of $(NH_4)_2SO_4$, $CaCl_2$, KCl, $K2SO_4$, $MgCl_2$ and methanol solutions. The lipase activity, methanol tolerance and operational stability of biodiesel production were significantly improved by using 1 mM salt solutions of $CaCl_2$, and $MgCl_2$ as a pretreatment solution. The result showed that pretreated lipase could be reusability for 9 batches with higher than 50% of the fatty acid methyl ester yield [10].

Li et al., (2012) investigated transesterification of *Pistacia* chinensis bge seed oil (PCO) with methanol catalyzed by different immobilized enzyme as immobilized

Rhizopus oryzae lipases on macroporous resin (MI-ROL) and immobilized *Rhizopus oryzae* lipase on anion exchange resin (AI-ROL) in a solvent free system. It could be seen that the highest biodiesel yields of 92% and 94% was achieved with AI-ROL dosage of 25 IU/g PCO and MI-ROL dosage of 7 IU/g PCO, respectively. However, AI-ROL could be reused for 5 cycles while MI-ROL could be reused only 4 cycles [39].

Chanprasert (2011) studied the production of butyloleate in solvent-free system by esterification of oleic acid with butanol using immobilized lipase Novozym 435 as biocatalyst in a batch system. The conversion of free fatty acid (FFA) could reach 91.0% at reaction temperature of 45°C, oleic acid/butanol molar ratio of 1:2, Novozym 435 loading based on FFA weight of 5%, a shaking rate of 250 rpm, and a reaction period of 24 h. The removal of water that was produced during the enzymatic esterification by the addition of molecular sieves could enhance the FFA conversion to 96.1%. Novozym 435 having been used for five cycles still remained active with only slightly loss of catalytic activity [40].

Sena (2011) studied the biolubricant production from oleic acid and propanol catalyzed by Novozym 435 in a batch system at solvent-free condition. Effects of reaction time, alcohol structure (propanol vs. isopropanol), enzyme loading, rotation speed, molar ratio of propanol to oleic acid, and reaction temperature were investigated. The optimal operating conditions were 45°C, molar ratio of Propanol to oleic acid of 2:1, Novozym 435 loading at 5% based on oleic acid weight and 250

rpm, in which the maximal FFA conversion at 88.9% was obtained. It was shown that the FFA conversion could be increased to 94.7% (or 6.5% increases) by removal of water during the reaction using molecular sieve. Novozym 435 could be reused at least 5 cycles without considerable change in the conversion for propyloleate production [41]

Zheng et al., (2012) investigated and characterized the protein-coated micro-crystals (PCMCs) from *Pseudomonas cepacia* lipase (PS) and K_2SO_4 of their application in biodiesel synthesis. They found that at the optimal condition; 40 $^{\circ}$C, FFA and ethanol molar ratio of 1:4, and 200 rpm for 12 h, the average biodiesel yield of 83% was obtained for seven oils (soybean oil, sunflower seed oil, olive oil, camellia oil, corn oil, rapeseed oil, and stillingia oil). Moreover, The PCMC-PS which was washed by hexane was actively stable for 8 batch cycles, with only 16% reduction in conversion [42].

Srisuwan (2013) studied the esterification of fatty acid distillates from rice bran, palm and coconut and short chain alcohols (methanol, ethanol, propanol, and butanol) catalyzed by Novozym 435 at conditions of; 45°C, fatty acid distillate to alcohol molar ratio of 1:2, Novozym 435 loading at 5% based on fatty acid distillates weight, 250 rpm and 8 h of reaction time. The experimental studies were conducted to investigate effects of fatty acid distillates, alcohols, water and reusability of Novozym435 on the esterification reaction. The FFA conversion of all fatty acid

distillates and short chain alcohols of >86% were obtained. The FFA conversion tended to decrease with the length of carbon chain in alcohols. The amount of water in alcohols affected on enzyme activity and the highest activity of Novozym 435 was achieved at 2% of water content based on fatty acid distillate weight. Novozym 435 could be reused at least 10 cycles in the esterification of rice bran and palm fatty acid distillates with 95.0% ethanol; only slightly change in the conversion for biodiesel production was observed [43].

3.2 Enzymatic biodiesel production in a continuous process

Dossat et al., (1999) studied the continuous enzymatic transesterification of high oleic acid sunflower oil with butanol in a packed bed reactor immobilized by Lipozyme [28]. It was found that glycerol which occurred during the reaction absorbed onto the enzymatic support and led to a drastic decrease in enzymatic activity. Therefore, the addition of other chemical substances to eliminated produced glycerol as silica gel, n-hexane and tertiary alcohol was studied. The results did not show any improvement from the addition of silica gel or n-hexane. However, the rinsing of catalyst bed with tertiary alcohol solution could eliminate the glycerol from the reactor and the high conversion of FFA was also maintained. The scheme of this reaction has shown below;

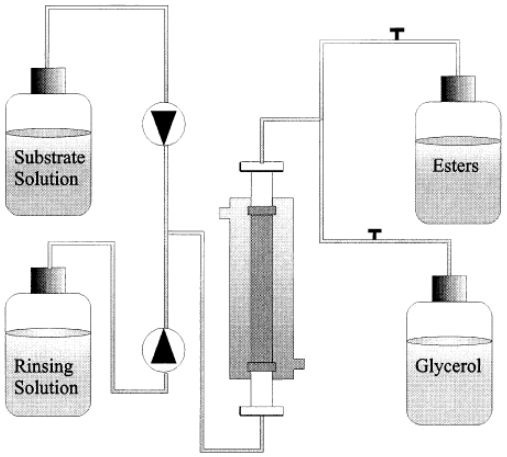


Figure 5 Semi-continuous reactor for the production/fractionation of ester and glycerol [28].

From Figure 4, reactor was a fixed bed type. It consisted of a thermostated column (40°C) with a diameter of 9 mm containing the immobilized enzyme of 4 cm/g. The reaction medium containing high oleic sunflower oil of 20 mM and butanol of 100 mM in n-hexane was pumped into the reactor at flow rate of 0.35 ml/min. Samples were collected and analyzed at the outlet. The highest conversion of 95% was obtained and no mono- and di-glyceride were detected at the outflow of the reactor [28].

Chen et al., (2009) synthesized biodiesel from waste cooking oil and methanol catalyzed by immobilized *Candida sp. 99-125* lipase in a three-step fixed bed reactor. The effects of lipase, solvent, water, temperature and flow of the

reaction mixture on the synthesis of biodiesel were analyzed [44]. The schematic diagram has shown below;

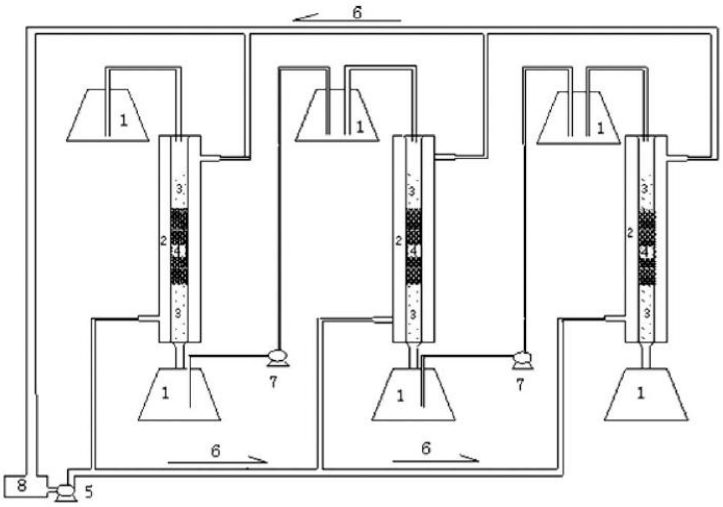
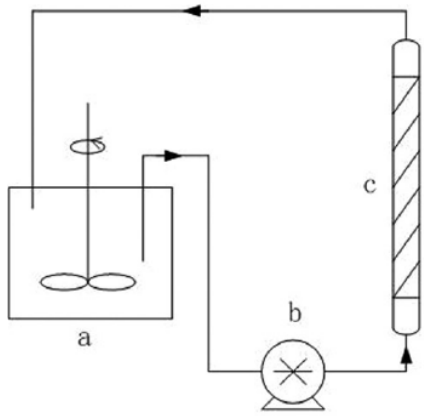


Figure 6 Schematic diagram of three-step reactor. 1. conical flask; 2. reactor; 3. glass beads; 4. immobilized lipase; 5. water pump; 6. circulating water; 7. peristaltic pump; 8. water bath.

The immobilized lipase with specific activity of 222.5 Unit/mg, was placed in the reactor columns which were kept at constant temperature by a water jacket connected with water bath and separated by glass beads. After being fully mixed using a magnetic stirring apparatus, the mixture of WCO, methanol, solvent hexane and water was pumped into the reactors from the top. Three reactors are connected in series to form a three-step reaction system and glycerol was separated at each

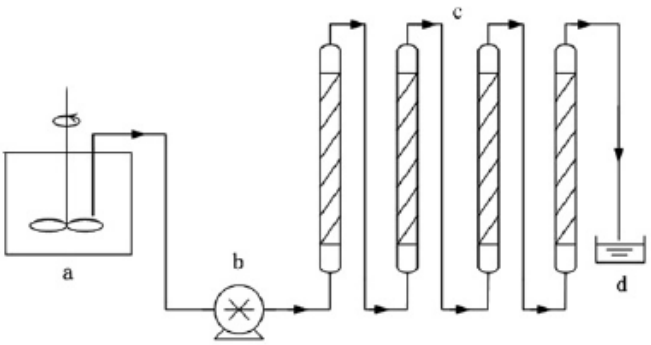
step. In order to reduce the toxicity to lipase activity from methanol, the WCO/methanol molar ratio was kept 1:1 in each reaction step. At the end of the reactions, the glycerol rich-phase was separated from the methyl ester layer in a decantation funnel. The latter phase was washed with water twice and then in distillate at 70 $^{\circ}$C under normal pressure. The purified methyl esters were then dried through molecular sieves and filtered under vacuum to produce crude biodiesel. The results indicated that a 91% of fatty acid methyl ester could be achieved in the end product under optimal conditions [44].

Wang et al., (2011) developed and investigated biodiesel production of soybean oil with methanol catalyzed with lipase-Fe_3O_4 nanoparticle biocomposite catalyst in two types of reactor as a single packed bed reactor and a four packed bed reactor, respectively [45]. A schematic diagram of biodiesel production in the single - and four- packed bed reactor has shown in Figure 6, and Figure 7, respectively;



Note: a is inlet close tank, b is peristaltic pump, c is reactor, d is outlet open tank

Figure 7 A schematic diagram of biodiesel production in the single packed bed reactor [45].



Note: a is inlet close tank, b is peristaltic pump, c is reactor, d is outlet open tank

Figure 8 A schematic diagram of biodiesel production in the four packed bed reactor [45].

A glass column was used as the packed-bed reactor, in which the lipase–nanoparticle biocomposite (including 40 mg of lipase) loaded with cotton

(approximately10 g) served as a support. The inner radius and axial height of the packed-bed reactor were 1.6 and 20 cm, respectively. The volume of the packed-bed reactor was 160 mL. The weight of the packing material in the packed-bed reactor was 40 g, and the working volume was 136 mL. The result showed that a high conversion rate and great stability were obtained with the four packed bed reactor which showed the highest conversion of over 88% for 192 h [45].

Shibasaki-Kitakawa et al., (2010) proposed a simple continuous production process of biodiesel. Fatty acid ester from crude rice bran oil (RBO) was constructed using two heterogeneous ion-exchange resin catalysts. In the system, the expanded-bed reactor packed with the cation-exchange PK208LH resin catalyst of 35.4 g (wet) for esterification of the FFA and that with the anion-exchange PA306S resin catalyst of 55.5 g (wet) for transesterification of the triglyceride were connected in series [46, 47]. When the mixed solution of the crude rice bran oil and alcohol was supplied to the proposed system, the fatty acid esters with a high conversion of more than 98.6% could be continuously produced without any extra operation such as dewaxing/degumming of the raw oil, removal of the byproduct, and addition of alcohol [47]. The experimental conditions were; 50°C, crude RBO to ethanol molar ratio of 1:3.6 and flow rate of 0.1 mL/min. The schematic diagram of biodiesel production has shown in Figure 9.

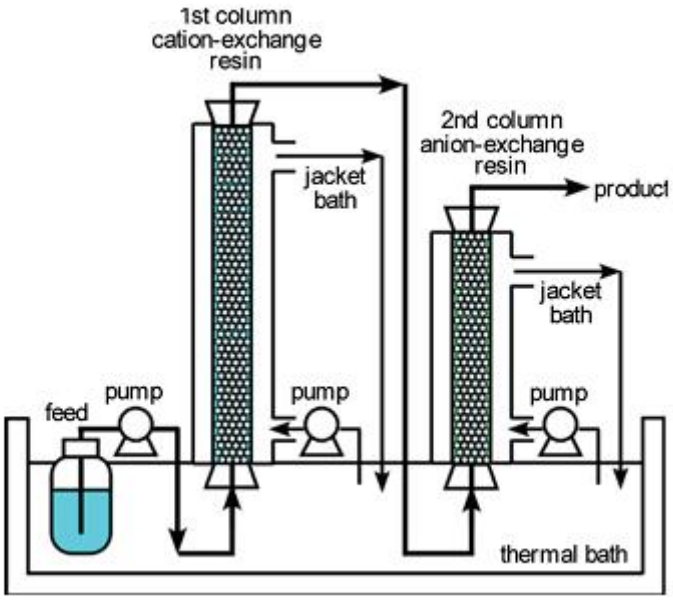


Figure 9 Schematic diagram of continuous production system of biodiesel fuel with cation- and anion-exchange resin catalysts [47].

CHAPTER IV

EXPERIMENTS

This chapter consists of experimental systems and procedures used in this research, which is divided into 5 main parts:

4.1 Chemicals and equipments

4.2 Enzymatic esterification

4.2.1 Batch esterification process

4.2.2 Continuous esterification process

4.2.2.1 A single expanded bed circulation reactor

4.2.2.2 Multiple expanded bed reactors in series

4.3 Biodiesel Analysis

4.4 Kinetic parameters (k, E_a) calculation

4.1 Chemicals and equipments

4.1.1 Chemicals

1. Short-Chain alcohols (methanol, ethanol, 1-propanol, 1-butanol)

2. Phenolphthalein

3. KOH

4. Oleic acid

5. Palm fatty acid distillates (PFAD) obtained from Patum Vegetable Oil Co., Ltd., Pathum Thani, Thailand.

6. Novozym 435 (Sigma-Aldrich Co. LLC, Canada), (lipase B from Candida antarctica, EC 3.1.1.3), a nonspecific lipase immobilized on macroporous acrylic resin was purchased from S.M. Chemical suppliers Co., Ltd, Bangkok, Thailand. The diameters of the particle beads are in a range of 0.3-0.9 mm with approximate density of 0.4 g/ml. The catalytic activity was 10,000 PLU/g.

4.1.2 Equipments

1. Hotplate stirrer with magnetic stirrer set

2. Vessel vial, flasks, Beaker

3. Burette

4. Centrifuge (5100, Kubota, Fujioka, Japan)

5. Incubator shaker (Innova 4000, ALT, Connecticut, USA)

6. Scanning electron microscope JSM-5410LV (Tokyo, Japan)

7. Nuclear Magnetic Resonance Spectrometer (NMR 500 MHz) with CP/MAS solid probe and Nano probe (Varian version INOVA, Lexington, USA).

4.2 Enzymatic esterification reaction

4.2.1Batch esterification process

The batch esterification process using 40 g of oleic acid and short-chain alcohols (methanol and ethanol) catalyzed by Novozym 435 was performed in a 250 ml Erlenmeyer flask. One milliliter samples were collected from the reaction mixture at 0, 0.5, 2, 4, 6, and 8 h. Water, a reaction byproduct and residual alcohols were removed from the samples via thermal evaporation. After then, the samples were centrifuged to re-check the left water and alcohol. The purified product was then analyzed using the titration method to determine the FFA conversion. On the study of the reusability of Novozym 435, after each cycle, the products and the remaining substrates were removed, and fresh substrates were added for the next cycle. From our previous study, the maximum oleic acid conversions of greater than 90.0% were obtained using a reaction temperature of 45°C, FFA to alcohol molar ratio of 1:2, and an enzyme loading of 5% (w/w of oleic acid) and shaking rate of 250 rpm. Hence, these optimal operating conditions were used for remainder of the studies reported in this research. The batch esterification of oleic acid with short-chain alcohols (methanol, ethanol) was studied to observe the effects of operating conditions on kinetics parameter (k, E_a), the reusability of Novozym 435, and the surface morphology of Novozym 435.

4.2.2 Continuous esterification process

For improvement of the biodiesel productivity, the study of continuous process of biodiesel production was investigated. Free fatty acids using in this study were oleic acid and palm fatty acid distillate (PFAD) and methanol catalyzed by Novozym 435

4.2.2.1 The single expanded bed circulation reactor

The cylindrical glass was used as the expanded bed reactor. The inner radius and the height of the single expanded bed reactor were 2.5 cm and 10.0 cm, respectively. The volume of the expanded bed reactor was 196 cm^3 and the maximum working volume at reaction zone was 50 cm^3. The bottom of the expanded bed reactor was used as a reservoir of 40 cm^3 with continuous mixing using a magnetic stirrer bar of length 1 cm at 600 rpm (No. 5) in order to continuously feed substrates flowing up through the bottom of the bed. The aluminium screen covered with fine cotton sheets was used as a support of the immobilized enzyme. The operating temperature was controlled at 45^{o}C (No.5) by a hotplate stirrer and thermometer set (IKAMAG, RO10 power, Japan). Feed of 1:2 molar ratio of FFA to methanol was mixed together in the feeding tank for 1 h before starting the experiment. The reaction mixture was then continuously fed into the

reservoir of the expanded bed reactor from the bottom through a peristaltic pump and the product flew out at the top of the reactor and flew to the feeding tank again. Thus, the mixture was continuously circulated into the reactor. The effluent liquid was collected at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 24 h to determine the FFA conversion. In this part, the effects of operating conditions on FFA conversion such as feed volumetric flow rate, bed to catalyst volume ratio, Novozym 435 loading were investigated. The usage of PFAD in this process was also examined. The schematic diagram of esterification from free fatty acid and methanol catalyzed by Novozym 435 in a single expanded bed circulation reactor has shown in Figure 10;

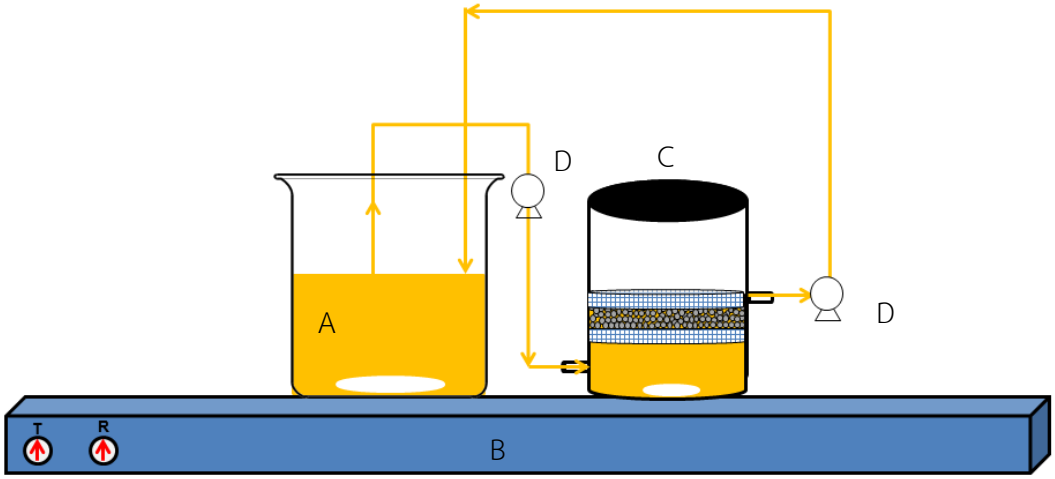


Figure 10 Schematic diagram of esterification from free fatty acid and methanol catalyzed by Novozym 435 in a single expanded bed circulation reactor. A, substrate mixture feeding tank; B, hotplate with multi stirrer; C, a single expanded bed circulation reactor; D, peristaltic pump; T, temperature control; and R, rotation speed control.

4.2.2.2 Multiple expanded bed reactors in series

For highly efficient use of lipase and to increase the productivity of fatty acid methyl ester production, the single expanded reactor was modified to the multiple expanded bed reactors in series. In this part, the optimal operating condition from the study of a single expanded bed circulation reactor was used to develop for the study of multiple expanded bed reactors in series. The use of PFAD

in this process was also examined. The yield of fatty acid methyl ester (FAME) was determined from ^{1}H-NMR method. The schematic diagram of esterification from free fatty acid and methanol catalyzed by Novozym 435 in multiple expanded bed reactors in series has shown in Figure 11.

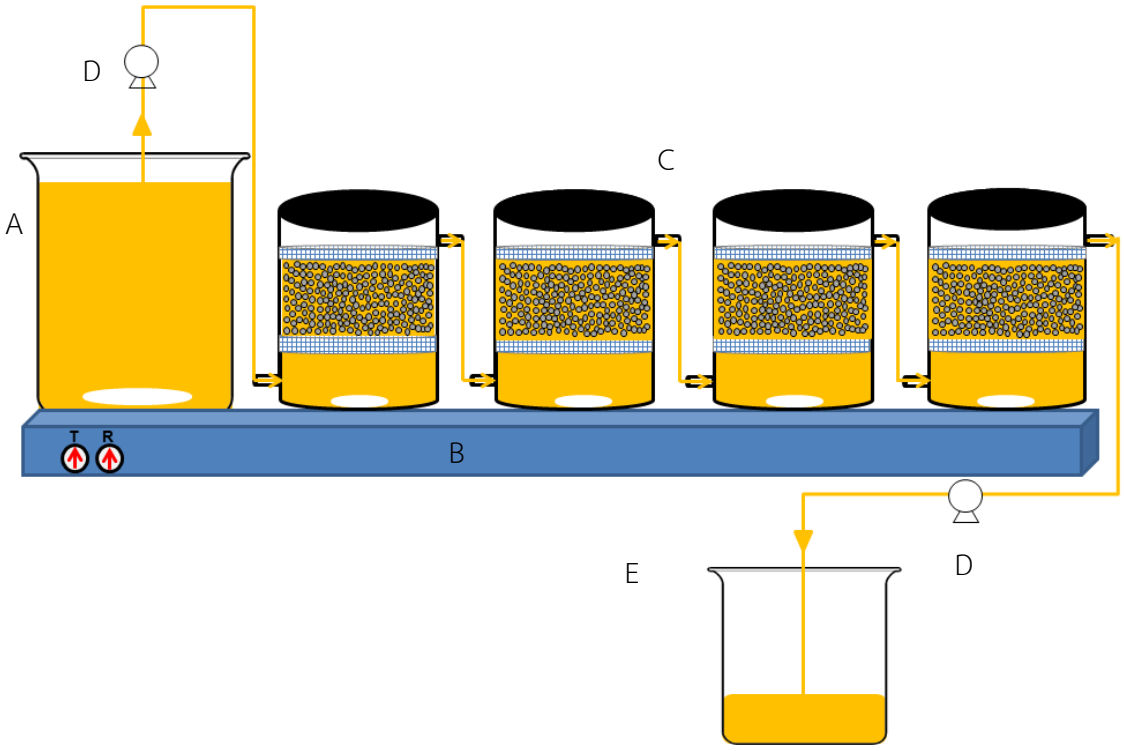


Figure 11 Schematic diagram of esterification from free fatty acid and methanol catalyzed by Novozym 435 in multiple expanded bed reactors in series; A, substrate mixture feeding tank; B, hotplate with multi stirrer; C, multiple expanded bed reactors in series; D, peristaltic pump; E, product tank; T, temperature control; and R, rotation speed control.

4.3 Biodiesel conversion analysis

The percentage of oleic acid conversion was determined by the titration method with 0.1 M KOH solution using phenolphthalein as the indicator. The oleic acid conversions were calculated from the titration volumes of the KOH solution. The reported values were the average values of each duplicate set.

4.4 Kinetic study of the esterification reaction

The esterification of oleic acid and alcohol can be represented by the following equation:

$$\text{Oleic acid} + \text{Alcohol} \leftrightarrow \text{Alkyl oleate} + \text{Water} \quad (5)$$

The rate for second order reaction (R) of the forward reaction is given by:

$$R = \frac{-d[\text{Oleic acid}]}{dt} = k[\text{oleic acid}][\text{alcohol}] \quad (6)$$

Where, k is the kinetic rate constant for the forward reaction. The concentrations of oleic acid and alcohol can be written in forms of the initial concentrations, the conversion of oleic acid (X) and the mole ratio of alcohol to oleic acid (M).

$$[\text{Oleic acid}] = [\text{Oleic acid}]_0 (1 - X_{\text{Oleic acid}}) \quad (7)$$

$$[\text{Alcohol}] = [\text{Alcohol}]_0 (1 - X_{\text{Alcohol}}) = [\text{Oleic acid}]_0 (M - X_{\text{Oleic acid}}) \quad (8)$$

By substitution of oleic acid and alcohol concentrations in Eq. (7) and Eq. (8) into Eq. (6) and integration, the kinetic rate constant (k) for the second order reaction could be determined as shown in Eq. (9);

$$\ln \frac{(M - X_{Oleic\ acid})}{M(1 - X_{Oleic\ acid})} = [\text{Oleic acid}]_0 (M - 1)kt \quad (9)$$

Quantitative analysis of the relationship between temperature (T) and the kinetic rate constant (k) can be determined by the Arrhenius equation. The activation energy is determined experimentally by carrying out the reaction at several temperatures. Taking the natural logarithm (ln) of the Arrhenius equation yields:

$$\ln k = \ln A - \frac{Ea}{RT} \quad (10)$$

The activation energy value (Ea) can be determined from the slope of a plot between ln k versus 1/T.

4.5 Novozym 435 morphology observation from scanning electron microscope (SEM)

Scanning electron microscope (SEM) was performed to observe morphology changes of the biocatalyst (Novozym 435) after being used in the esterification reaction. Excess oil and solution at the surface of the biocatalysts was blotted out with Kimwipes paper. The samples of biocatalysts were then sputtered with gold and

were examined for morphological structures by Scanning electron microscope JSM-5410LV (Tokyo, Japan).

4.6 Analysis of fatty acid methyl ester (FAME) by Nuclear Magnetic Resonance Spectrometer (^{1}H-NMR)

The fatty acid methyl ester (FAME) yield from esterification of free fatty acid and methanol catalyzed by Novozym 435 was analyzed by Nuclear Magnetic Resonance Spectrometer (NMR 500 MHz) with CP/MAS solid probe and Nano probe (Varian version INOVA, Lexington, USA). NMR analysis was performed by dissolving the sample in $CDCl_3$. The dissolved sample was transferred to an NMR tube. All solid material must be removed from the solution before it was placed in the NMR tube. Then, the NMR tube was inserted into a sample turbine. Spectra were recorded on a Varian Mercury-500 spectrometer operating at 500 MHz at room temperature. The FAME content was determined by the ratio of the area of peaks associated with the methyl ester (3.65 ppm) and methylene group protons (2.26 ppm) [48, 49]. The equation for FAME determination has shown below;

$$C\,(\%) = 100 \times \frac{2A_{Me}}{3A_{CH_2}} \qquad (11)$$

Where; C is the FAME yield percentage of esterification, A_{Me} is the integration value of the methoxy protons of the methyl esters, A_{CH_2} is the integration value of **α**-methylene protons and 2, 3 are proton numbers in the methylene and methoxy groups, respectively.

CHAPTER V

RESULTS AND DISCUSSION

In this part, results and discussions were individually described into two parts of batch and continuous systems as follows;

5.1 Esterification of oleic acid and short-chain alcohols by Novozym 435 in batch system

5.1.1 The optimal operating conditions for esterification of oleic acid and short-chain alcohols (methanol, ethanol, n-propanol, and n-butanol) using Novozym 435

5.1.2 Effects of operating conditions on kinetics of Novozym 435 for esterification of free fatty acids with short-chain alcohols (methanol, ethanol, n-propanol, and n-butanol)

5.1.3 Influences of operating conditions on biocatalytic activity and reusability of Novozym 435 for esterification of free fatty acids with short-chain alcohols

5.2 Esterification of oleic acid and short-chain alcohols by Novozym 435 in continuous system

5.2.1. The optimal operating conditions for esterification of oleic acid and methanol catalyzed by Novozym 435 in a single expanded bed circulation reactor

5.2.2 The optimal operating conditions for esterification of free fatty acid and methanol catalyzed by Novozym 435 in multiple expanded bed reactors in series

5.1 Esterification of oleic acid and short-chain alcohols by Novozym 435 in batch system

5.1.1 The optimal operating conditions for esterification of oleic acid and short-chain alcohols using Novozym 435

This part focused on the investigation of the optimal operating conditions for biodiesel production in a batch esterification process using oleic acid and short-chain alcohols (methanol and ethanol, n-propanol, and n-butanol) as substrates and using immobilized lipase (Novozym 435) as a biocatalyst. The optimal operating conditions of oleic acid and short-chain alcohols catalyzed by Novozym 435 were: 45°C, oleic acid to alcohol molar ratio of 1:2, Novozym 435 amount of 5% w/w of oleic acid, the rotation speed of 250 rpm. As shown in Figure 12, the FFA conversions of all reactions rapidly increased in 1 h. After then, the FFA conversions gradually increased and reached the equilibrium at 8h. The final FFA conversions of methyl oleate, ethyl oleate, propyl oleate, and butyl oleate productions were found at 94.82%, 91.27%, 89.08%, and 88.20%, respectively. These results suggested the possibility of the biodiesel production using Novozym 435 as a biocatalyst. Therefore, biodiesel production from esterification of oleic acid and short-chain alcohols catalyzed by Novozym 435 were additionally studied to observe the kinetics (k, E_a), reusability and

stability of Novozym 435, and the development of the reactor configuration for the continuous system.

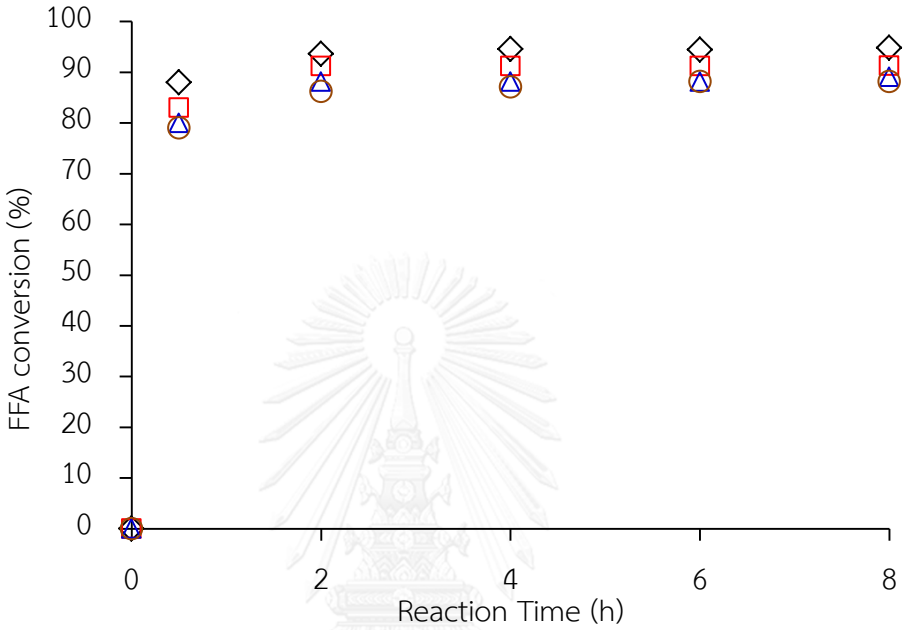


Figure 12 Esterification of oleic acid with short-chain alcohols catalyzed by Novozym 435 at Reaction conditions: Oleic acid to alcohol molar ratio=1:2, Novozym 435=5%wt of oleic acid, shaking rate=250 rpm and 8h. Alcohols (anhydrous): methanol (◊), ethanol (□), n-propanol (△), n-butanol (**O**)

5.1.2 Effects of operating conditions on kinetics of Novozym 435 for esterification of free fatty acids with short-chain alcohols

5.1.2.1 Effect of alcohols on the kinetic rate constant (k)

In an enzymatic production of biodiesel, the type of alcohol is one of the most significant factors that affect the activity, stability and reusability of the enzyme. Various studies have found that the enzyme can be inhibited by alcohols [34, 35, 50-52]. In addition, different alcohols can have different effects on the properties of biodiesel, such as viscosity [11]. In this study, the effect of various short-chain alcohols, including methanol, ethanol, n-propanol and n-butanol, on the esterification of oleic acid catalyzed by Novozym 435 was investigated. The concentrations of the (anhydrous) alcohols were not less than 99.9% v/v. The optimal operating conditions obtained from 5.1.1 were used in this study. The plots of FFA conversions during the first hour catalyzed by Novozym 435 using different short-chain alcohols and the plots for determining the kinetic rate constants (k) at the operating temperature of 45°C are shown in Figure 13 and Figure 14, respectively.

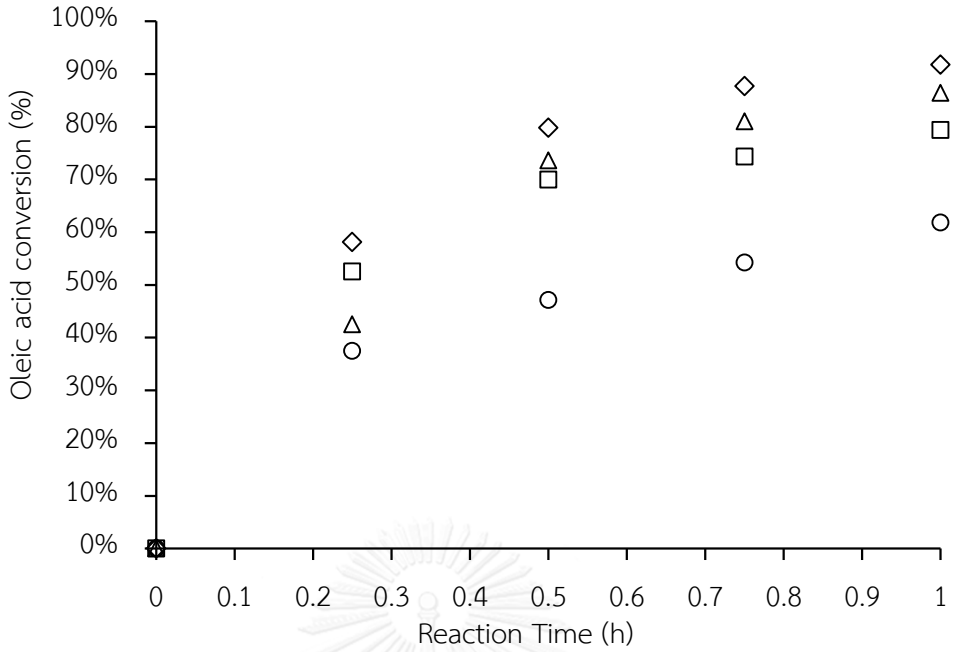


Figure 13 The effect of type of short chain alcohol on the esterification of oleic acid catalyzed by Novozym 435. Reaction conditions: molar ratio of alcohol to oleic acid of 1:2, enzyme loading at 5% (w/w of oleic acid), shaking rate of 250 rpm and temperature at 45°C. Alcohols (anhydrous): methanol (◊), ethanol (□), n-propanol (△), n-butanol (**O**)

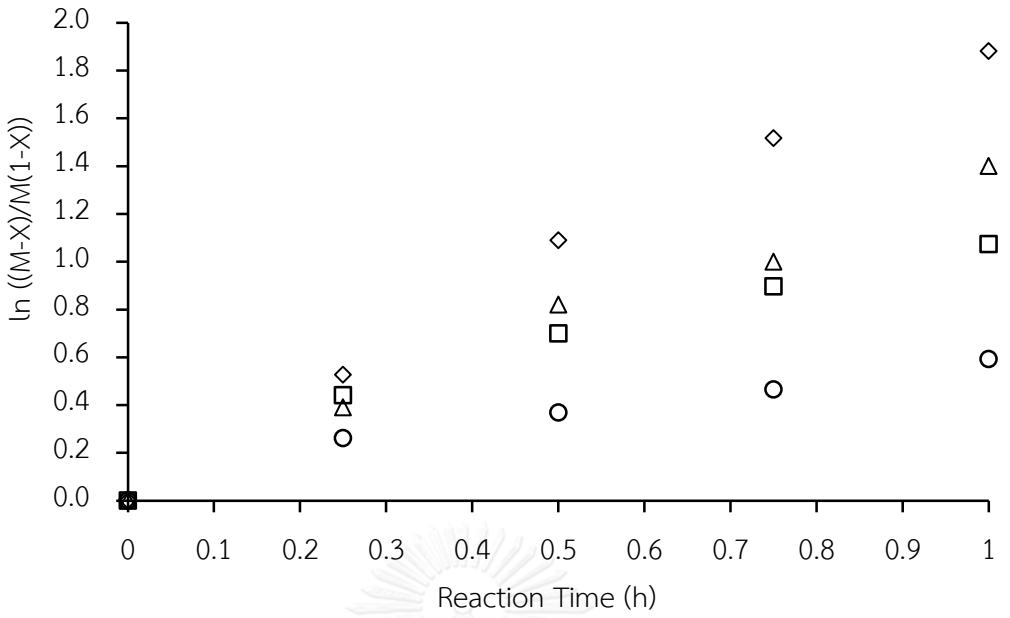


Figure 14 Plots for the determination of the rate constants (k) of the esterification of oleic acid catalyzed by Novozym 435. Alcohols (anhydrous): methanol (◊), ethanol (□), n-propanol (△), n-butanol (O)

Higher k values resulted in higher initial conversion rates. According to the initial rates of the forward reaction during the first hour, the esterification of oleic acid with methanol afforded the highest conversion, which was approximately 90.0% in 1 h, and the lowest conversion was observed in the production of butyl oleate (~ 60%). It was shown that during the first 15 min, the rate of ethyl oleate production was slightly higher than that of propyl oleate production. However, from 15 min – 1

h, the rate of propyl oleate production was higher than that of ethyl oleate production. This result might be due to the stronger inhibition of Novozym 435 by ethanol than by propanol. At the operating temperature of 45°C, the k values for the esterification of oleic acid with methanol, ethanol, propanol and butanol were 0.78, 0.52, 0.69 and 0.17 $m^3 \cdot h^{-1} \cdot kmol^{-1}$, respectively (The calculation is shown in Appendix B). It has previously been reported that propanol exhibits better miscibility with oils and fats and less inhibition of lipase activity than ethanol and butanol [53]. It was also suggested that Novozym 435 is not suitable for esterification with large short-chain alcohols, such as butanol. Only a 54% conversion of sunflower oil was obtained in the esterification with 1-butanol catalyzed by Novozym 435 at 40°C for 24 hours, whereas the conversions of the reaction with smaller alcohols were greater than 90% [53]. According to the previous study by Osuna and Rivero (2012), the rates of enzymatic esterification of lauric acid with short chain alcohols were depending on type of alcohol as follows: methanol > ethanol > propanol > butanol [54].

In this work, Novozym 435 exhibited the best catalytic activity in the production of methyl oleate. The final conversions of oleic acid after 8 hours, depending on type of alcohol as follows: methanol > ethanol > propanol > butanol. Because the two most common types of alcohol in use for the production of biodiesel are methanol and ethanol, the subsequent studies primarily focused on the esterification of oleic acid with methanol and ethanol.

5.1.2.2 Effect of temperature on the kinetic rate constant (k)

The kinetic rate constants (k) for the production of alkyl ester were determined from the slope of a linear equation, as shown in Eq. 9. The k values of the reactions with methanol and ethanol in the temperature range of 40 °C to 55 °C are shown in Figure 15. The k values for the production of methyl oleate and ethyl oleate were observed to increase when the operating temperatures increased, which could be explained by the Arrhenius theory (Eq.2).

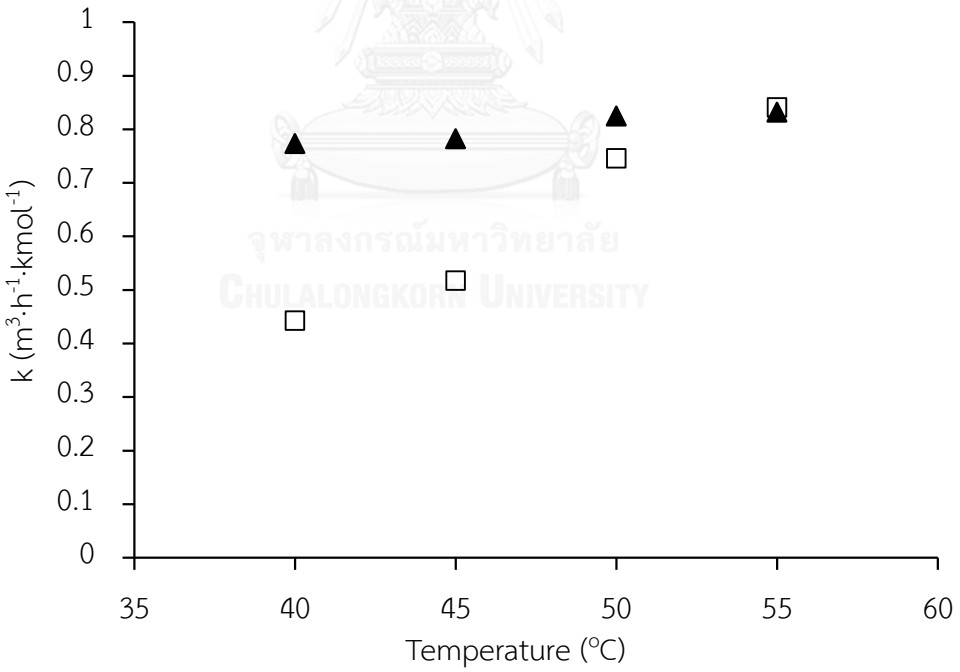


Figure 15 The rate constants (k) for the esterification of oleic acid catalyzed by Novozym 435 for a range of temperatures of 40 - 55°C. Alcohol (anhydrous): methanol (▲) and ethanol (□).

On the other hand, the optimal high temperature for the enzymatic production of biodiesel was also affected by the thermal deactivation of the biocatalyst at high operating temperatures. High temperatures could affect the stability and activity of reused enzymes [14]. It was found that the k values of the enzymatic esterification with short-chain alcohols, such as propanol and butanol, were slightly changed in the temperature range of 45 ℃ to 55 ℃, in which the k values were approximately 0.7 and 0.2 $m^3 \cdot h^{-1} \cdot kmol^{-1}$, respectively (Figure not shown). It has previously been reported that the activation energy (E_a) is dependent on the molecular weight of the alcohol [55]. A longer molecule generally requires more activation energy to overcome the intermolecular interaction force. In this study, the activation energy (E_a) value for the forward reaction of the production of methyl oleate was 4.7 kJ/mol, and the E_a value for the forward reaction of the production of ethyl oleate was 39.1 kJ/mol (The calculation has shown in Appendix B). Pogaku et al., (2012) reported that the E_a value for the transesterification of palm oil with methanol catalyzed by *Burkholderia cepacia* lipase was 4 kJ/mol [56]. A lower E_a value of 1.3 kJ/mol was reported for the esterification of palmitic acid with propanol in MTBE solvent catalyzed by *Rhizopus oryzae* lipase [57]. Higher E_a values in the range of 25–60 kJ/mol were required by the use of chemical catalysts, such as H_2SO_4 and sulfated zirconia [58-60].

5.1.3 Influences of operating conditions on biocatalytic activity and reusability of Novozym 435 for esterification of free fatty acids with short-chain alcohols

In this part, we focused on the study of effects of the operating conditions such as temperature, types of alcohol such as methanol and ethanol, and initial water content (in ethanol) on biocatalytic activity and reusability of Novozym 435 for the esterification of oleic acid with short-chain.

5.1.3.1 Effects of operating conditions on catalytic activity and reusability of Novozym 435

5.1.3.1.1 Effect of operating temperature

Higher operating temperature results in higher initial conversion rate and according to the high rates, the reaction reaches equilibrium sooner. However, thermal deactivation of enzymes might occur, thereby negatively impacting the activity, stability and reusability of enzymes. Therefore, the selection of an optimal operating temperature is another important issue for the enzymatic production of biodiesel. The optimum active temperature for Novozym 435 is between 40°C and 60°C. Our previous study showed that there was no significant difference in the FFA conversion and the reaction rate at the operating temperature

between 45 and 60 °C [61]. As shown in Figure 16, with the use of methanol, the initial FFA conversion slightly increased as the temperature increased from 45°C to 50°C. However, it was found that the final FFA conversion was not significantly affected by temperature changes in this temperature range. The effect of the thermal deactivation of Novozym 435 at 50°C was more clearly observed in the esterification of oleic acid with ethanol as shown in Figure 17. In the first batch with the fresh enzyme, the rates of FFA conversion and the final FFA conversions at the operating temperature of 45°C and 50°C were quite similar. However, from the second batch to the fifth batch, the rates of FFA conversion significantly decreased as the temperature increased from 45°C to 50°C. At the operating temperature of 50°C, the FFA conversion after 8 h was approximately 91% in the first batch, and it was less than 80% in the fifth batch, whereas no significant drop in FFA conversion was observed with the operating temperature at 45°C. The results indicated that for the long-term use of this biocatalyst, the optimal temperature for the esterification of oleic acid with methanol or ethanol by Novozym 435 should be 45°C for maintaining high catalytic activity and reusability of the enzyme.

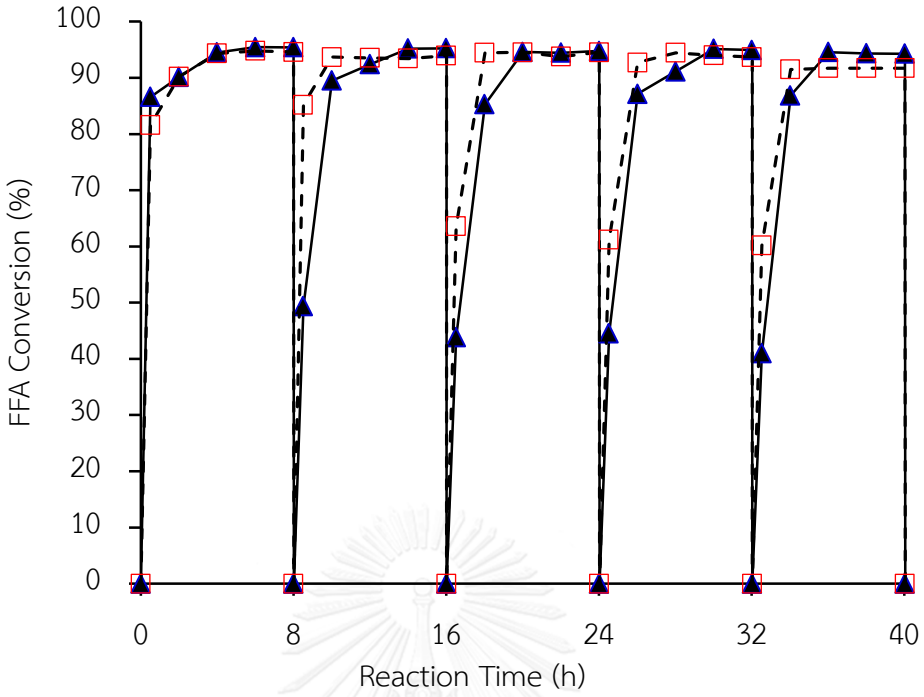


Figure 16 The effect of operating temperature on the reusability of Novozym 435 in esterification of the oleic acid with 99.9% methanol. The cycle time is 8 h and the operating temperatures are 45°C (▲) and 50°C (□)

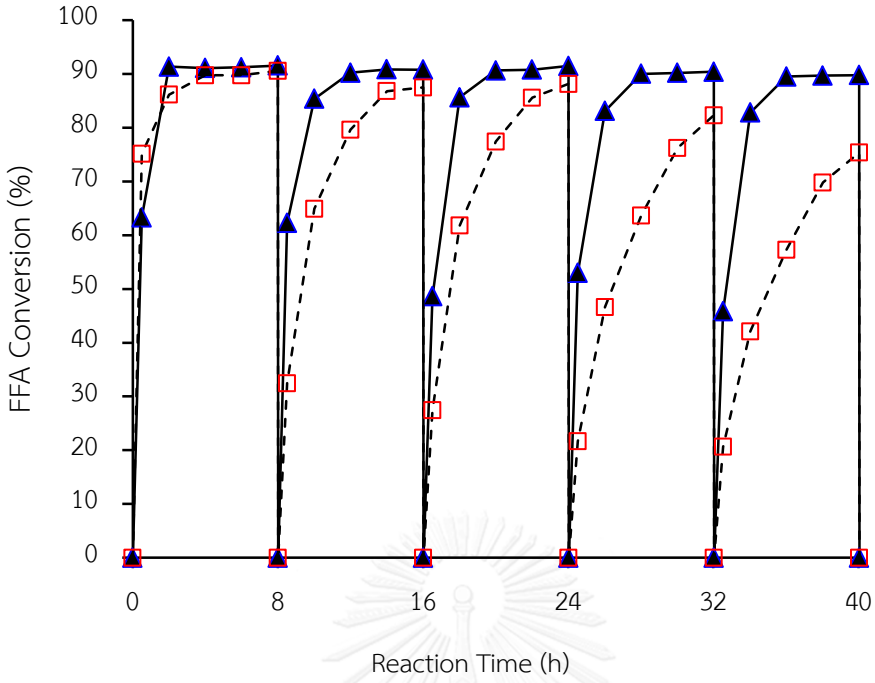


Figure 17 The effect of operating temperature on the reusability of Novozym 435 in the esterification of oleic acid with 99.9% ethanol. The cycle time is 8 h and the operating temperatures are 45°C (▲) and 50°C (□)

5.1.3.1.2 Effect of types of alcohol

The effect of alcohols on the enzyme activity in acyl transfer reactions includes reversible inhibition and irreversible inactivation [62]. The type of alcohol might directly affect the reusability of enzymes. In this part, the effects of alcohols, methanol (99.9%) and ethanol (99.9%), on the reusability of Novozym 435 in the esterification of oleic acid were investigated. On 5.1.3.1.1, to reduce the effects of thermal deactivation on

the activity and stability of Novozym 435, the reactions were performed at a constant operating temperature of 45°C. As shown in Figure 18, the FFA conversion rates to produce methyl oleate were higher than those of ethyl oleate. During the production of methyl oleate, the conversion rate slightly decreased as the number of enzyme reuse cycles increased. However, a gradual decrease in the FFA conversion rate with increasing number of Novozym 435 reuse cycles was more clearly seen during the production of ethyl oleate. Nevertheless, for both reactions, Novozym 435 could be reused for 13 repeated batches (104 h) with FFA conversions of at least 90%. Previously, it was reported that among all enzymes tested, Novozym 435 was the most effective biocatalyst for the methanolysis in a continuous process [34]. However, the deactivation of Novozym 435 in the esterification could occur because of the interaction of alcohols with the surface of Novozym 435 through the adsorption of alcohols [63]. The effect of alcohols on the activity of Novozym 435 in acyl transfer reactions included reversible inhibition and irreversible inactivation was previously observed [62]. Therefore, progressive deactivation after several reuses of the biocatalyst during the esterification also occurred [63]. In previous reports, 85% of the initial Novozym 435 activity was maintained after 9 batches in the transesterification of vegetable oils and ethanol [64], and 90% of the activity of Novozym 435 was maintained over 7 batch reactions in the transesterification of vegetable oils and short-chain

alcohols [26]. For the recovery of enzymatic activity, Tan et al., (2010) reported that the treatment of reused immobilized biocatalyst (Candida sp. 99-125) by the use of 1 mM salt solutions of $CaCl_2$, and $MgCl_2$ as a pretreatment solution can recover the biocatalyst activity which more than 50% of the fatty acid methyl ester yield could be obtained for 9 reused batches [10].

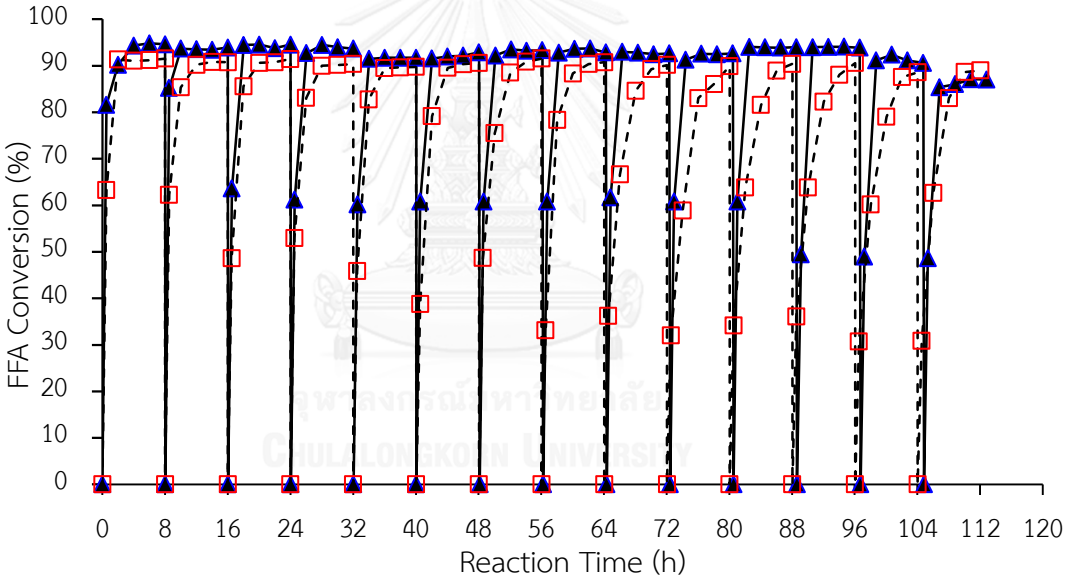


Figure 18 The effect of type of alcohol on the reusability of Novozym 435 in the esterification of oleic acid at 45°C with 99.9% methanol (▲) and 99.9% ethanol (□).

5.1.3.1.3 Effect of initial water content in alcohol

Influence of the initial water content on the esterification and transesterification reactions is another important issue. It was reported that transesterification reactions catalyzed by C. rugosa, P. cepacia, and P. fluorescens lipases could not occur in the water-free system [11]. However, Novozym 435 exhibited the highest activity with a low availability of water content [35, 53]. The presence of water during the reaction could have a negative effect on the conversion of FFAs due to the occurrence of a hydrolysis reaction during transesterification [65] and a reversible reaction effect on esterification [6]. Methanol, mostly produced from coal or natural gas, is easily available as an anhydrous alcohol. Ethanol is considerably less toxic and contains more energy than methanol. Ethanol is mostly produced from renewable materials via fermentation processes. During the separation of ethanol by distillation, ethanol forms an azeotrope with water with a composition of 96.6% ethanol and 3.4% water (by volume). Therefore, hydrated ethanol (95-96% v/v) is more available at a relatively lower price than anhydrous ethanol (99.9% v/v). Thus, in this study, the effect of the initial water content in ethanol on the reusability of Novozym 435 during the esterification of oleic acid was investigated.

The initial water content in ethanol was varied by using 99.9% (v/v) (anhydrous), 96.0% (v/v) and 95.0% (v/v) ethanol. During the first hour of the reaction, the initial FFA conversions during the production of ethyl oleate with 96.0% and 95.0% ethanol were slightly higher than that with 99.9% ethanol. However, the final conversion reached the same level of about 90% (Figure 19). A higher initial rate of esterification catalyzed by an enzymatic catalyst in a system containing some amount of water was previously reported during the production of biodiesel from soybean oil catalyzed by various lipases, such as Rhizopus oryzae, Candida rugosa, Pseudomonas fluorescens, Candida antarctica and Burkholderia cepacia [66]. It was suggested that a certain amount of water was required for many of enzymes to work [66, 67].

The presence of water of 4-5 % in ethanol did not considerably affect the FFA conversion catalyzed by Novozym 435 in Batch 1 to Batch 9; however it exhibited a negative effect on the reusability of Novozym 435. In this study, it was shown that the reusability of the enzyme decreased with increasing water content from 4% to 5%. As shown in Figure 19, when 96.0% ethanol and 95.0% ethanol were used, to retain a conversion of at least 90%, the number of Novozym 435 reuse cycles should not be greater than 10 cycles and 8 cycles, respectively, whereas the number of Novozym 435 reuse cycles in the system using anhydrous ethanol was 13 cycles. Previously, in a solvent-free system, the deposition of water on the surface of

the lipase could reduce the conversion yield of biodiesel [68]. A fast interaction between water and enzymes might deactivate enzymes and the presence of water as a byproduct of the reaction could lower the equilibrium conversion [6]. However, it was suggested that the inhibition of the enzyme by water is reversible, which could be solved by the removal of water [34]. It was reported that the enzyme recovered its catalytic activity after being dried to its initial water content [68]. Sena, 2011 reported that the removal of water during the process by the addition of molecular sieve could significantly improve the FFA conversion from the esterification of oleic acid with propanol catalyzed by Novozym 435 from 88.9 to 94.7 [41].

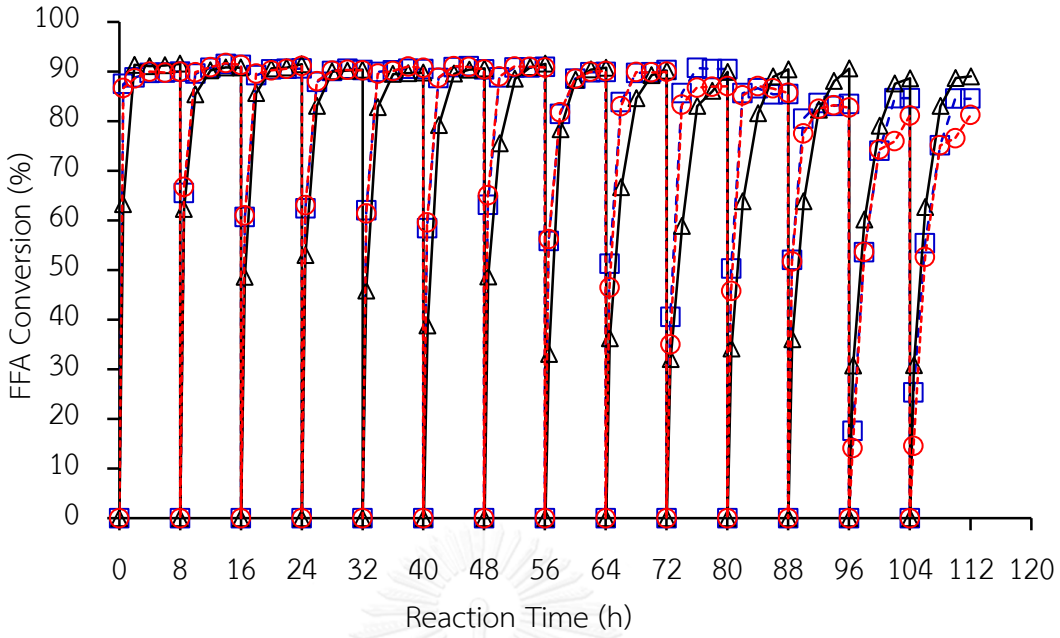


Figure 19 The effect of initial water content in ethanol on the reusability of Novozym 435 in the esterification of oleic acid at 45°C with 99.9% ethanol (Δ), 96.0% ethanol (□) and 95.0% ethanol (○).

5.1.4 Effects of operating conditions on the surface morphology of Novozym 435

Loss of the enzyme activity could also be caused by deactivation of the enzyme from the adsorption of alcohols on the surface. The experimental results in this study showed that in a solvent-free system, the deactivation of

Novozym 435 during the esterification of oleic acid with ethanol tended to be stronger than that observed in the system with methanol.

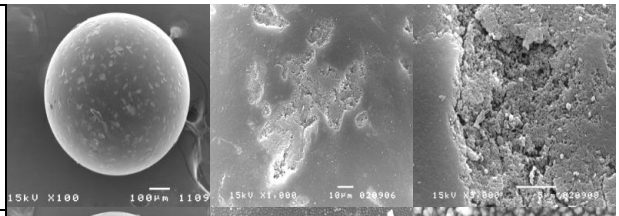
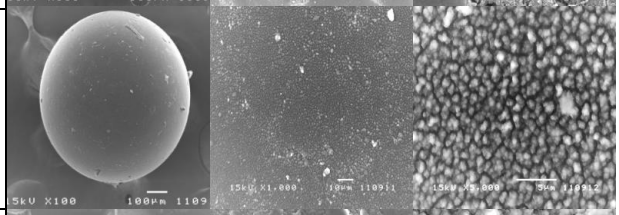
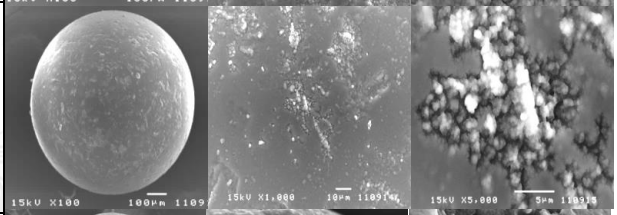
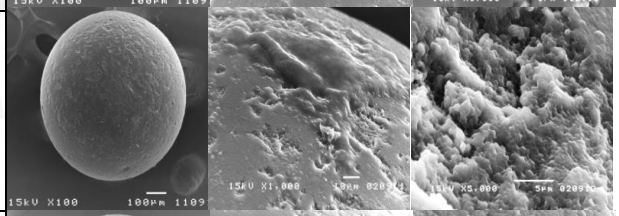
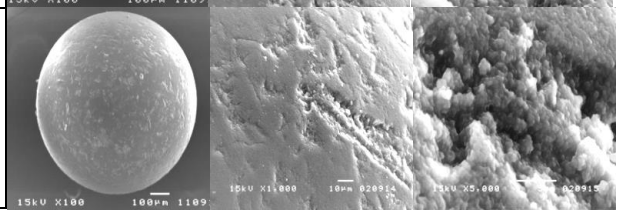
A) Fresh	
(B) 5 reused cycles with methanol 99.9%, 45°C	
(C) 5 reused cycles with methanol 99.9%, 50°C	
(D) 5 reused cycles with ethanol 99.9%, 45°C	
(E) 5 reused cycles with ethanol 99.9%, 50°C	
(F)13 reused cycles with ethanol 95.0%, 45°C	

Figure 20 SEM micrographs of Novozym 435 in the esterification of oleic acid with short-chain alcohols at various operating conditions.

Under SEM observation of the surface morphology of fresh Novozym 435 (Figure 20A) compared to that after being used in the system using 99.9% methanol at operating temperature of 45°C for 5 repeated batches (Figure 20B), slightly change in the surface morphology was noticed. On the other hand, the partial swelling of the catalyst surface was clearly observed after being used for 5 repeated batches in the system using 99.9% ethanol at 45°C (Figure 20D). A significant increase in the degree of swelling was observed on the surface of Novozym 435 as the operating temperature was increased to 50°C. Compared to the system using methanol at 50°C for 5-repeated batches (Figure 20C), considerably increase in the degree of swelling was observed in the system using ethanol at 50°C (Figure 20E). From the experimental results, it was noticed that the reduction in catalytic activity or the degree of deactivation of Novozym 435 was related to the swelling degree of the catalyst surface. From the SEM observation, it was also suggested that the reaction at a high operating temperature might enhance the adsorption of alcohols, especially in the system using ethanol, resulting in significant reduction in the reusability of Novozym 435.

Novozym 435 catalyzed the reaction with high activity under highly water-deficient conditions [1, 35, 53]. Previously, it was suggested that the agglomeration of water might flood the enzyme pores, causing decreases in the reaction rates [69]. In this study, it was also shown that the presence of water at 4-5 % in ethanol cause

some negative effects on the reusability of the biocatalyst. Considerable swelling of the catalyst surface was also observed after Novozym 435 being used for 13 repeated batches in the system using 95% ethanol at 45°C (Figure 20F).

5.2 Esterification of oleic acid and short-chain alcohols by Novozym 435 in continuous systems

Our objective in this part is to develop continuous process for the esterification of free fatty acids (oleic acid and PFAD) and methanol using Novozym 435 as a biocatalyst from the batch process in the previous work in order to improve biodiesel productivity. A single expanded bed circulation reactor and multiple expanded bed reactors in series were studied as follows;

5.2.1. The optimal operating conditions for esterification of oleic acid and methanol catalyzed by Novozym 435 in a single expanded bed circulation reactor

From results of batch experiment, the catalytic activity and reusability of Novozym 435 performed the best when using methanol 99.9% in the biodiesel production, therefore, methanol 99.9% was selected for the study of the continuous processes. For experimental planning in continuous processes, the important operating factors affecting on FFA conversion as the feed volumetric flow rate, the

enzyme amount and the reaction bed volume ratio were observed in this section. Other operating conditions such as temperature of 45^{o}C, FFA to methanol molar ratio of 1:2 were kept as a constant. The schematic diagram is shown in Figure 10.

5.2.1.1 Effect of flow rate

One of the most important factors for the reaction in packed bed reactor is the feed flow rate, therefore the effect of feed volumetric flow rate on FFA conversion was investigated to reduce the mass transfer inhibition effect by increasing circulation flow rate from 4 to 5 and 6 mL/min (cm^{3}/min), respectively in the single expanded bed circulation reactor at the optimal operating condition described earlier. In terms of velocity through the surface area of reactor (19.63 cm^{2}), it was denoted that the velocity increased with the enhanced flow rate from 0.20 to 0.25 and 0.31 cm/min, respectively.

Figure 21 shows the effect of the circulation flow rate on the FFA conversion of oleic acid and methanol by Novozym 435 in a single expanded bed circulation reactor.

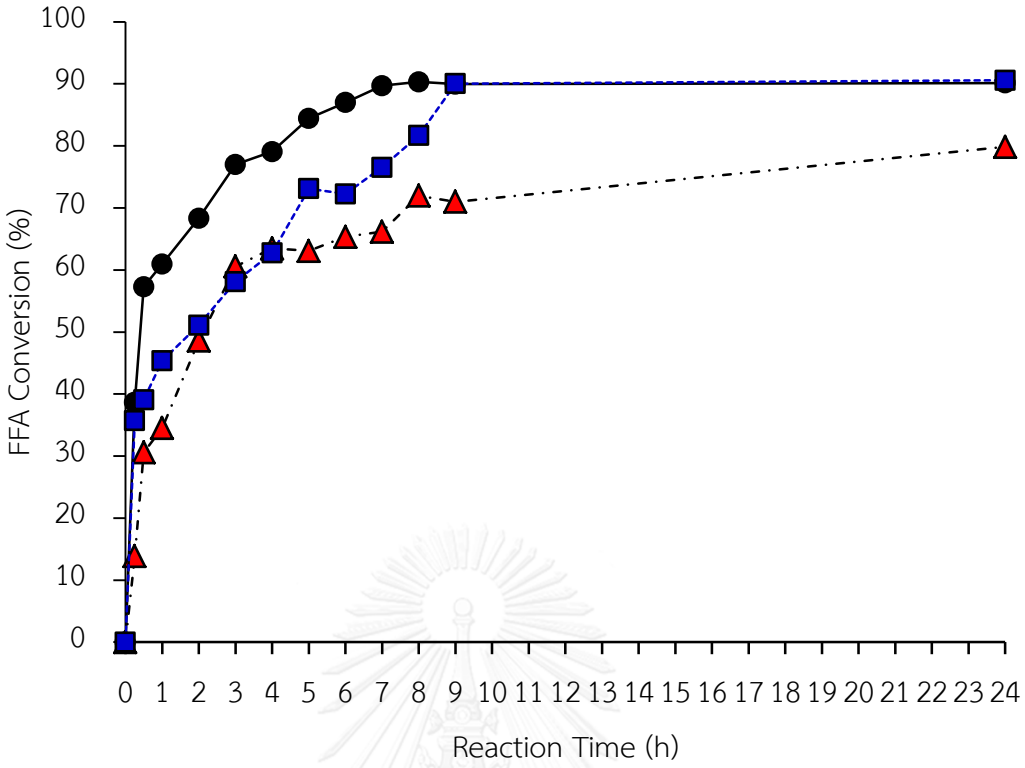


Figure 21 Effect of circulation flow rate on FFA conversion of esterification from oleic acid and methanol catalyzed by Novozym 435 in the single expanded bed circulation reactor. Reaction conditions: oleic acid to methanol molar ratio of 1:2, 45°C, Novozym 435 of 5%wt of oleic acid, rotation speed of 600 rpm; (▲) 4 mL/min, (●) 5 mL/min, (■) 6 mL/min.

The FFA conversion rate increased as the circulation flow rate was increased from 4 mL/min to 5 mL/min due to the consequence of higher rate of mass transfer [70]. The maximum FFA conversion of 90% was obtained at the circulation flow rate of 5 mL/min within 8 h. However, when circulation flow rate was further increased to

6 mL/min, the reaction rate decreased and the equilibrium time was slightly increased from 8 to 9 h compared to that of 5 mL/min. This could be explained that higher shear rate could cause the loss of enzyme activity. Previously, on the study of transesterification from canola oil and methanol by Novozym 435 using circulation process in the packed bed reactor, the optimal flow rate was at 6.3 mL/min while other operating conditions were kept constant at 38°C, FFA to methanol molar ratio of 4.3. Methyl ester yield of >98% was obtained within 72 h [71]. Furthermore, the effect of feed flow rate on FFA conversion was also observed in the study of the biodiesel production from soybean oil and methanol catalyzed by a mixture of immobilized *Candida rugosa* and *Rhizopus oryzae* lipases in a packed-bed reactor using circulation method [72]. The feed flow rate was varied from 0.2-1.0 mL/min. The maximum FFA conversion of 97.98% was obtained at the feed flow rate of 0.8 mL/min within 3 h. However, at 0.6 and 1.0 mL/min of the feed flow rate, reaction rate for accomplishment of maximum conversion yield increased to 7 and 8 h, respectively [72]. At low flow rate, FFA conversion was limited due to the mass transfer resistance at liquid film layer. However, at too high flow rate, the contact between the substrate and enzyme active sites was insufficient lowering FAME yield [73]. Wang et al., (2011) also found that the shear stress of the high fluid flow could damage the immobilized biocatalyst [45]. From the experimental results, the optimal circulation flow rate of 5 mL/min was used in the subsequent experiments.

5.2.1.2 Effect of volume ratios of the reaction beds

The effect of volume ratios of reaction bed was investigated in this section. At the volume ratio of bed column to catalyst volume of 1:1, the reaction bed could be considered as a packed bed, while that at the ratio of 2:1 could be considered as an expanded bed. The experiments were carried out with the volume of the reactants (initial liquid volume) of 140 mL. The amount of Novozym 435 was 5%wt of FFA (5 g) and the recirculated flow rate was 5 mL/min. When the volume ratio of reaction bed (reaction zone) was increased from 1:1 to 2:1, the contact time in reaction zone doubly increased from 2.5 to 5 min/circulating cycle. In term of the volume ratio of reaction zone to total volume, the ratio was increased from 0.09:1 to 0.18:1 [see Appendix B3 for details] which meant that in each cycle, the substrate was in the reaction zone for longer time as shown in Figure 22. Moreover, compared to the packed bed, the expanded bed should be more agitated and the catalysts could move more easily. Thus, this should enhance mixing between substrates and catalysts and reduce the external mass and heat transfer effects. As shown in Figure 23, the initial rate of the esterification using the bed to catalyst volume ratio of 2:1 was higher than that of 1:1. This could be explained in terms of the increase of contact surface area and the contact time between substrate and enzymatic catalyst. However, the equilibrium conversions were found at the same level. There were studies that suggested the use of expanded bed reactor for the biodiesel production [46, 47]. The ion-exchange resin catalyst was packed in the expanded bed reactor for

the continuous production of ethyl oleate from crude rice bran oil and ethanol. The FFA conversion of >98.6% could be obtained without any extra operation [47]. Therefore, the application of the expanded bed reactor using the bed to catalyst volume ratio of 2:1 for the esterification of free fatty acids and methanol by Novozym 435 was selected for the further studies.

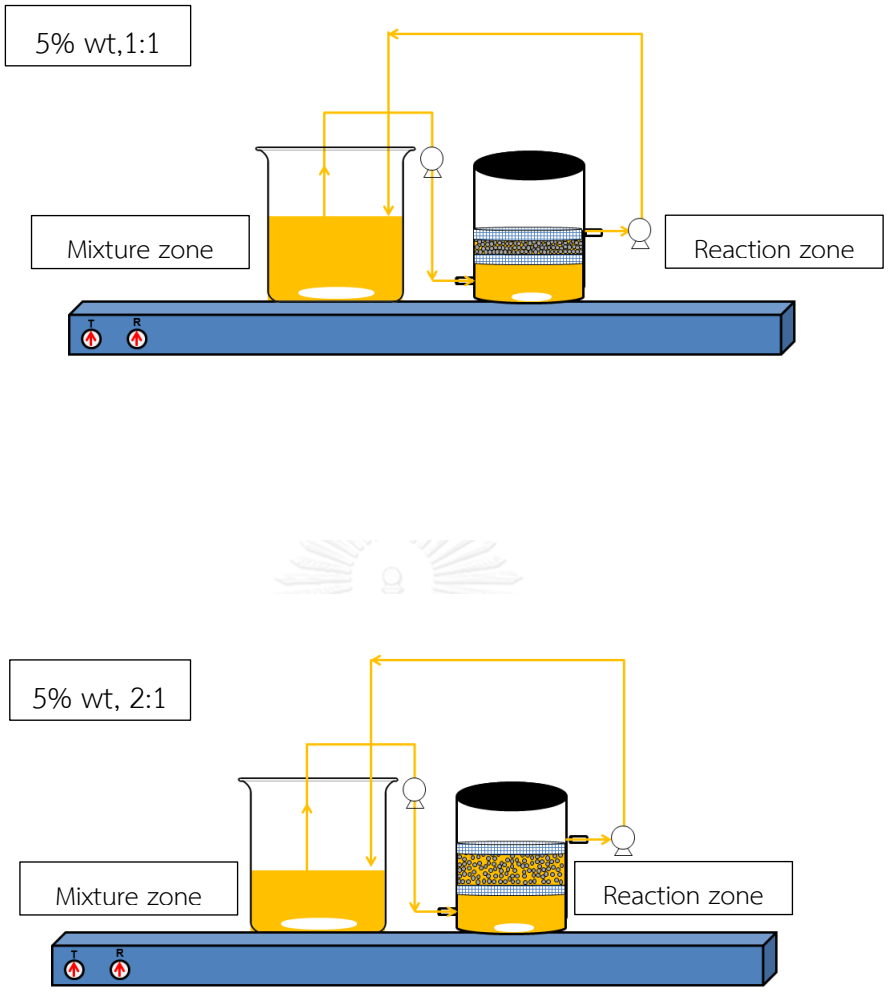


Figure 22 Schematic diagram of esterification from oleic acid and methanol catalyzed by Novozym 435 in a single expanded bed circulation reactor. Reaction conditions: oleic acid to methanol molar ratio of 1:2, 45°C, Novozym 435 of 5%wt of oleic acid, rotation of speed of 600 rpm, circulation flow rate of 5 mL/min, and the bed to catalyst volume ratio were 1:1 and 2:1.

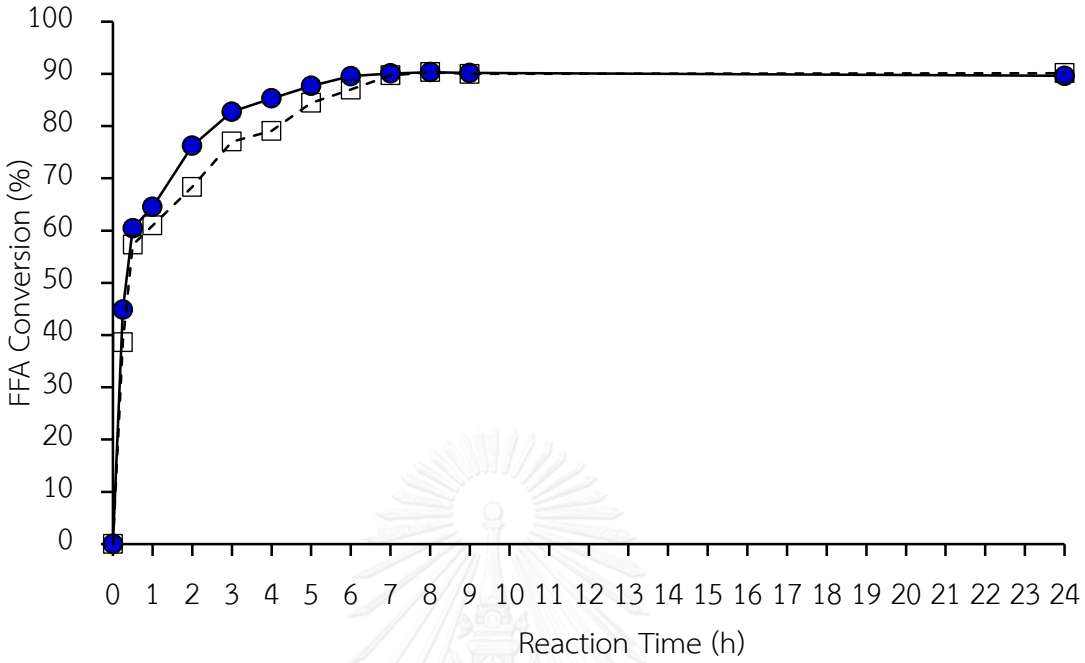


Figure 23 Effect of bed to catalyst volume ratio on FFA conversion of esterification from oleic acid and methanol catalyzed by Novozym 435 in a single expanded bed circulation reactor. Reaction conditions: oleic acid to methanol molar ratio of 1:2, 45^{o}C, Novozym 435 of 5%wt of oleic acid, rotation of speed of 600 rpm, and circulation flow rate of 5 mL/min; (□) 1:1 (●) 2:1.

5.2.1.3 Effect of Novozym 435 loading

The effect of Novozym 435 loading in the expanded bed reactor was further investigated in this study. In our previous study in batch experiment [61], we found that the optimal enzyme amount in the batch reaction was at 5% w/w of FFA. Compared to 5.2.1.2., the enzyme amount in this part was doubled from 5% to 10% w/w of FFA (or 5g to 10g) and the bed to catalyst volume in reaction zone was at 2:1. The schematic diagram has shown in Figure 24. It could be seen that when enzyme amount was doubly increased and the bed to catalyst volume ratio was at 2:1, the contact time in reaction zone also doubly increased from 5 min to 10 min/circulating cycle. In this case, the volume ratio of the reaction zone to the total volume was increased to 0.36: 1. As shown in Figure 25, with a double increase of the amount of enzyme and volume ratio of the reaction zone, the initial conversion rate was significantly enhanced and the reaction reached the equilibrium faster. The FFA conversion of the esterification using 10 %wt of Novozym 435 was 96% within 5h. Previously, it was also reported for the improved reaction performance with the increase of the ratio between enzyme and raw materials in the expanded bed reactor [1, 2]. In this study, it was shown that by using the expanded bed reactor, the maximum FFA conversion was higher than that obtained from the conventional batch reactor. The result in this study was in good agreement with those previously reported on the biosynthesis of hexyl laurate by a packed bed bioreactor [74].

Therefore, Novozym 435 amount of 10% (w/w of free fatty acid) or 10 g of Novozym 435 loaded in the reactor was selected for further studies.

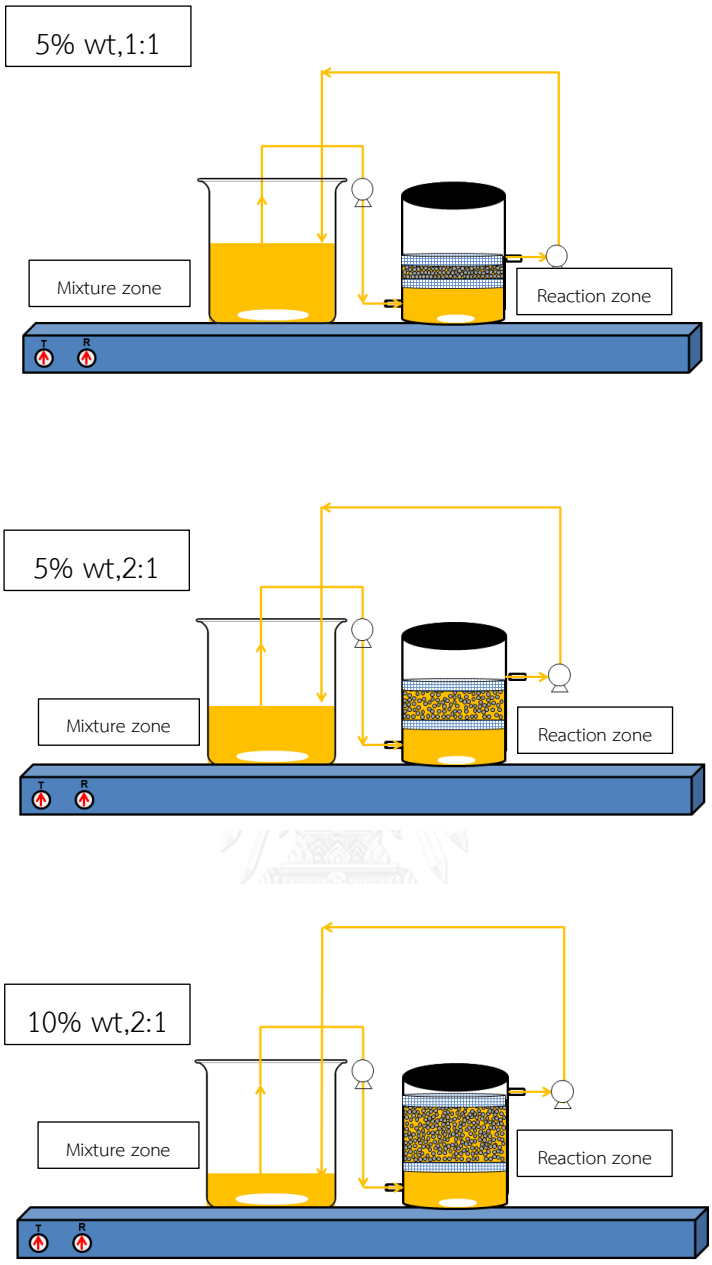


Figure 24 Schematic diagram of esterification from oleic acid and methanol catalyzed by Novozym 435 in the a single expanded circulation reactor. Reaction conditions: oleic acid to methanol molar ratio of 1:2, 45°C, rotation of speed of 600 rpm, and circulation flow rate of 5 mL/min; bed to catalyst volume ratio =1:1 and 2:1, and Novozym 435 loading of 5% wt and 10% wt.

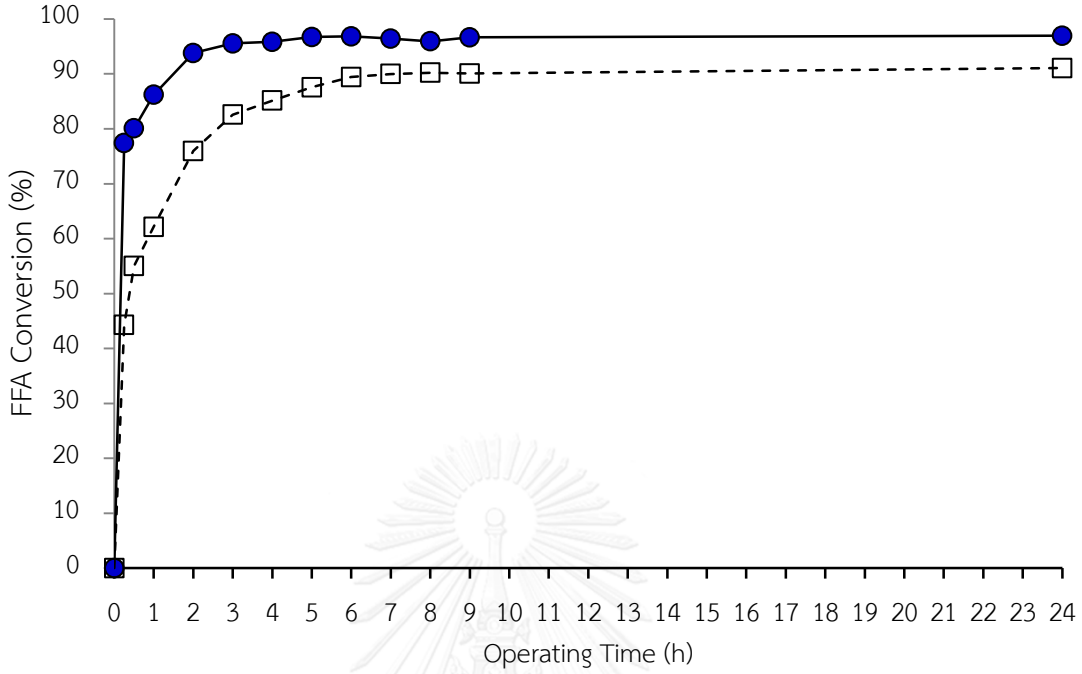


Figure 25 Effect of Novozym 435 loading on FFA conversion of esterification from oleic acid and methanol catalyzed by Novozym 435 in a single expanded circulation reactor. Reaction conditions: oleic acid to methanol molar ratio of 1:2, 45°C, bed to catalyst volume ratio of 2:1, rotation of speed of 600 rpm, and circulation flow rate of 5 mL/min; (□) 5% wt (●) 10% wt.

5.2.1. Reusability and stability of Novozym 435 in the esterification of oleic acid and methanol in the single expanded bed circulation reactor

For long term operation and up-scaled purpose, the reusability and stability of Novozym 435 using in the single expanded bed circulation reactor was also investigated. Figure 26 shows the reusability of Novozym 435 catalyzed in the esterification of oleic acid and methanol by Novozym 435 in a single expanded circulation bed reactor at 45°C, FFA to methanol molar ratio of 1:2, enzyme amount of 10% by weight of oleic acid (10 g), the bed to catalyst volume ratio of 2:1, rotation speed of 600 rpm, the circulation flow rate of 5 mL/min and the circulation time of 5h. The results showed that the FFA conversion could be maintained >90% for longer than 110 h or it could be reused for at least 21 cycles (Figure 27). These results are relatively better than those previously reported in the packed bed reactor [75]. In the transesterification of *Jatropha curcas* oil and methanol by Lipozyme® IM in the circulated batch packed bed reactor (CBPBR), the immobilized lipase showed stability in CBPBR by maintaining 70% of its relative activity up to 10 cycles [75]. Another work from Hajar et al., (2009) showed that methyl ester yield constant around 97% for six cycles was obtained in the circulating packed-bed reactor system of canola oil and methanol by Novozym 435 [71]. Therefore, this process has the potential for application to industrial biodiesel production.

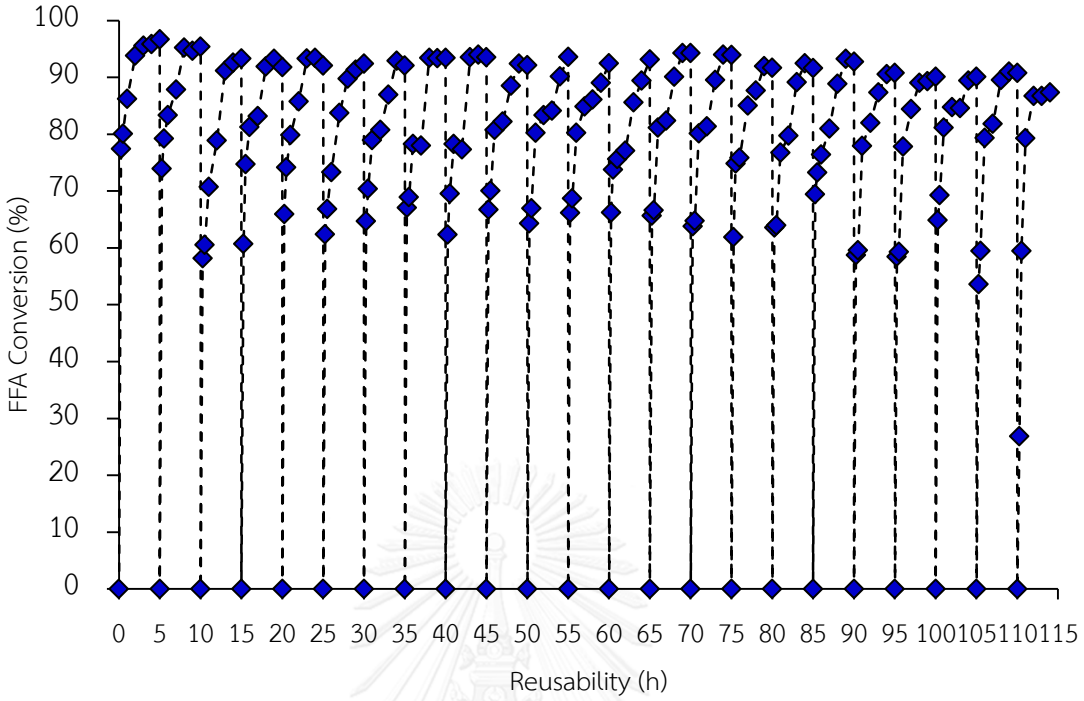


Figure 26 Reusability and stability of Novozym 435 of esterification from oleic acid and methanol catalyzed by Novozym 435 in a single expanded bed circulation reactor. Reaction conditions: oleic acid to methanol molar ratio of 1:2, 45oC, Novozym 435= 10% wt of oleic acid (10g), bed to catalyst volume ratio of 2:1, circulation flow rate of 5 ml/min, rotation of speed of 600 rpm, and at 5 h.

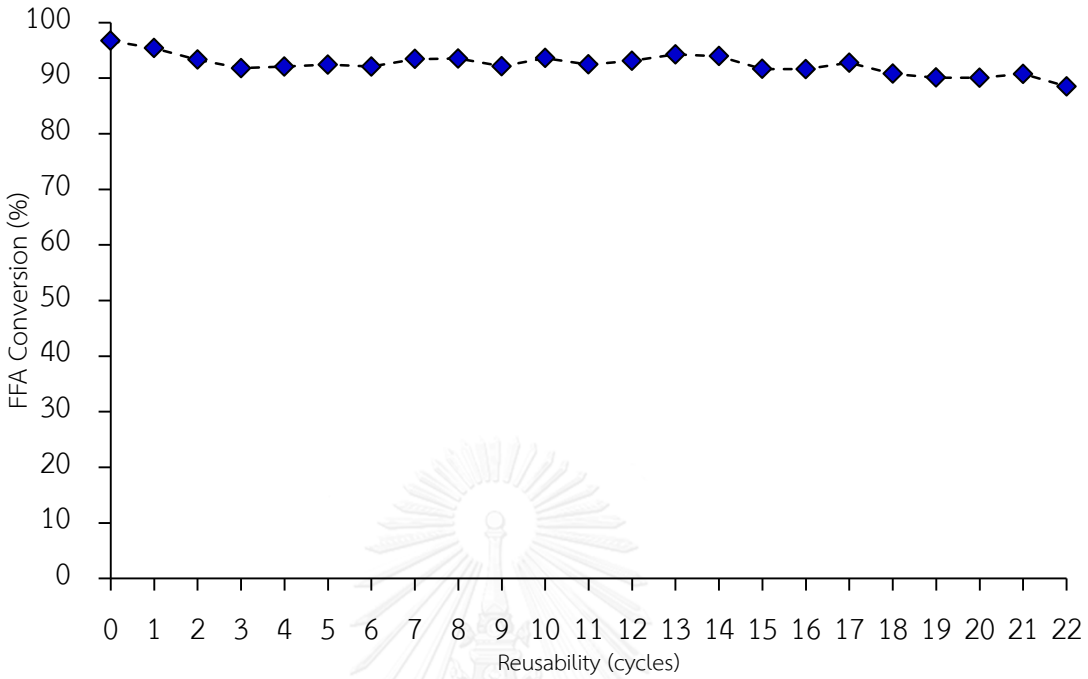


Figure 27 Reusability cycles of Novozym 435 of esterification from oleic acid and methanol catalyzed by Novozym 435 in a single expanded bed circulation reactor. Reaction conditions: oleic acid to methanol molar ratio of 1:2, 45°C, Novozym 435= 10% wt of oleic acid (10g), bed to catalyst volume ratio of 2:1, circulation flow rate of 5 mL/min, rotation of speed of 600 rpm, and at 5 h.

5.2.2. Esterification of free fatty acid and methanol catalyzed by Novozym 435 in the multiple expanded bed reactors in series.

In order to reduce the operating time and increase the productivity of biodiesel production, the continuous process of esterification in the expanded bed reactor in series was studied. The multiple expanded bed reactors were investigated in order to determine the optimal series for the expanded bed reactors. The optimal operating conditions obtaining from 5.2.1 at 45^{o}C in each reactor were: FFA to methanol molar ratio of 1:2, enzyme amount of 10 g, the volume ratio of bed to catalyst of 2:1, rotation speed of 600 rpm and the circulation flow rate of 5 mL/min.

5.2.2.1 The optimal Novozym 435 amount for the esterification using multiple expanded bed reactors in series.

It was shown that, when the multiple expanded bed reactors in series were used for the biodiesel production, the FFA conversion subsequently increased. From Figure 28, four expanded bed reactors were connected in series following schematic diagram as shown in Figure 11, and the FFA conversions of the 1st, 2nd, 3rd, and 4th reactor were 47.40%, 75.63%, 92.56%, and 93.51%, respectively. From these results, the maximum FFA conversion in the expanded bed reactors for biodiesel

production was obtained by using the four expanded bed reactors in series, in which the total Novozym 435 amount of 40 g was used. Previously, some other enzymatic biodiesel productions with packed bed reactors connected in series were also reported. Wang et al., (2011) studied the biodiesel production in the four packed bed reactors connected in series using Pseudomonas cepacia-Fe_3O_4 nanoparticle biocomposite. The FFA conversion of over 88% for 192 h was maintained while the use of circulation process in the single packed bed reactor performed FFA conversion only 75% for 132 h [45]. Chen et al., (2009) synthesized biodiesel from waste cooking oil and methanol using Novozym 435 in the three fixed bed reactors. The result showed that 75%-91% of FAME yield for 100 h could be obtained at the optimal operating conditions [44]. Chattopadhyay and Sen developed a continuous stirred tank reactor to the two packed bed reactors connected in series for biodiesel production from FFA and methanol using IIT-SARKZYME [70, 76]. The results showed that 80% of relative yield of biodiesel could be maintained up to 45 cycles [70].

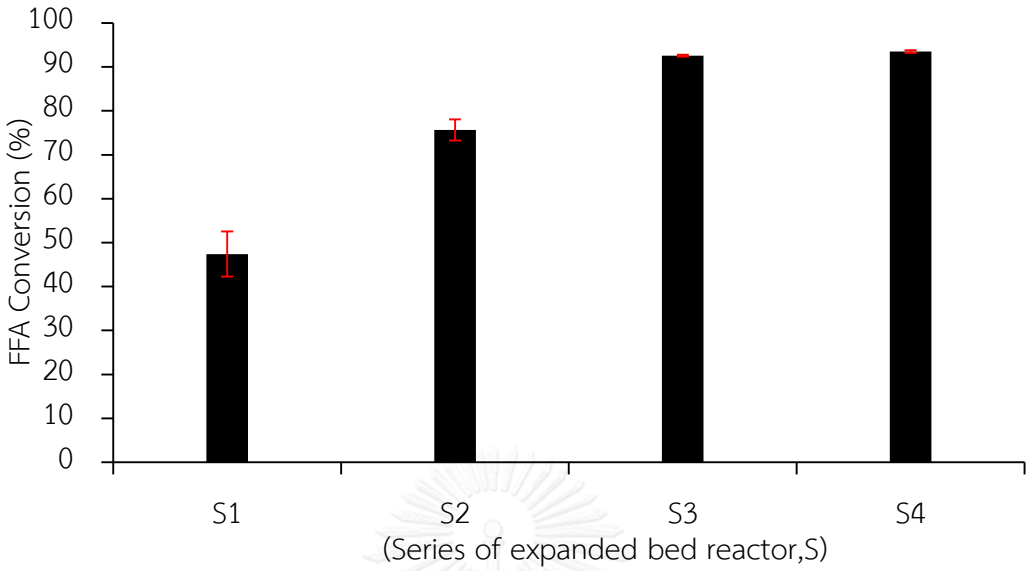


Figure 28 FFA conversion the esterification from oleic acid and methanol catalyzed by Novozym 435 in multiple expanded bed reactors in series (1st series=S1, 2nd series=S2, 3rd series=S3 and 4th series=S4). Reaction conditions: Oleic acid to methanol molar ratio of 1:2, 45°C , Novozym 435 of 10 g/reactor, bed to catalyst volume ratio of 2:1, circulation flow rate of 5 mL/min, rotation of speed of 600 rpm and contact time of 40 min.

5.2.2.2 Esterification of PFAD and methanol by Novozym 435 in the multiple expanded bed reactors in series.

The requirement of low production cost is a very important issue for the enzymatic biodiesel production. The use of low cost feedstock is another effective way to minimize the production cost. Therefore, this part was focused on the esterification of PFAD and methanol by Novozym 435 in the four expanded bed reactors in series. It was shown that when PFAD was used to produce biodiesel in the four expanded bed reactors in series, FFA conversion of >88% could be achieved as shown in Figure 29. However, the FFA conversion from the use of PFAD was relatively lower than that of using oleic acid in this continuous process. It might be due to the effect of triglyceride which appeared as one of the compositions in PFAD which could convert to glycerol as a by-product from the transesterification process [28, 36, 77]. Previously, coating of glycerol on the immobilized enzyme could lower enzymes activity [28, 36]. However, this might be solved by the solvent addition such as acetone [44] or rinsing of the immobilized lipase catalyst by tert-alcohol [28] which has minor effect on enzyme activity . Moreover, other impurities in PFAD might also affect the Novozym 435 activity. Therefore, purification of PFAD prior to esterification should be further considered.

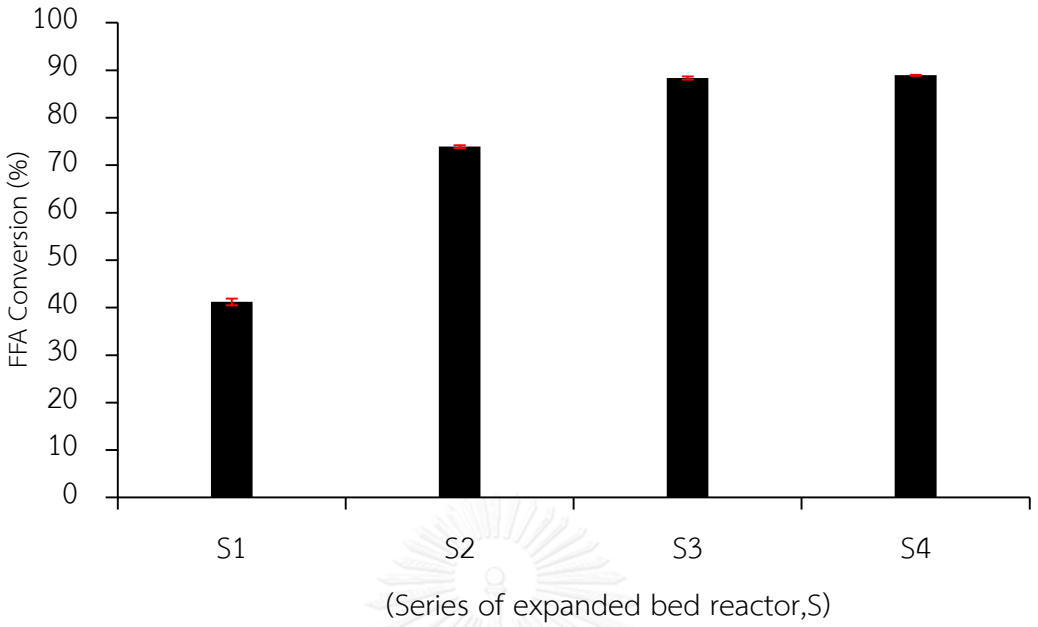


Figure 29 FFA conversion the esterification from palm fatty acid distillate (PFAD) and methanol catalyzed by Novozym 435 in multiple expanded bed reactors in series (1st series=S1, 2nd series=S2, 3rd series=S3 and 4th series=S4). Reaction conditions: Oleic acid to methanol molar ratio of 1:2, 45°C , Novozym 435 of 10 g/reactor, bed to catalyst volume ratio of 2:1, circulation flow rate of 5 mL/min, rotation of speed of 600 rpm and contact time of 40 min.

5.2.2.3 The determination of fatty acid methyl ester (FAME) yield with ^{1}H-NMR method.

In order to confirm the accurate conversion yield of biodiesel production, ^{1}H-NMR method was used to determine yield of fatty acid methyl ester (FAME). Proton NMR provides a good probe for biodiesel since ^{1}H is the most naturally abundant and most sensitive NMR active isotope [78]. The possible peaks in ^{1}H-NMR for quantitating unsaturated fatty acids are those of the olefinic protons (5.3 5.4 ppm), protons attached to the bis-allylic carbons (2.7–2.8 ppm), protons attached to the allylic carbons (2.0–2.1 ppm) and the terminal methyl group protons (0.8–0.9 ppm). Furthermore, the amounts of saturated fatty acids can be determined by utilizing the signal of the methylene (CH_2) protons at 1.2–1.4 ppm [79].

The yield percentage of FAME could be calculated from the ratio of the area of peaks associated with the methyl ester (3.65 ppm) and methylene group protons (2.26 ppm) from Figure 30 and Figure 31 by using Eq. (11). The result showed that FAME of the esterification from two types of free fatty acids such as oleic acid and palm fatty acid distillate (PFAD) and methanol by Novozym 435 in the four expanded bed reactors in series of 93.46 and 88.50% % were obtained. The relative lower of PFAD conversion compared to that of oleic acid was observed in the appearance of triglycerides peak (4.1-4.4 ppm) [36]. This was quite in good agreement with the observed FFA conversion of 93.51± 1.1% and 88.94 ± 2.8% from titration method,

respectively as shown Figure 32. This could be suggested the possibility of enzymatic biodiesel production in the four expanded bed reactors in series. Moreover, in terms of productivity calculation (g FAME·h^{-1}·g $Enzyme^{-1}$), at PFAD to methanol molar ratio of 1:2, a flow rate of 5 ml/min, Novozym 435 of 40 g and FAME yield of 88.50%, the productivity of biodiesel production of 4.68 g FAME·h^{-1}·g $Enzyme^{-1}$ could be obtained. The productivity of biodiesel in this process was higher than that of the batch process (The productivity calculation has shown in Appendix B). Moreover, this was comparable with the study of the enzymatic production of biodiesel by methanolysis of cottonseed oil using immobilized Candida antarctica lipase as a catalyst in t-butanol solvent which the productivity of 4 g FAME·h^{-1}·g $enzyme^{-1}$ was achieved [36]. Moreover, the productivity of biodiesel from the use of oleic acid and PFAD in this process was 1048.16 and 936.71 g FAME·L^{-1}·h^{-1}, respectively, which was higher than those from previous studies as shown in Table 7 [70]. Therefore, the continuous process being developed in this study has good potential to be applied for effective biodiesel production from low cost feed stocks and short chain alcohols.

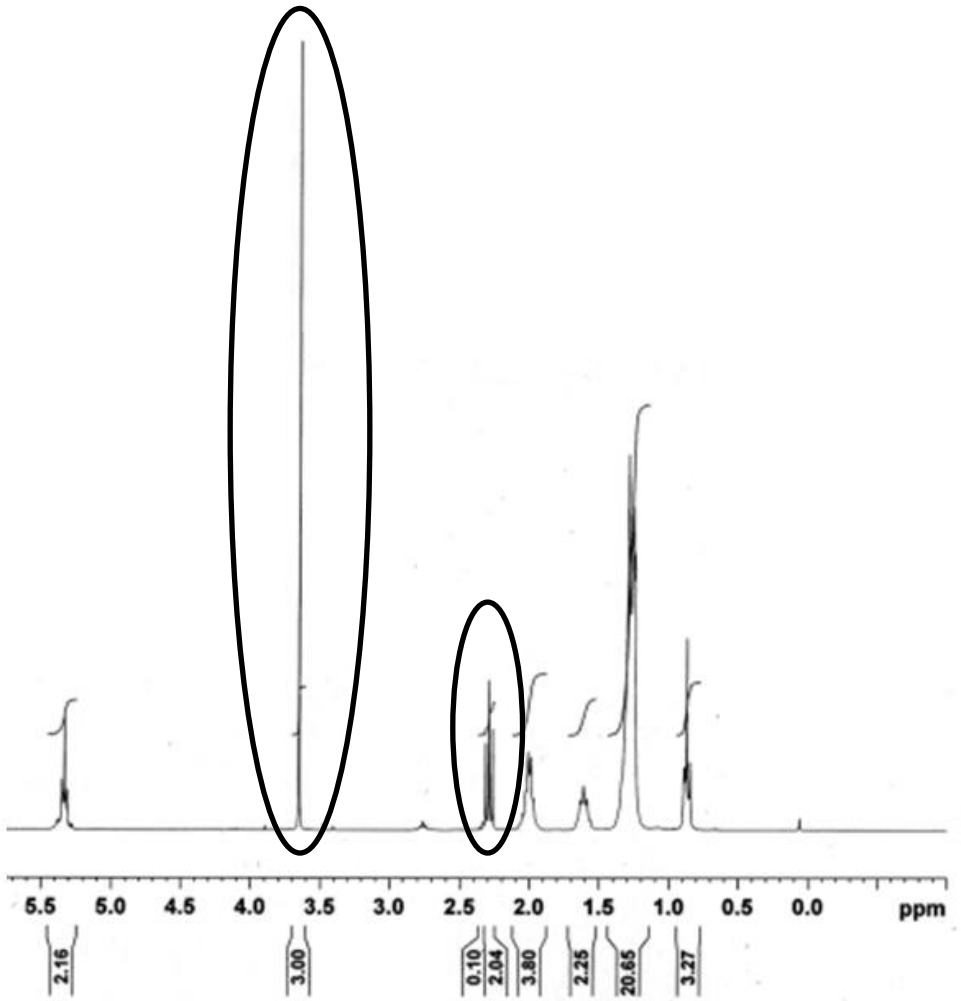


Figure 30 ^{1}H-NMR spectrum of biodiesel production from oleic acid and methanol catalyzed by Novozym 435 in four expanded bed reactors in series; Reaction conditions: FFA to methanol molar ratio of 1:2, 45°C, Novozym 435= 10 g/series, bed to catalyst volumetric ratio=2:1, feed volumetric flow rate of 5ml/min, rotation of speed of 600 rpm and reaction time of 40 min.

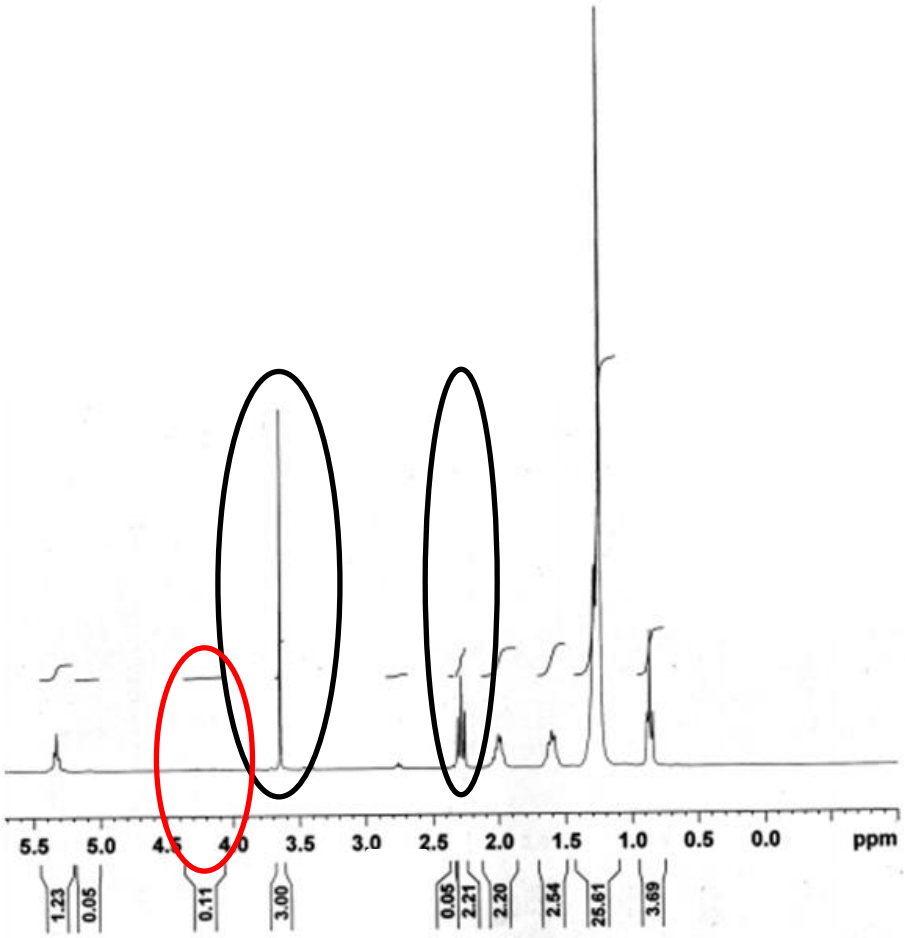


Figure 31 ^{1}H-NMR spectrum of biodiesel production from palm fatty acid distillate (PFAD) and methanol catalyzed by Novozym 435 in four expanded bed reactors in series; Reaction conditions: FFA to methanol molar ratio of 1:2, 45°C, Novozym 435= 10 g/series, bed to catalyst volumetric ratio=2:1, feed volumetric flow rate of 5ml/min, rotation of speed of 600 rpm and retention time of 40 min.

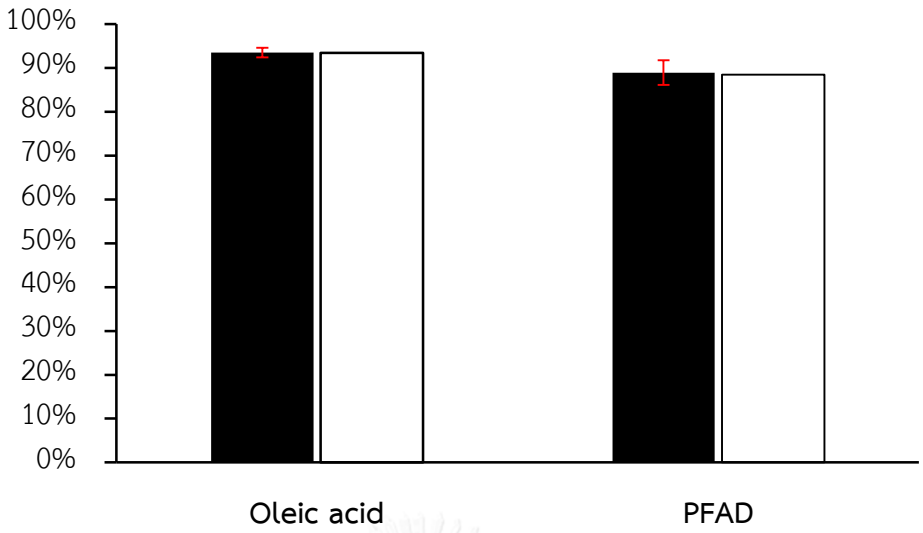


Figure 32 FFA conversion and FAME yield of esterification from free fatty acids (oleic acid and palm fatty acid distillate (PFAD)) and methanol catalyzed by Novozym 435 in four expanded bed reactors in series; Reaction conditions: FFA to methanol molar ratio of 1:2, 45°C, Novozym 435= 10 g/series, bed to catalyst volumetric ratio=2:1, feed volumetric flow rate of 5ml/min, rotation of speed of 600 rpm and reaction time of 40 min; FFA from titration method (■) and FAME yield from ^{1}H-NMR method (□).

Table 7 Comparative analysis of continuous process [70]

Effective reactor volume (mL)	Productivity (g FAME·L^{-1}·h^{-1})	Remark on the process	Reference
5	79.3	Low reaction rate, use of organic solvent	Royon et al. (2007)
1.2	40.7	Low reaction rate, use of organic solvent	Shaw et al. (2008)
18	9.5	Three step methanolysis, recycled up to 72 h	Hajar et al. (2009)
21	247.8	High enzyme loading and use of organic solvent	Rosa et al. (2009)
8	151.5	Use of organic solvent and expensive Novozyme 435	Halim et al. (2009)
23	82.5	Two step methanolysis, methanol addition at 7 h	Salum et al. (2010)
40	28.7	High reaction time, use of organic solvent	Wang et al. (2011)
100	137.2	High reaction rate, perfectly continuous, no organic solvent, green, no intermittent methanol addition	Chattopadhyay and Sen (2013)
200 (50mL/reactor)	936.71	4-Expanded bed reactors in series in the esterification of PFAD and methanol catalyzed by Novozym 435	This work

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

The kinetics and reusability of Novozym® 435 for the production of biodiesel from oleic acid with short-chain alcohols (methanol, ethanol, propanol and butanol) were studied under the following conditions: 45^{o}C, molar ratio of oleic acid to alcohol of 1:2, enzyme loading of 5% (w/w of oleic acid), stirring rate of 250 rpm and reaction time of 8 h. Novozym 435 exhibited the best performance in the production of methyl oleate, with FFA conversion of 94.82%. The final FFA conversion decreased as the length of the carbon chain of the alcohols increased. The order of the initial rate of FFA conversion during the first hour using different alcohols was as follows: methanol > 1-propanol > ethanol > 1- butanol. At the optimal temperature (45°C), the rate constants (k values) for the production of methyl oleate, ethyl oleate, propyl oleate and butyl oleate by Novozym 435 were 0.78, 0.52, 0.69 and 0.17 $m^{3}\cdot h^{-1}\cdot kmol^{-1}$, respectively. The activation energies for the production of methyl oleate and ethyl oleate over the temperature range of 40 °C to 55 °C were 4.7 and 39.1 kJ/mol, respectively.

Temperature, type of alcohol, and water content considerably affected the reusability of Novozym 435. The effect of thermal deactivation on the reusability of

Novozym 435 in the esterification of oleic acid with ethanol at 50°C was greater than that with methanol. It was shown that under the optimal conditions, Novozym 435 could be reused in the production of methyl oleate and ethyl oleate for 13 cycles with FFA conversions of at least 90%. However, when 96.0% ethanol and 95.0% ethanol were used, to retain a conversion of at least 90%, the number of Novozym 435 reuse cycles should not be greater than 10 cycles and 8 cycles, respectively. Changes in the surface morphology related to the degree of deactivation of Novozym 435 during the esterification with various conditions were observed.

The effective development of esterification from free fatty acids and methanol catalyzed by Novozym 435 from the batch experiment to the continuous process was achieved in this study. The optimal operating condition for the continuous process was primarily obtained in the single expanded bed circulation reactor at; FFA to methanol molar ratio of 1:2, 45°C, rotation speed of 600 rpm, circulation flow rate of 5 mL/min, bed to catalyst volume ratio of 2:1, Novozym 435 of 10% w/w of free fatty acid and 5h. The reusability of Novozym 435 using in this operation was 22 cycles which the FFA conversion>90% could be maintained. Furthermore, this operating condition was developed to the continuous process using the four expanded bed reactors in series. The FAME yields from free fatty acids (oleic acid and palm fatty acid distillate) and methanol catalyzed by Novozym 435 in four expanded bed reactors in series were 93.46% and 88.50%, respectively. The

productivity of biodiesel from the use of oleic acid and PFAD without any pretreatment or solvent addition were 5.24 and 4.68 g FAME•h^{-1}•g $enzyme^{-1}$, respective or 1,048.16 and 936.71 g FAME·L^{-1}·h^{-1}, respectively.

Recommendation

- The study of water removal during the esterification to improve FFA conversion, such as the addition of molecular sieve or pervaporation unit within the biodiesel production process.
- The pretreatment of palm fatty acid distillate (PFAD) to remove impurities before using in the biodiesel production.
- The regeneration of used Novozym 435 in the methyl ester production to increase reusability of the immobilized biocatalyst, such as the use of 1 mM salt solutions of $CaCl_2$, and $MgCl_2$ as a pretreatment solution.
- The study of life cycle assessment to evaluate the enzymatic biodiesel production in this work.

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APPENDIX

Appendix A

A1 Data sheet of Novozym 435

Novozym® 435

Valid from **2011-09-14**

Product Characteristics:

Declared enzyme	Lipase
Declared activity	10000 PLU/g
Colour	Off-white Colour can vary from batch to batch. Colour intensity is not an indication of enzyme activity.
Physical form	Immobilized Granulate
Approximate density (g/ml)	0.40
Carriers	Acrylic resin
Production organism	Aspergillus niger
Production method	Produced by submerged fermentation of a genetically modified micro organism. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Product Specification:

	Lower Limit	Upper Limit	Unit
Propyl Laurate Unit PLU	10000		/g
Loss on Drying 105 C	-	3	%

Packaging: See the standard packaging list for more information.

A2 Palm fatty acid distillates (PFAD) composition from GC-MS analytical and its molecular weight

Fatty Acids	**Formula**	**MW (g/mol)**	**Compositions (% wt)**
Caprylic Acid	$C_8H_{16}O_2$	144.21	-
Capric Acid	$C_{10}H_{20}O_3$	172.27	1.0
Lauric Acid	$C_{12}H_{24}O_2$	200.3	0.1
Myristic acid	$C_{14}H_{28}O_2$	228.37	0.8
Palmitic acid	$C_{16}H_{32}O_2$	256.42	50.7
Stearic acid	$C_{18}H_{36}O_2$	284.48	0.9
Oleic acid	$C_{18}H_{34}O_2$	286.46	41.0
Linoleic acid	$C_{18}H_{32}O_2$	280.45	6.5
Linolenic acid	$C_{18}H_{30}O_2$	278.44	-
Arachidic acid	$C_{20}H_{40}O_2$	304.5	-
Behenic acid	$C_{22}H_{44}O_2$	340.58	-

Note: calculated average molecular weight of PFAD = (((∑%Area from GC × MW of fatty acids)))/((Total % Area from GC)) = **223.64 g/mol**

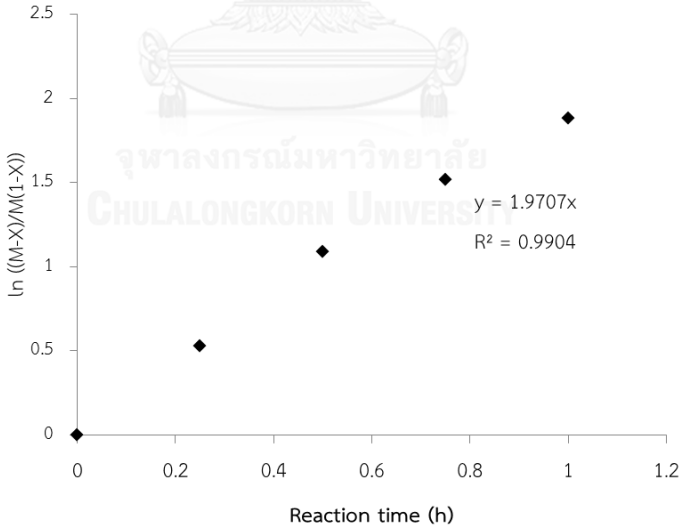
APPENDIX B

B1 The determination of kinetics (k, E_a) of methyl oleate production

Reaction conditions; molar ratio of alcohol to oleic acid of 1:2, enzyme loading at 5% (w/w of oleic acid), shaking rate of 250 rpm and temperature at 45°C.

The kinetic rate constant (k) for the second order reaction could be determined as shown in Eq. (1);

$$\ln \frac{(M - X_{Oleic\ acid})}{M(1 - X_{Oleic\ acid})} = [\text{Oleic acid}]_0 (M - 1)kt \quad (1)$$



The slope of the linear plot of eq. (1) is;

$$m = [\text{Oleic acid}]_0 (M - 1)k$$

$$\text{Therefore,} \quad k = \frac{m}{[\text{Oleic acid}_0](M - 1)}$$

(2)

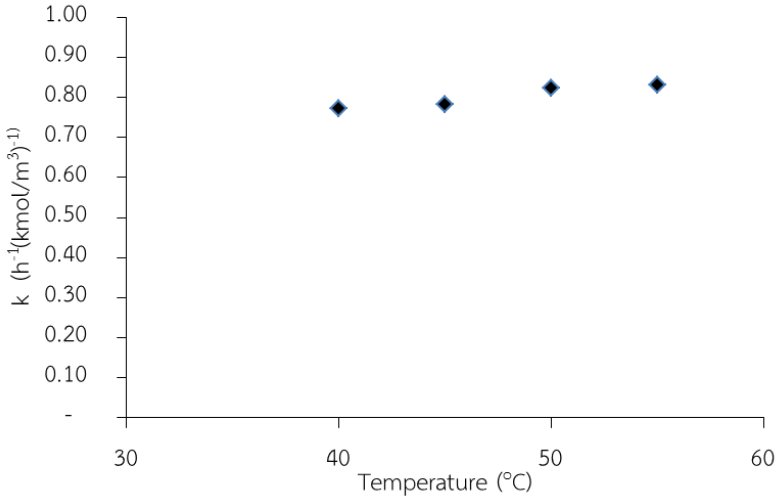
Since $[\text{Oleic acid}]_0 = 2.52\ \text{kmol/m}^3$, $M = \frac{[\text{Alcohol}]_0}{[\text{Oleic acid}]_0} = 2$

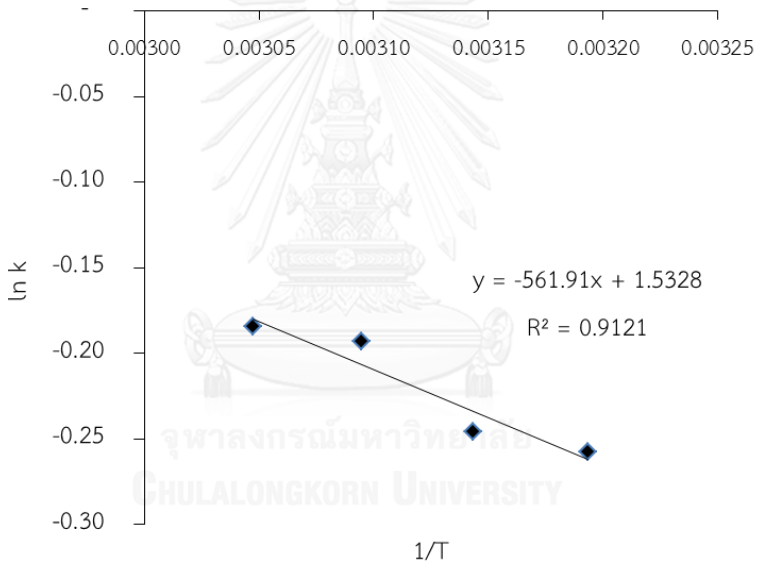
So, $k = (1.9707/2.52/(2-1)) = 0.78\ \text{m}^3 \cdot \text{h}^{-1} \cdot \text{kmol}^{-1}$ #

The activation energy is determined experimentally by carrying out the reaction at several temperatures. Taking the natural logarithm (ln) of the Arrhenius equation yields:

$$\ln k = \ln a - \frac{Ea}{RT} \qquad (2)$$

The activation energy value (Ea) can be determined from the slope of a plot between ln k versus 1/T.





Since slope is; m= -Ea/R = -561.91

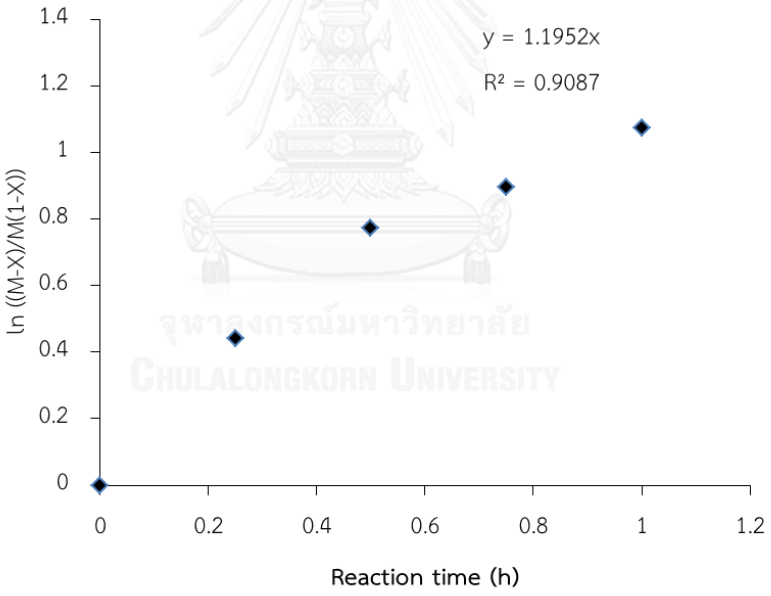
Therefore, $E_a = (561.91 \times 8.31) \times 10^{-3} = 4.67$ kJ/mol #

B2 The determination of kinetics (k, E_a) of ethyl oleate production

Reaction conditions; molar ratio of alcohol to oleic acid of 1:2, enzyme loading at 5% (w/w of oleic acid), shaking rate of 250 rpm and temperature at 45°C.

The kinetic rate constant (k) for the second order reaction could be determined as shown in Eq. (1);

$$\ln \frac{(M - X_{Oleic\ acid})}{M(1 - X_{Oleic\ acid})} = [Oleic\ acid]_0 (M - 1)kt \quad (1)$$

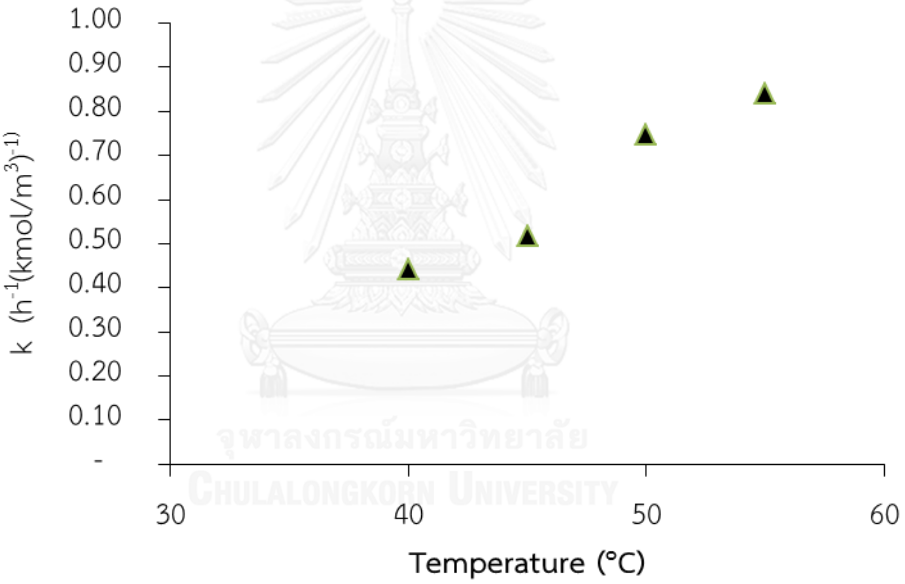


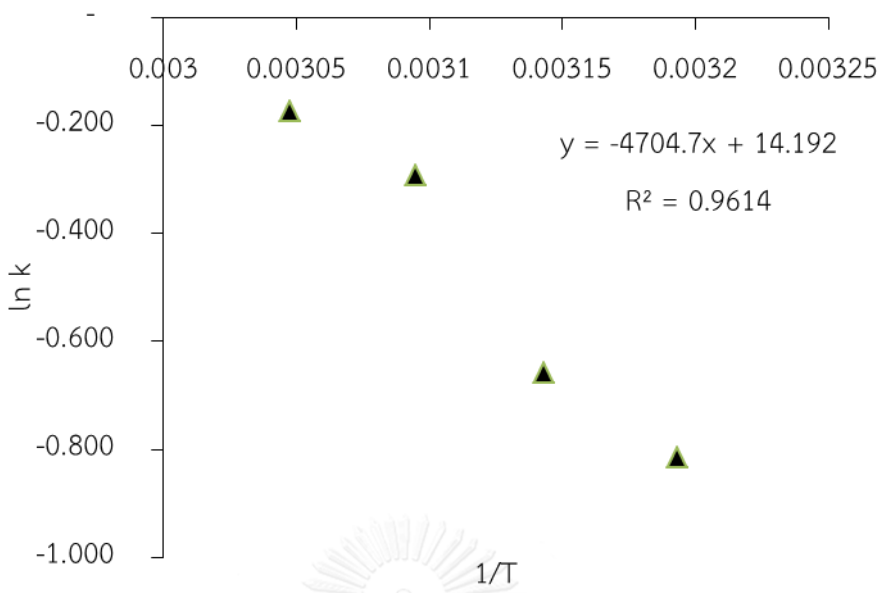
From the slope of the linear plot of eq. (1) , $[\text{Oleic acid}]_0 = 2.52\ \text{kmol/m}^3$, M=

$$\frac{[\text{Alcohol}]_0}{[\text{Oleic acid}]_0} = 2$$

So, $k = (1.1952/2.31/(2-1)) = 0.52\ \text{m}^3 \cdot \text{h}^{-1} \cdot \text{kmol}^{-1}$ #

The activation energy value (Ea) can be determined from the slope of a plot between ln k versus 1/T.





Since slope is; m= -Ea/R = -4,704.7

Therefore, $E_a = (4{,}704.7 \times 8.31) \times 10^{-3} = 39.11$ kJ/mol #

B3: Calculation of the volumetric ratio of working volume at reaction zone to mixture zone

Since, total mixture feed in Tank 140 mL, and volumetric ratio of bed to catalyst was 1:1, the volumetric ratio of the reaction zone to the total volume was

12.5:140 or 0.09:1

And when volumetric ratio of bed to catalyst was increased to 2:1, the volumetric ratio of the reaction zone to the total volume was

25:140 or 0.18:1

Moreover, when doubly increased enzyme amount at bed to catalyst volumetric ratio of 2:1, the volumetric ratio of reaction zone to the total volume was

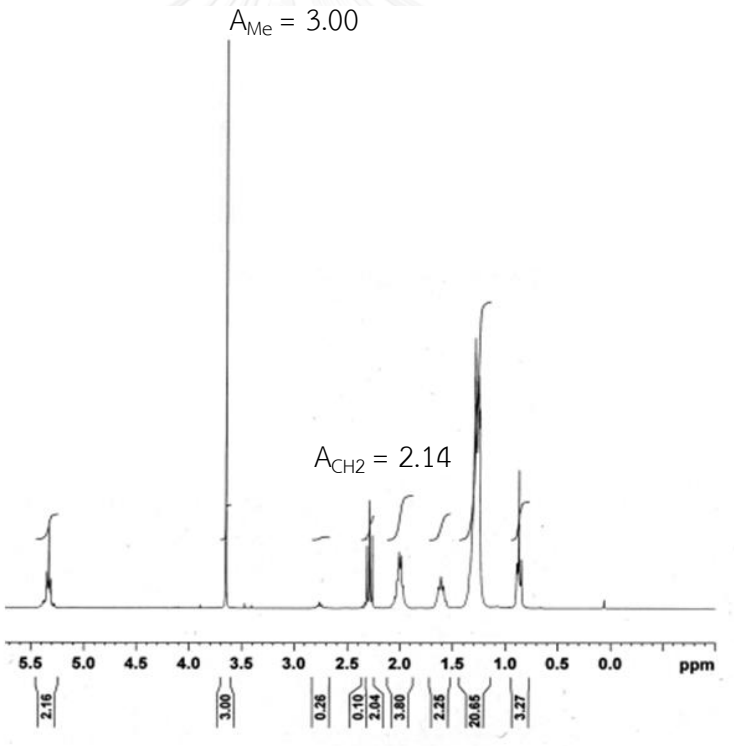
50:140 or 0.36:1

B4 Yield of fatty acid methyl ester (FAME, %) calculation from ^{1}H-NMR method

B4-1 Yield of FAME from esterification of oleic acid and methanol catalyzed by Novozym 435 in four expanded bed reactors in series

The FAME content was determined by the ratio of the area of peaks associated with the methyl ester (3.65 ppm) and methylene group protons (2.26 ppm). The equation for FAME determination has shown below;

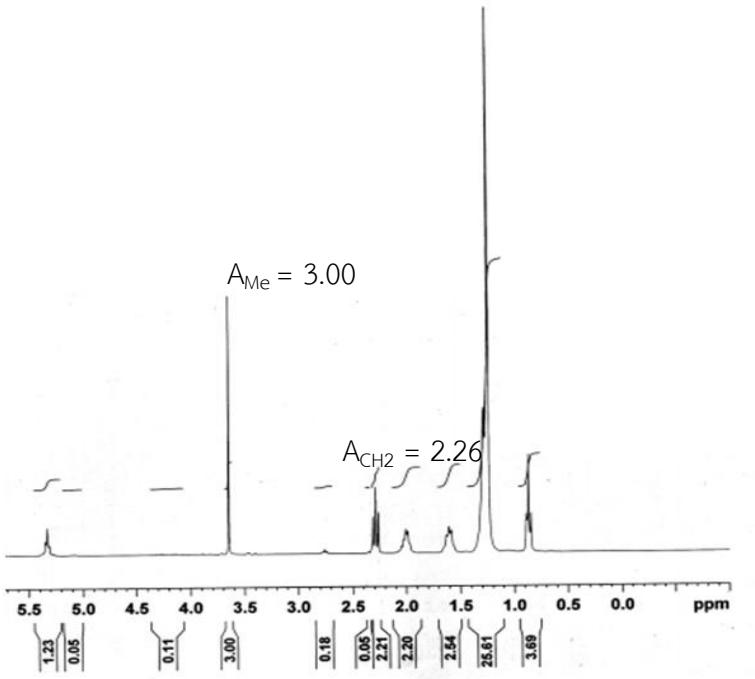
$$C(\%) = 100 \times \frac{2A_{Me}}{3A_{CH_2}} = \frac{100 \times 2(3)}{3 \times 2.14} = 93.46\,\%$$



B4-2 Yield of FAME from esterification of palm fatty acid distillate and methanol catalyzed by Novozym 435 in four expanded bed reactors in series

The FAME content was determined by the ratio of the area of peaks associated with the methyl ester (3.65 ppm) and methylene group protons (2.26 ppm). The equation for FAME determination has shown below;

$$C\,(\%) = 100 \times \frac{2A_{Me}}{3A_{CH_2}} = \frac{100 \times 2(3)}{3 \times 2.26} = 88.50\,\%$$



B5 Productivity calculation of biodiesel production from free fatty acids (oleic acid and palm fatty acid distillate (PFAD)) and methanol catalyzed by Novozym 435 in the four expanded bed reactors in series

Information data

- **Chemicals property**

Name	Formula	MW (g/mol)	Density (g/cm^3)
Oleic acid	$C_{18}H_{32}O_2$	282.41	0.895
Palm fatty acid distillate	-	223.64	0.874
Methanol	CH_3OH	32.04	0.792
Water	H_2O	18.02	0.997
Novozym 435	-	-	0.4

B5.1 Productivity of biodiesel production from oleic acid and methanol catalyzed by Novozym 435 in four expanded bed reactors in series

- **Substrate ratio**

Molar ratio:	FFA: methanol (mol)	1	:	2
Mass ratio:	FFA: methanol (g)	282.41	:	64.08
Volumetric ratio:	FFA: methanol (ml)	315.55	:	80.92

Feed volumetric ratio in a continuous system, mixture feed flow rate = 5 ml/min

Therefore, PFAD flow rate = 3.98 ml/min

- **Reactor design**

Series of expanded bed reactors	= 4
Enzyme usage (g/series)	=10
Therefore, total enzyme usage (g)	= 40
Yield (% FAME) from ^{1}H-NMR	=93.46%

- **Mole and Mass balance**

Since esterification is PFAD + Methanol = FAME + H_20 (by mol)

Therefore, 282.41 + 32.04 = 296.44 + 18.02 (by mass)

- **Productivity**

Since PFAD flow rate = 3.98 ml/min or 3.56 g/min, Novozym 435 = 40 g, and FAME = 93.46%

Therefore, productivity of biodiesel production

$$= \frac{(\frac{296.44}{282.41} \times 3.98 \times 93.46\%) \text{ g of FAME / min}}{40 \text{ g of Novozym 435}}$$

= 0.09 g of FAME/min/g of Novozym 435

Biodiesel productivity from oleic acid and methanol catalyzed by Novozym 435 in the four expanded bed reactors in series is **0.09** g of FAME/min/g of Novozym 435 or **5.24** g of FAME/h/g of Novozym 435 #

Since the reaction volume in four-expanded bed reactor was 200 cm^3 and Novozym 435 loading was 40 g, the productivity in unit of g of FAME/L/h was at **1,048.16** g of FAME·L^{-1}·h^{-1}#

B5.2 Productivity of biodiesel production from palm fatty acid distillate (PFAD) and methanol catalyzed by Novozym 435 in four expanded bed reactors in series

- **Substrate ratio**

Molar ratio:	FFA: methanol (mol)	1	:	2
Mass ratio:	FFA: methanol (g)	223.64	:	64.08
Volumetric ratio:	FFA: methanol (ml)	255.88	:	80.92

Feed volumetric ratio in a continuous system, mixture feed flow rate = 5 ml/min

Therefore, PFAD flow rate = 3.80 ml/min

- **Reactor design**

Reactors design	Series of expanded bed reactors	= 4
	Enzyme usage (g/series)	=10
	Therefore, total enzyme usage (g)	= 40
	Yield (% FAME) from ^{1}H-NMR	=88.50%

- **Mole and Mass balance**

Since esterification is PFAD + Methanol = FAME + H_20 (by mol)

Therefore, 223.64 + 32.04 = 237.66 + 18.02 (by mass)

- **Productivity**

Since PFAD flow rate = 3.80 ml/min or 3.32 g/min, Novozym 435 = 40 g, and FAME = 88.50%

Therefore, productivity of biodiesel production

$$= \frac{(\frac{237.66}{223.64} \times 3.32 \times 88.50\%) \text{ g of FAME / min}}{40 \text{ g of Novozym 435}}$$

$$= 0.08 \text{ g of FAME/min/g of Novozym 435}$$

Biodiesel productivity from PFAD and methanol catalyzed by Novozym 435 in the four expanded bed reactors in series is **0.08** g of FAME/min/g of Novozym 435 or **4.68** g of FAME/h/g of Novozym 435 #

Since the reaction volume in four-expanded bed reactor was 200 cm^3 and Novozym 435 loading was 40 g, the productivity in unit of g of FAME/L/h was at **936.71** g of FAME·L^{-1}·h^{-1}#

APPENDIX C

EXPERIMENTAL RAW DATA

Table C1 Raw data of Figure 12

Reaction time (h)	FFA conversion (%)			
	Methanol (99.9%)	Ethanol (99.9%)	Propanol (99.9%)	Butanol (99.9%
0	-	-	-	-
0.5	88.00	83.00	80.00	79.00
2	93.58	91.21	88.10	86.22
4	94.56	91.21	88.10	87.13
6	94.44	91.21	88.10	88.15
8	94.82	91.27	89.08	88.20

Table C2 Raw data of Figure 13

Reaction time (h)	FFA conversion (%)	
	Methanol (99.9%)	Ethanol (99.9%)
0	0%	0%
0.25	58%	53%
0.5	80%	70%
0.75	88%	74%
1	92%	79%

Table C3 Raw data of Figure 14

Reaction time (h)	ln ((M-X)/M(1-X))	
	Methanol (99.9%)	Ethanol (99.9%)
0	-	-
0.25	0.53	0.44
0.5	1.09	0.70
0.75	1.52	0.90
1	1.88	1.07

Table C4 Raw data of Figure 15

Temperature (°C)	k ($m^3 \cdot h^{-1} \cdot kmol^{-1}$)	
	Methanol (99.9%)	Ethanol (99.9%)
40	0.77	0.44
45	0.78	0.52
50	0.82	0.75
55	0.83	0.84

Table C5 Raw data of Figure 16

Reaction time (h)	FFA Conversion (%)	
	45°C	50°C
-	-	-
0.5	86.60	81.61
2.0	90.15	90.21
4.0	94.52	94.34
6.0	95.44	94.80
8.0	95.44	94.61
8.0	-End of Cycle-	
8.5	49.37	85.21

10.0	89.49	93.69
12.0	92.39	93.55
14.0	95.21	93.43
16.0	95.26	93.93
16.0	-End of Cycle-	
16.5	43.85	63.59
18.0	85.30	94.46
20.0	94.60	94.53
22.0	94.46	93.77
24.0	94.76	94.54
24.0	-End of Cycle-	
24.5	44.53	61.22
26.0	87.15	92.72
28.0	91.13	94.46
30.0	95.13	93.99
32.0	94.94	93.68
32.0	-End of Cycle-	
32.5	40.96	60.16
34.0	86.86	91.49
36.0	94.57	91.72

38.0	94.34	91.72
40.0	94.28	91.71

Table C6 Raw data of Figure 17

Reaction time (h)	FFA Conversion (%)	
	45°C	50°C
-	-	-
0.5	63.28	75.11
2.0	91.32	86.11
4.0	91.07	89.73
6.0	91.22	89.71
8.0	91.54	90.54
8.0	-End of Cycle-	
8.5	62.26	32.46
10.0	85.36	64.89
12.0	90.21	79.59
14.0	90.81	86.76
16.0	90.76	87.46
16.0	-End of Cycle-	

16.5	48.62	27.42
18.0	85.60	61.78
20.0	90.62	77.39
22.0	90.76	85.54
24.0	91.45	88.12
24.0	-End of Cycle-	
24.5	52.97	21.66
26.0	83.08	46.63
28.0	90.00	63.62
30.0	90.16	76.22
32.0	90.37	82.29
32.0	-End of Cycle-	
32.5	45.86	20.65
34.0	82.78	42.10
36.0	89.48	57.29
38.0	89.65	69.79
40.0	89.74	75.33

Table C7 Raw data of Figure 18

Reaction time (h)	FFA Conversion (%)	
	Methanol	Ethanol
-	-	-
0.5	81.61	63.28
2.0	90.21	91.32
4.0	94.34	91.07
6.0	94.80	91.22
8.0	94.61	91.54
8.0	-End of Cycle-	
8.5	85.21	62.26
10.0	93.69	85.36
12.0	93.55	90.21
14.0	93.43	90.81
16.0	93.93	90.76
16.0	-End of Cycle-	
16.5	63.59	48.62
18.0	94.46	85.60
20.0	94.53	90.62

22.0	93.77	90.76
24.0	94.54	91.45
24.0	-End of Cycle-	
24.5	61.22	21.66
26.0	92.72	46.63
28.0	94.46	63.62
30.0	93.99	76.22
32.0	93.68	82.29
32.0	-End of Cycle-	
32.5	60.16	20.65
34.0	91.49	42.10
36.0	91.72	57.29
38.0	91.72	69.79
40.0	91.71	75.33
40.0	-End of Cycle-	
40.50	60.77	38.80
42.00	91.53	79.17
44.00	92.00	89.55
46.00	92.17	90.35
48.00	92.89	90.62

48.00	-End of Cycle-	
48.50	60.77	48.67
50.00	92.21	75.50
52.00	93.50	88.52
54.00	93.21	90.84
56.00	93.34	91.56
56.00	-End of Cycle-	
56.50	60.77	33.10
58.00	92.83	78.37
60.00	93.66	88.35
62.00	93.72	90.37
64.00	92.84	90.74
64.00	-End of Cycle-	
64.50	61.72	36.21
66.00	92.98	66.65
68.00	92.80	84.60
70.00	92.48	89.26
72.00	92.64	90.17
72.00	-End of Cycle-	
72.50	60.77	32.01

74.00	91.30	58.85
76.00	92.59	83.03
78.00	92.48	86.06
80.00	92.64	89.91
80.00	-End of Cycle-	
80.50	60.77	34.16
82.00	94.05	63.86
84.00	93.97	81.55
86.00	93.88	88.98
88.00	94.00	90.41
88.00	-End of Cycle-	
88.50	49.46	36.06
90.00	93.99	63.81
92.00	94.06	82.27
94.00	94.09	88.08
96.00	93.90	90.59
96.00	-End of Cycle-	
96.50	48.93	30.73
98.00	91.18	60.18
100.00	92.39	79.03

102.00	91.13	87.57
104.00	90.61	88.60
104.00	-End of Cycle-	
104.50	48.64	30.83
106.00	85.37	62.67
108.00	86.03	83.03
110.00	87.10	88.66
112.00	87.04	89.00
112.00	-End of Cycle-	

Table C8 Raw data of Figure 19

Reaction time (h)	**FFA Conversion (%)**		
	Ethanol 99.9%	**Ethanol 96.0%**	**Ethanol 95.0%**
-	-	-	-
0.5	63.28	87.48	86.65
2.0	91.32	88.76	88.59
4.0	91.07	89.71	89.96
6.0	91.22	89.76	89.78

8.0	91.54	89.97	89.97
8.0	-End of Cycle-		
8.5	62.26	65.50	66.74
10.0	85.36	89.56	89.85
12.0	90.21	90.76	90.88
14.0	90.81	91.53	91.68
16.0	90.76	91.28	91.32
16.0	-End of Cycle-		
16.5	48.62	60.65	60.96
18.0	85.60	89.22	89.56
20.0	90.62	90.43	90.22
22.0	90.76	90.56	90.56
24.0	91.45	90.58	91.10
24.0	-End of Cycle-		
24.5	52.97	62.38	63.05
26.0	83.08	87.89	88.02
28.0	90.00	90.08	90.12
30.0	90.16	90.56	90.32
32.0	90.37	90.30	90.21
32.0	-End of Cycle-		

32.5	45.86	62.01	61.35
34.0	82.78	89.90	89.56
36.0	89.48	90.23	90.11
38.0	89.65	90.56	91.01
40.0	89.74	90.50	90.71
40.0	-End of Cycle-		
40.50	38.80	58.44	59.55
42.00	79.17	88.67	88.76
44.00	89.55	90.86	91.07
46.00	90.35	91.02	90.91
48.00	90.62	90.39	90.41
48.00	-End of Cycle-		
48.50	48.67	63.11	65.04
50.00	75.50	88.80	88.98
52.00	88.52	90.79	90.99
54.00	90.84	90.77	90.93
56.00	91.56	90.94	90.80
56.00	-End of Cycle-		
56.50	33.10	55.85	56.22
58.00	78.37	81.47	81.78

60.00	88.35	88.56	88.54
62.00	90.37	89.84	89.95
64.00	90.74	89.88	90.10
64.00	-End of Cycle-		
64.50	36.21	51.27	46.49
66.00	66.65	83.86	83.01
68.00	84.60	89.70	89.87
70.00	89.26	89.80	89.80
72.00	90.17	90.27	89.93
72.00	-End of Cycle-		
72.50	32.01	40.55	35.03
74.00	58.85	85.63	83.28
76.00	83.03	90.72	86.74
78.00	86.06	90.48	86.94
80.00	89.91	90.48	87.02
80.00	-End of Cycle-		
80.50	34.16	50.31	45.77
82.00	63.86	85.60	85.15
84.00	81.55	86.62	87.07
86.00	88.98	85.38	86.57

88.00	90.41	85.45	85.69
88.00	-End of Cycle-		
88.50	36.06	52.00	51.70
90.00	63.81	80.50	77.54
92.00	82.27	83.63	82.50
94.00	88.08	83.06	83.19
96.00	90.59	83.44	82.73
96.00	-End of Cycle-		
96.50	30.73	17.60	14.11
98.00	60.18	53.59	53.67
100.00	79.03	74.02	74.26
102.00	87.57	84.60	75.94
104.00	88.60	84.60	81.12
104.00	-End of Cycle-		
104.50	30.83	25.31	14.58
106.00	62.67	55.40	52.61
108.00	83.03	75.10	75.10
110.00	88.66	84.52	76.55
112.00	89.00	84.52	81.24

Table C9 Raw data of Figure 21

Reaction time (h)	FFA Conversion (%)		
	4 ml/min	5 ml/min	6 ml/min
-	-	-	-
0.25	13.9	38.6	35.7
0.5	30.6	57.3	39.1
1.0	34.5	61.0	45.4
2.0	48.6	68.3	51.1
3.0	60.6	77.0	58.1
4.0	63.5	79.1	62.7
5.0	63.1	84.4	73.1
6.0	65.4	87.0	72.3
7.0	66.2	89.7	76.5
8.0	72.0	90.3	81.7
9.0	71.0	90.0	90.1
24.0	79.8	90.1	90.6

Table C10 Raw data of Figure 22

Reaction time (h)	Bed to catalyst volumetric ratio	
	1:1	2:1
-	-	-
0.25	38.6	44.9
0.5	57.3	60.4
1.0	61.0	64.5
2.0	68.3	76.2
3.0	77.0	82.8
4.0	79.1	85.3
5.0	84.4	87.7
6.0	87.0	89.5
7.0	89.7	90.1
8.0	90.3	90.3
9.0	90.0	90.2

24.0	90.1	89.6

Table C11 Raw data of Figure 23

Reaction time (h)	FFA conversion (%)	
	5 %wt	10%wt
-	-	-
0.25	44.3	77.4
0.5	55.0	80.1
1.0	62.1	86.2
2.0	76.0	93.8
3.0	82.6	95.6
4.0	85.2	95.8
5.0	87.6	96.7
6.0	89.4	96.8
7.0	90.0	96.4

8.0	90.2	95.9
9.0	90.1	96.7
24.0	91.1	96.9

Table C12 Raw data of Figure 24

Reaction time (h)	FFA Conversion (%)
-	-
0.3	77.42
0.5	80.07
1.0	86.20
2.0	93.80
3.0	95.55
4.0	95.84
5.0	96.71
5.0	-End of cycle-
5.3	73.95
5.5	79.24

6.0	83.35
7.0	87.86
8.0	95.20
9.0	94.68
10.0	95.40
10.0	-End of cycle-
10.3	58.17
10.5	60.54
11.0	70.70
12.0	78.90
13.0	91.19
14.0	92.58
15.0	93.32
15.0	-End of cycle-
15.3	60.68
15.5	74.70
16.0	81.23
17.0	83.15
18.0	91.89
19.0	93.26

20.0	91.80
20.0	-End of cycle-
20.3	65.94
20.5	74.16
21.0	79.83
22.0	85.73
23.0	93.30
24.0	93.43
25.0	92.09
25.0	-End of cycle-
25.3	62.41
25.5	66.84
26.0	73.31
27.0	83.71
28.0	89.76
29.0	91.36
30.0	92.43
30.0	-End of cycle-
30.3	64.69
30.5	70.37

31.0	78.94
32.0	80.77
33.0	86.92
34.0	92.92
35.0	92.08
35.0	-End of cycle-
35.3	67.06
35.5	68.89
36.0	78.27
37.0	77.98
38.0	93.44
39.0	93.37
40.0	93.43
40.0	-End of cycle-
40.3	62.36
40.5	69.55
41.0	78.26
42.0	77.33
43.0	93.52
44.0	94.00

45.0	93.51
45.0	-End of cycle-
45.3	66.74
45.5	70.04
46.0	80.74
47.0	82.20
48.0	88.53
49.0	92.42
50.0	92.14
50.0	-End of cycle-
50.3	64.29
50.5	66.95
51.0	80.23
52.0	83.34
53.0	84.14
54.0	90.17
55.0	93.61
55.0	-End of cycle-
55.3	66.13
55.5	68.67

56.0	80.24
57.0	84.86
58.0	86.07
59.0	89.01
60.0	92.46
60.0	-End of cycle-
60.3	66.20
60.5	73.78
61.0	75.61
62.0	77.09
63.0	85.58
64.0	89.43
65.0	93.12
65.0	-End of cycle-
65.3	65.67
65.5	66.58
66.0	81.17
67.0	82.37
68.0	90.10
69.0	94.29

70.0	94.27
70.0	-End of cycle-
70.3	63.79
70.5	64.74
71.0	80.13
72.0	81.39
73.0	89.54
74.0	93.97
75.0	93.95
75.0	-End of cycle-
75.3	61.87
75.5	74.81
76.0	75.85
77.0	85.00
78.0	87.66
79.0	91.94
80.0	91.65
80.0	-End of cycle-
80.3	63.56
80.5	63.95

81.0	76.72
82.0	79.72
83.0	89.12
84.0	92.45
85.0	91.61
85.0	-End of cycle-
85.3	69.45
85.5	73.28
86.0	76.41
87.0	80.95
88.0	88.89
89.0	93.23
90.0	92.75
90.0	-End of cycle-
90.3	58.70
90.5	59.55
91.0	77.94
92.0	82.00
93.0	87.34
94.0	90.55

95.0	90.78
95.0	-End of cycle-
95.3	58.44
95.5	59.30
96.0	77.80
97.0	84.38
98.0	89.02
99.0	89.32
100.0	90.08
100.0	-End of cycle-
100.3	64.87
100.5	69.28
101.0	81.16
102.0	84.76
103.0	84.57
104.0	89.44
105.0	90.07
105.0	-End of cycle-
105.3	53.58
105.5	59.43

106.0	79.34
107.0	81.81
108.0	89.55
109.0	91.02
110.0	90.76
110.0	-End of cycle-
110.3	26.85
110.5	59.45
111.0	79.30
112.0	86.72
113.0	86.73
114.0	87.35
115.0	88.50

Table C13 Raw data of Figure 25

Reusability (Cycles)	FFA Conversion (%)
0	96.71
1	95.40
2	93.32
3	91.80
4	92.09
5	92.43
6	92.08
7	93.43
8	93.51
9	92.14
10	93.61

11	92.46
12	93.12
13	94.27
14	93.95
15	91.65
16	91.61
17	92.75
18	90.78
19	90.08
20	90.07
21	90.76
22	88.50
23	88.500

Table C15 Raw data of Figure 26

Series of reactor	Conversion (%)	SD (%)
S1	41.21	5.16
S2	73.89	2.39
S3	88.34	0.20
S4	88.94	0.28

Table C14 Raw data of Figure 27

Series of reactor	Conversion (%)	SD (%)
S1	-	0.98
S2	47.40	0.42
S3	75.63	0.45
S4	92.56	0.15

Table C14 Raw data of Figure 30

	Oleic acid	PFAD
Conversion	93.51%	88.94%
Yield	93.46%	88.50%
SD (titration)	1.10%	2.80%

VITA

Miss Sawittree Mulalee was born on September 14th, 1983 in Udon Thani, Thailand. She received her Bachelor's Degree of Chemical Engineering at Thammasat University in June 2006 and Master's degree in chemical engineering at Chulalongkorn University in May, 2008. She has been studied Ph.D. degree in chemical engineering at Chulalongkorn University since December, 2011 and received funds from the 90th anniversary of Chulalongkorn University (Ratchadaphiseksomphot Endowment Fund) and Chemical Engineering Research Unit for Value Adding of Bioresources, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University during her study.

Her academic publications and conferences are as follows;

Academic publications

1. Esterification of oleic acid and bioalcohols using immobilized lipase.

(Mulalee, S., et al., Adv Mat Res, 2013. 724-725: p. 1154-1157.)

2. Enzymatic esterification of oleic acid and propanol by Novozym 435.

(Mulalee, S., K. Sena, and M. Phisalaphong, Applied Mechanics and Materials, 2014.) (Accepted)

3. Influences of operating conditions on biocatalytic activity and reusability of Novozym 435 for esterification of free fatty acids with short-chain alcohols: a case study of palm fatty acid distillate (PFAD).

(Mulalee, S., P. Srisuwan, and M. Phisalaphong, Chinese Journal of Chemical Engineering, 2014.) (Minor revision)

Conferences

1. 2013/2nd International Conference on Energy and Environmental Protection (ICEEP 2013) in Guilin, China, April 20-21, 2013.

2. 2014 International Conference on Renewable Energy Technologies (ICRET 2014), in Hong Kong during November 7-9, 2014 and won "the outstanding paper presentation" award.