การผลิตไบโอดีเซลแบบกึ่งต่อเนื่องจากกรดไขมันปาล์มโดยใช้ โนโวไซม์ 435

นางสาวสุกัญญา ทรัพย์เกิด

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

SEMI-CONTINUOUS PRODUCTION OF BIODIESEL FROM PALM FATTY ACID USING NOVOZYM 435

Miss Sukanya Subkerd

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2008

Thesis Title	SEMI-CONTINUOUS PRODUCTION OF BIODIESEL FROM AND PALM FATTY ACID USING NOVOZYM 435
By	Miss Sukanya Subkerd
Field of study	Petrochemistry and Polymer Science
Thesis Advisor	Associate Professor Surachai Pornpakakul, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

S. Hannongbua
.....Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

..... Chairman
(Associate Professor Supawan Tantayanon, Ph.D.)

Surachai Pornpakakul
..... Advisor
(Associate Professor Surachai Pornpakakul, Ph.D.)

..... Examiner
(Associate Professor Amorn Petsom, Ph.D.)

W. Trakarnpruk
..... Examiner
(Associate Professor Wimonrat Trakarnpruk, Ph.D.)

P. Khorphueng
..... External examiner
(Prapas Khorphueng, Ph.D.)

สุกัญญา ทรัพย์เกิด : การผลิตไบโอดีเซลแบบกึ่งต่อเนื่องจากกรดไขมันปาล์มโดยใช้โนโวไซม์ 435 (SEMI-CONTINUOUS PRODUCTION OF BIODIESEL FROM PALM FATTY ACID USING NOVOZYM 435) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.สุรชัย พรภคกุล, 86 หน้า.

การผลิตไบโอดีเซลแบบกึ่งต่อเนื่องจากกรดไขมันปาล์มซึ่งเป็นผลพลอยได้จากกระบวนการกลั่นน้ำมันปาล์มให้บริสุทธิ์ โดยใช้โนโวไซม์ 435 เป็นตัวเร่งปฏิกิริยา พบว่าการใช้เทอร์เชียรี่บิวทานอลเป็นตัวทำละลายร่วมจะรักษาสภาพของเอนไซม์ถึงแม้จะมีเมทานอลปริมาณมากในระบบ และทำให้ผลิตภัณฑ์เมทิลเอสเทอร์สูงขึ้น ในกระบวนการผลิตไบโอดีเซลแบบกึ่งต่อเนื่อง สัดส่วนของกรดไขมัน: เมทานอล, ปริมาณตัวทำละลายร่วม และ อัตราการไหลมีผลต่อเปอร์เซ็นต์เมทิลเอสเทอร์ ภาวะที่เหมาะสมในการสังเคราะห์ไบโอดีเซลคือ อุณหภูมิ 50 องศาเซลเซียส, อัตราการไหล 1.67 มิลลิกรัมต่อวินาที และอัตราส่วนโดยโมลของกรดไขมันปาล์ม:เมทานอลที่ 1:2 และใช้เทอร์เชียรี่บิวทานอล 29 เปอร์เซ็นต์ ของน้ำหนักกรดไขมันปาล์ม กระบวนการแบบขั้นเดียวและแบบ 3 ขั้น พบว่าให้ค่าร้อยละเมทิลเอสเทอร์สูงสุด 80 และ 92 เปอร์เซ็นต์ตามลำดับ เอนไซม์ไลเปสสามารถใช้งานได้มากกว่า 240 ชั่วโมง โดยไม่เสียสภาพ

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....
ปีการศึกษา .....2551.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

4972531723 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORDS: BIODIESEL / NOVOZYM 435 / ESTERIFICATION

SUKANYA SUBKERD: SEMI-CONTINUOUS PRODUCTION OF BIODIESEL FROM PALM FATTY ACID USING NOVOZYM 435. THESIS ADVISOR: ASSOC. PROF. SURACHAI PORNPAKAKUL, Ph.D., 86 pp.

Production of biodiesel from palm fatty acid, which was a by-product from refining palm oil production, was investigated semi-continuous process catalyzed by Novozym 435. It was found that, using *tert*-butanol as a cosolvent could maintain activity of lipase even high amount of methanol was present in the system and could improve yield of fatty acid methyl ester (FAME). A molar ratio of palm fatty acid and methanol, *tert*-butanol quantity and flow rate had significant effects on the percent molar conversion in semi-continuous process. The appropriate conditions for synthesis were as follows: reaction temperature 50°C, flow rate 1.87 mg/sec, and methanol to palm fatty acid ratio 2:1 and *tert*-butanol 29% based on palm fatty acid weight. One-step and three-step process were investigated. The highest conversion ratio of the FAME from one-step and three-step under the optimal condition was 80% and 92%, respectively. The lipase catalyst could be used for >240 hours without decrease of the activity.

Field of Study : Petrochemistry and Polymer Science Student's signature....S. Subkerd
Academic Year.....2008..... Advisor's signature.....S. Pornpakakul

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and gratitude to my advisor, Associate Professor Dr. Surachai Pornpakakul, for his excellent suggestion, guidance, encouragement and supportiveness throughout the entire period of conducting this thesis.

I would also like to extend to Associate Professor Dr. Supawan Tantayanon, Associate Professor Dr. Amorn Petsom, Associate Professor Dr. Wimonrat Trakarnpruk and Dr. Prapas Khorphueng, attending as the chairman and members of my thesis committee, respectively, for their kind guidance, helpful discussions and valuable suggestions throughout my study.

I thank Center for Petroleum, Petrochemicals, and Advanced Materials and Graduate School of Chulalongkorn University for partial financial support to conduct this research.

I am very grateful to Dr. Aphichart Karnchanatat, Miss Natta Rattanapanya, Miss Sunisa Suwanjaroen, Mr. Yoottana Jampahom, Miss Watsamon Saetae, Mr. Prachya Watasit, Nipawan Thadkad and all members on Research Center for Bioorganic Chemistry (RCBC) for their kind gratitude of finding me the information, their friendship, support and helpfulness.

I thank the ministry of energy for determining chemical properties of biodiesel. I also thank Oleen Co., Ltd. for palm fatty acid.

Finally, I would like to express thanks to my family for their care and supports to make my study successful. Thanks are also due to everyone who has contributed suggestions and supports throughout my research.

CONTENTS

PAGE

CONTENTS

PAGE

PAGE

PAGE

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

FIGURE **PAGE**

LIST OF SCHEMES

SCHEME **PAGE**

LIST OF ABBREVIATIONS

CO_2	=	Carbondioxide
No.	=	Number
°C	=	Degree Celsius
°F	=	Degree Fahrenheit
ASTM	=	American Society for Testing and Materials
		Methods of Analysis of AOAC
FAME	=	Fatty acid methyl ester
EN	=	European Standards
% wt	=	Percent by weight
wt	=	Weight
NO_x	=	Nitrogen oxides
SO_2	=	Sulphurdioxide
h	=	Hour
KOH	=	Potassium hydroxide
NaOH	=	Sodium hydroxide
FFAs	=	Free fatty acid
TLC	=	Thin Layer Chromatography
% yield	=	Percent yield
% conversion	=	Percent conversion
^{1}H-NMR	=	Proton Nuclear Magnetic Resonance

CHAPTER I

INTRODUCTION

Nowadays, energy consumption in Thailand has been increasing substantially, especially diesel fuel to meet with rising demand. Presently, the country's demand for diesel fuel is about 18,000 million liters per year [1]. This type of fuel widely used in transportation, agriculture, industry, etc. However continued and increasing use of petroleum will intensify local air pollution and magnify the global warming problems caused by carbondioxide. Consequently, the search for alternative energy which is renewable and environment-friendly has been carried out. One of the extensively researched renewable around the world is biodiesel. This fuel is gaining more attention due to the depleting fossil fuel resource, its renewable, low emission particles, biodegradable and nontoxic [2].

There are four primary ways to produce biodiesel, direct use and blending, microemulsions, thermal cracking (pyrolysis) and transesterifications [3]. The most commonly used method is transesterification in which oil or fat is reacted with a monohydric alcohol in presence of catalyst. The chemical process for biodiesel production, alkali or acid is usually adopted as a catalyst. Alkali – catalyzed process has been established that gives high conversion levels of oils to methyl esters. However, it has several drawbacks, including difficulty of recycling glycerol and the need for either removal of the catalyst or the wastewater treatment. Recently, enzymatic processes have been developed. There are many advantages of using lipases as a catalyst such as easy separation of product, environmental friendly and reuse of the immobilized enzyme, etc [4].

Biodiesel is monoalkyl ester of long chain fatty acids from vegetable oils and animal fats. The types of vegetable oils used to produce biodiesel are palm oil, rapeseed, soybean, etc [5]. Most of biodiesel in Thailand are produced from palm oil because it has high yield crops in the south of the country with the lowest price. Palm oil like natural fats and oils comprises mainly of triglycerides, mono- and diglycerides.

Recently, biodiesel production from palm fatty acid, which was a by-product from palm oil production, by NOVOZYME A/S lipase in batch process gave high conversion and required short reaction time [6]. This research focuses on the development of continuous production of biodiesel from palm fatty acid using Novozym 435.

1.1 Objectives of the research

- To study continuous production of biodiesel from palm fatty acid using Novozym 435.
- To study the parameter of biodiesel production from palm fatty acid by continuous process.

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Background

Majority of the world energy needs are supplied through petrochemical sources, coal and natural gases, with the exception of hydroelectricity and nuclear energy, of all, these sources are finite and at current usage rates will be consumed shortly. Diesel fuels have an essential function in the industrial economy of a developing country and used for transport of industrial and agricultural goods and operation of diesel tractor and pump sets in agricultural sector. Economic growth is always accompanied by commensurate increase in the transport. The high energy demand in the industrialized world as well as in the domestic sector and pollution problems caused due to the widespread use of fossil fuels make it increasingly necessary to develop the renewable energy sources of limitless duration and smaller environmental impact than the traditional one. This has stimulated recent interest in alternative sources for petroleum-based fuels. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable and readily available. One possible alternative to fossil fuel is the use of oils of plant origin like vegetable oils and tree borne oil seeds. This alternative diesel fuel can be termed as biodiesel. This fuel is biodegradable and non-toxic and has low emission profiles as compared to petroleum diesel. Usage of biodiesel will allow a balance to be sought between agriculture, economic development and the environment [5].

2.1.1 Biodiesel production [2, 3]

There are four primary ways to make biodiesel, direct use and dilution, microemulsification, thermal cracking (pyrolysis) and transesterification. The most commonly used method is transesterification.

1. Dilution

Dilution of vegetable oils can be accomplished with such materials as diesel fuels, a solvent or ethanol. The dilution of sunflower oil with diesel fuels in the ratio of 1:3 by volume has been studied and engine tests. The viscosity of this blend was 4.88 cSt at 40°C. They concluded that the blend could not be recommended for long-term use in the direct injection diesel engines because of severe injector nozzle coking and sticking. A comparable blend with high oleic safflower oil was also tested and it gave satisfactory results, but its use in the long term is not applicable as it leads to thickening of lubricant.

2. Microemulsion

Microemulsions are defined as a colloidal equilibrium dispersions of optically isotropic fluid microstructures, with dimensions generally in the 1-150 nm range. These are formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphiphile. A microemulsion is designed to tackle the problem of the high viscosity of pure vegetable oils by reducing the viscosity of oils with solvents such as simple alcohols.

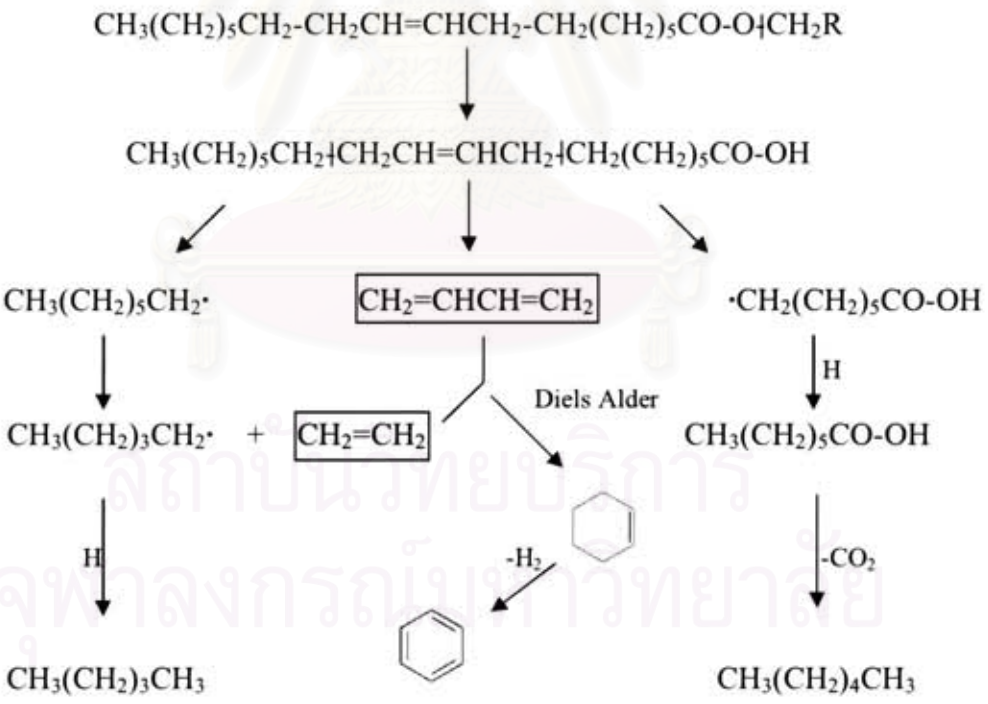
The performances of ionic and non-ionic microemulsions where found to be similar to diesel fuel, over short term testing. They also achieved good spray characteristics, with explosive vaporization which improved the combustion characteristics. In longer term testing no significant deterioration in performance was observed, however significant injector needle sticking, carbon deposits, incomplete combustion and increasing viscosity of lubricating oils.

3. Pyrolysis

Pyrolysis refers to a chemical change caused by the application of thermal energy in the presence of air or nitrogen sparge. Many investigators have studied the pyrolysis of triglycerides to obtain products suitable for diesel engines. These studies include the effect of temperature on the type of products obtained, the use of catalysts, largely metallic salts, to obtain paraffins and olefins similar to those present in hydrocarbon-based diesel fuels, the characterization of the thermal decomposition products.

Thermal decomposition of triglycerides produces the compounds of classes including alkanes, alkenes, alkadienes, aromatics and carboxylic acids. Different types of vegetable oils produce large differences in the composition of the thermally decomposed oil. The mechanism of thermal decomposition is shown in

Scheme 2.1. Mechanisms for the thermal decomposition of triglycerides are likely to be complex because of many structures and multiplicity of possible reactions of mixed triglycerides. Generally, thermal decomposition of these structures proceeds through either a free-radical or carbonium ion mechanism. Formation of homologous series of alkanes and alkenes is accountable from the generation of the RCOO radical from the triglyceride cleavage and subsequent loss of carbon dioxide. The R radical, upon disproportionation and ethylene elimination, gives the odd-numbered carbon alkanes and alkenes. The presence of unsaturation enhances cleavage at a position α, β to the unsaturation. Thermal positional isomerization and subsequent cleavage could account for the higher amounts of C_5 to C_{10} alkanes obtained from safflower compared with soybean oil. The formation of aromatics is supported by a Diels-Alder addition of ethylene to a conjugated diene formed in the pyrolysis reaction. Carboxylic acids formed during the pyrolysis of vegetable oils probably result from cleavage of the glyceride moiety.



Scheme 2.1 The mechanism of thermal decomposition of triglycerides

4. Transesterification

Transesterification, also called alcoholysis, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis, except than an alcohol is used instead of water. This process has been widely used to reduce the viscosity of triglycerides. Transesterification of triglyceride with alcohol shown in Figure 2.1.

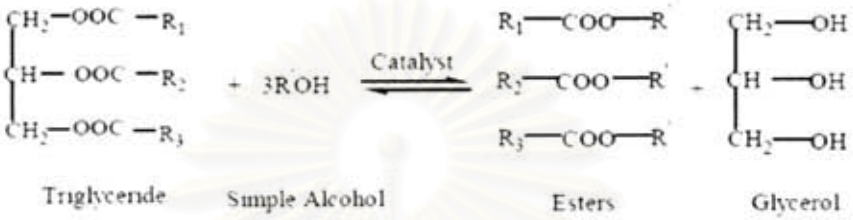


Figure 2.1 Transesterification of triglycerides with alcohol.

2.2 Biodiesel

Biodiesel, an alternative diesel fuel, is made from renewable biological sources such as vegetable oils and animal fats. It is biodegradable and nontoxic which has low emission profiles and so is environmentally beneficial. Biodiesel has been defined as the monoalkyl esters of long-chain fatty acids derived from renewable feedstocks, such as vegetable oils or animal fats, for use in compression-ignition (diesel) engines. The biodiesel that is considered as a possible substitute or extender of conventional diesel fuel is commonly composed of fatty acid methyl esters that are prepared from the triglycerides in vegetable oils by transesterification with methanol. The resulting biodiesel is quite similar to conventional diesel fuel in its main characteristics. Biodiesel is compatible with conventional diesel and both can be blended in any proportion. A number of plants are manufacturing biodiesel worldwide. These units are using sunflower oil, used-frying oil, jatropha oil, etc. as a source of triglycerides [3].

2.2.1 Biodiesel advantages [2]

1. Conventional diesel engines can be operated without much, if any, modification on biodiesel.

2. Biodiesel can be used pure or in a mixture with hydrocarbon-based diesel fuels.

3. Biodiesel is nontoxic, safe to handle and biodegradable.

4. No evaporation of low-boiling components takes place.

5. Exhaust gas is free of SO_2 and halogens.

6. There is substantial reduction of soot, unburnt hydrocarbons, and also of carbon monoxide (when an oxidation catalyst is used) in the exhaust gases.

7. NO_X emissions increase slightly if there are no changes in the engine setting.

8. Good performance in auto-ignition of fatty esters results in a smooth running diesel engine.

9. Biodiesel consumption is similar to hydrocarbon-based diesel fuels.

2.3 Raw materials for biodiesel [7]

A major source of fuel energy comes from nonrenewable, potentially exhaustible, resources such as fossil fuels (natural gas, petroleum, and coal). These sources have environmental and toxic problems and they are not biodegradable.

1. Plant-Derived Oils

Plant-derived oils (TAG) as a substitute raw material for diesel were considered a good energy supply because they are carbon dioxide neutral. This stems from the fact that green plants grow through the photosynthesis process with CO_2 as a carbon source. Therefore, the combustion of plant-derived oils will release carbon dioxide which has previously been fixed through photosynthesis. Plant-derived fuels are renewable, inexhaustible, nontoxic and biodegradable, with an energy content similar to that of fossil diesel fuel. The fatty acid composition of the source oil or fat is important in biodiesel because, in the winter, oils containing more saturated fatty acids than unsaturated fatty acids may solidify and clog the fuel lines. The fatty acids found in vegetable oils and typical fatty acid compositions of common oil sources are summarized in Table 2.1 and Table 2.2, respectively. Table 2.3 showed the names, in a variety of formats, of common fatty acids.

Refined oils are more expensive but have a low production scale. Of all the vegetable oils available, high oleic acid containing oils are preferred because of the increased stability of their alkyl esters on storage and improved fuel properties. Soybean, palm kernel, cottonseed, sunflower, safflower, rapeseed, peanut, groundnut and castor bean oils are the more commonly used oils in biodiesel production.

Table 2.1 Percentage of fatty acid type for different oils [8]

Vegetable oil	Fatty acid composition, % by weight								
	16:0	18:0	20:0	22:0	24:0	18:1	18:2	18:3	22:1
Corn	12	2	1	0	0	25	61	1	0
Cottonseed	28	1	0	0	0	13	58	0	0
Crambe	2	1	2	1	1	19	9	7	59
Rapeseed	4	1	0	0	0	64	22	8	0
Soybean	12	3	0	0	0	23	56	7	0
Sunflower seed	6	3	0	0	0	17	74	0	0
Canola oil	6	2	1	1	0	55	24	9	1
Palm	44	4	1	0	0	40	10	0	0
Butter	30	30	2	1	0	30	3	0	2
Peanut	6	6	10	10	0	66	38	0	0
Linseed	9	1	0	0	0	9	8	45	0
Tung	0	0	0	0	0	13	15	72	0

Table 2.2 Typical fatty acid composition-common oil sources [9]

Fatty acid	Soybean	Cotton	Palm	Lard	Tallow	Coconut seed
Lauric, C12:0	0.1	0.1	0.1	0.1	0.1	46.5
Myristic, C14:0	0.1	0.7	1.0	1.4	2.8	19.2
Palmitic, C16:0	10.2	20.1	42.8	23.6	23.3	9.8
Stearic, C18:0	3.7	2.6	4.5	14.2	19.4	3.0
Oleic, C18:1	22.8	19.2	40.5	44.2	42.4	6.9
Linoleic, C18:2	53.7	55.2	10.1	10.7	2.9	2.2
Linolenic, C18:3	8.6	0.6	0.2	0.4	0.9	0.0

Table 2.3 Names and structures of the most common fatty acids [10]

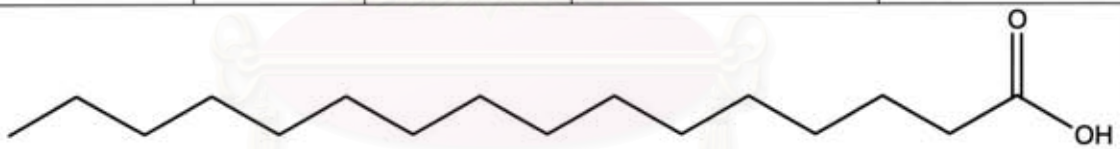
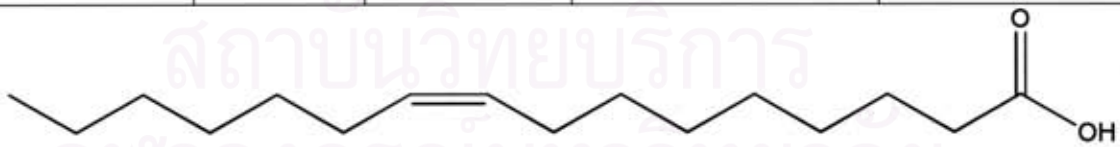
No. of C:No. of double bound	Molecular formular	Molecular mass	Systematic name	Other names
C12:0	$C_{12}H_{24}O_2$	200.32	Dodecanoic acid	Lauric acid, n-Dodecanoic acid
				
C14:0	$C_{14}H_{28}0_2$	228.38	Tetradecanoic acid	Myristic acid
				
C16:0	$C_{16}H_{32}O_2$	256.43	Hexadecanoic acid	Palmitic acid, Hexadecylic acid, Cetylic acid
				
C16:1	$C_{16}H_{30}O_2$	254.41	9-hexadecanoic acid	Palmitoleic acid
				

Table 2.3 Names and structures of the most common fatty acids (*Continued*)

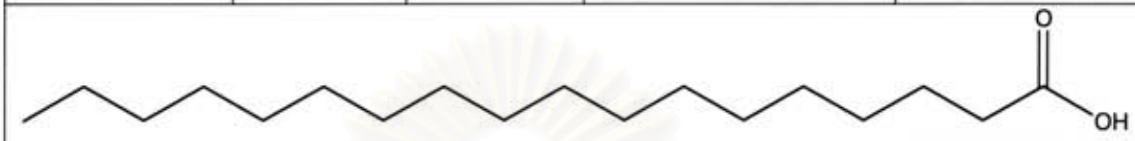
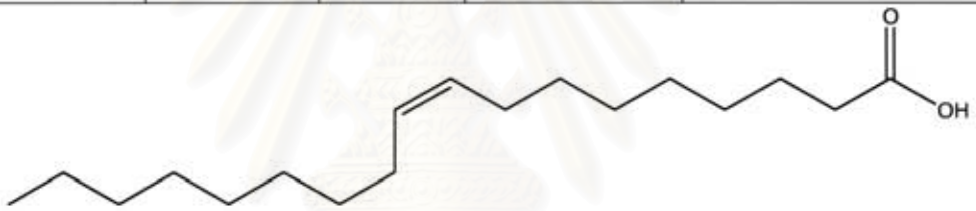
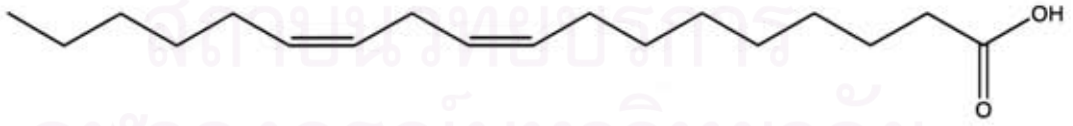
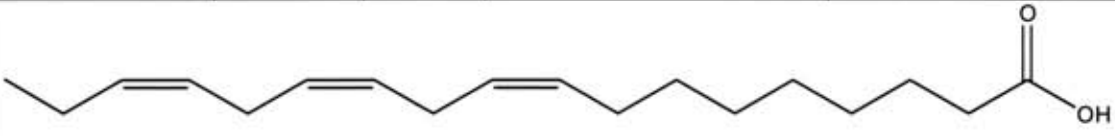
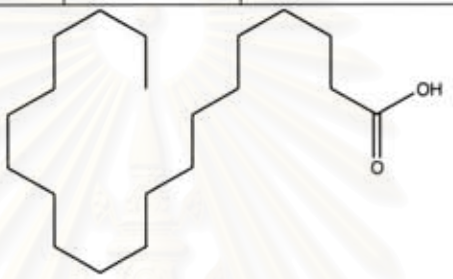
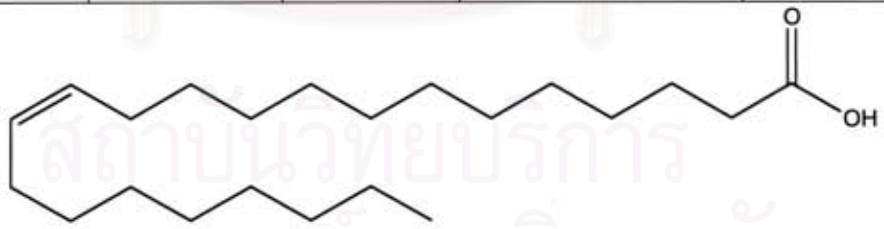
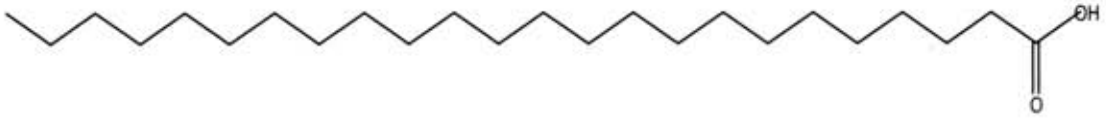
No. of C:No. of double bound	Molecular formular	Molecular mass	Systematic name	Other names
C18:0	$C_{18}H_{36}O_2$	284.48	Octadecanoic acid	Stearic acid
				
C18:1$^{\Delta 9}$	$C_{18}H_{34}0_2$	282.46	9-octadenoic acid	Oleic acid, (9Z)-Octadecenoic acid, (Z)-Octadec-9-enoic acid, *Cis*-9-octadecenoic acid, Cis-Δ9-octadecenoic acid
				
C18:2$^{\Delta 9,12}$	$C_{18}H_{32}O_2$	280.46	(9,12)-Octadecadienoic acid	Linoleic acid, Cis-cis-9,12-Octadecadienoic acid
				
C18:3$^{\Delta 9,12,15}$	$C_{18}H_{30}0_2$	278.44	(9,12,15)-Octadecatrienoic acid	α-Linolenic acid, Cis,cis,cis-9,12,15-Octadecatrienoic acid
				

Table 2.3 Names and structures of the most common fatty acids (*Continued*)

No. of C:No. of double bound	**Molecular formular**	**Molecular mass**	**Systematic name**	**Other names**
C20:0	$C_{20}H_{40}O_2$	312.54	Eicosanoic acid	Arachidic acid, Arachic acid Eicosanoic acid, *n*-eicosanoic acid
				
C22:0	$C_{22}H_{44}0_2$	340.60	Docosanoic acid	Behenic acid
				
C22:1	$C_{22}H_{42}0_2$	338.58	13-Docosenoic	Erucic acid *Cis*-13-Docosenoic
				
C24:0	$C_{22}H_{48}O_3$	368.63	Tetracosanoic acid	Lignoceric acid
				

2. Waste Oils and Fats [7]

Waste oils and fats, used frying oils, lard, beef tallow, yellow grease and other hard stock fats can also be used in preparing biodiesel. Used frying oils, while cheap, may have some disadvantages because of the contents of high polymerization products, high free fatty acid contents, susceptibility to oxidation and high viscosity. Therefore, preliminary treatment such as the use of adsorbent materials (such as magnesium silicates) to reduce the free fatty acid content and polar contaminants may be necessary to improve the oil quality prior to transesterification to produce biodiesel catalyzed by a basic catalyst.

3. Microbial Oils

Microalgal oils represent another cheap source of renewable raw materials for biodiesel production that has received little or no attention. Algal oils are largely produced through substrate feeding and heterotrophic fermentation. Li et al.[11] reported the production of biodiesel on a large scale using the oils from microalga, *Chlorella* protothecoids, in bioreactors. The lipid content of the microalga was increased up to 44-48% of the cell dry weight. The oils were then used to produce biodiesel (98% conversion to FAME) by a reaction catalyzed by immobilized *Candida* sp. lipase at a substrate molar ratio of 3:1 and a reaction time of 12 hours. The product was said to be comparable to conventional biodiesel in physical properties. Table 2.4 showed the fatty acids compositions of microorganism oil.

Table 2.4 Fatty acid compositions of microorganism oil

Microorganism oil	**Fatty acid composition**								
	14:0	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**	**20:4**	**20:5**	**22:1**
Algae	-	/	/	/	/	/	-	-	-
Yeast	/	/	/	/	/	/	/	-	-
Fungus	/	/	/	/	/	/	/	/	-

Remark ; / = the fatty acids were found in microorganism oil.

- = the fatty acids were not found in microorganism oil.

2.4 Palm oil

The oil palm is a monocotyledon belonging to the genus *Elaeis*. It is a perennial tree crop and the highest oil producing plant. The crop is unique in that it produces two types of oil. The fleshy mesocarp produces palm oil, which is used mainly for its edible properties and the kernel produces palm kernel oil, which has wide application in the oleochemical industry. The genus *Elaeis* comprises two species, namely *E. guineensis*, *E. oleifera*. Currently, most of the world's production of palm oil comes from South-East Asia, in particular Malaysia and Indonesia.

Like all oils, TGs are the major constituents of palm oil. Over 95% of palm oil consists of mixtures of TGs. During oil extraction from the mesocarp, the hydrophobic TGs attract other fat- or oil-soluble cellular components. These are the minor components of palm oil such as phosphatides, sterols, pigments, tocopherols, tocotrienols and trace metals. Other components in palm oil are the metabolites in the biosynthesis of TGs and products from lipolytic activity. These include the monoglycerols (MGs), diglycerols (DGs) and free fatty acids (FFAs). The oil palm fruit is a drupe, which forms in a tight bunch. The pericarp comprises three layers: the exocarp (skin); mesocarp (outer pulp containing palm oil); and endocarp (a hard shell enclosing the kernel (the endosperm) which contains oil and carbohydrate reserves for the embryo). Composition of palm oil fruit is shown in Figure 2.2.

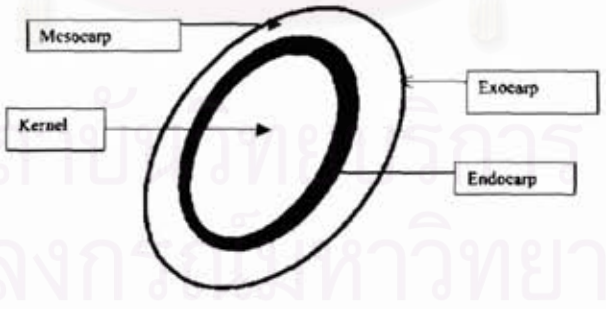
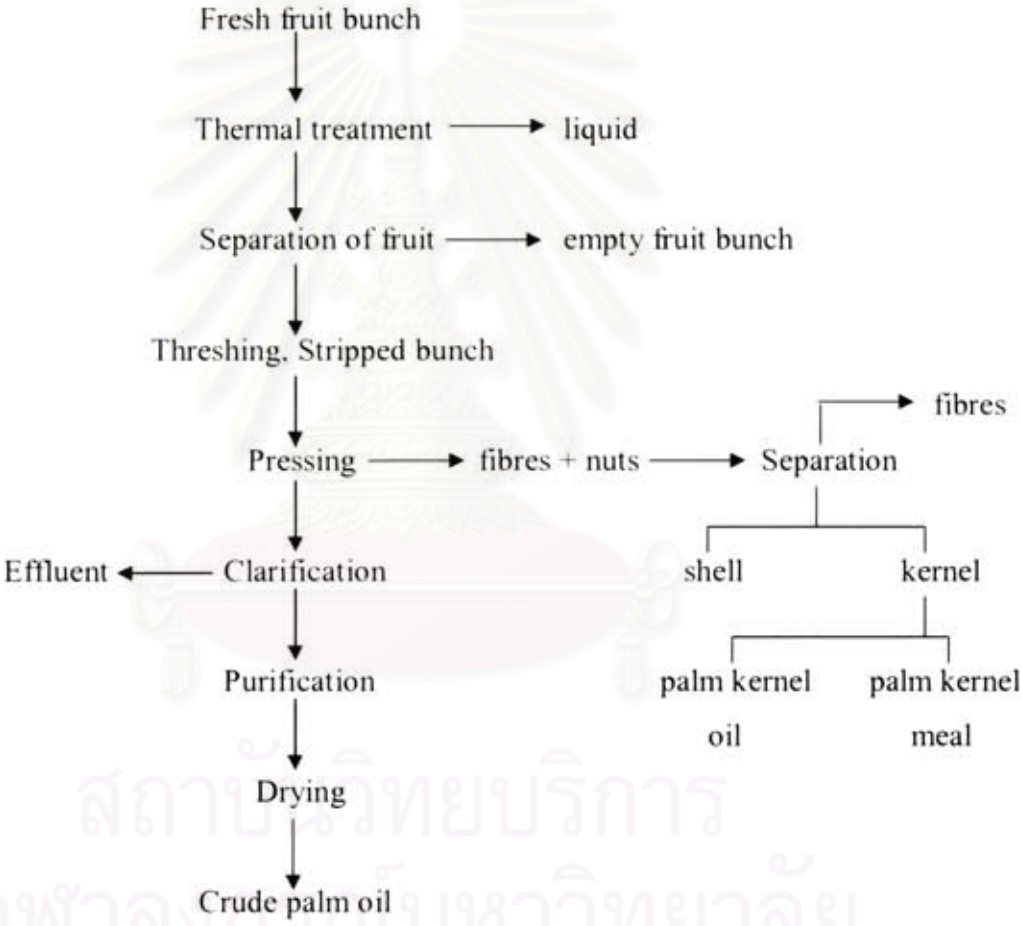


Figure 2.2 Composition of palm oil fruit.

Palm oil is available in a variety of forms: crude palm oil (CPO, the production of crude palm oil is shown in Scheme 2.2), palm olein, refined, bleached and deodorized (RBD) palm oil, fractionated palm olein, palm stearin and palm midfraction. The extracted oil is known as CPO. The oil palm being extracted can be used in two different ways, the first part which contains 90% is used for food and food derivatives and the rest is used for non-food products. The use of non-food product is rather small but it has high value [12].



Scheme 2.2 Diagram of the production of crude palm oil [13]

Biodiesel typically contains up to 14 different types of fatty acids that are chemically transformed into fatty acid alkyl ester. Compositions of palm oil are within the range of those types of fatty acids shown in Table 2.5.

Table 2.5 Typical (%) fatty acid composition of palm oil and palm kernel oil [14]

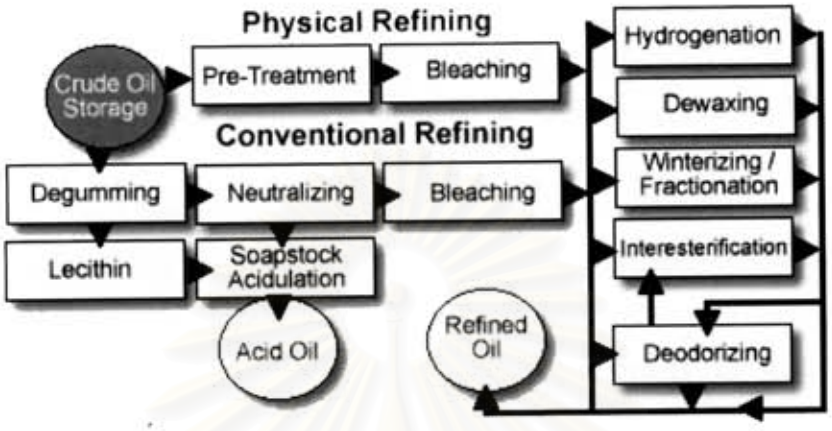
Fatty acids oil	Carbon & Double bond	%Fatty acid content	
		Palm oil	Palm kernel
Caprylic	C8		2-4
Capric	C10		3-7
Lauric	C12		45-52
Myristic	C14	1-6	14-19
Palmitic	C16:0	32-47	6-9
Palmitoleic	C16:1		0-1
Stearic	C18:0	1-6	1-3
Oleic	C18:1	40-52	10-18
Linoleic	C18:2	2-11	1-2
Linolenic	C18:3		
Arachidic	C20:0		1-2
Eicosenoic	C20:1		
Behenic	C22:0		1-2
Euricic	C22:1		

CPO has a rich orange-red colour because of its high content of carotene (700-800 ppm). The major carotenoids in palm oil are β- and α-carotene, which account for 90% of the total carotenoids. Carotenoids are the precursors of vitamin A, with β-carotene having the highest provitamin A activity.

2.4.1 Refining process

Refining of oil is carried out or to remove solid material such as phospholipids, free fatty acid and colored impurities. A number of processes are employed for this purpose, including treatment with alkali and absorbent materials. A further process, known as deodorization, may be applied to edible oils. It consists of treatment with steam at high temperatures and under low pressure to remove volatile material such as residual solvent, certain free acids and other substances which would give undesirable tastes or odors. A final treatment for edible oils may consist of

hydrogenation and blending of the hydrogenated product with other oils to obtain a product of the desired characteristics. Vegetable oil physical refining process is shown in Scheme 2.3.



Scheme 2.3 Vegetable oil physical refining process

Fatty acid is a by product of the refining of edible vegetable oil process. The price of fatty acid is less than refined vegetable oil.

2.4.1.1 Step of refining vegetable oil [15]

1. Degumming

Gums in vegetable oil need to be removed to avoid colour and taste reversion during subsequent refining steps. Stage phosphoric acid treatment and a stage hot water treatment followed by continuous removal of the hydrated gums in a degumming centrifuge. The process is applied to many oils that contain phospholipids in significant amounts. Since the separated phospholipids are rather waxy or gummy solids, the term degumming was quite naturally applied to the separation. The aqueous phase can be removed from the lipids and phospholipids can be removed from the oil.

2. Neutralizing

The neutralization step is necessary to remove free fatty acids from the oil. This can be done in one of two ways: alkali (chemical) or steam stripping (physical) means.

(a) Alkali/chemical method: caustic soda (alkali) is mixed in the proper amounts and the aqueous solution is removed, leaving the neutral oil behind.

(b) Steam stripping: This is done under vacuum, to remove moisture, free fatty acids, odor bodies and other impurities from the oil. As it is performed under vacuum conditions, the oil can be kept at a low temperature, preserving its chemical structure by not subjecting it to temperatures in which undesirable dehydration reactions can occur.

3. Bleaching

Bleaching earth (adsorbent natural or activated earth, mixed with activated carbon if necessary): high-activity clay is added into oil, mix and heat mixture of oil and clay to make the small particle of pigment absorbed on the crystal of clay. At last filtering the oil, perfect mechanism, liable performance, well configured equipment. Bleaching results in the removal of coloring materials, phospholipids and oxidation products.

4. Dewaxing

Dewaxing process includes slow chilling of the oil to temperatures sufficient to crystallize the waxy components from the crude oil, preferably under gentle agitation. The crystallized components are then generally removed by a cold filtration step. Dewaxing can improve oil transparency and brightness.

5. Deodorizing

Deodorization results in the removal of odor from the oil. Most heat of bleached oil is recovered by heat exchangers, the bleached oil is heated to the process temperature by mineral oil or high pressure steam and then the oil enters into the combined deodorizer, the deodorizer is a combined type: the upper is packing

structure, which is used to remove odor components like FFA, the lower is plate type used for heat bleaching and making product quality more consistent. Oil coming from the deodorizer is cooled and stored after series of heat exchange; volatile like FFA is collected and stored as by products.

6. Winterizing

For refining products to be bottled as edible oils (e.g. rice bran oil, sunflower seed oil or corn oil) winterization is required to achieve the necessary cold stability. Winterization prevents crystallization and clouding of the waxes contained in the oil at ambient temperature. It is often desirable to remove the traces of waxes (*e.g.*, cuticle wax from seed coats) and the higher-melting glycerides from fats. Waxes can generally be removed by rapid chilling and filtering. Separation of high-melting glycerides, or stearine, usually requires very slow cooling in order to form crystals that are large enough to be removed by filtration or centrifuging.

2.5 Biodiesel catalyst [16]

2.5.1 Acid-catalyst

The processes are catalyzed by Bronsted acids, preferably by sulfonic acids, sulfuric acid and hydrochloric acid. These catalysts give very high yields in alkyl esters but the reactions are slow and requiring temperature above 100°C and more than 3 hours to reach the complete conversion. Transesterification process under acid-catalyzed condition needs to be done in absences of water because the water reduces the yield alkyl ester. The alcohol/oil molar ratio is one of the main factors that influence the transesterification. An excess alcohol favors the formation of products. On the other hand, an excessive amount of alcohol makes the recovery of glycerol difficult. Mechanism of the acid catalyst transesterification is shown in Figure 2.3.

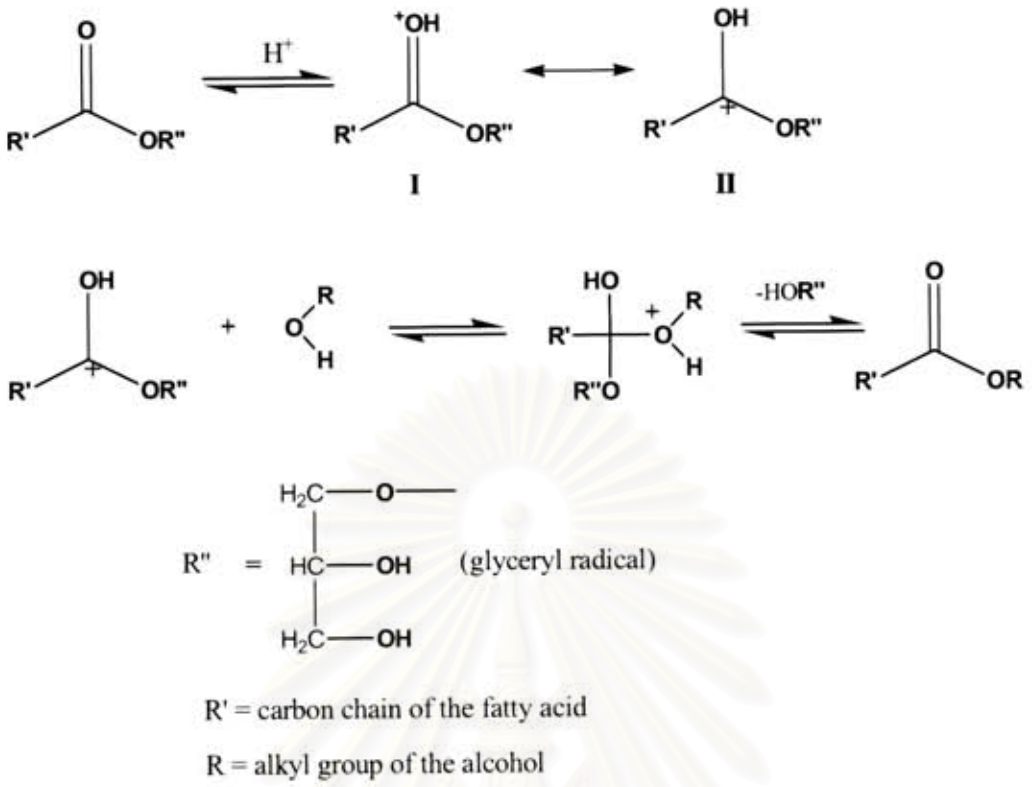


Figure 2.3 Mechanism of the acid catalyst transesterification.

2.5.2 Base-catalyst

This reaction is base catalysed. Commonly the base (KOH and NaOH) is dissolved in the alcohol to make a convenient method of dispersing the otherwise solid catalyst into the oil. The ROH needs to be very dry. Any water in the process promotes the saponification reaction and inhibits the transesterification reaction. Mechanism of the base catalyst transesterification is shown in Figure 2.4.

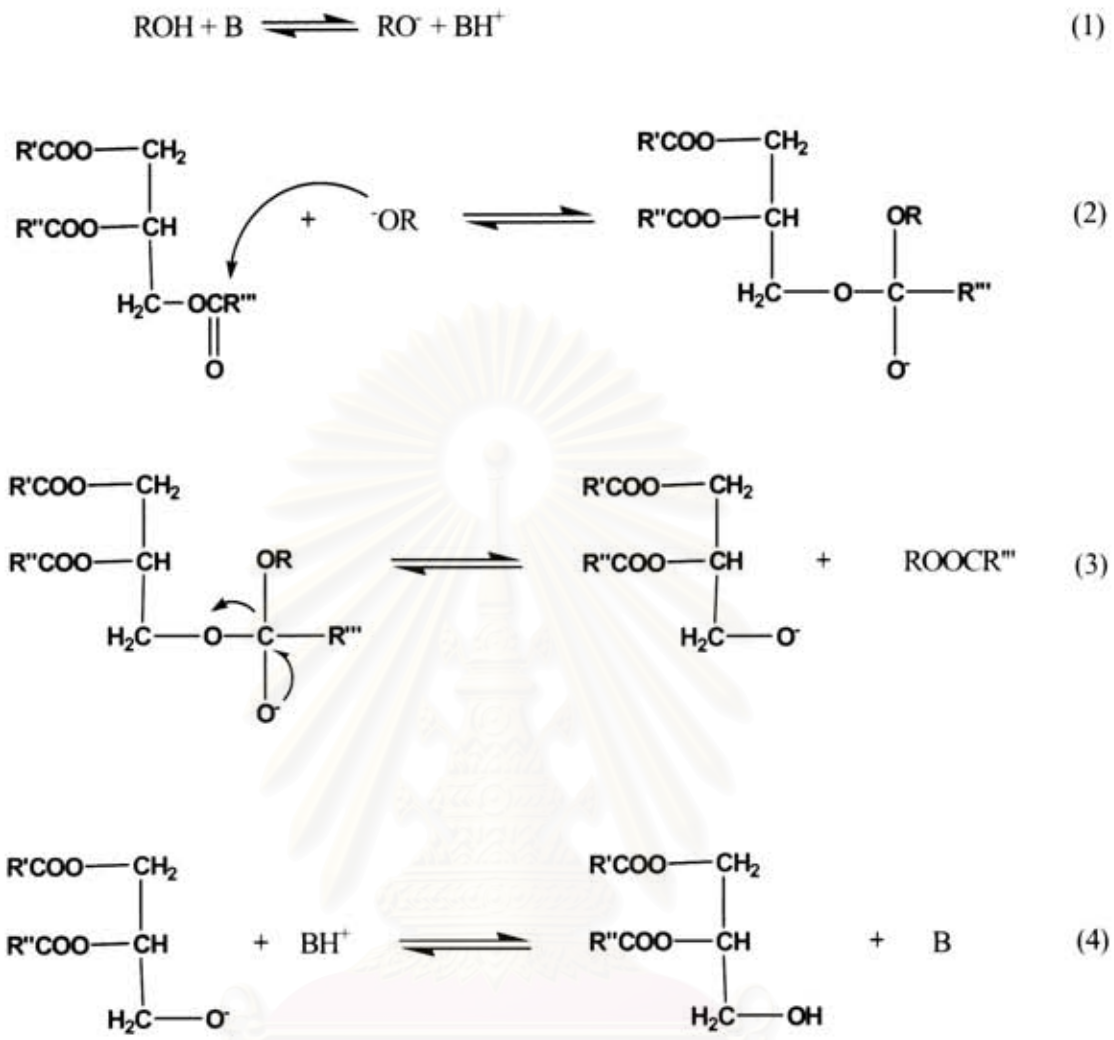


Figure 2.4 Mechanism of the base catalyst transesterification.

2.5.3 Enzyme catalyst

Although chemical transesterification using an alkali-catalysis process gives high conversion levels of triglycerides to their corresponding methyl esters in short reaction times, the reaction has several drawbacks: it is energy intensive, recovery of glycerol is difficult, the acidic or alkaline catalyst has to be removed from the product, alkaline wastewater requires treatment and free fatty acids and water interfere with the reaction. Both extracellular and intracellular lipases are also able to effectively catalyze the transesteritication of triglycerides in either aqueous or nonaqueous systems is shown in Table 2.6, enzymatic transesterification methods can overcome the problems mentioned above. In particular, it should be noted that the by-product, glycerol, can be easily recovered without any complex process and also that free fatty

acids contained in waste oils and fats can be completely converted to methyl esters by esterification. On the other hand, in general the production cost of a lipase catalyst is significantly greater than that of an alkaline one. Lipases catalyze not only transesterification, but also esterification. Esterification of free fatty acid is shown in Figure 2.5.

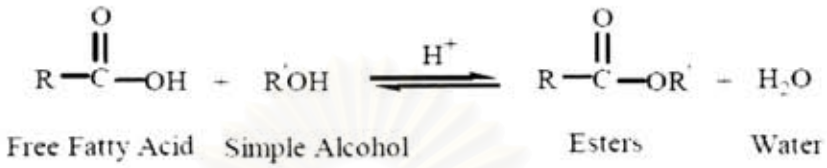


Figure 2.5 Esterification of free fatty acid.

Table 2.6 Comparison between alkali-catalysis and lipase-catalysis methods for biodiesel fuel production [4]

	Alkali-catalysis process	**Lipase-catalysis process**
Reaction temperature	60-70°C	30-40°C
Free fatty acids in raw materials	Saponified products	Methyl esters
Water in raw materials	Interference with the reaction	No influence
Yield of methyl esters	Normal	Higher
Recovery of glycerol	Difficult	Easy
Purification of methyl esters	Repeated washing	None
Production cost of catalyst	Cheap	Relatively expensive

2.6 Immobilized enzyme [17]

Lipases (triacylglycerol acylhydrolases E.C.3.1.1.3) are enzymes that hydrolyse fatty acyl ester bonds of acylglycerols at the interface between oil and water. Lipases have been extensively studied because of their actual and potential applications in the detergent, oil and food industries. Recently, various strategies in the pharmaceutical and chemical industries have used lipases in the synthesis of optically pure drugs and

agrochemicals that are more effective and produce fewer side effects compared with their racemates.

Immobilized enzymes are becoming increasingly popular as reusable, selective analytical chemical reagents in solid-phase flow-through reactors, as membranes in sensors and as films in dry reagent kits. Immobilized enzymes are becoming increasingly popular as reusable, selective analytical chemical reagents in solid-phase flow-through reactors, as membranes in sensors and as films in dry reagent kits.

Principal method of immobilization is shown in Figure 2.6. There is a variety of methods by which enzymes can be localized, ranging from covalent chemical bonding to physical entrapment however they can be broadly classified as follows:

1. Covalent bonding of the enzyme to a derivatized, water-insoluble matrix.
2. Intermolecular cross-linking of enzyme molecules using multi-functional reagents.
3. Adsorption of the enzyme onto a water-insoluble matrix.
4. Entrapment of the enzyme inside a water-insoluble polymer lattice or semi-permeable membrane.

2.6.1 Advantages of immobilized enzyme

1. The enzyme is easily removed.
2. The enzyme can be packed into columns and used over a long period.
3. Speedy separation of products reduces feedback inhibition.

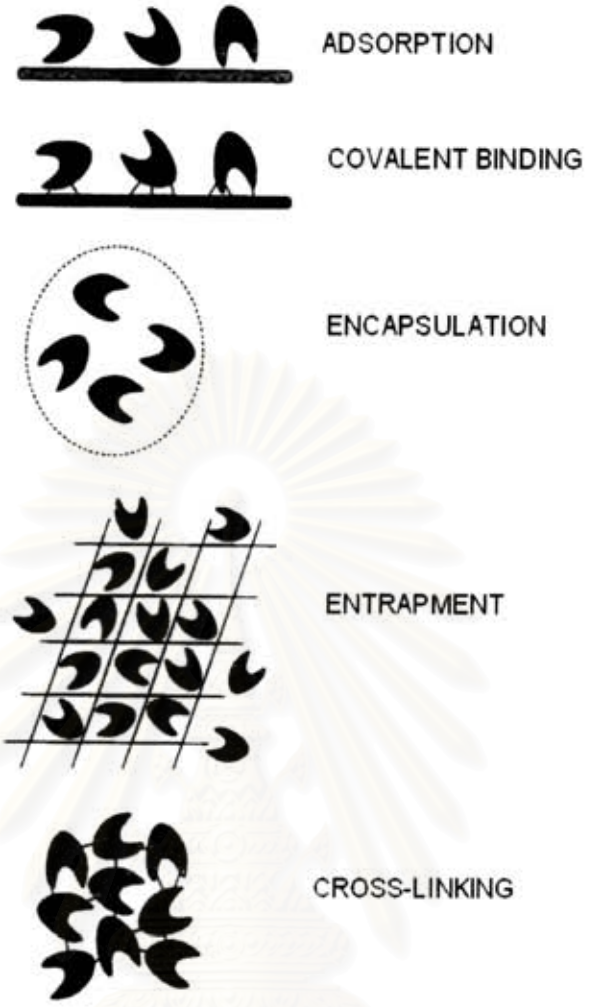


Figure 2.6 Principal method of immobilization [16].

2.7 Biodiesel property

Biodiesel is made up of fourteen different types of fatty acids, which are transformed into fatty acid methyl esters (FAME) by transesterification. Different fractions of each type of FAME present in various feedstocks influence some properties of fuels. Table 2.7 shows some of the properties defined in the ASTM standards for diesel and biodiesel. For Thailand, it has set legislative assembly characteristic and quality of biodiesel shown in Table 2.8.

Table 2.7 Comparison of fuel properties between diesel and biodiesel [18]

Fuel property	Diesel	Biodiesel
Fuel standard	ASTM D975	ASTM PS 121
Fuel composition	C10-C21 HC	C12-C22 FAME
Lower heating value, Btu/gal	131,295	117,093
Viscosity, @ 40° C	1.3-4.1	1.9-6.0
Specific gravity kg/l @ 60° F	0.85	0.88
Density, lb/gal @ 15° C	7.079	7.328
Water, ppm by wt	161	0.05% max
Carbon, wt %	87	77
Hydrogen, wt %	13	12
Oxygen, by dif. wt %	0	11
Sulfur, wt %	0.05 max	0.0 - 0.0024
Boiling point (°C)	188-343	182-338
Flash point (°C)	60-80	100-170
Cloud point (°C)	-15 to 5	-3 to 12
Pour point (°C)	-35 to -15	-15 to 10
Cetane number	40-55	48-65
Stoichiometric air/fuel ratio wt./wt.	15	13.8
BOCLE Scuff, grams	3,600	>7,000

Table 2.8 Characteristic and quality of biodiesel (methyl ester of fatty acids) in Thailand [19]

Characteristic	Value	Method of standard
Methyl ester, %wt.	>96.5	EN 14103
Density at 15°C, kg/m^3	860-900	ASTM D 1298
Viscosity at 40°C, cSt	3.5-5.0	ASTM D445
Flash point, °C	>120	ASTM D 93
Sulphur,%wt.	<0.0010	ASTM D 2622
Carbon residue, on 10% distillation residue, %wt	<0.30	ASTM D 4530
Cetane number	>51	ASTM D 613
Sulfated ash, %wt.	<0.02	ASTM D 874
Water, %wt.	<0.050	ASTM D 2709
Total contaminate, %wt.	<0.0024	ASTM D 5452
Copper strip corrosion	<96.5	ASTM D 130
Oxidation stability at 110°C, hours	>6	EN 14112
Acid value, mg KOH/g	<0.50	ASTM D 664
Iodine value, g Iodine/100 g	<120	EN 14111
Linolenic acid methyl ester, %wt.	<12.0	EN 14103
Methanol, %wt.	<0.20	EN 14110
Monoglyceride, %wt.	<0.80	EN 14105
Diglyceride, %wt.	<0.20	EN 14105
Triglyceride, %wt.	<0.20	EN 14105
Free glycerin, %wt.	<0.02	EN 14105
Total glycerin, %wt.	<0.25	EN 14105
Group I metals (Na+K)	<5.0	EN 14108 and EN 14109
Group II metals (Ca+Mg)	<5.0	EN 14538
Phosphorus, %wt.	<0.0010	ASTM D 4951

2.8 Literature reviews

2.8.1 Batch process

In 1999 Watanabe and coworkers [20] studied stepwise ethanolysis of tuna oil using immobilized *Candida antractica* lipase. The first step was carried out at 40°C for 12 hours in a mixture of tuna oil and l/3 molar equivalent of ethanol with 4% immobilized lipase; the second step was performed for 36 hours after adding 2/3 molar equivalent of ethanol. The three-step reaction was conducted as follows: the first step was conducted under the same conditions as those in the two-step ethanolysis; in the second and third steps, l/3 molar equivalent of ethanol was added after 12 and 24 hours, respectively; and in the third step, the mixture was shaken for 24 hours. Both types of ethanolysis achieved the conversion of 95%. The two- and three-step reactions maintained over 95% of the conversion for 70 d and over 100 d, respectively.

In 2002 Kose and coworkers [21] studied immobilized *Candida antarctica* lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium. The refined cotton seed oil of Turkish origin with primary and secondary alcohols was investigated in the presence of an immobilized enzyme from *Candida antarctica*, commercially called Novozym 435 in a solvent-free medium. The optimum conditions of the methanolysis were as follows: 30% enzyme based on oil weight; oil/alcohol molar ratio 1:4; temperature: 50°C and reaction time: 7 hours. Maximum methyl esters (ME) yield was 91.5%. At the same conditions cotton seed oil was converted with short-chain primary and secondary alcohols to its corresponding esters with conversions between 72% and 94%. The results indicated that alcoholysis products of cotton seed oil could be used as valuable intermediates in oleochemistry.

In 2002 Shimada and coworkers [22] studied enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. Two-step batch methanolysis: the first-step reaction was conducted in the presence of 1/3 molar equivalent of MeOH for the stoichiometric amount, and the second-step reaction was performed by adding 2/3 molar equivalent of MeOH. If the immobilized carrier is destroyed by agitation in a reactor with impeller, three-step flow reaction will be

available: the first-step substrates were waste oil and 1/3 molar equivalent of MeOH; the second-step, the first-step eluate and 1/3 molar equivalent of MeOH; the third-step, the second-step eluate and 1/3 molar equivalent of MeOH. The conversion of waste oil to biodiesel fuel reached >90% in the two reaction systems, and the lipase catalyst could be used for >100 days without decrease of the activity.

In 2003 Chen and coworkers [23] studied regeneration of immobilized *Candida antarctica* lipase for transesterification. Immobilized lipase is frequently deactivated by lower alcohols with deactivation being caused by the immiscibility between triglycerides and methanol or ethanol. When the lower alcohol is adsorbed to the immobilized enzyme, the entry of triglycerides is blocked, which causes the reaction to stop. An alcohol with three or more carbon atoms, preferably 2-butanol or *tert*-butanol, can regenerate the deactivated immobilized enzyme. The present work established that the activity of immobilized lipase could be significantly increased when such alcohols were used for an immersion pretreatment of the enzyme. The activity of the commercially available immobilized enzyme, Novozym 435, increased about tenfold in comparison to the enzyme not subjected to any pretreatment. Following complete deactivation of the enzyme by methanol, washing with 2-butanol and *tert*-butanol successfully regenerated the enzyme and restored it to about 56% and 75% of its original activity level, respectively.

In 2005 Hass and coworkers [24] studied improving the economics of biodiesel production through the use of low value lipids as feedstocks: vegetable oil soapstock. The most effective method involved the complete saponification of the soapstock followed by acidulation using methods similar to those presently employed in industry. This resulted in an acid oil with a free fatty acid (FFA) converted to methyl esters by acid-catalyzed esterification. Following a simple washing protocol, this preparation met the established specifications for biodiesel of the ASTM standard. Engine emissions and performance during operation on soy soapstock biodiesel were comparable to those on biodiesel from soy oil. An economic analysis suggested that the production cost of soapstock biodiesel would be approximately US$ 0.41/L, a 25% reduction relative to the estimated cost of biodiesel produced from soy oil.

In 2006 Li and coworkers [25] studied Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium. Combined use of Lipozyme TL IM and Novozym 435 was proposed further to catalyze the methanolysis and the highest biodiesel yield of 95% could be achieved under the optimum conditions (*tert*-butanol/oil volume ratio 1:1; methanol/oil molar ratio 4:1; 3% Lipozyme TL IM and 1% Novozym 435 based on the oil weight; temperature 35°C; 130 rpm, 12 hours). There was no obvious loss in lipase activity even after being repeatedly used for 200 cycles with *tert*-butanol as the reaction medium. Furthermore, waste oil was also explored for biodiesel production and it has been found that lipase also showed good stability in this novel system.

In 2006 Saifon [6] studied biodiesel using NOVOZYMS A/S from *antractica* lipase. The optimized methyl ester biodiesel production was determined from palm soapstock. The experiment reaction was catalyzed by immobilized lipase, NOVOZYMS A/S from *antractica* lipase. The optimum condition consisted of the palm soapstock and methanol in the ratio of 1:2 and NOVOZYMS A/S 15% (w/w) of palm soapstock at 60°C for one hour. Besides the previous NOVOZYMS A/S was also used in the next batch with the same condition for two hours. The synthesized methyl ester was 89.53%.

In 2007 Watanabe and coworkers [26] studied conversion of acid oil by-product in vegetable oil refining to biodiesel fuel by immobilized *Candida antractica* lipase. The first-step reaction was conducted by shaking a mixture of 66 wt% acid oil (77.9 wt% FFAs, 10.8 wt% acylglycerols) and 34 wt% MeOH with 1 wt% immobilized lipase, to convert FFAs to their methyl esters. The second-step reaction was performed by shaking a mixture of 52.3 wt% dehydrated first-step product (79.7 wt% FAMEs, 9.7 wt% acylglycerols), 42.2 wt% rapeseed oil, and 5.5 wt% MeOH using 6 wt% immobilized lipase in the presence of additional 10 wt% glycerol, to convert acylglycerols to FAMEs. The resulting product was composed of 91.1 wt% FAMEs, 0.6 wt% FFAs, 0.8 wt% triacylglycerols, 2.3 wt% diacylglycerols, and 5.2 wt% other compounds. Even though each step of reaction was repeated every 24 hours by transferring the immobilized lipase to the fresh substrate mixture, the composition was maintained for >100 cycles.

In 2008 Jeong and coworkers [27] studied lipase-catalyzed transesterification of rapeseed oil for biodiesel production with *tert*-butanol. The application of Novozym 435 was determined to catalyze the transesterification process and a conversion of 76.1% was achieved under selected conditions (reaction temperature 40°C, methanol/oil molar ratio 3:1, 5% (w/w) Novozym 435 based on the oil weight, water content 1% (w/w), and reaction time of 24 hours). Under these reaction conditions, a conversion of approximately 76.1% was achieved. It has also been determined that rapeseed oil can be converted to fatty acid methyl ester using this system and the results of this study contribute to the basic data relevant to the development of continuous enzymatic processes.

2.8.2 Continuous process

In 1999 Dossat and coworkers [28] studied continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor: influence of the glycerol production. The transesterification of high oleic sunflower oil with butanol by the immobilized Lipozyme in *n*-hexane was carried out in a continuous packed bed reactor, oleic acid, butyl ester and glycerol being formed as the main products. It was found that glycerol, insoluble in *n*-hexane, remained in the reactor adsorbed onto the enzymatic support, leading to a drastic decrease in enzymatic activity. The phenomenon involved in this loss of activity was attributed to the formation of an hydrophilic hindrance around the enzyme resulting in diffusion limitations of the hydrophobic substrate from the organic phase to the enzyme. To recover enzymatic activity, several solutions are proposed. The addition of silica gel into the enzymatic bed to adsorb the produced glycerol did not enable this loss of activity to be avoided. In order to enhance the solubility of glycerol in the reaction medium as soon as it was produced, *n*-hexane amended acetone was used as solvent, but high conversion of sunflower oil was not restored. Finally, by intermittent rinsing of the catalyst bed with a solution of tertiary alcohol amended with water to obtain the optimal thermodynamic water activity of 0.54, glycerol was eliminated from the reactor, and high conversion was maintained.

In 2006 Nie and coworkers [29] studied lipase catalyzed methanolysis to produce biodiesel: optimization of the biodiesel production. Continuous reaction in a fixed bed reactor in three-step transesterification with methanol of oil was conducted by using a series of nine columns packed with immobilized *Candida* sp. 99–125 lipase. As substrate of the first reaction step, plant or waste oil was used together with 1/3 molar equivalent of methanol against total fatty acids in the oil. Mixtures of the first- and second-step eluates and 1/3 molar equivalent of methanol were used for the second- and third-reaction steps. A hydrocyclone was used in order to on-line separate the by-product glycerol after every 1/3 molar equivalent of methanol was added. Petroleum ether was used as solvent (3/2, v/v of oil) and the pump was operated with a flow rate of 15 L/hour giving an annual throughput of 100 t. The final conversion ratio of the FAME from plant oil and waste oil under the optimal condition was 90% and 92%, respectively. The life of the immobilized lipase was more than 10 days.

In 2007 Royon and coworkers [30] studied enzymatic production of biodiesel from cotton seed oil using *t*-butanol as a solvent. The enzymatic production of biodiesel by methanolysis of cottonseed oil was studied using immobilized *Candida antarctica* lipase as catalyst in *t*-butanol solvent. It was found, using a batch system, that enzyme inhibition caused by undissolved methanol was eliminated by adding *t*-butanol to the reaction medium, which also gave a noticeable increase of reaction rate and ester yield. The effect of *t*-butanol, methanol concentration and temperature on this system was determined. A methanolysis yield of 97% was observed after 24 hours at 50°C with a reaction mixture containing 32.5% *t*-butanol, 13.5% methanol, 54% oil and 0.017 g enzyme $(\text{g oil})^{-1}$. With the same mixture, a 95% ester yield was obtained using a one step fixed bed continuous reactor with a flow rate of 9.6 ml h^{-1} $(\text{g enzyme})^{-1}$. Experiments with the continuous reactor over 500 hours did not show any appreciable decrease in ester yields.

In 2007 Shaw and coworkers [31] studied continuous enzymatic synthesis of biodiesel with Novozym 435. Novozym 435 was packed in a packed-bed reactor and used to catalyze the alcoholysis of methanol and soybean oil to produce FAMEs in a cosolvent system. Reaction temperature and flow rate had significant effects on the percent molar conversion. On the basis of ridge max analysis, the optimum conditions for synthesis were as follows: reaction temperature 52°C, flow rate 0.1 ml/min and

substrate molar ratio 4.3:1. The predicted value was 74.2% and the actual experimental value was 75.2% molar conversion.

In 2008 Mcneff and coworkers [32] studied continuous catalytic system for biodiesel production. A novel continuous fixed bed reactor process has been developed for the production of biodiesel using a metal oxide-based catalyst. Porous zirconia, titania and alumina micro-particulate heterogeneous catalysts are shown to be capable of continuous rapid esterification and transesterfication reactions under high pressure (ca. 2500 psi) and elevated temperature (300-450°C). The continuous transesterification of triglycerides and simultaneous esterification of free fatty acids with residence times as low as 5.4 s was described. Biodiesel produced from soybean oil, acidulated soapstock, tall oil, algae oil and corn oil with different alcohols to make different alkyl esters using this new process pass all current ASTM testing specifications. Furthermore, the economics of this novel process is much more cost competitive due to the use of inexpensive lipid feedstocks that often contain high levels of free fatty acids. The process has been shown to easily scale up a factor of 49 for more than 115 hours of continuous operation without loss of conversion efficiency.

In 2009 Chongkhong and coworkers [33] studied continuous esterification for biodiesel production from palm fatty acid distillate using economical process. An overflow system for continuous esterification of palm fatty acid distillate (PFAD) using an economical process was developed using a continuous stirred tank reactor (CSTR). Continuous production compared to batch production at the same condition had higher product purity. The optimum condition for the esterification process was a 8.8:1:0.05 molar ratio of methanol to PFAD to sulfuric acid catalyst, 60 min of residence time at 75°C under its own pressure. The free fatty acid (FFA) content in the PFAD was reduced from 93 to less than 1.5 %wt by optimum esterification. The esterified product had to be neutralized with 10.24 %wt of 3 M sodium hydroxide in water solution at a reaction temperature of 80°C for 20 min to reduce the residual FFA and glycerides.

In 2009 Halim and coworkers [34] studied continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: Optimization using response surface methodology (RSM) and mass transfer. Response surface methodology (RSM) based on central composite rotatable design (CCRD) was used to optimize the two important reaction variables packed bed height (cm) and substrate flow rate(ml/min) for the transesterification of waste cooking palm oil in a continuous packed bed reactor. The optimum condition for the transesterification of waste cooking palm oil was as follows: 10.53 cm packed bed height and 0.57 ml/min substrate flow rate. The optimum predicted fatty acid methyl ester (FAME) yield was 80.3% and the actual value was 79%.

CHAPTER III

EXPERIMENTAL

3.1 Materials and equipments

3.1.1 Chemicals

1. Methanol; analytical grade; Merck
2. Ethanol; absolute GR for analysis grade; Merck
3. Propanol; analytical grade; Fisher scientific
4. Butanol; analytical grade; Fisher scientific
5. 2-Propanol; analytical grade; Merck
6. *tert*-Butanol; for synthesis grade; Merck
7. 2-Butanol; analytical grade; Merck
8. Hexane; analytical grade; Merck
9. Ethyl acetate; analytical grade; Fisher scientific
10. Novozym 435 (Lipase B from *C. antarctica*, EC 3.1.1.3, a nonspecific lipase immobilized on macroporous acrylic resin); S.M. Chemical Suppliers Co., Ltd.
11. Vanillin solution (containing vanillin (1%) and conc.H_2SO_4 (4.5%) in ethanol)
12. Palm fatty acid; Oleen Co., Ltd.
13. Crude palm oil; Thai Eastern Group Co., Ltd.
14. Chloroform-d ($CDCl_3$); NMR spectroscopy grade; Merck KGaA Darmstadt, Germany
15. Molecular sieve 4 Å; Merck KGaA Darmstadt, Germany
16. Nitrogen gas industry grade

3.1.2 Equipments

1. Hotplate stirrer with magnetic stirrer set
2. Thermometer
3. Vessel vial, round bottom flask, volumetric flask and erlenmeyer flask
4. Beaker

5. Filter paper
6. Centrifuge
7. Rotary evaporator
8. Stainless steel tank
9. Stainless steel column; id.4.57 mm

3.2 Chemical properties of palm fatty acid

3.2.1 Fatty acid compositions were determined by gas chromatography (EN 14103)

3.3 Batch process

3.3.1 Biodiesel production from palm fatty acid using Novozym 435 in a solvent free medium

3.3.1.1 Optimization of process parameter

1. Effect of reaction time

In the esterification reaction, biodiesel was produced by using a 2:1 ratio of methanol and palm fatty acid and 15% w/w of Novozym 435. A hot plate with a magnetic stirrer was used for heating the mixture at 55°C. In order to investigate the reaction time, the reactions were conducted at 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hours. All reactions were monitored by TLC developed by hexane:ethyl acetate (90:10 v/v) and visualized by vanillin solution. The methyl ester (5 mg) was subjected to ^{1}H-NMR analysis.

2. Effect of alcohol type

Palm fatty acid with primary (methanol, ethanol, propanol, butanol), secondary (2-propanol, 2-butanol) and tertiary (*tert*-butanol) alcohols were conducted at 55°C. The reaction mixture consisted of a 2:1 ratio of methanol and palm fatty acid and 15% w/w of Novozym 435. A hot plate with a magnetic stirrer was used for heating the mixture in the round bottom. The operation condition during the whole reaction process was fixed at reaction time 4 hours.

3. Effect of temperature

The effect of temperature on the ester content and on the esterification of palm fatty acid was investigated with its reaction temperature varying at 55°C, 60°C and 65°C. The operation conditions during the whole reaction process were fixed at 2:1 molar ratio of methanol to FFA, 15% w/w of Novozym 435 and reaction time for 4 hours.

4. Effect of the absorbent quantity on the esterification

In the esterification, biodiesel was produced by using a 2:1 ratio of methanol and palm fatty acid and 15% w/w of Novozym 435. A hot plate with a magnetic stirrer was used for heating the mixture at 60°C. In order to investigate the amount of molecular sieves, the reactions were conducted at 0, 15, 30, 50, 80% based on palm fatty acid weight. The operation condition during the whole reaction process was fixed at reaction time 4 hours.

3.3.2 Esterification of palm fatty acid for biodiesel production in organic solvent medium

3.3.2.1 Optimization of process parameter

1. Effect of temperature

The effect of temperature on the ester content and on the esterification of palm fatty acid was investigated with its reaction temperature varying at 30°C, 40°C, 50°C and 60°C. The operation conditions during the whole reaction process were fixed at 2:1 ratio of methanol and palm fatty acid and *tert*-butanol 29% based on palm fatty acid weight and enzyme to palm fatty acid ratio 15% w/w. A hot plate with a magnetic stirrer was used for heating the mixture in the round bottom. The operation condition during the whole reaction process was fixed at reaction time 4 hours.

2. Effect of *tert*-butanol quantity on the esterification

In the esterification reaction, biodiesel was produced by using a 2:1 ratio of methanol and palm fatty acid and enzyme to palm fatty acid ratio 15% w/w. A hot plate with a magnetic stirrer was used for heating the mixture in the round bottom. In order to investigate the amount of *tert*-butanol, the reactions were conducted at 0, 5, 10, 20, 40 and 80% based on palm fatty acid weight and reaction time for 4 hours.

3.4 Continuous process

3.4.1 Biodiesel production from palm fatty acid using Novozym 435 in a solvent free medium

3.4.1.1 Optimization of process parameter

1. Effect of temperature

A 1:2 molar ratio of palm fatty acid and methanol were mixed well in a feeding tank. The esterification reaction was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435 (Figure 3.1) . The mixture was pumped through a column with flow rate 1.67 mg/sec. The effect of temperature on the ester content of palm fatty acid was investigated with its reaction temperature varying at 50°C, 55°C, 60°C and 65°C. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

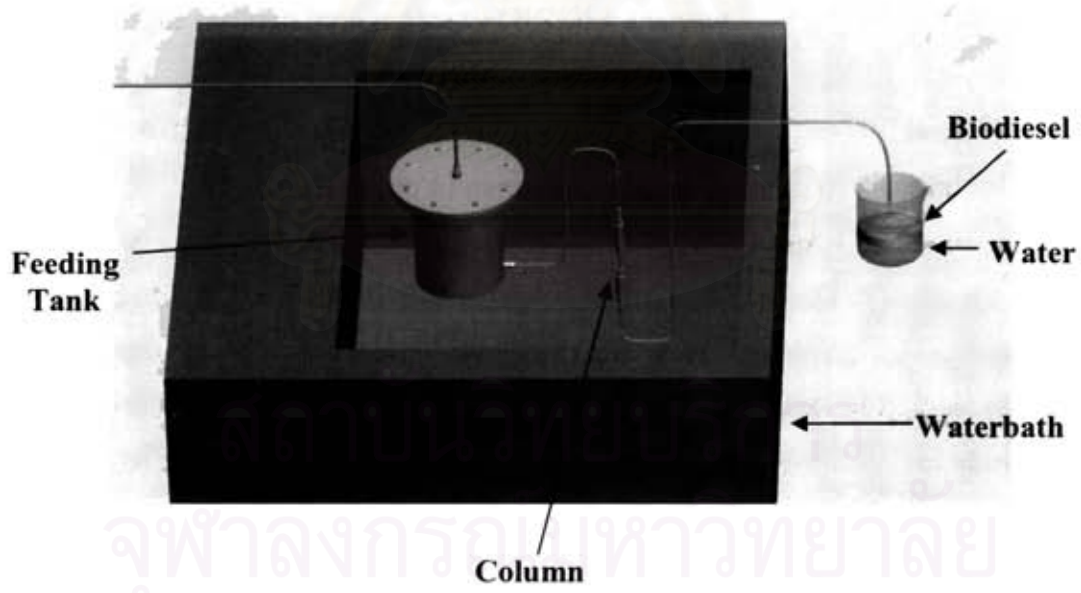


Figure 3.1 Continuous biodiesel production from palm fatty acid using Novozym 435.

2. Effect of a molar ratio of palm fatty acid and methanol

The esterification reaction was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column with flow rate 1.67 mg/sec. The reaction was performed at 50°C. The different methanol to oil ratios 1:1, 1:1.5, 1:2 and 1:3 were used to investigate their influence on the percentage of methyl ester. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

3. Effect of flow rate

A 1:2 molar ratio of palm fatty acid and methanol were mixed well in a feeding tank. The esterification reaction was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column. The reaction was performed at 50°C. To investigate the effect of flow rate, the reactions were conducted at 1.67, 3.33 and 5.01 mg/sec. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

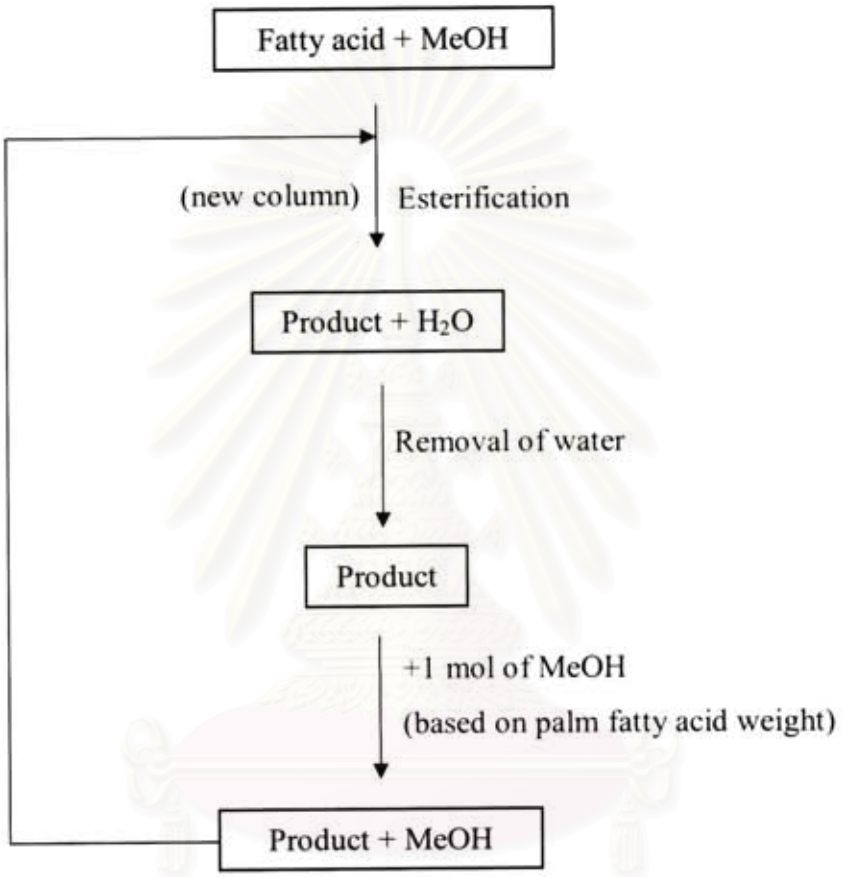
4. Effect of amount of Novozym 435

A 1:2 molar ratio of palm fatty acid and methanol were mixed well in a feeding tank. To investigate the effect of amount of Novozym 435, the reaction was conducted at 0.328 and 0.656 g. The esterification was carried out using a 5, 10 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328, 0.656 g of Novozym 435. The mixture was pumped through a column with flow rate 1.67 mg/sec. The reaction was performed at 50°C. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

3.4.1.2 Three column esterification process

Continuous flow esterification of palm fatty acid using Novozym 435 was shown in Scheme 3.1. The reaction was conduced at 50°C with three columns (5 cm×4.57 mm) containing 0.328 g of Novozym 435. A 1:2 ratio palm fatty acid and methanol were mixed well and then fed into the first column at a constant flow rate of 1.67 mg/sec. The product, in which water/FAMEs mixture, was separated completely

by centrifuge. In the second step, 1 molar equivalent of methanol was added in the water-free of the product, and then fed into the second column at the same condition. The third step was performed in similar manner by feeding a mixture of the second step. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.



Scheme 3.1 Diagram of three column esterification process

3.4.1.3 Operational stability of the immobilized lipase

The operational stability of the immobilized lipase is an important parameter in an industrial process, since it directly affects the cost. A 1:2 ratio palm fatty acid and methanol were mixed well in a feeding tank. The esterification reaction was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column with flow rate 1.67 mg/sec. The reaction was operated at 50°C during 240 hours. The methyl ester (5 mg) was subjected to ^{1}H-NMR analysis.

3.4.2 Esterification of palm fatty acid for biodiesel production in organic solvent medium

3.4.2.1 Optimization of process parameter

1. Effect of temperature

A 1:2 molar ratio of palm fatty acid and methanol were mixed well with *tert*-butanol (29% based on palm fatty acid weight) as cosolvent in a feeding tank. The esterification reaction was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column with flow rate 1.67 mg/sec. In order to investigate the temperature effect, the reactions were conducted at 30, 40, 50, 60 and 65°C. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

2. Effect of a molar ratio of the palm fatty acid and methanol

The esterification was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column with flow rate 1.67 mg/sec. The reaction was performed at 50°C. The different methanol to oil ratios 1:1, 1:1.5, 1:2 and 1:3 using *tert*-butanol (29% based on palm fatty acid weight) as cosolvent were used to investigate their influence on the percentage of methyl ester. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

3. Effect of cosolvent on the esterification

A 1:2 molar ratio of palm fatty acid and methanol were mixed well with *tert*-butanol as cosolvent in a feeding tank. The esterification was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column with flow rate 1.67 mg/sec. The effect of *tert*-butanol quantity on the esterification were conducted at 0, 14.5, 29 and 58% based on palm fatty acid weight at 50°C. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

4. Effect of flow rate

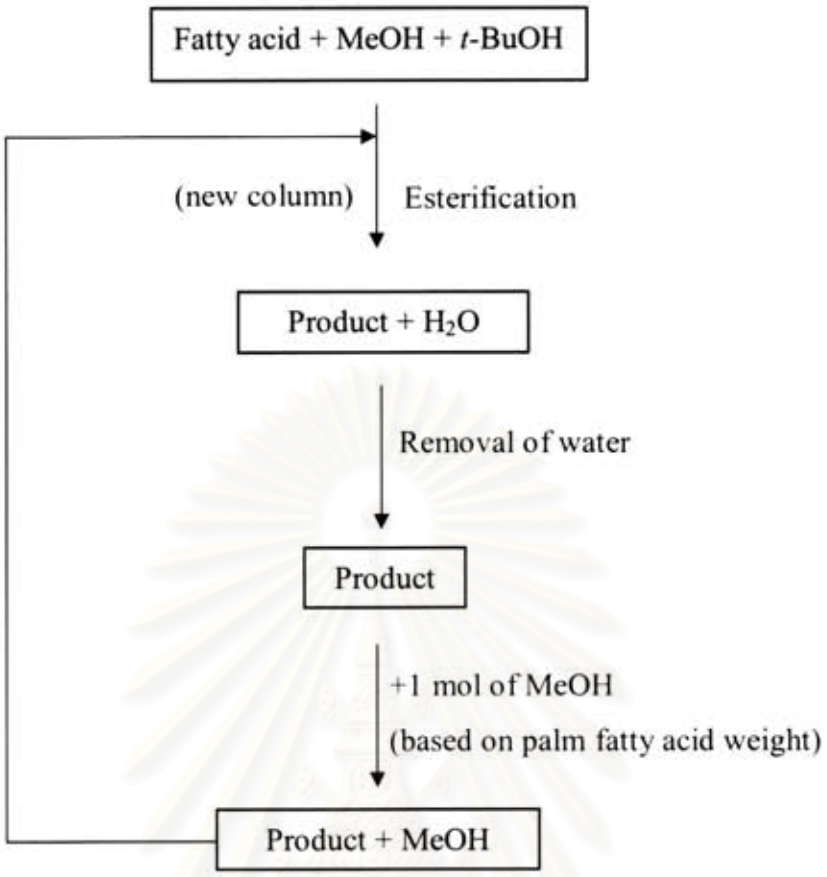
A 1:2 molar ratio of palm fatty acid and methanol were mixed well with *tert*-butanol (29% based on palm fatty acid weight) as cosolvent in a feeding tank. The esterification was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column. The reaction was performed at 50°C. To investigate the effect of flow rate, the reactions were conducted at 0.83, 1.67 and 3.33 mg/sec. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

5. Effect of amount of Novozym 435

A 1:2 molar ratio of palm fatty acid and methanol were mixed well with *tert*-butanol (29% based on palm fatty acid weight) as cosolvent in a feeding tank. To investigate the effect of amount of Novozym 435, the reaction was conducted at 0.328 and 0.656 g. The esterification was carried out using a 5, 10 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328, 0.656 g of Novozym 435. The mixture was pumped through a column with flow rate 0.83, 1.67 mg/sec. The reaction was performed at 50°C. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.

3.4.2.2 Three column esterification process

Continuous flow esterification of palm fatty acid using Novozym 435 was shown in Scheme 3.2. The reaction was conduced at 50°C with three columns (5 cm×4.57 mm) in which 0.328 g of immobilized lipase was packed. A 1:2 ratio palm fatty acid and methanol and *tert*-butanol (29% based on palm fatty acid weight) were mixed well and then fed into the first column at a constant flow rate of 1.67 mg/sec. The product, in which water/FAMEs mixture, was separated completely by centrifuge. In the second step, 1 molar equivalent of methanol was added in the water-free of the product, and then fed into the second column at the same condition. The third step was performed in similar manner by feeding a mixture of the second step. All products were collected at the outlet of the reaction tube after 24, 48 and 72 hours of the reaction time for further FAMEs analysis.



Scheme 3.2 Diagram of three column esterification process

3.4.2.3 Operational stability of the immobilized lipase

The operational stability of the immobilized lipase is an important parameter in an industrial process, since it directly affects the cost. A 1:2 molar ratio of palm fatty acid and methanol and *tert*-butanol (29% based on palm fatty acid weight) were mixed well in a feeding tank. The esterification reaction was carried using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column with flow rate 1.67 mg/sec. The reaction was operated at 50°C during 240 hours. The methyl ester (5 mg) was subjected to ^{1}H-NMR analysis.

3.5 Properties of biodiesel

The percentage of methyl ester, flash point and density tested by the Department of Energy Business, Ministry of Energy, following EN14103, ASTM D93 and ASTM D 1298.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Compositions of palm fatty acid

The palm fatty acid obtained from Oleen Co., Ltd. was used for the synthesis of the biodiesel. The compositions of free fatty acid (FFA) of palm fatty acid were determined by EN14103 and the results (Table 4.1) indicated that palmitic acid and oleic acid were major components.

Table 4.1 Compositions of fatty acids in palm fatty acid (see details in Appendix B)

Fatty acid composition	Total fatty acid (%)
Palmitic acid, C16:0	44.66
Stearic acid, C18:0	4.13
Oleic acid, C18:1	37.67
Linoleic acid, C18:2	9.80
Linolenic acid, C18:3	0.48
Arachidic acid, C20:0	0.32
Eicosenoic acid, C20:1	0.15
Eicosapentaenoic acid, C20:5	0.06
Lignoceric acid, C24:0	0.14
Others	2.59

The percentages of palmitic acid, stearic acid, arachidic acid and ligoceric acid were 44.66, 4.13, 0.32 and 0.14%, respectively. The total of saturated fatty acids was 49.25%. The percentages of oleic acid, linoleic acid, linolenic acid, eicosenoic acid and eicosapentaenoic acid were 37.67, 9.80, 0.48, 0.15 and 0.06%, respectively. The total of unsaturated fatty acids was 48.16%. The calculated average molecular weight of palm fatty acid was 270.44 g/mole (see details in Appendix B).

4.2 Batch process

4.2.1 Biodiesel production from fatty acid using Novozym 435 in a solvent free medium

4.2.1.1 Optimization of process parameter

1. Effect of reaction time

In the process of biodiesel production, reaction temperature, methanol quantity and reaction time were found to be significant operating parameters, which are closely associated with energy costs from an economic perspective. In the esterification reaction, biodiesel was performed at 55°C using a 2:1 ratio of methanol and palm fatty acid and enzyme 15% (based on palm fatty acid weight).

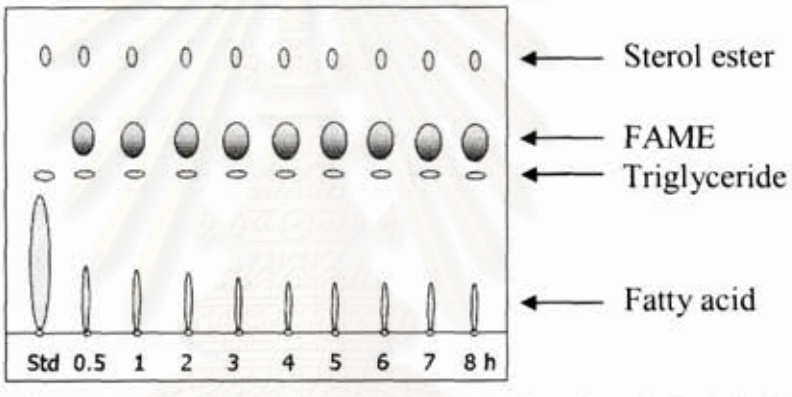


Figure 4.1 TLC for monitoring the methyl ester at reaction time 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hours.

The results analyzed by TLC were shown in Figure 4.1. The changes in product compositions with reaction time and the distribution of various components in the reaction system can be clearly seen. TLC analysis showed that the free fatty acid was converted rapidly to FAME within 30 minutes while the conversion was relatively constant after 2 hours. The ester content increased when increasing reaction time.

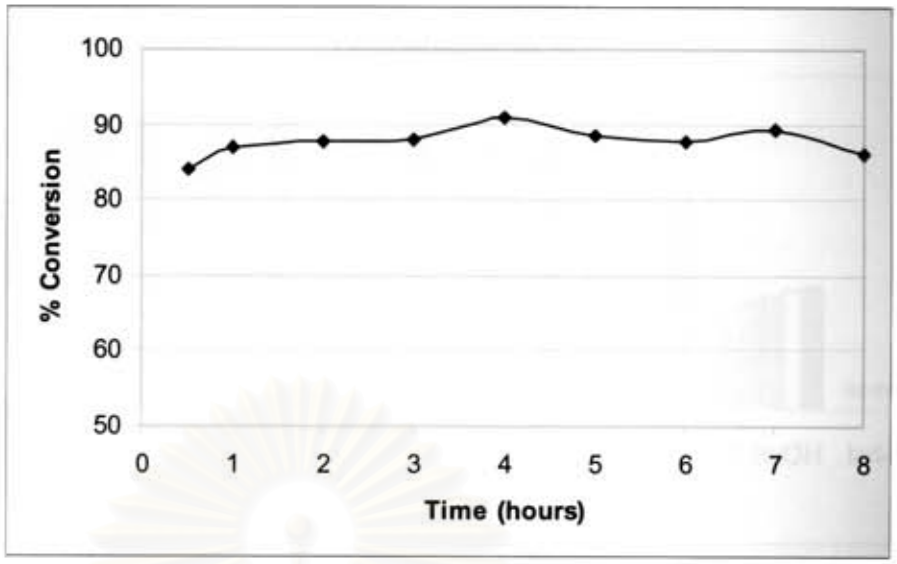


Figure 4.2 Effects of reaction time on esterification at 55°C.

Conversion of palm fatty acid analyzed by ^{1}H-NMR were shown in Figure 4.2. The results showed that the reaction was very fast in a few minutes, more than 80% ester content was formed within 30 minutes. After that, the reaction slowed down and entered a slow rate stage till the reaction equilibrium was reached eventually. The highest percentage of conversion reached 90.9% at 4 hours .The conversion of FAME was decreased after 4 hours. FFA increased again from reversible reaction. The appropriate reaction time was 4 hours. Thus, the other experiments were carried out at this appropriate time.

2. Effect of alcohol type

Esterification of the palm fatty acid with primary (methanol, ethanol, propanol, butanol) secondary (2-propanol, 2-butanol) and tertiary (*tert*-butanol) alcohols was investigated. The conditions of the esterification were as follows: 15% enzyme based on palm fatty acid weight; a 1:2 molar ratio of palm fatty acid and alcohol and temperature 55°C. Conversion of palm fatty acid analyzed by ^{1}H-NMR were shown in Figure 4.3 (see details in Appendix B).

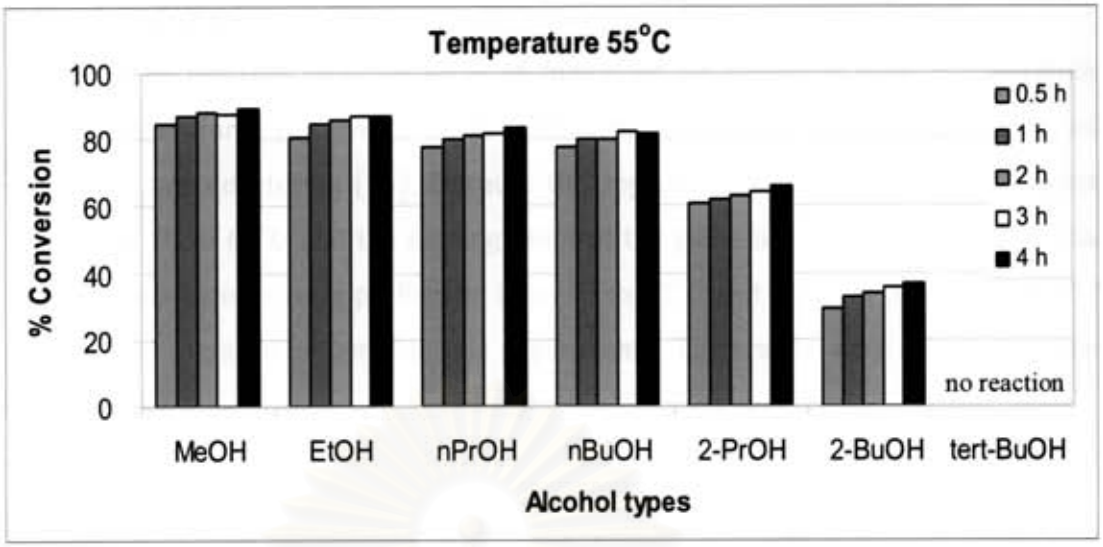


Figure 4.3 Effect of alcohol types on the esterification of palm fatty acid. The conditions of the esterification were as follows: 15% enzyme based on palm fatty acid weight; 1:2 molar ratio of palm fatty acid and alcohol; temperature 55°C.

Esterification of methanol and the palm fatty acids progressed faster than other alcohol. The highest percentage of conversion in the reaction reached 90.9%. 2-Propanol and 2-butanol are less active than methanol and ethanol for biodiesel production. The percentage of conversion reached 66.3 and 36.6%, respectively. Due to steric effect of secondary alcohols to enter the active site of enzyme was more difficult than primary alcohols. For bulky alcohol such as *tert*-butanol was unreacted. Increasing the carbon numbers of primary alcohols and secondary alcohols decreased the percentage of conversion. Due to small size of molecule and higher polarity, methanol may easily diffuse and access the lipase enzyme resulting in higher reaction rate [35].

3. Effect of temperature

The reaction temperature is an important parameter in enzymatic catalysis. Higher temperatures can give a faster transformation, but too high temperature will lead to enzyme denaturing [36]. Because the appropriate temperature of the enzyme range from 40 to 60°C and the melting point of the palm fatty acid is about 50°C, the reaction temperatures were performed from 55 to 65°C and reaction times (0.5, 1, 2, 3 and 4 hours) were investigated in this experiments. The results were shown in Figure 4.3, 4.4 and 4.5.

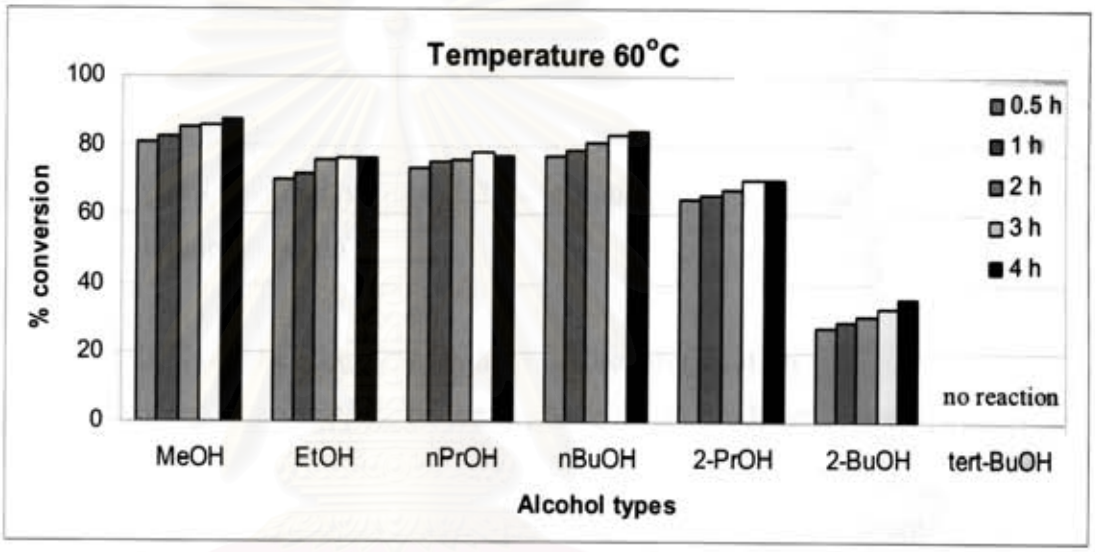


Figure 4.4 Effect of temperature on the esterification of palm fatty acid. The conditions of the esterification were as follows: 15% enzyme based on palm fatty acid weight; 1:2 molar ratio of palm fatty acid and alcohol; temperature 60°C.

At different temperatures, highest percentage of FAME (90.9%) was obtained at 55°C after the reaction time for 4 hours. The optimal reaction temperature of primary alcohols is lower than secondary alcohols. Due to the steric effect of secondary alcohols, the percentage of conversion was low. Higher temperature can improve the efficiency of reaction. Therefore, the optimal temperatures of primary and secondary alcohols were 55°C and 65°C, respectively.

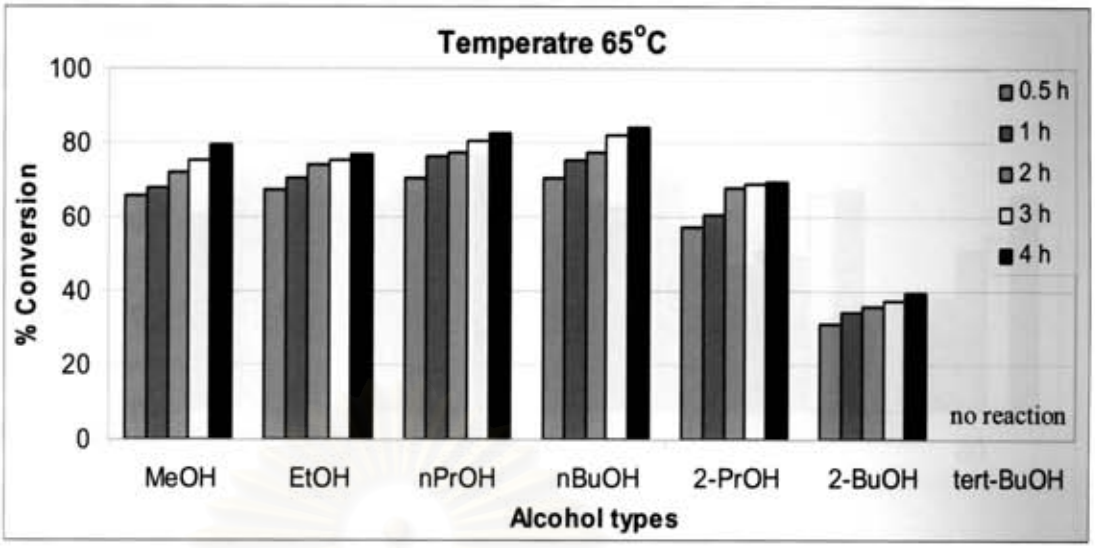


Figure 4.5 Effect of temperature on the esterification of palm fatty acid. The conditions of the esterification were as follows: 15% enzyme based on palm fatty acid weight; 1:2 molar ratio of palm fatty acid and alcohol; temperature: 65°C.

4. Effect of the adsorbent quantity on the esterification

Since esterification of palm fatty acid produced water, which cause reversible of the reaction, molecular sieve was used as adsorbents. Effect of different amount of adsorbents on the esterification was studied. The amount of molecular sieves were conducted at 0, 15, 30, 50, 80% based on palm fatty acid. In the esterification reaction, biodiesel was produced by using methanol to palm fatty acid ratio 2:1, enzyme to palm fatty acid ratio 15% w/w and temperature 60°C. The results were shown in Figure 4.6.

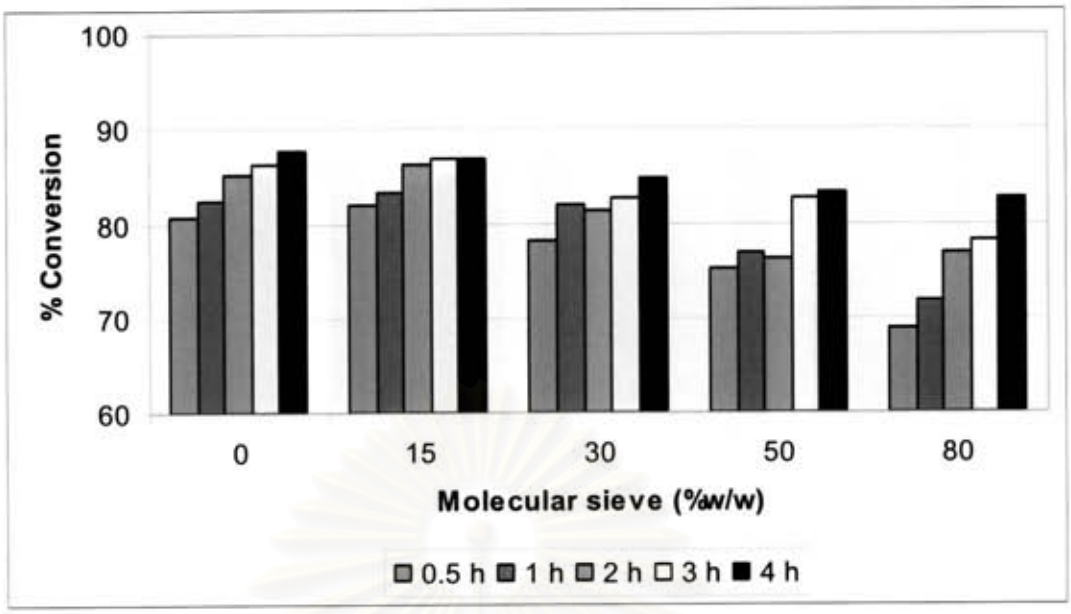


Figure 4.6 Effect of the absorbent quantity on the esterification. The conditions of the esterification were as follows: 15% enzyme based on palm fatty acid weight; 1:2 molar ratio of palm fatty acid and alcohol; temperature: 65°C.

In the batch process, a little amount of water in the reaction did not affected to the percentage conversion significantly. There was no much change in the percentage conversion with molecular sieve 15% and percentage conversion decreased when amount of molecular sieve more than 15%. The percentage conversion decreased when increasing the amount of molecular sieve. Because molecular sieve can absorb methanol in the reaction, it results in the lower percentage of conversion [27].

4.2.2 Esterification of palm fatty acid for biodiesel production with organic solvent

4.2.2.1 Optimization of process parameter

1. Effect of temperature

In the esterification, biodiesel was produced by using methanol to palm fatty acid ratio 2:1 and 29% *tert*-butanol (based on palm fatty acid weight) and enzyme 15% w/w (based on palm fatty acid weight). The reactions were conducted at 30, 40, 50 and 60°C. The results were shown in Figure 4.7.

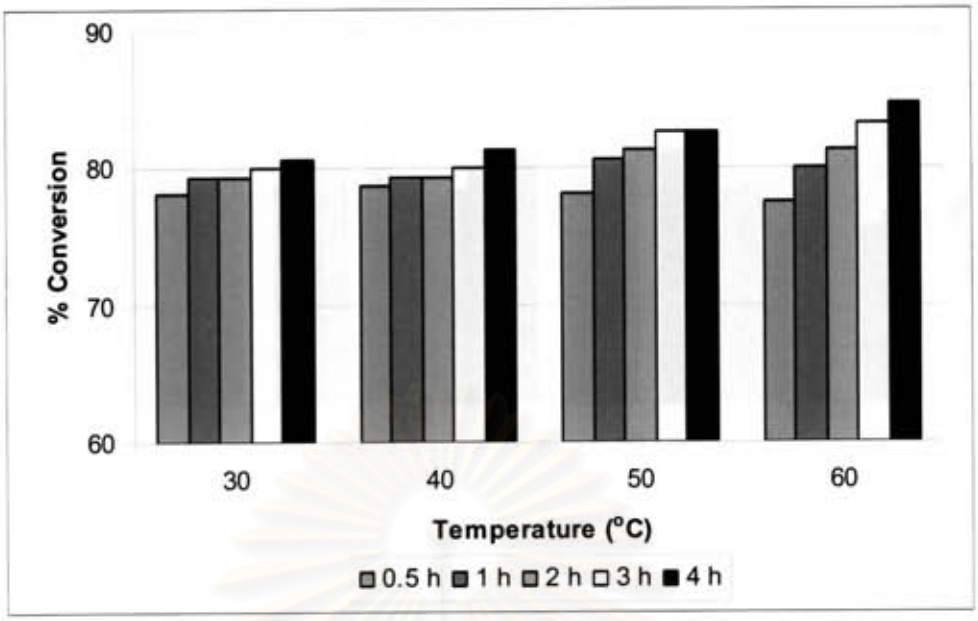


Figure 4.7 Effect of temperature on the esterification of palm fatty acid. The conditions of the esterification were as follows: 15% enzyme and *tert*-butanol 29% based on palm fatty acid weight; 1:2 molar ratio of palm fatty acid and alcohol.

At different temperatures, highest percentage of FAME (84.8%) was obtained at 60°C after the reaction time for 4 hours. When using *tert*-butanol as a solvent, reaction temperature does not have much effect on increasing the percentage of FAME. The percentage of FAME increased when increasing temperature and higher temperature gave a faster transformation. Therefore, the optimal reaction temperature is 60°C.

2. Effect of *tert*-butanol quantity on the esterification

It has been confirmed in our study that *tert*-butanol is inert in the esterification of palm fatty acid for biodiesel production. In the esterification reaction, biodiesel was produced by using a 1:2 molar ratio of palm fatty acid and alcohol and enzyme 15% w/w (based on palm fatty acid weight). Different amounts of *tert*-butanol were conducted at 0, 5, 10, 20, 40 and 80% based on palm fatty acid weight. The results were shown in Figure 4.8.

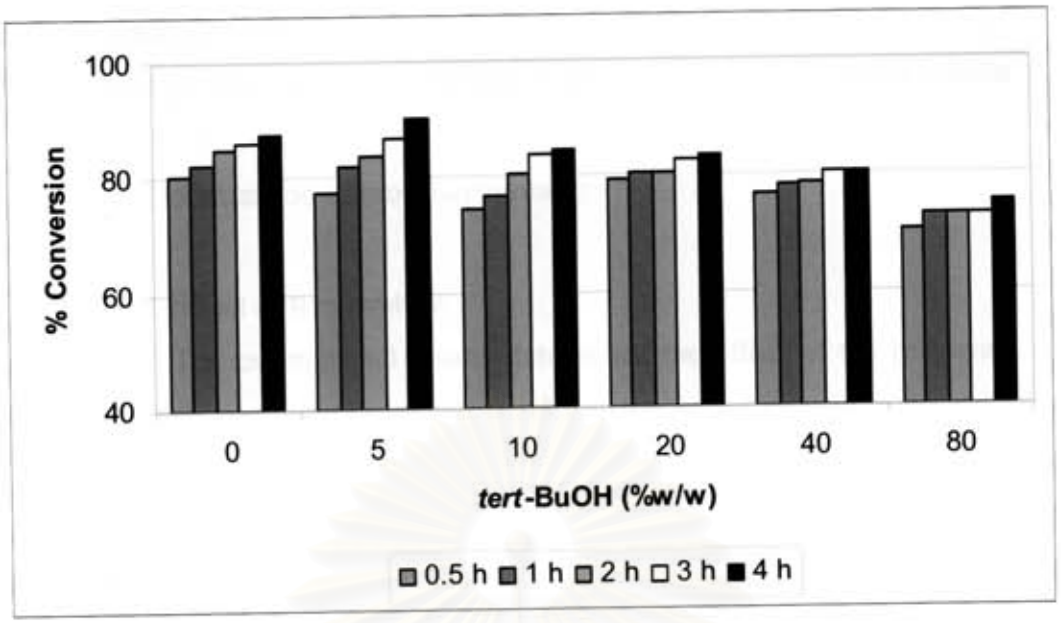


Figure 4.8 Effect of *tert*-butanol quantity on the esterification. The conditions of the esterification were as follows: 15% enzyme based on palm fatty acid weight; palm fatty acid /alcohol molar ratio 1:2 and temperature 60°C.

At different amounts of *tert*-butanol, highest percentage of FAME (90.1%) was obtained under the reaction time for 4 hours at 5% *tert*-butanol. *tert*-Butanol could improve the solubility of methanol and reaction mixture. However, there was no much increase in the percentage of FAME. A further increase of the *tert*-butanol quantity would decrease the percentage FAME gradually, which might be due to the dilution effect of the substrate with too much *tert*-butanol presenting in the system [37].

4.3 Continuous process

4.3.1 Biodiesel production from palm fatty acid using Novozym 435 in a solvent free medium

4.3.1.1 Optimization of process parameter

1. Effect of temperature

The experimental results determined the effect of the temperature on the reaction. The appropriate temperature to operate of the enzyme in the range from 40 to 60°C and the melting point of the palm fatty acid is about 50°C, the reaction temperatures were performed from 50 to 65°C and reaction times (24, 48 and 72 hours) were investigated.

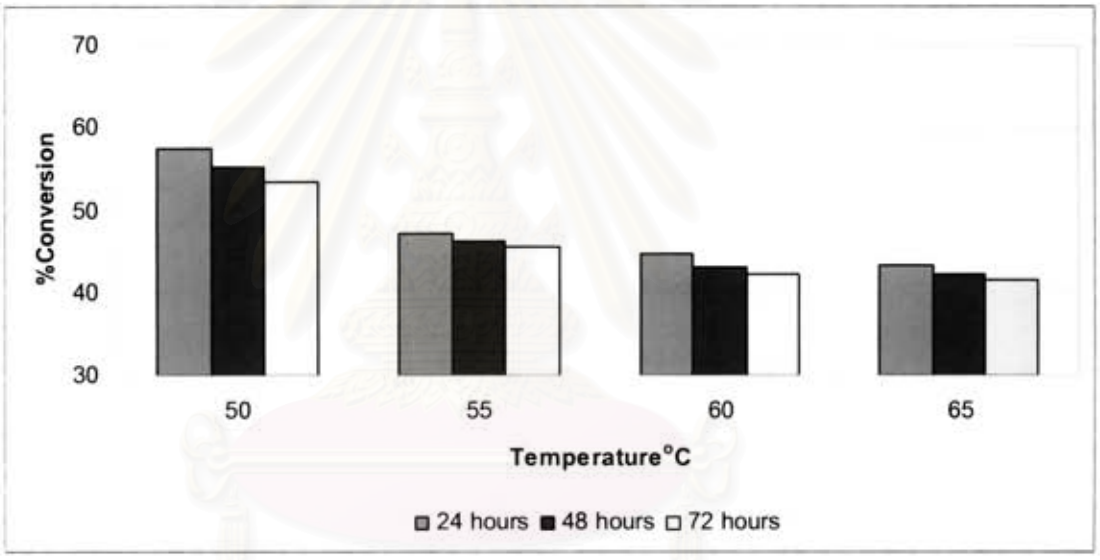


Figure 4.9 Conversion of fatty acid versus reaction temperature at 50°C, 55°C, 60°C and 65°C during 24, 48 and 72 hours in a solvent free system.

From ^{1}H-NMR analysis the results were shown in Figure 4.9. At different temperatures, the highest percentage of FAME (57.5%) was obtained at 50°C after the reaction time for 24 hours. Conversions of fatty acid performed at 60 and 65°C were similar. The conversion decreased when increasing temperature. Therefore, the optimal reaction temperature was 50°C. At 50°C, the reaction time at 24, 48 and 72 hours gave 57.5%, 55.3% and 53.5% of conversion to methyl ester, respectively. The conversion significantly reduced with time, which means that water produced by the reaction stays in the column. This trend was expected because the presence of higher

concentrations of water than needed by the enzyme drives the reaction toward hydrolysis rather than esterification [28, 38].

2. Effect of a molar ratio of palm fatty acid and methanol

The results also showed that the optimal reaction temperature was 50°C. An important variable which affected % conversion of methyl ester was the molar ratio of palm fatty acid to methanol. From ^{1}H-NMR analysis the results were shown in Figure 4.10.

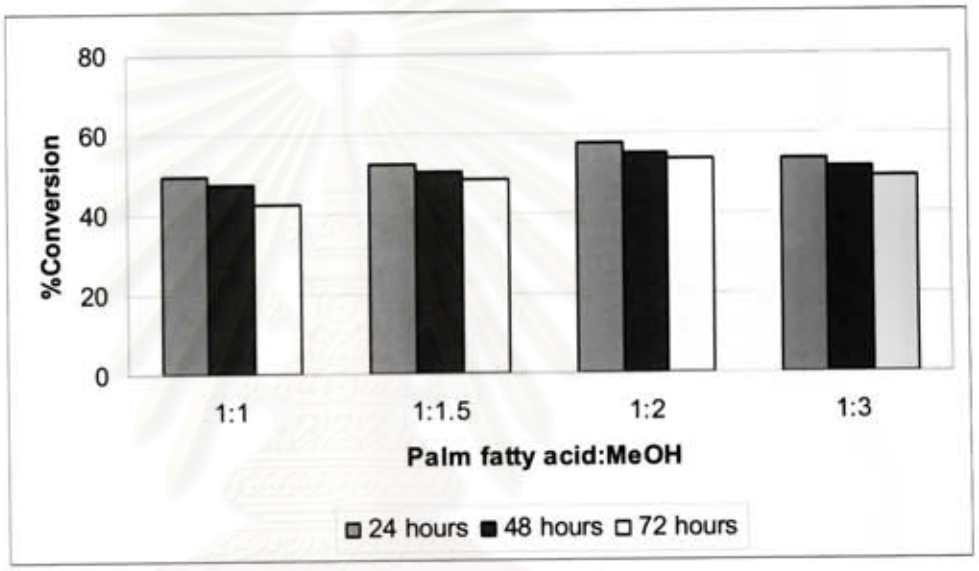


Figure 4.10 Conversion of palm fatty acid versus molar ratio of methanol and palm fatty acid at 1:1, 1:1.5, 1:2 and 1:3 during 24, 48 and 72 hours in a solvent free system.

After esterification was performed for 24 hours at 1:2 molar ratio, the highest percentage of conversion (57.5%) was obtained. The FAME was enhanced with the increase of methanol molar ratio. At the 2 molar equivalents, the reaction gave higher conversion than at one molar equivalent. Although the reaction at 3 molar equivalents gave also higher conversion than at 1 molar equivalent, the conversion was less than at 2 molar equivalents. This results indicated that more excess methanol caused deactivation of enzyme. This result corresponds with the results obtained by Watanabe et al. [26] who conducted the methanolysis of vegetable oil using the same lipase and the lipase was inactivated irreversibly in the presence of >2 molar

equivalent MeOH. Therefore, the appropriate molar ratio of palm fatty acid and methanol was 1:2.

3. Effect of flow rate

The effect of flow rate on conversion of palm fatty acid to FAME was investigated at 1.67, 3.33 and 5.01 mg/sec using an optimal reaction temperature 50°C and 2:1 molar ratio of methanol and palm fatty acid and the reactions were monitored by ^{1}H-NMR analysis.

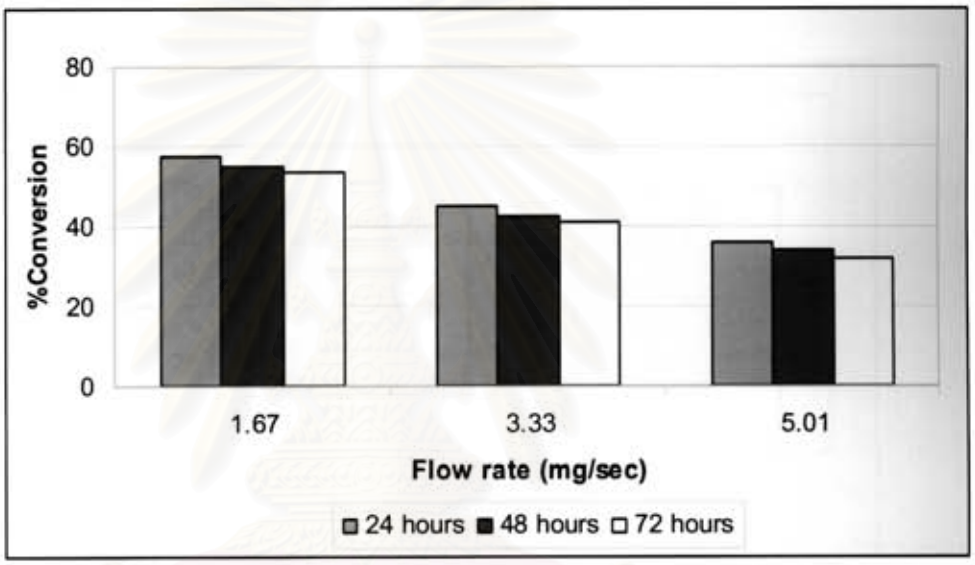


Figure 4.11 Conversion of palm fatty acid versus flow rate 1.67, 3.33 and 5.01 mg/sec during 24, 48 and 72 hours in a solvent free system.

From ^{1}H-NMR analysis the results were shown in Figure 4.11. After esterification was occurred for 24 hours at flow rate 1.67 mg/sec, the highest percentage of conversion was obtained. The reaction time at 24, 48 and 72 hours gave 57.47%, 55.25% and 53.48% of conversion to methyl ester, respectively. The FAME was increased with decreasing flow rate, indicating that a lower flow rate might extend the contact time between substrate and enzyme and result in higher conversion FAME. At flow rate 3.33 mg/sec, reaction time 24, 48 and 72 hours gave 44.8%, 42.6% and 40.9% of conversion to methyl ester, respectively. At increasing flow rate, the substrate will only pass through the enzyme with less interaction with the enzyme, consequently failing to bind at the enzyme active site. Hence, there is less contact

between the substrate and enzyme active sites yielding lower FAME [34]. Therefore, the appropriate flow rate was 1.67 mg/sec.

4. Effect of amount of Novozym 435

The effect of amount of Novozyme 435 on conversion of palm fatty acid to FAME was investigated at 0.328 and 0.656 g using an optimal reaction temperature 50°C, and 2:1 molar ratio of methanol and palm fatty acid. The reactions were monitored by ^{1}H-NMR analysis. From ^{1}H-NMR analysis the results were shown in Figure 4.12.

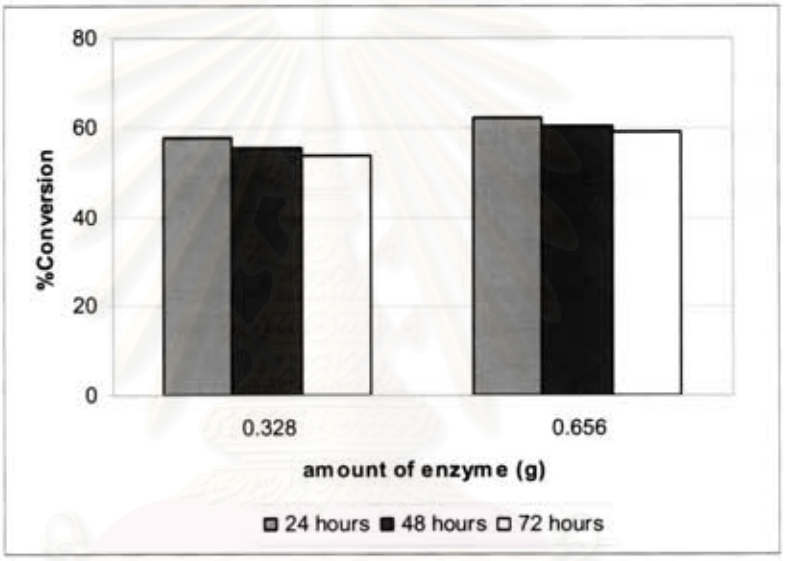


Figure 4.12 Conversion of palm fatty acid versus amount of enzyme 0.328 and 0.656 mg/sec during 24, 48 and 72 hours in a solvent free system.

At 0.328 (5 cm column with inner diameter 4.57 mm) and 0.656 g (10 cm with inner diameter 4.57 mm) of Novozym 435, the highest percentage of FAME was 57.5 and 62.1%, respectively. The percentage of FAME obtained from the amount of 0.656 g enzyme was higher than 0.328 g by approximately 5%. It was observed that increasing the column height does not have much effect on increasing the percentage of FAME. The reason may be that water, which was the by-product from the reaction, was produced in the column. When using a longer column, water quantity would increase and was longer retain the column, thus inducing the hydrolysis reaction at the end of the column. The hydrolysis reaction was shown in Figure 4.13.

$$R^1CO_2CH_3 + H_2O \rightleftharpoons R^1CO_2H + CH_3OH$$

Methyl Ester water Fatty acid methanol

Figure 4.13 Hydrolysis of fatty acid methyl ester

4.3.1.2 Three-step esterification process

The reaction was conduced at 50°C with three columns (5 cm×4.57 mm) in which 0.328 g of immobilized lipase was packed. A mixture of palm fatty acid and methanol with 1:2 molar ratio were fed into the first column at a constant flow rate of 1.67 mg/sec. The product, in which water/FAMEs mixture, was separated completely by centrifuge. In the second step 1 molar equivalent of methanol was added in the water-free of the product and the substrate mixture was fed into the second column at the same flow rate. The third step was similarly operated by feeding a mixture of the second step. The reactions were monitored by ^{1}H-NMR analysis.

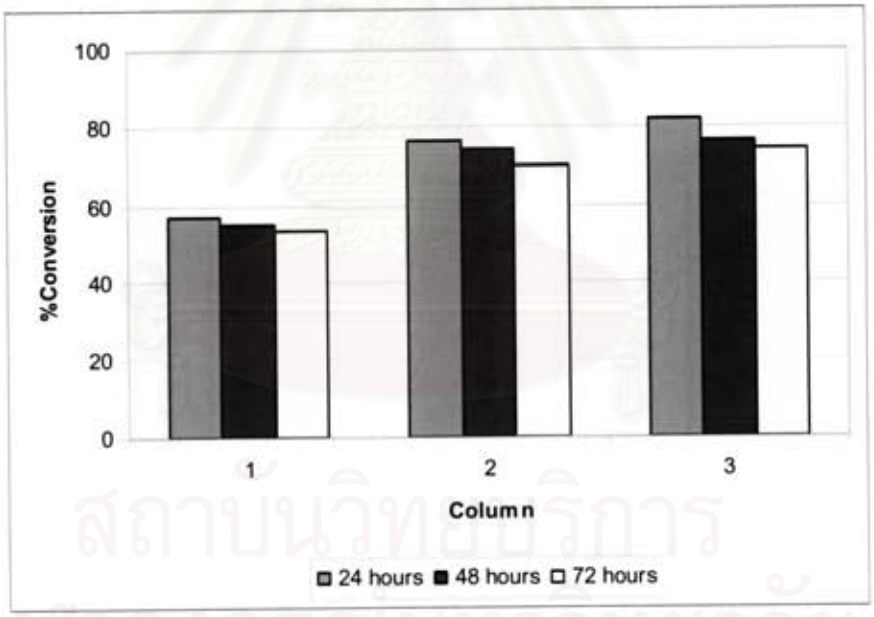


Figure 4.14 Conversion of palm fatty acid versus number of column during 24, 48 and 72 hours in a solvent free system.

From ^{1}H-NMR the results were shown in Figure 4.14. The percentage of conversion in the first, second and third column eluates at 24 hours of the reaction was 57.5, 76.3 and 82.0%, respectively. The percentage of FAME from the second and the third column increased about 20% and 5%, respectively. The cost of lipase

accounts for a large part in the total cost of biodiesel production. Therefore, the optimal condition for biodiesel production in a solvent free medium were temperature at 50°C with three columns (5 cm×4.57 mm) in which 0.328 g of immobilized lipase was packed, flow rate 1.67 mg/sec, palm fatty acid and methanol with 1:2 molar ratio in the first column and 1:1 in the second and third column.

4.3.2 Esterification of palm fatty acid for biodiesel production with organic solvent

4.3.2.1 Optimization of process parameter

1. Effect of temperature

The experimental results determined the effect of the temperature on the reaction. In the esterification reaction, biodiesel was produced by using methanol to palm fatty acid ratio 2:1 and 29% *tert*-butanol (based on palm fatty acid weight). The reaction temperatures were performed from 40 to 65°C and reaction times (24, 48 and 72 hours) were investigated. The reactions were monitored by ^{1}H-NMR analysis.

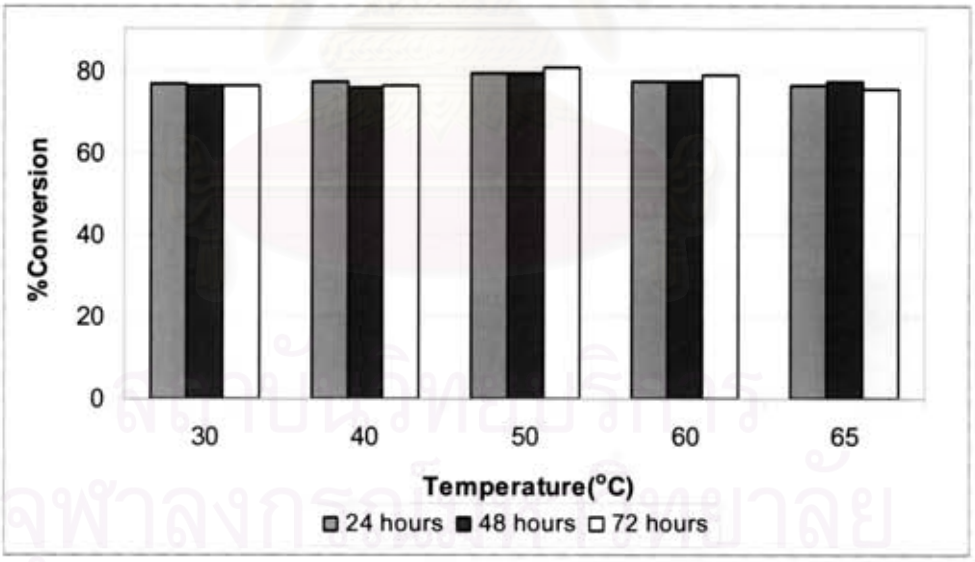


Figure 4.15 Conversion of palm fatty acid versus reaction temperature 30°C, 40°C, 50°C, 60°C and 65°C during 24, 48 and 72 hours in a cosolvent system.

From ^{1}H-NMR analysis the results were shown in Figure 4.15. It has also been found in our study that with *tert*-butanol existing in the system, much better stability of the lipase could be achieved. At different temperatures, highest percentage of

FAME (about 80%) was obtained at 50°C. Conversions of fatty acid performed at 30 and 40°C were similar while the conversion at reaction temperatures exceeding 50°C was decreased. This indicated that conversion decreased with increasing temperature. This result corresponds with the results obtained by Shimada et al. [36] who conducted the methanolysis of vegetable oil using the same lipase. The optimal reaction temperature was 50–60°C. The difference of conversion from reaction temperature was analyzed via statistics in T-test. Based on a 95% confidence level, the temperature at 40°C and 50°C were significant. At 50°C, the reaction time at 24, 48 and 72 hours gave 79.4%, 79.4% and 80.6% of conversion to methyl ester, respectively. This demonstrated that the conversion was non-significant with increasing time. Because water is soluble in the *tert*-butanol, the negative effect caused by water on lipase catalytic activity could be eliminated. Thus, the optimal reaction temperature is 50°C.

2. Effect of a molar ratio of palm fatty acid and methanol

Additionally, the results showed that the optimal reaction temperature was 50°C and important variable which affected %conversion of methyl ester was the molar ratio of palm fatty acid to methanol. The reactions were conducted at 1:1, 1:1.5, 1:2, 1:3 and 29% *tert*-butanol (based on palm fatty acid weight).

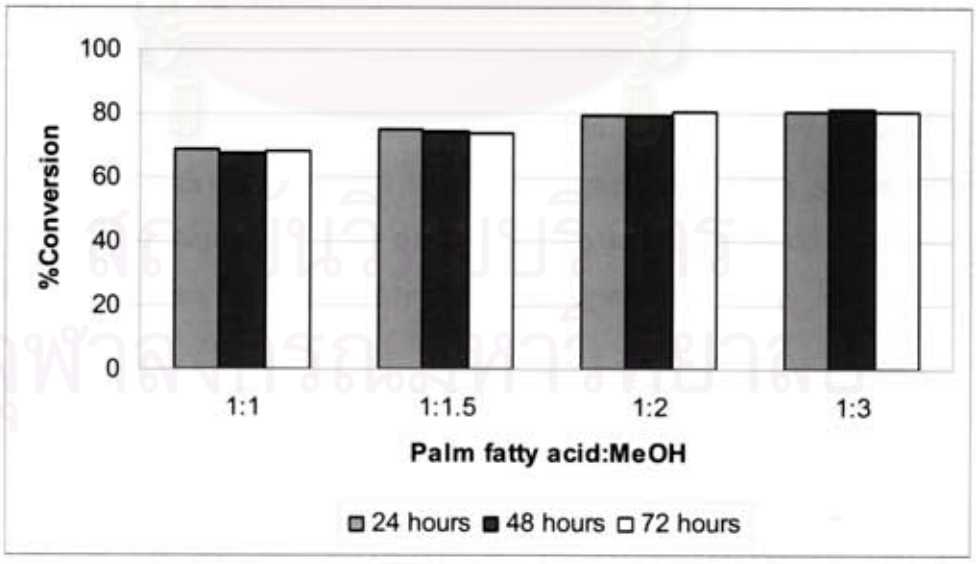


Figure 4.16 Conversion of palm fatty acid versus molar ratio of methanol and palm fatty acid at 1:1, 1:1.5, 1:2 and 1:3 during 24, 48 and 72 hours in a cosolvent system.

From ^{1}H-NMR analysis the results were plotted as shown in Figure 4.16. The FAME was enhanced with the increase of methanol molar ratio. The percentage of FAME obtained from a molar ratio of methanol and palm fatty acid at 1:2 and 1:3 were similar. The difference of conversion from molar ratio of methanol and palm fatty acid was analyzed via statistics in T-test. Based on a 95% confidence level, the molar ratio of palm fatty acid to methanol 1:2 and 1:3 were non-significant and the percentage of FAME was about 80%. This study showed that one molar equivalent of methanol to FFA has less capability compared with the excess methanol to protect reversible reaction. At the 2 molar equivalents protected reversible reaction. The presence of *tert*-butanol could improve the solubility of methanol in the reaction mixture, so lipase still maintained high activity even with much methanol present in the system. For economical reasons, the appropriate condition for this process was 1:2 molar ratio of palm fatty acid to methanol which caused decreasing in production cost and separated excess methanol.

3. Effect of *tert*-butanol quantity on the esterification

It has been demonstrated that more than 1/2 molar equivalent methanol are insoluble in vegetable oils and the immobilized lipases are easily inactivated by contacting with insoluble methanol existing as drops in the oils. By-product water is hydrophilic and insoluble in the fatty acid, the presence of higher concentrations of water than needed by the enzyme drives the reaction toward hydrolysis rather than esterification. *tert*-Butanol has been demonstrated in our study as an ideal solvent for lipase-catalyzed biodiesel production. With a certain amount of *tert*-butanol as the reaction medium, both methanol and by-product water are soluble, so the negative effect caused by methanol and water on lipase catalytic activity could be eliminated. From ^{1}H-NMR analysis the results were shown in Figure 4.17.

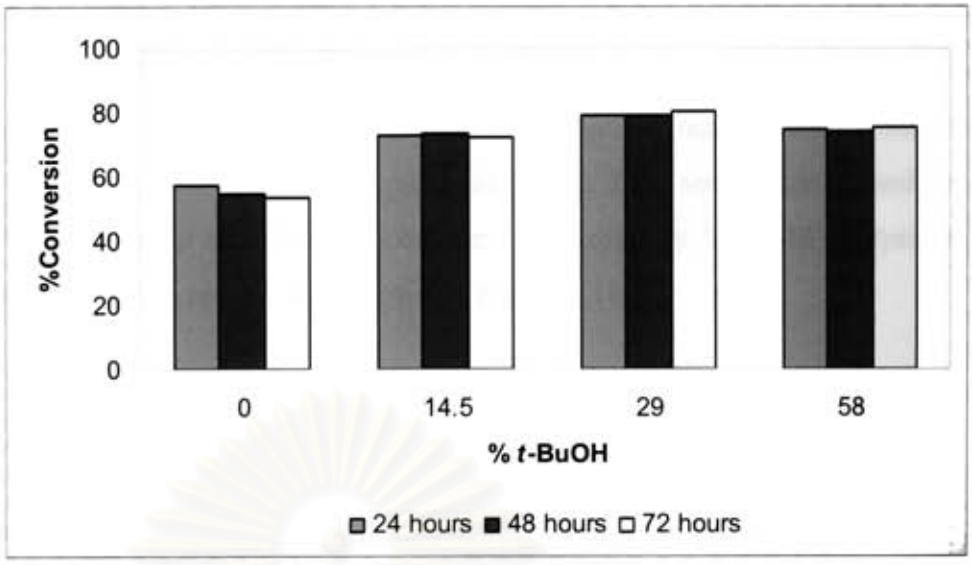


Figure 4.17 Conversion of palm fatty acid versus amount of *tert*-butanol at 0, 14.5, 29 and 58% during 24, 48 and 72 hours in a cosolvent system.

In lipase-catalyzed esterification of palm fatty acid for biodiesel production, different amounts of *tert*-butanol were examined. Without *tert*-butanol, the reaction time at 24, 48 and 72 hours gave 57.5%, 55.3% and 53.5% of conversion, respectively. The conversion was significantly reduced with time, which may be caused by water remained in the column. This trend was expected because the presence of higher concentrations of water than needed by the enzyme drives the reaction toward hydrolysis rather than esterification. The percentage FAME increased by adding *tert*- butanol into the reaction mixture. When the *tert*-butanol reached 29% (based on the palm fatty acid weight), the highest conversion of 80% was obtained. The presence of *tert*-butanol could improve the solubility of methanol in the reaction mixture, so acitivity of lipase was still maintained even with much methanol present in the system. When more than 29% *tert*-butanol was used, the percentage of conversion decreased gradually, which was caused by the dilution of the reactants and excessive *tert*-butanol. In comparison to a solvent free system, the stability of enzyme was obviously improved in a *tert*-butanol system. Therefore, the appropriatel amount of *tert*-butanol was 29%.

4. Effect of flow rate

The effect of flow rate on conversion of palm fatty acid to FAME was investigated at 0.83, 1.67 and 3.33 mg/sec using an optimal reaction temperature 50°C and 2:1 molar ratio of methanol to palm fatty acid, 29% *tert*-butanol based on the palm fatty acid weight and the reactions were monitored by ^{1}H-NMR analysis. From ^{1}H-NMR analysis the results were shown in Figure 4.18.

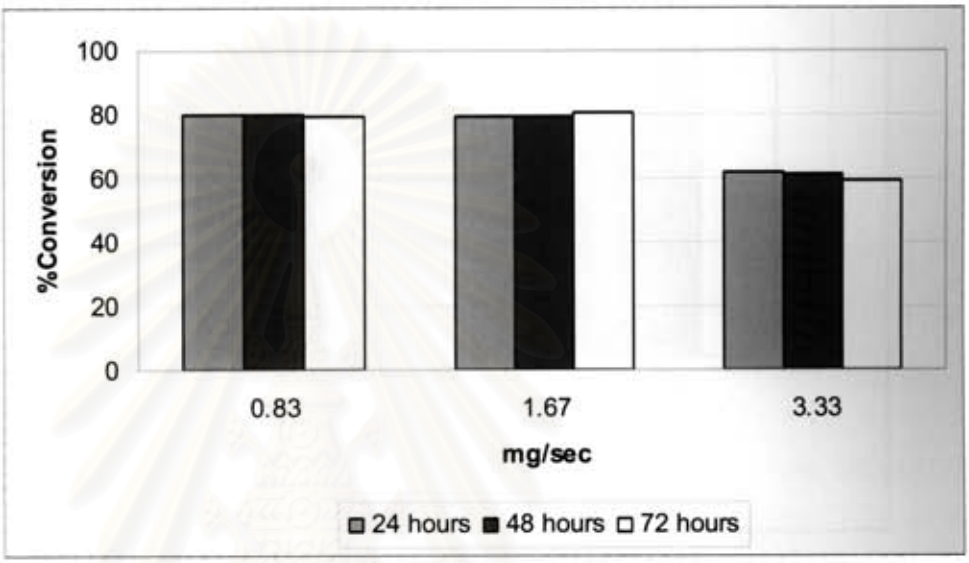


Figure 4.18 Conversion of palm fatty acid versus flow rate 0.83, 1.67 and 3.33 mg/sec during 24, 48 and 72 hours in a cosolvent system.

The velocity of substrates flow is an important parameter in the experiments. The difference of conversion from flow rate was analyzed via statistics in T-test. Based on a 95% confidence level, the flow rate 0.83 and 1.67 mg/sec were non-significant and the percentage of FAME was about 80%. The FAME was increased with decreasing flow rate, indicating that a lower flow rate might extend the contact time between substrate and enzyme and result in higher conversion FAME. The flow rate of 3.33 mg/sec gave about 60% of conversion to methyl ester. With the increase of flow rate, the substrate will only pass through the enzyme with less interaction with the enzyme, consequently failing to bind at the enzyme active site. Hence, there is less contact between the substrate and enzyme active sites yielding lower FAME. If the flow rate is too high, the contact time of substrate on lipase will be too short and the reaction will be incomplete. If the velocity is too low, the throughput of the reaction will be little. Therefore, the optimal flow rate was 1.67 mg/sec.

5. Effect of amount of Novozym 435

The effect of amount of Novozyme 435 on conversion of palm fatty acid to FAME was investigated at 0.328 and 0.656 g using an optimal reaction temperature 50°C, 2:1 molar ratio of methanol to palm fatty acid and 29% *tert*-butanol (based on palm fatty acid weight). The reactions were monitored by ^{1}H-NMR analysis.

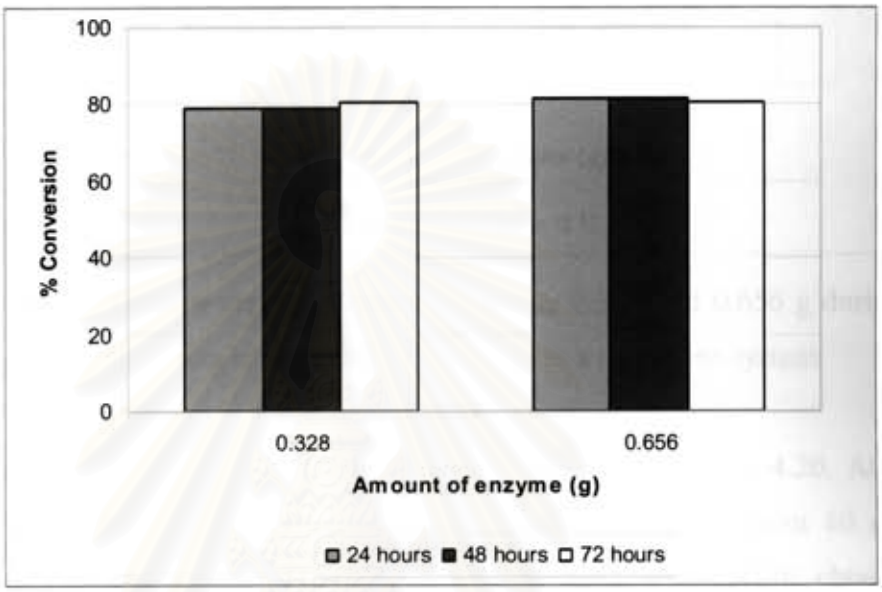


Figure 4.19 Conversion of palm fatty acid versus amount of enzyme 0.328 and 0.656 g during 24, 48 and 72 hours at flow rate 1.67mg/sec in a cosolvent system.

From ^{1}H-NMR analysis the results were shown in Figure 4.19. The percentage of FAME obtained from amount of enzyme 0.328 and 0.656 g were similar. The difference of conversion from amount of enzyme was analyzed via statistics in T-test. Based on a 95% confidence level, the amount of enzyme 0.328 and 0.656 g at flow rate 1.67mg/sec were non-significant and the percentage of FAME was about 80%. It was observed that increasing amount of enzyme does not have much effect on increasing the percentage of FAME at flow rate 1.67mg/sec.

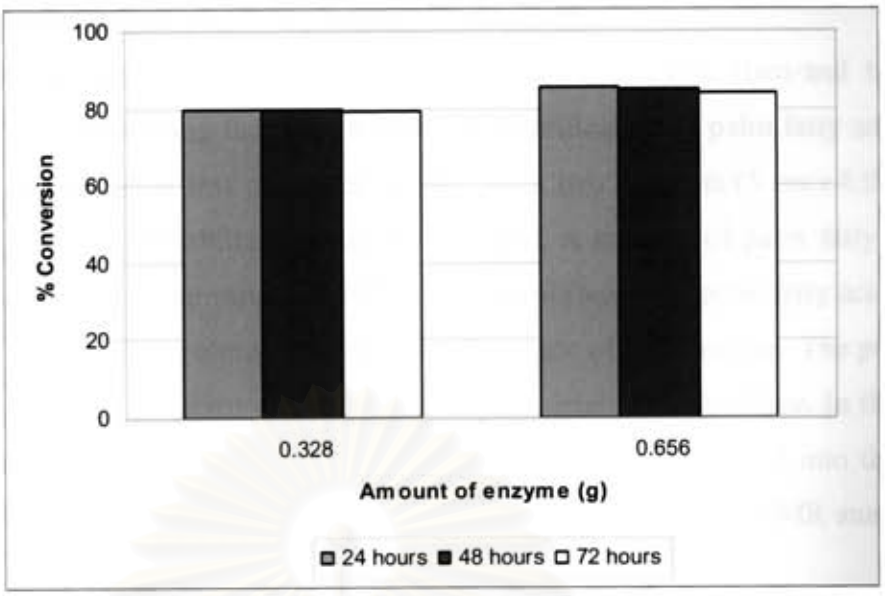


Figure 4.20 Conversion versus amount of enzyme 0.328 and 0.656 g during 24, 48 and 72 hours at flow rate 0.83mg/sec in a cosolvent system.

From ^{1}H-NMR analysis the results were shown in Figure 4.20. At the flow rate 0.83 mg/sec, amount of enzyme 0.328 and 0.656 g gave about 80 and 85 of conversion to methyl ester, respectively. The percentage of FAME obtained from amount of enzyme 0.656 g was higher than 0.328 g about 5%. It was observed that the ester content increased when increasing amount of enzyme. Based on a 95% confidence level, the amount of enzyme 0.328 and 0.656 g at flow rate 0.83 mg/sec were significant. It can be clearly seen effect of amount of Novozym 435 at flow rate 0.83 mg/sec because the lower flow rate might extend the contact time between substrate and enzyme and result in higher conversion FAME. Amount of enzyme related with production cost. Therefore, this study used 0.328 g of Novozym 435 as optimal condition.

4.3.2.2 Three-step esterification process

We attemped to carry out two types of the reaction (two-and three step reactions) by considering the factors affecting esterification of palm fatty acid. In the two-step esterification was conduced at 50°C with two columns (5 cm×4.57 mm) in which 0.328 g of immobilized lipase was packed. A mixture of palm fatty acid and methanol with 1:2 molar ratio and 29% *tert*-butanol (based on palm fatty acid weight) were fed into the first column at a constant flow rate of 1.67 mg/sec. The product, in which water/FAMEs mixture, was separated completely by centrifuge. In the second step the water-free of the product and the substrate mixture was fed into the second column at the same flow rate. The reactions were monitored by ^{1}H-NMR analysis.

From ^{1}H-NMR analysis in Table 4.2, the percentage conversion in the first and second column eluates of the reaction was about 80 and 76%, respectively. The percentage conversion from the second column decreased about 4%. This maybe caused by the reversible reaction.

Table 4.2 The effect of two-step esterification on conversion by ^{1}H-NMR analysis in a cosolvent system

Time (hours)	column	
	1	2
24	79.4%	76.9%
48	79.4%	76.3%
72	80.7%	76.4%

From two-step esterification (without methanol added in the second step), the percentage of conversion decreased because of the reversible reaction causing a little remaining methanol in the reaction mixture. Therefore the three step esterification was investigated. The reaction was conduced at 50°C with three columns (5 cm×4.57 mm) in which 0.328 g of immobilized lipase was packed. A mixture of palm fatty acid and methanol with 1:2 molar ratio and 29% *tert*-butanol (based on palm fatty acid weight) were fed into the first column at a constant flow rate of 1.67 mg/sec. The product, in which water/FAMEs mixture, was separated completely by centrifuge.
In the second step 1 molar equivalent of methanol was added in the water-free of the product and the substrate mixture was fed into the second column at the same flow

rate. The third step was similarly operated by feeding a mixture of the second step. The reactions were monitored by ^{1}H-NMR analysis.

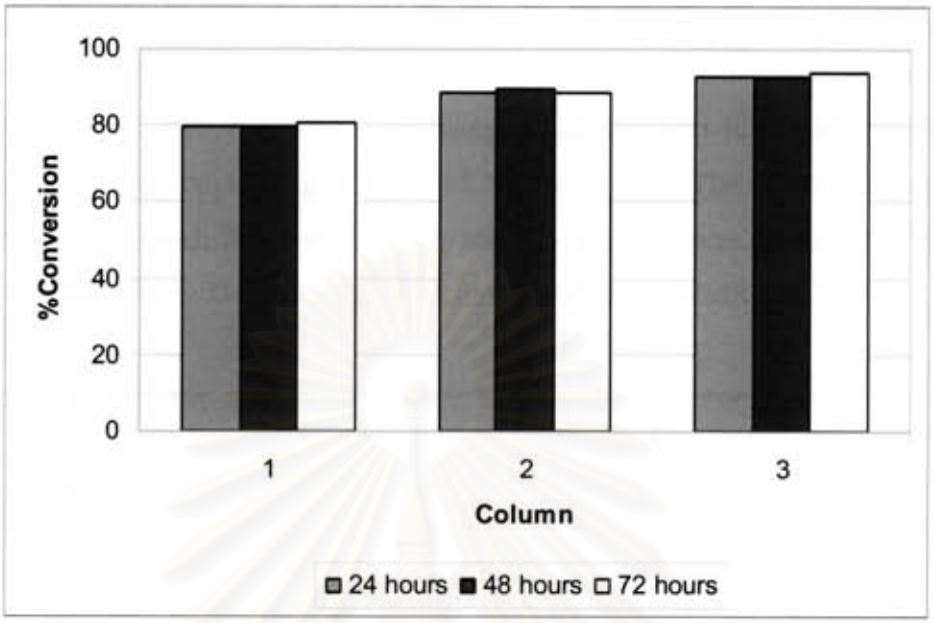


Figure 4.21 Conversion versus number of column during 24, 48 and 72 hours in a cosolvent system.

From ^{1}H-NMR analysis the results were shown in Figure 4.21. The percentage conversion in the first, second and third column eluates of the reaction was about 80, 88 and 92%, respectively. The percentage of FAME from the second and the third column increased about 8% and 4%, respectively. The cost of lipase accounts for a large part in the total cost of biodiesel production. Therefore, the optimal condition for biodiesel production in a solvent medium were temperature at 50°C with three columns (5 cm×4.57 mm) in which 0.328 g of immobilized lipase was packed, flow rate 1.67 mg/sec, palm fatty acid and methanol with a 1:2 molar ratio in the first column and 1:1 in the second and third column.

4.4 Operational stability of the immobilized lipase

The operational stability of the immobilized lipase is an important parameter in an industrial process, since it directly affects the cost. To compare the stability of the immobilized lipase in a solvent free system and a cosolvent system was investigated. Palm fatty acid and methanol with a 1:2 molar ratio (using *t*-BuOH 29% based on palm fatty acid weight in a cosolvent system) were mixed well in a feeding tank. The esterification was carried out using a 5 cm stainless tube reactor with a 4.57 mm inner diameter containing 0.328 g of Novozym 435. The mixture was pumped through a column with flow rate 1.67 mg/sec. The reaction was operated at 50°C with stir during 240 hours. The reactions were monitored by ^{1}H-NMR analysis.

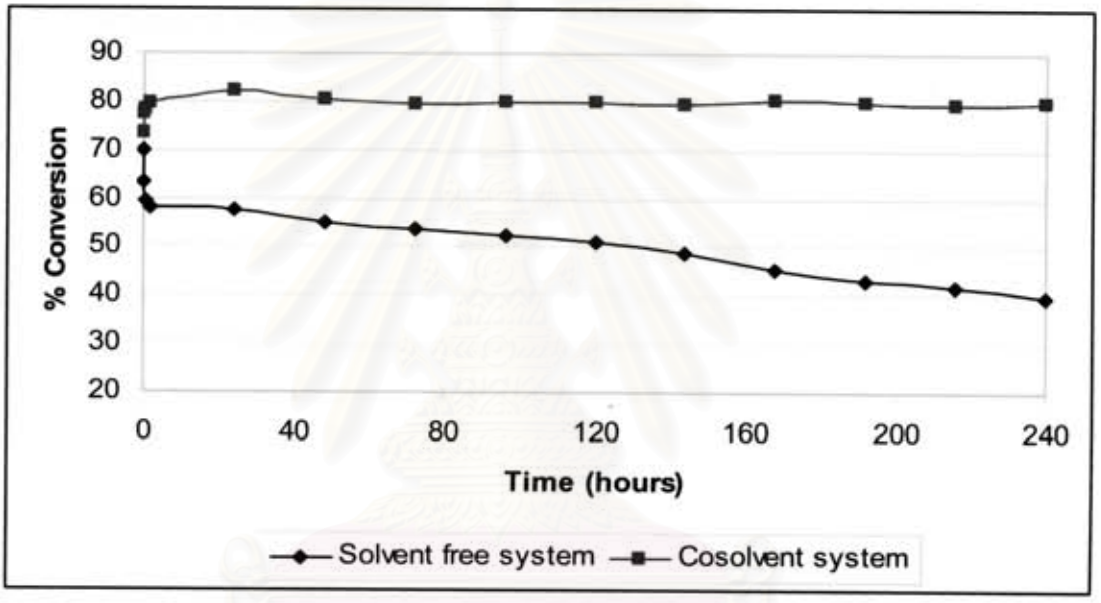


Figure 4.22 Conversion versus reaction time during 240 hours in a solvent free system and a cosolvent system.

From ^{1}H-NMR analysis, the results were plotted as shown in Figure 4.22. In a solvent free system, the esterification reached equilibrium at 1 hour and the percentage of conversion was about 57%. After that the percentage of conversion significantly reduced with time. In a cosolvent system, the esterification reached equilibrium at 1 hour and the percentage of conversion was about 80%. The operational stability of the immobilized lipase in a cosolvent system is longer than 240 hours, which maintained 79–81% of conversion to methyl ester during all experiment. In the solvent free system, water produced by the reaction stays in the

column. The presence of higher concentrations of water than needed by the enzyme drives the reaction toward hydrolysis rather than esterification. In *tert*-butanol system which is a moderate polar solvent, both water and MeOH are soluble in *tert*-butanol solvent, the negative effect caused by water on lipase catalytic activity could be eliminated. This result corresponds with the results obtained by Royon et al. (2007) also reported the stability of immobilized lipase is longer than 500 hours without appreciable loss in substrate conversion, for the transesterification of cotton seed oil with methanol in *tert*-butanol system using. A thermostatized column containing a mixture of Novozyme 435 plus mill glass was used as the fixed bed reactor.

4.5 Properties of biodiesel

The characteristics of biodiesel produced from palm fatty acid by three-step esterification. In a solvent free and cosolvent system were comparable to that of standard biodiesel as shown in Table 4.3. The comparison of these properties with American standards for biodiesel (ASTM D6751-02) shows the density and flash point are in range of fuel properties prescribed in standard biodiesel, except percentage of methyl ester (See details in Appendix A).

Table 4.3 Properties of biodiesel

Fuel property	Standard biodiesel ASTM D6751-02	Biodiesel in a solvent free	Biodiesel in a cosolvent
Methyl ester, %wt	> 96.5	77.5	91.7
Density at 15°C, kg/m^3	0.86-0.90	0.8784	0.8780
Flash Point (°C)	100-170	>130	>130

CHAPTER V

CONCLUSION AND SUGGESTION

5.1 Conclusion

Lipase-catalyzed biodiesel production from palm fatty acid with the linear and branched alcohols was investigated. Among several short chain alcohols, methanol gave the highest conversion. Primary alcohols gave higher conversion than secondary alcohols. The reason could be due to secondary alcohols reacted slower than primary alcohol. Increasing the carbon numbers of primary alcohols and secondary alcohols decreased the percent conversion of the free fatty acids. Due to small size of molecule and higher polarity, methanol might easily diffuse and access to active site of the lipase enzyme resulting in higher reaction rate. For bulky alcohol such as *tert*-butanol as bulky alcohol, there was no reaction.

The amount of molecular sieves were conducted at 0, 15, 30, 50, 80% based on palm fatty acid. The results showed that a small amount of water in the reaction did not affected to the percentage conversion significantly. There was no much change in the percent conversion with molecular sieve. The percent conversion decreased when increasing the amount of molecular sieve. Because molecular sieve may absorb methanol in the reaction, it results in the lower percent conversion.

Activity of *Candida antarctica* lipase may be reduced in the continuous production of biodiesel from palm fatty acid in a solvent free system and FAME was obtained 62.1% at the appropriate condition (reaction temperature 50°C, flow rate 1.67 mg/sec, palm fatty acid/methanol molar ratio of 1:2 and Novozym 435 0.656 g (column length 10cm).

tert-Butanol could improve the solubility of methanol and reaction mixture, so lipase still maintained high activity even high amount of methanol present in the system. In the presence of this solvent, high reaction rates are obtained. The quantity of enzyme needed to catalyze the reaction within a reasonable time periods is lower

than the solvent free system. The appropriate conditions for synthesis were as follows: reaction temperature 50°C, flow rate 1.87 mg/sec, and methanol to palm fatty acid ratio 2:1 and *tert*-butanol 29% based on palm fatty acid weight. The highest conversion ratio of the FAME from one-step and three-step under the optimal condition was 80% and 92%, respectively. The lipase catalyst could be used for >240 hours without decrease of the activity.

Biodiesel properties were analyzed including %methyl ester, flash point and density which were in range of fuel properties prescribed in standard biodiesel, except percentage of methyl ester which less than limit of fuel property.

The enzymatic process established in this study has the following advantages: the immobilized lipase can be used for a long period so that the production costs are reduced. In addition, the process is advantageous in the following respects: the reaction temperature of lipase-catalysis process is lower than alkali-catalysis process, therefore the energy for heating can be saved; a process to remove the acid catalyst is not necessary; and by product, water, is easily separated from the products. These advantages indicate that this enzymatic system is eco-friendly and may be applicable to an industrial process for the production of biodiesel fuel from palm fatty acid.

5.2 Suggestion

Palm fatty acid which was a by-product from palm oil production is an interesting raw material for use in the industrial biodiesel production. However, the production cost of lipase catalyst is significantly higher than an alkali. Reducing the cost of lipase can use genetic engineering technology, such as by developing lipases with high levels of expression or stability towards methanol.

REFERENCES

[1] EPPO Department Operation Centre. Ministry of Energy, Thailand. Energy Statistics. Available from: http://www.eppo.go.th, INTERNET.

[2] Srivastava, A. and Prasad, R. Triglyceride-based diesel fuels. 2000. *Renewable and Sustainable Energy Review* 4:111-133.

[3] Ma, F. and Hanna, M.A. Biodiesel production: a review. 1999. *Bioresource Technology* 70: 1-15

[4] Fukuda, H., Kondo, A. and Nodo, H. 2001. Biodiesel fuel production by transesterification of oils. *Bioscience and Bioengineering* 92: 405-416.

[5] Meher, L.C., Sagar, D.V. and Naik, S.N. 2006. Technical aspects of biodiesel production by transesterifiaction-a review. *Renewable and Sustainable Energy Review* 10: 248-268.

[6] สายฝน เกลียวสัมพันธ์. 2549. การเปลี่ยนไขสบู่เป็นไบโอดีเซลด้วย NOVOZYMES A/S จาก *Candida antarctica* lipase. วิทยานิพนธ์ปริญญามหาบัณฑิต. สาขาวิชาเทคโนโลยีชีวภาพ จุฬาลงกรณ์มหาวิทยาลัย.

[7] Akoh, C.C., Chang, S.W. and Shaw, J.F. 2007.Enzymatic approach to biodiesel production. *Agricultural and Food Chemistry* 55: 8995-9005.

[8] Small scale biodiesel production. *Waste management and research center* 4-5.

[9] Marchetti, J.M., Miguel, A.F. 2005. Possible methods for biodiesel production. *Renewable and Sustainable Energy Review* 11: 1300-1311.

[10] Fatty acid Index, http://www.lipomics.com/fatty_acids INTERNET.

[11] Li, X., Xu, H. and Wu, Q. 2007. Large-scale biodiesel production from microalga *Chlorella* protothecoids through heterotrophic cultivation in bioreactors. *Biotechnology and Bioengineering* 98: 764-771.

[12] Sundram, K., Sambanthamurthi, R. and Yew, A.T. 2003. Palm fruit chemistry and nutrition. *Asia Pacific Journal of Clinical Nutrition* 12: 355-362.

[13] Sarakoon, N., Sinthurahat, S.and Dansakulphon, S. 1998. Agro-ecological Zoning Analysis for Oil Palm in the South of Thailand. Department of Agriculture. The Ministry of Agriculture and Cooperatives. Bangkok.. [in Thai]

[14] Tyson, K.S. Biodiesel Handling and Use Guidelines. National Renewable Energy Laboratory, Department of Energy, CO. USA. (2001), NREL/TP-580-30004, Available from: http://www.agriculture.state.ia.us, INTERNET.

[15] Swern, D. 1973. *Bailey's Industrial oil and fat products 4 th ed.* New York: John wiley&Sons.

[16] Schuchardf, U., Sercheli, R. and Vargas, R.M. 1998. Transesterification of vegetable oils: a review. *Journal of the Brazilian Chemical Society* 9: 199-210.

[17] Chibata, I., Tosa, T., Sato, T. and Mori, T. 1978. *Immobilized enzymes research and development.* Tokyo: Kodansha Ltd.

[18] Tyson, K.S. Biodiesel Handling and Use Guidelines. National Renewable Energy Laboratory, Department of Energy, CO.USA. (2001), NREL/TP-580-30004, Available from: http://www.agriculture.state.ia.us, INTERNET.

[19] ประกาศกรมธุรกิจพลังงาน เรื่อง กำหนดลักษณะและคุณภาพของไบโอดีเซลประเภทเมทิลเอสเทอร์ของกรดไขมัน. *ราชกิจจานุเบกษา* (2548), 122 (ตอนพิเศษ 70 ง).

[20] Watanabe, Y., Shimada, Y., Sugihara, A. and Tominaga, Y. 1999. Stepwise ethanolysis of tuna oil using immobilized *Candida antractica* lipase. *Journal of Bioscience and bioengineering* 88: 622-626.

[21] Kose, O., Tuter, M. and Aksoy, H.A. 2002. Immobilized *Candida antarctica* lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium. *Bioresource Technology* 83: 125-129.

[22] Shimada, Y., Watanabe, Y., Sugihara, A. and Tominaga, Y. 2002. Enzymatic alcoholysis for biodiesel fuel production and application of the reaction to oil processing. *Journal of Molecular Catalysis B: Enzymatic* 17: 133-142.

[23] Chen, J.W. and Wu, W.T. 2003. Regeneration of immobilized *Candida antarctica* lipase for transesterification. *Bioscience and bioengineering* 95: 466-469.

[24] Hass, M.J. 2005. Improving the economics of biodiesel production through the use of low value lipids as feedstocks : vegetable oil soapstock. *Fuel Processing Technology* 86: 1087-1096.

[25] Li, L., Du, Wei., Liu, D. and Wang, L. 2006. Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium. *Journal of Molecular Catalysis B: Enzymatic* 43: 58-62.

[26] Watanabe, Y., Pinsirodom, P., Nagao, T., Yamauchi, A., Kobayashi, T., Nishida, Y. and Shimada, Y. 2007. Conversion of acid oil by-produced in vegetable oil refining to biodiesel fuel by immobilized *Candida antractica* lipase. *Journal of Molecular Catalysis B: Enzymatic* 44: 99-105.

[27] Jeong, T.G. and Park, D.H. 2008. Lipase-Catalyzed Transesterification of Rapeseed Oil for Biodiesel Production with *tert* –Butanol. *Applied Biochemistry and Biotechnology* 148: 131-139.

[28] Dossat, V., Combes, D. and Marty, A. Continuous enzymatic transesterification of high oleic sunflower oil in a packed bed reactor: influence of the glycerol production. *Enzyme and Microbial Technology* 25: 194-200.

[29] Nie, K., Xie, F., Wang, F. and Tan, T. 2006. Lipase catalyzed methanolysis to produce biodiesel: Optimization of the biodiesel production. *Journal of Molecular Catalysis B: Enzymatic* 43: 142-147.

[30] Royon, D., Daz, M. and Ellenrieder. 2007. Enzymatic production of biodiesel from cotton seed oil using *t*-butanol as a solvent. *Bioresource Technology* 98: 648-653.

[31] Shaw, J.F., Chang, S.W., Lin, S.C. and Wu, T.T. 2008. Continuous enzymatic synthesis of biodiesel with Novozym 435. *Energy and fuels* 22: 840-844.

[32] Mcneff, C.V., Mcneff, L.C., Yan, B. and Nowlan, D.T. 2008. A continuous catalytic system for biodiesel production. *Applied Catalysis A* 343: 39-48.

[33] Chongkhong, S., Thongurai, C. and Chetpattananondh, P. 2008. Continuous esterification for biodiesel production from palm fatty acid distillate using economical process. *Renewable Energy* 34: 1059-1063.

[34] Halim, S.F.A., Kamaruddin, A.H. and Fernando, W.J.N. 2009. Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: Optimization using response surface methodology (RSM) and mass transfer studies. *Bioresource Technology* 100: 710-716.

[35] Tamalampudi, S., Talukder, M.R., Hamad, S. and Numata, T. 2008. Enzymatic production of biodiesel from Jatropha oil: A comparative study of immobilized-whole cell and commercial lipases as a biocatalyst. *Biochemical Engineering Journal* 39:185–189

[36] Watanabe, Y., Shimada, Y., Sugihara, A. and Tominaga, Y. 2002. Conversion of degummed soybean oil to biodiesel fuel with immobilized *Candida antractica* lipase. *Journal of Molecular Catalysis B: Enzymatic* 17: 151-155.

[37] Wang, L., Du, W. and Liu, Dehua. 2006. Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in *tert*-butanol system. *Journal of Molecular Catalysis B: Enzymatic* 43: 29-32.

[38] Balcao, V.C., Paiva A.L. and Malcata F.X. 1996. Bioreactor with immobilized lipases: State of art. *Enzyme and Microbial Technology* 18: 392-416.

[39] Talukder, M.M.R., Beatrice, K.L.M. and Song, O.P. 2008. Improved method for efficient production of biodiesel from palm oil. *Energy and Fuels* 22: 141-144.

[40] Chen, J.W. and Wu, W.T. 2003. Regeneration of immobilized *Candida antarctica* lipase or transesterification. *Bioscience and Bioengineering* 95:466-469.

APPENDICES

Appendix A

1. Report of analysis of biodiesel three step esterification from palm fatty acid in a solvent free system.

กรมธุรกิจพลังงาน
กระทรวงพลังงาน
ส่วนตรวจวิเคราะห์ สำนักคุณภาพน้ำมันเชื้อเพลิง กรมธุรกิจพลังงาน กระทรวงพลังงาน
44/100 ถนนนนทบุรี ตำบลบางกระสอ อำเภอเมือง จังหวัดนนทบุรี 11000
โทรศัพท์ 02-5474317-20 โทรสาร 02-5474319

เลขที่ CE-0027-51
แผ่นที่ 1/3

ใบรับรองผลการตรวจสอบคุณภาพ
สำหรับ

ตัวอย่างเลขที่: CU 0029/52
ชนิดน้ำมัน: ไบโอดีเซลประเภทเมทิลเอสเตอร์ของกรดไขมัน
Biodiesel in a solvent free system
รหัสอ้างอิง: -
ชื่อผู้ประกอบการ: ตัวอย่างน้ำมันฯ จากนิสิตจุฬาลงกรณ์มหาวิทยาลัย
ที่อยู่: จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร

ผู้จัดส่ง: -
ตามเลขที่หนังสือ: -
ลงวันที่: -
สภาพภาชนะบรรจุ: ปกติ
วันที่รับตัวอย่าง: 27/03/2552
วันที่ออกใบรับรองผลการตรวจสอบคุณภาพ: 02/04/2552

สรุปผลการตรวจสอบ: ( ) ถูกต้องตามมาตรฐานฯ ( ) ไม่ถูกต้องตามมาตรฐานฯ (/) อื่นๆ.....

ตรวจสอบโดย .....
(นางสาวประภาศรี สุนทโรดม)
ตำแหน่ง นักวิทยาศาสตร์ 7ว

กรมธุรกิจพลังงาน
สถาบันวิจัยพลังงาน
สวนตรวจวิเคราะห์ สำนักคุณภาพน้ำมันเชื้อเพลิง กรมธุรกิจพลังงาน กระทรวงพลังงาน
44/100 ถนนนนทบุรี ตำบลบางกระสอ อำเภอเมือง จังหวัดนนทบุรี 11000
โทรศัพท์ 02-5474317-20 โทรสาร 02-5474319

เลขที่ CE-0027-51
แผ่นที่ 2/3

บัญชีสรุปผลการตรวจสอบคุณภาพน้ำมันเชื้อเพลิง
สำหรับ

ตัวอย่างเลขที่: CU 0029/52 **ชนิดน้ำมัน:** ไบโอดีเซลประเภทเมทิลเอสเตอร์ของกรดไขมัน

วันที่ตรวจสอบ: 02/04/2552

รายละเอียดการตรวจสอบ

ข้อกำหนดคุณภาพ	อัตราสูงต่ำ (ตามประกาศกรมธุรกิจพลังงาน)	วิธีมาตรฐานที่ใช้ตรวจสอบ	ผลการตรวจสอบ
1. เมทิลเอสเตอร์ (ร้อยละโดยน้ำหนัก)	ไม่ต่ำกว่า 96.5	EN 14103	77.53
2. ความหนาแน่น ณ อุณหภูมิ 15 °ซ (กิโลกรัม/ลูกบาศก์เมตร)	ไม่ต่ำกว่า 860 และ ไม่สูงกว่า 900	ASTM D 1298	878.4
3. ความหนืด ณ อุณหภูมิ 40 °ซ (เซนติสโตกส์)	ไม่ต่ำกว่า 3.5 และ ไม่สูงกว่า 5.0	ASTM D 445	-
4. จุดวาบไฟ (°ซ)		ASTM D 93	>130
5. ก้ำมะถัน (ร้อยละโดยน้ำหนัก)	ไม่ต่ำกว่า 120	ASTM D 2622	-
6. กากถ่าน (ร้อยละ 10 ของกากที่เหลือจากการกลั่น) (ร้อยละโดยน้ำหนัก)	ไม่สูงกว่า 0.0010 ไม่สูงกว่า 0.30	ASTM D 4530	-
7. จำนวนซีเทน		ASTM D 613	-
8. เถ้าซัลเฟต (ร้อยละโดยน้ำหนัก)	ไม่ต่ำกว่า 51	ASTM D 874	-
9. น้ำ (ร้อยละโดยน้ำหนัก)	ไม่สูงกว่า 0.02	EN ISO 12937	-
10. สิ่งปนเปื้อนทั้งหมด (ร้อยละโดยน้ำหนัก)	ไม่สูงกว่า 0.050	EN 12662	-
11. การกัดกร่อนแผ่นทองแดง	ไม่สูงกว่า 0.0024 ไม่สูงกว่า หมายเลข 1	ASTM D 130	-

สรุปผลการตรวจสอบ: ◯ ถูกต้องตามมาตรฐานฯ ⊘ ไม่ถูกต้องตามมาตรฐาน ◯ อื่นๆ.....

ตรวจสอบโดย:.....

(นางสาวประภาศรี สุนทโรดม)

ตำแหน่ง นักวิทยาศาสตร์ 7ว

2. Report of analysis of biodiesel three step esterification from palm fatty acid in a cosolvent system.

กรมธุรกิจพลังงาน
กระทรวงพลังงาน
สวนตรวจวิเคราะห์ สำนักคุณภาพน้ำมันเชื้อเพลิง กรมธุรกิจพลังงาน กระทรวงพลังงาน
44/100 ถนนนนทบุรี ตำบลบางกระสอ อำเภอเมือง จังหวัดนนทบุรี 11000
โทรศัพท์ 02-5474317-20 โทรสาร 02-5474319

เลขที่ CE-0027-51
แผ่นที่ 1/3

ใบรับรองผลการตรวจสอบคุณภาพ
สำหรับ

ตัวอย่างเลขที่: CU 0030/52
ชนิดน้ำมัน: ไบโอดีเซลประเภทเมทิลเอสเตอร์ของกรดไขมัน
Biodiesel in a cosolvent (t-BuOH)
รหัสอ้างอิง: -
ชื่อผู้ประกอบการ: ตัวอย่างน้ำมันฯ จากนิสิตจุฬาลงกรณ์มหาวิทยาลัย
ที่อยู่: จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร

ผู้จัดส่ง: -
ตามเลขที่หนังสือ: -
ลงวันที่: -
สภาพภาชนะบรรจุ: ปกติ
วันที่รับตัวอย่าง: 27/03/2552
วันที่ออกใบรับรองผลการตรวจสอบคุณภาพ: 2/04/2552

สรุปผลการตรวจสอบ: ◯ ถูกต้องตามมาตรฐานฯ ◯ ไม่ถูกต้องตามมาตรฐานฯ (/) อื่นๆ.....

ตรวจสอบโดย: .....
(นางสาวประภาศรี สุนทโรดม)
ตำแหน่ง นักวิทยาศาสตร์ 7ว

กรมธุรกิจพลังงาน
กระทรวงพลังงาน
สวนตรวจวิเคราะห์ สำนักคุณภาพน้ำมันเชื้อเพลิง กรมธุรกิจพลังงาน กระทรวงพลังงาน
44/100 ถนนนนทบุรี ตำบลบางกระสอ อำเภอเมือง จังหวัดนนทบุรี 11000
โทรศัพท์ 02-5474317-20 โทรสาร 02-5474319

เลขที่ CE-0027-51
แผ่นที่ 2/3

บัญชีสรุปผลการตรวจสอบคุณภาพน้ำมันเชื้อเพลิง
สำหรับ

ตัวอย่างเลขที่: CU 0030/52 **ชนิดน้ำมัน:** ไบโอดีเซลประเภทเมทิลเอสเตอร์ของกรดไขมัน

วันที่ตรวจสอบ: 2/04/2552

รายละเอียดการตรวจสอบ

ข้อกำหนดคุณภาพ	อัตราสูงต่ำ (ตามประกาศกรมธุรกิจพลังงาน)	วิธีมาตรฐานที่ใช้ตรวจสอบ	ผลการตรวจสอบ
1. เมทิลเอสเตอร์ (ร้อยละโดยน้ำหนัก)	ไม่ต่ำกว่า 96.5	EN 14103	91.71
2. ความหนาแน่น ณ อุณหภูมิ 15 °ซ (กิโลกรัม/ลูกบาศก์เมตร)	ไม่ต่ำกว่า 860 และ ไม่สูงกว่า 900	ASTM D 1298	878
3. ความหนืด ณ อุณหภูมิ 40 °ซ (เซนติสโตกส์)	ไม่ต่ำกว่า 3.5 และ ไม่สูงกว่า 5.0	ASTM D 445	-
4. จุดวาบไฟ (°ซ)		ASTM D 93	>130
5. กำมะถัน (ร้อยละโดยน้ำหนัก)	ไม่ต่ำกว่า 120	ASTM D 2622	-
6. กากถ่าน (ร้อยละ 10 ของกากที่เหลือจากการกลั่น) (ร้อยละโดยน้ำหนัก)	ไม่สูงกว่า 0.0010 ไม่สูงกว่า 0.30	ASTM D 4530	-
7. จำนวนซีเทน		ASTM D 613	-
8. เถ้าซัลเฟต (ร้อยละโดยน้ำหนัก)	ไม่ต่ำกว่า 51	ASTM D 874	-
9. น้ำ (ร้อยละโดยน้ำหนัก)	ไม่สูงกว่า 0.02	EN ISO 12937	-
10. สิ่งปนเปื้อนทั้งหมด (ร้อยละโดยน้ำหนัก)	ไม่สูงกว่า 0.050	EN 12662	-
11. การกัดกร่อนแผ่นทองแดง	ไม่สูงกว่า 0.0024 ไม่สูงกว่า หมายเลข 1	ASTM D 130	-

สรุปผลการตรวจสอบ: ◯ ถูกต้องตามมาตรฐานฯ ⊘ ไม่ถูกต้องตามมาตรฐาน ◯ อื่นๆ.....

ตรวจสอบโดย:.....

(นางสาวประภาศรี สุนทโรดม)

ตำแหน่ง นักวิทยาศาสตร์ 7ว

3. Data sheet of Novozym 435

Product Data Sheet

novozymes

Novozym® 435

Valid from 13-Oct-2006

Product Characteristics:

Enzyme Class	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
Carrier	Acrylic resin
Preservatives	Potassium sorbate Sodium benzoate
Production organism	Aspergillus niger 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	-	3.0	%

Packaging: See the standard packaging list for more information.

Recommended Storage:

Best before	When stored as recommended, the product is best used within 6 months from date of delivery.
Storage temperature	0-25°C (32°F-77°F)
Storage Conditions	In unbroken packaging - dry and protected from the sun. The product has been formulated for optimal stability. Extended storage or adverse conditions such as higher temperature or higher humidity may lead to a higher dosage requirement.

novozymes

Safety and handling precautions:

Enzymes are proteins. Inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes and mucous membranes upon prolonged contact. Powdered enzymes are readily inhaled and should be handled only with specific precautions to prevent inhalation of dust. All equipment and handling procedures must be designed to control airborne dust. Personal respiratory protection is recommended in all cases where full dust control is not secured . All spills, however minor, should be removed immediately. Use respiratory protection. Major spills should be carefully shovelled into plastic-lined containers. Minor spills and the remains of major spills should be removed by vacuum cleaning or flushing with water (avoid splashing). Vacuum cleaners and central vacuum systems should be equipped with HEPA filters. Wear suitable protective clothing, gloves and eye/face protection as prescribed on the warning label. Wash contaminated clothes. A Material Safety Data Sheet is supplied with all products. See the Safety Manual for further information regarding how to handle the product safely.

Novozymes A/S
Krogshoejve, 36
2880 Bagsvaerd
Denmark

Tel +45 8824 9999
Fax +45 8824 9998

For more information and addresses of international offices please see www.novozymes.com or contact info@novozymes.com

Appendix B

CALCULATIONS

1. Composition of fatty acid in palm fatty acid

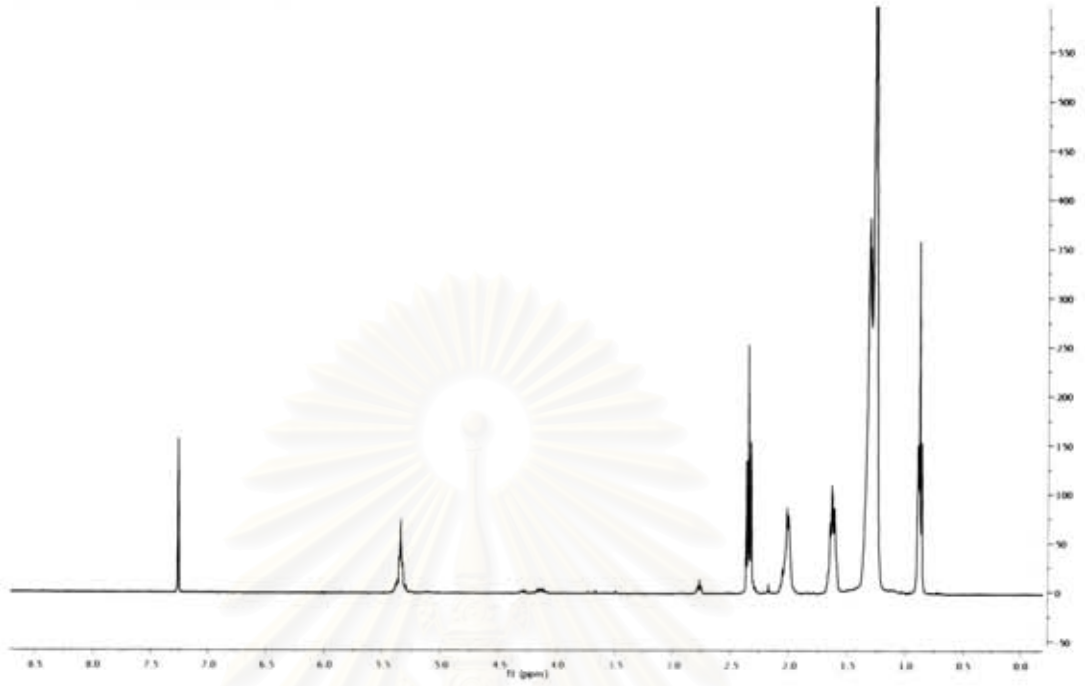
where

$$M_{FFA} = \frac{\Sigma\,[(\text{MW of fatty acids} \times \%\ \text{fatty acids})}{\text{total of fatty acids (only fatty acid)}}$$

So,
$$M_{FFA} = \frac{[(256.43\times44.66) + (284.48\times4.13) + (282.46\times37.56) + ...]}{97.41}$$

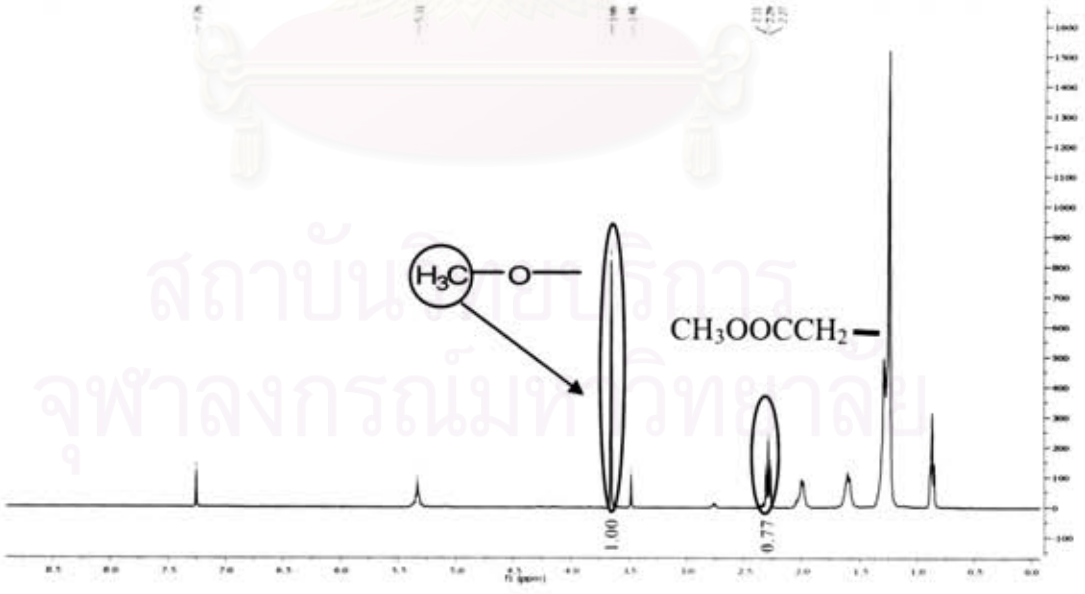
$$= 270.44\ \text{g/mol}$$

2. Calculation of %conversion of biodiesel from palm fatty acid with primary and secondary alcohols



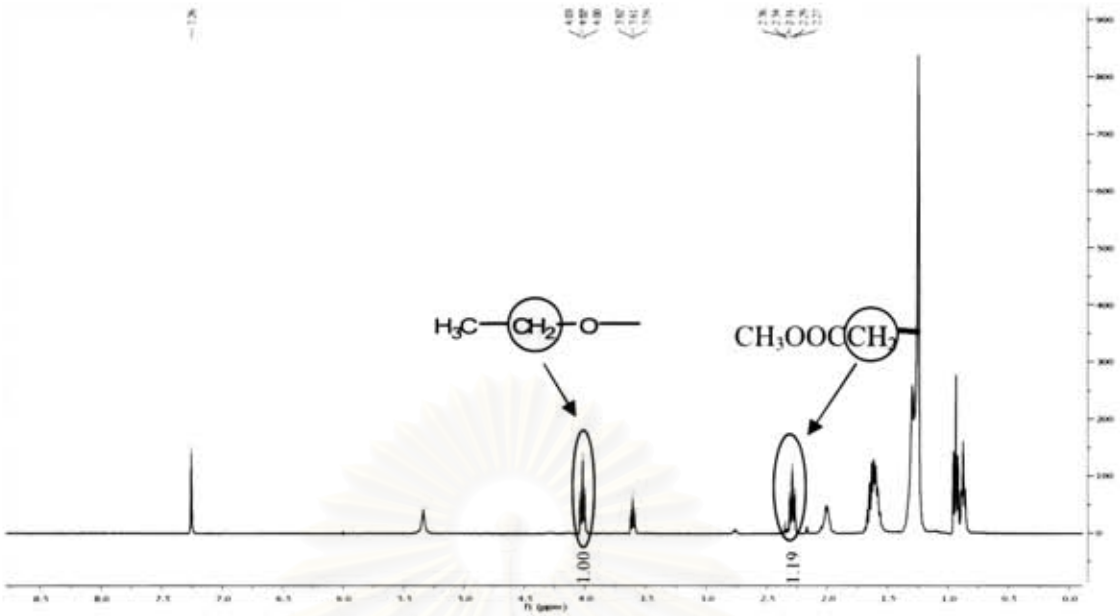
^{1}H NMR spectrum of palm fatty acid

$$\%\text{conversion} = \frac{\text{integration of methoxy group per one mole equivalent}}{\text{integration of methylene group per one mole equivalent}}$$



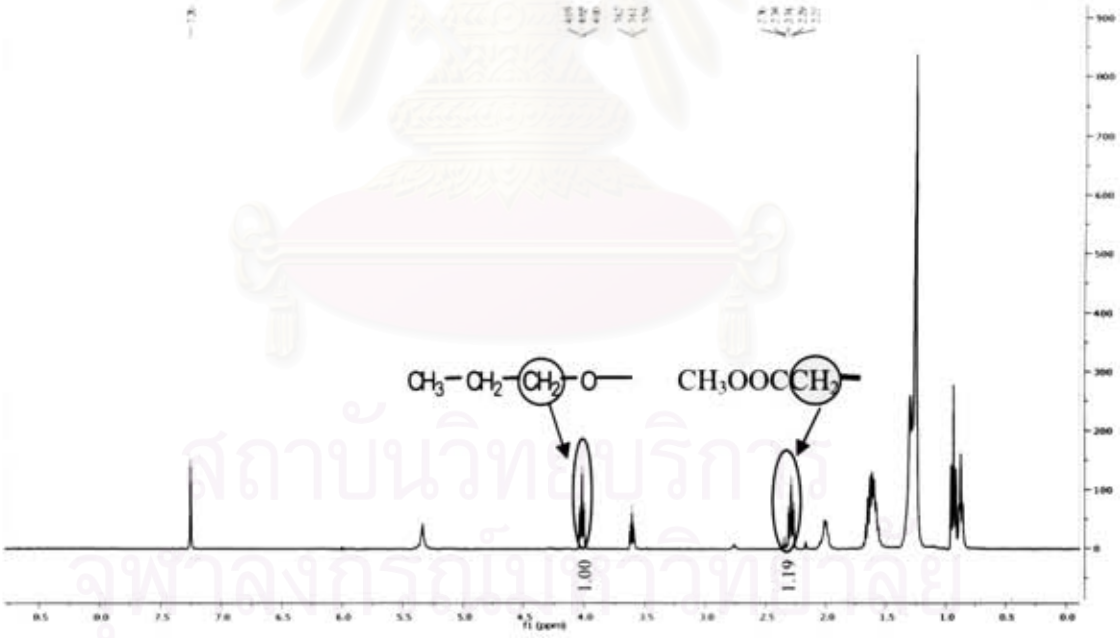
^{1}H-NMR spectrum of the reaction mixture of palm fatty acid and MeOH

%conversion = $(1/3)/(0.77/2) \times 100 = 86.84\%$



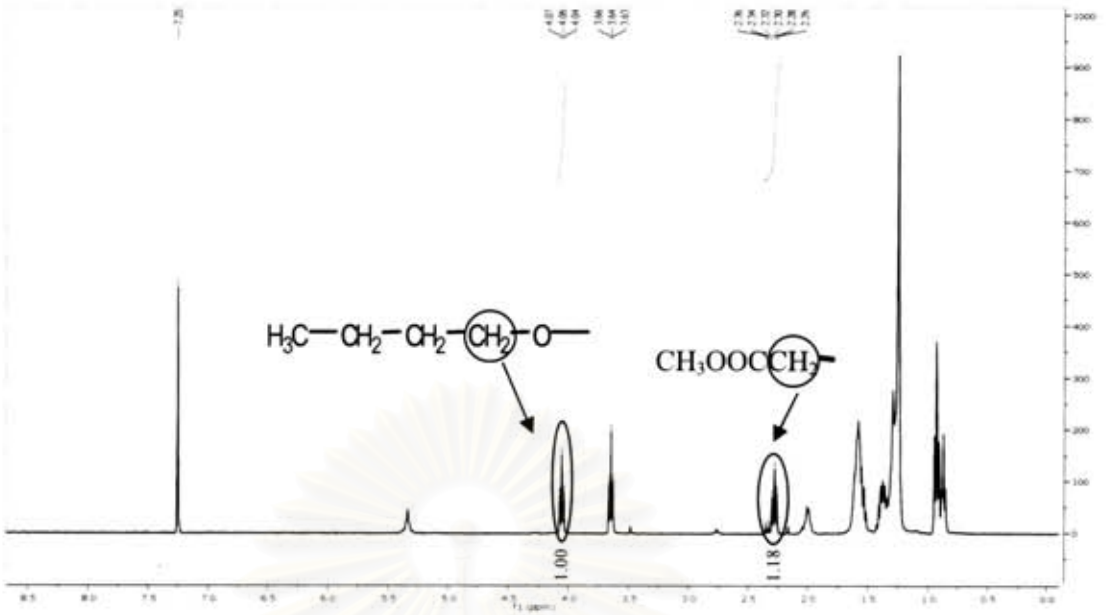
^{1}H-NMR spectrum of the reaction mixture of palm fatty acid and EtOH

%conversion = (1/1.19)×100 = 84.03%



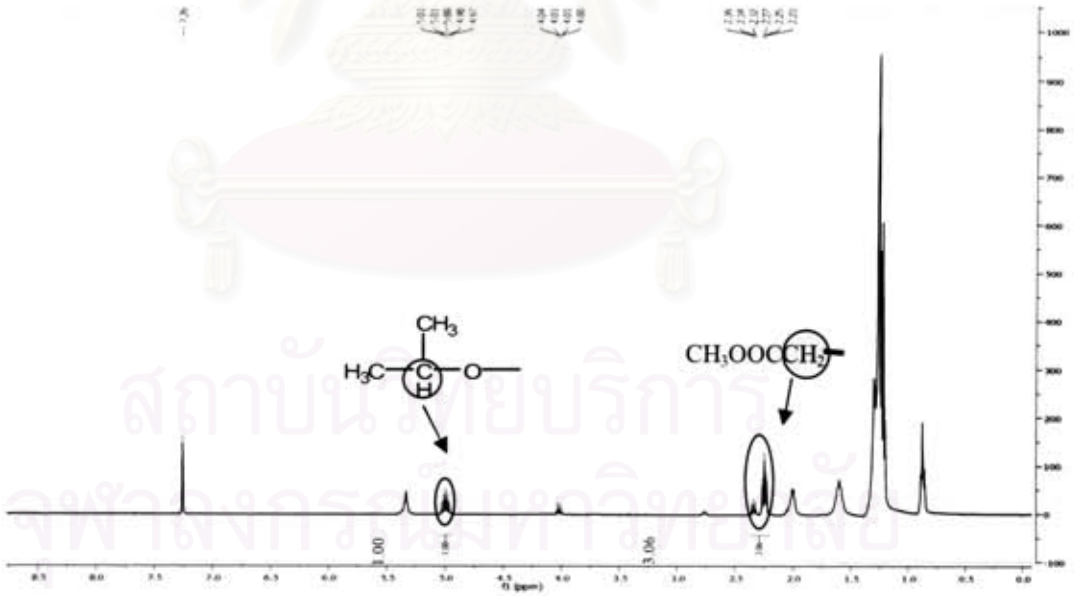
^{1}H-NMR spectrum of the reaction mixture of palm fatty acid and PrOH

%conversion = (1/1.19)×100 = 84.03%



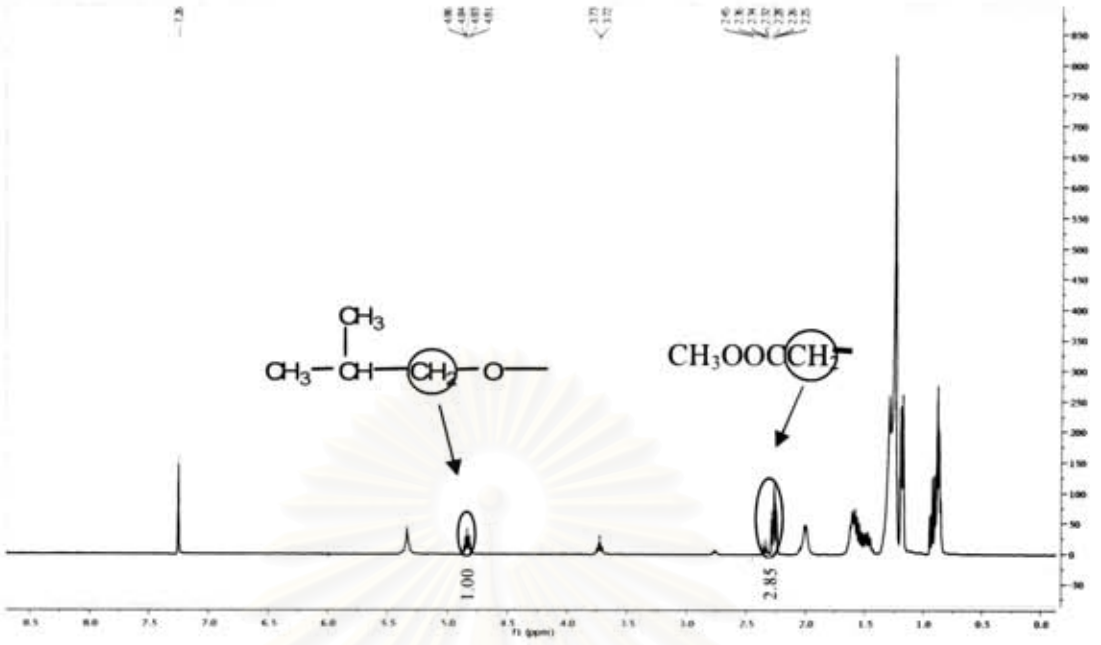
^{1}H-NMR spectrum of the reaction mixture of palm fatty acid and BuOH

%conversion = (1/1.18)×100 = 84.74%



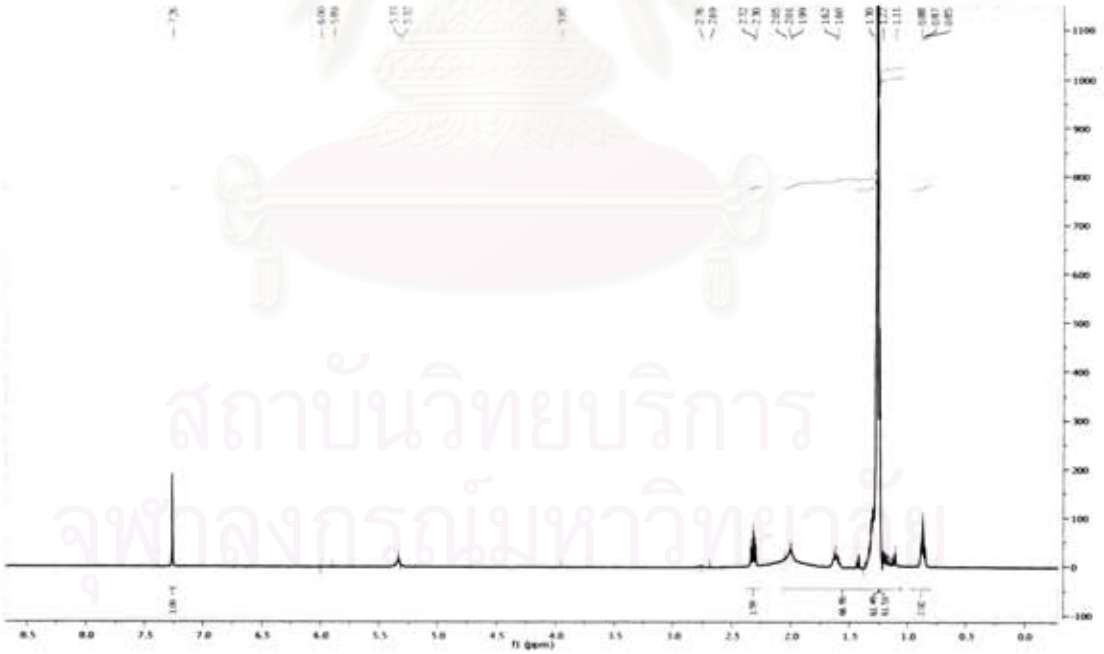
^{1}H-NMR spectrum of the reaction mixture of palm fatty acid and *i*-PrOH

%conversion = 1/(3.06/2)×100 = 65.36%



^{1}H-NMR spectrum of the reaction mixture of palm fatty acid and *i*-BuOH

%conversion = (1/2.85)×100 = 32.68%



^{1}H-NMR spectrum of the reaction mixture of palm fatty acid and *tert*-BuOH

It was no reaction

3. Calculation amount of enzyme in the column

Density of enzyme = 0.4 g/cm^3

Inner diameter 0f the column = 0.4572 cm.

In the column 5 cm. (height)

$$V = \prod r^2 h$$
$$= 3.14 \times (0.2286)^2 \times 5$$
$$= 0.82\ cm^3$$

Enzyme 1 ml use 0.4 g

Enzyme 0.82 ml use $0.4 \times 0.82 = 0.328$ g

$\therefore$ In the column 5 cm. has 0.328 g of enzyme

In the column 10 cm. (height)

$$V = \prod r^2 h$$
$$= 3.14 \times (0.2286)^2 \times 10$$
$$= 1.64\ cm^3$$

Enzyme 1 ml use 0.4 g

Enzyme 1.64 ml use $0.4 \times 1.64 = 0.656$g

$\therefore$ In the column 10 cm. has 0.656 g of enzyme

2. %Methyl ester content from GC

%Methyl ester content from GC of palm fatty acid can be calculated this equation

$$\left[\frac{\Sigma A - Ai}{Ai}\right] \times \frac{C \times V}{m} \times 100$$

C = Concentration of standard

V = Volume of standard

m = Amount of sample

Ai = Peak area of internal standard

ΣA = Total peak area

$$= \left[\frac{12034.7 - 2468.71}{2468.71}\right] \times \frac{10.085 \times 5}{251.68} \times 100$$
$$= 77.53\%$$

VITA

Miss Sukanya Subkerd was born on April 20, 1984 in Bangkok, Thailand. She graduated at Nawamintrachutit School in 2001. She received the Bachelor Degree of Science in biology, Kasetsart University in 2006. She continued her Master study in Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkom University in 2006 and completed the program in 2009.

Conference

19-21 November 2008 "Enzymatic production of biodiesel from palm fatty acid"
The 1st PSU Phuket Research Conference (2008)
PRINCE OF SONGKLA UNIVERSITY, PHUKET CAMPUS