A Study of Two-Stage Transesterification: Base Homogeneous Catalyst and Acid Heterogeneous Catalyst

Dang Nguyen Thoai

A Thesis Submitted in Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in Chemical Engineering
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Author Mr. Dang Nguyen Thoai
Major Program Chemical Engineering

Major Advisor

………………………………………
(Assoc. Prof. Dr. Chakrit Tongurai)

Co-advisor

………………………………………
(Assoc. Prof. Dr. Kulchanat Prasertsit)

Examining Committee:

…………………………..........Chairperson
(Assoc. Prof. Dr. Penjit Srinophakun)

…………………………………Committee
(Assoc. Prof. Dr. Chakrit Tongurai)

…………………………………Committee
(Assoc. Prof. Dr. Kulchanat Prasertsit)

…………………………………Committee
(Assoc. Prof. Dr. Pakamas Chetpattananondh)

…………………………………Committee
(Assoc. Prof. Dr. Sukritthira Ratanawilai)

The Graduate School, Prince of Songkla University, has approved this thesis as fulfillment of the requirements for the Doctor of Philosophy Degree in Chemical Engineering.

....
(Assoc. Prof. Dr. Damrongsak Faroongsarng)
Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate’s own investigations. Due acknowledgment has been made of any assistance received.

....Signature
(Assoc. Prof. Dr. Chakrit Tongurai)
Major Advisor

....Signature
(Assoc. Prof. Dr. Kulchanat Prasertsit)
Co-advisor

....Signature
(Mr. Dang Nguyen Thoai)
Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

....Signature
(Mr. Dang Nguyen Thoai)
Candidate

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ABSTRACT

The utilization of base catalyst for two-stage transesterification prompts soap formation and yield loss in biodiesel production. In order to overcome this difficulty, the two-stage transesterification process catalyzed by heterogeneous acid catalyst in the second stage is a good recommendation to reduce the soap formation. The aim of this thesis is to develop the two-stage transesterification process with homogeneous base catalyst in the first stage and heterogeneous acid catalyst in the second stage.

A novel chemical method for determining the ester content in biodiesel was demonstrated as an effective method. Moreover, evaluation of two-stage transesterification process as per the determining of the total glycerol content in biodiesel was also a suitable solution in this present study.

The first stage transesterification catalyzed by homogeneous base catalyst was studied in a batch reactor. Experimental factors were investigated; including MeOH/RPO molar ratio (5:1-6:1), CH_3ONa catalyst content (0.30-0.70 wt% to RPO), reaction time (20-60 min) and reaction temperature (45-65 ^oC). The Composite Central Design (CCD) was applied to investigate the influences of the experimental variables on the ester content and total glycerol content; and to find the optimum conditions for the requested ester content. The requested ester content of 85% was obtained under optimum condition: 5.48 of MeOH/RPO molar ratio, 0.32 wt% of CH_3ONa, 40 min and 55 ^oC.

Response surface methodology (RSM) has been applied in modeling and optimization for the first stage transesterification. This model investigated that the CH_3ONa catalyst content is the most significant factor for this stage. Polynomial regression equation for the first stage transesterification was also established as per the analysis of variance (ANOVA).

The second stage transesterification catalyzed by heterogeneous acid catalyst was carried out in high pressure apparatus. Experimental runs were changed following to reaction conditions; including MeOH/oil molar ratio (8:1-12:1), Amberlyst-15 catalyst content (4-16 wt%), reaction time (3-12 h) and reaction temperature (115 ^{o}C). The factorial design was used to conduct the effects of the experimental factors on the ester content and total glycerol content; and to find the optimum conditions for the requested ester content. The requested ester content of 98% was obtained under optimum condition: 10 of MeOH/oil molar ratio, 12 wt% of Amberlyst-15 catalyst, 9 h and 115 ^{o}C.

RSM has been applied in modeling and optimizing for the second stage transesterification. In the present study, the polynomial regression equation for the second stage transesterification was also established as per the analysis of variance (ANOVA).

As a remarkable point of this thesis, application of the present two-step transesterification technology has led to decrease the soap and the total amount of sodium methoxide. The soap content decreased 50% (by mol%) from one-step transesterification and amount of base catalyst used was 33% (by mol%) as compared to one-step transesterification process.

In an effort to enhance the present two-stage transesterification process in this thesis, a process development of two-stage transesterification was also studied. By using the ester phase directly for the second stage, there was a decrease of 20 wt% fresh MeOH used for the second stage transesterification. This decreasing along with not applying the washing and drying process after the first stage transesterification lead to a good solution for the cost reducing of the biodiesel production.

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LIST OF ABBREVIATIONS

RPO	Refined Palm Oil
WCO	Waste Cooking Oil
FFA	Free Fatty Acid
MG	Monoglyceride
DG	Diglycerdie
TG	Triglyceride
FAME	Fatty Acid Methyl Ester
GC	Gas Chromatography
GC-FID	GC equipped with Flame Ionization Detector
NMR	Nuclear Magnetic Resonance
AOCS	American Oil Chemists' Society
ASTM	American Society for Testing and Materials
EN	European Nations
RSM	Response Surface Methodology
CCD	Central Composite Design
ANOVA	Analysis of Variance
EED	Essential Experimental Design
SEM	Scanning Electron Microscope

LIST OF PUBLICATIONS

1. D.N. Thoai, C. Tongurai, K. Prasertsit, S. Photaworn. *Optimization of the first step transesterification of refined palm oil using solid sodium methoxide catalyst*. The 23rd Regional Symposium on Chemical Engineering (RSCE2016), Vung Tau, Vietnam, Volume: ISBN: 978-604-73-4690-5 (book); 978-604-73-4690-8 (CD disk); Section 3: 137 - 144. DOI: 10.13140/RG.2.2.17398.19521.
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5. D.N. Thoai, C. Tongurai, K. Prasertsit, A. Kumar. *A novel two-step transesterification process catalyzed by homogeneous base catalyst in the first step and heterogeneous acid catalyst in the second step*. Fuel Processing Technology 168 (2017) 97–104. DOI: 10.1016/j.fuproc.2017.08.014 (ISI, Q1, IF = 3.752).
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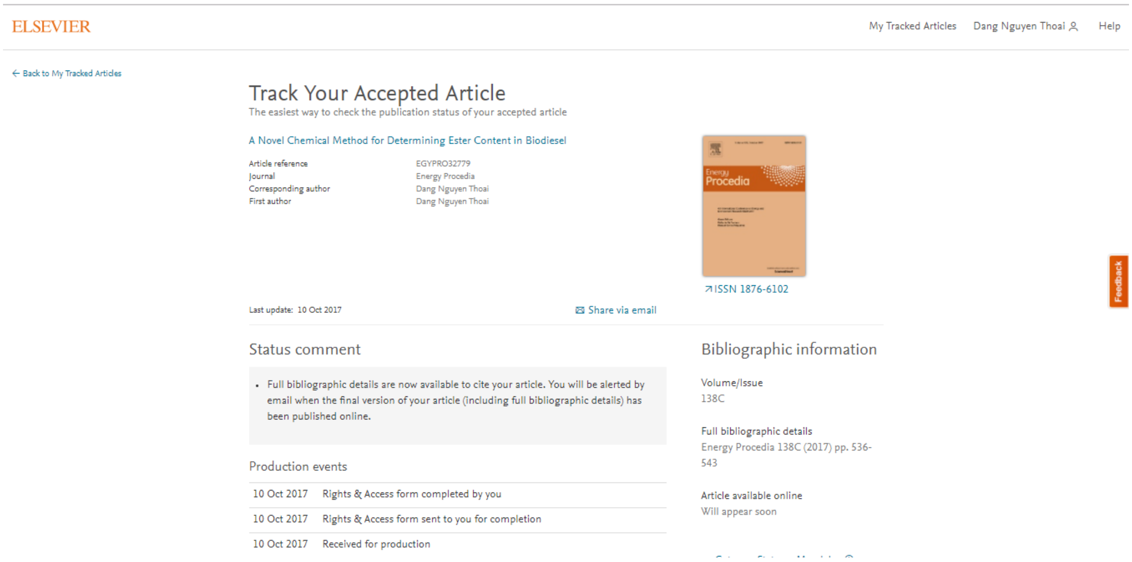

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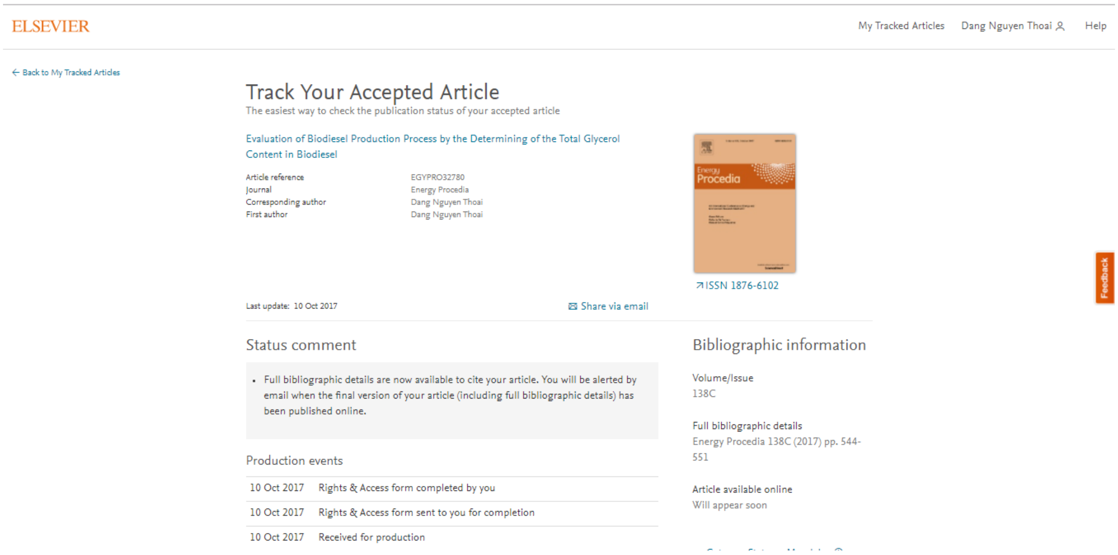

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1. INTRODUCTION

1.1. Rationale/Problem Statement

Nowadays in the modern era, the development of the human society is essentially dependent on energy resources. Up to 2017, fossil fuel was still the biggest source of energy (79.5%), whereas, the percentage of nuclear power and renewable energy sources were only 9.5% and 11%, respectively [1]. The fossil fuel is a serious root of greenhouse gas emissions and leads to the environmental hazards and the global warning [2]. Therefore, the most remarkable issue is to produce energy from non-fossil and eco-friendly energy sources. The renewable energy is a good solution for this problem. The renewable energy resources include hydroelectric power, geothermal, solar, wind and biomass [1]. These energy resources play noteworthy roles in the future. Biodiesel is one of the most promising liquid fuel of high quality, derived from renewable resources, which is suitable to substitute for petroleum-based diesel without engine modification [3,4]. In comparison with petroleum diesel, biodiesel has proved many outstanding advantages; such as lower emissions, renewability, biodegradability, lower toxic, higher safety, higher cetane index and lubricity [5,6]. For these reasons, biodiesel has become an alternative fuel for petroleum-based diesel [3-6].

Biodiesel is mostly obtained from transesterification of renewable resources (edible oil, inedible oil, algae) in the presence of suitable alcohol (methanol or ethanol) and catalyst [7]. The economy efficiency of the biodiesel depends on many factors, including kind of feedstock oils, alcohol, catalyst as well as applying the appropriate technologies.

In the effort to reduce the cost for the biodiesel production process, some technologies have been studied and developed to produce biodiesel from various feedstocks, such as one-stage reaction [8,9] and two-stage reaction [10-14]. The technology of one-stage reaction, transesterification reaction, is suitable for a low free fatty acid (FFA)-feedstock oil (less than 1 wt%). If the FFA level exceeds this amount, the soap formation will inhibit the separation of the ester from glycerol and also reduce the reaction rate. Especially, transesterification reaction does not take place if FFA content in feedstock oils is more than 3 wt% [15]. The drawback of the one-stage reaction is to consume a larger amount of catalyst and alcohol in comparison with the two-stage reaction [8,16]. The two-stage reaction, including first stage esterification and second stage transesterification [13,17-21] and two-stage transesterification [11-13,14,16,22,23].

The two-stage reaction technology has proven its superiority over one-stage reaction process in biodiesel production, such as using various feedstocks, decreasing of alcohol and catalyst content, smoother conditions, higher conversion, higher ester content and biodiesel yield [11-17,21-23].

However, most present two-stage technologies are using base catalyst for the second stage, especially for the two-stage transesterification [14,16,23-25]. The advantage of this application is to increase the conversion and lead to improve the ester content. Nevertheless, the base catalyst also accretes the saponification reaction, especially for homogeneous base catalyst. Soap formation prevents from doing the separation of biodiesel, glycerol and washed water and is also crucial reason of biodiesel loss. In order to overtake on this unexpected problem, H_2SO_4 was also considered as a homogeneous acid catalyst for the second step in some previous studies [11,12,22]. However, using homogeneous acid catalyst causes corrosion on equipment and is unable for reusing. Therefore, it is a good recommendation that studies on heterogeneous acid catalyst should be carried out extensively to develop this two-stage transesterification process in biodiesel production.

Regarding to these considerations, the scope of this research is to study a two-stage transesterification process catalyzed by homogeneous base catalyst in the first stage and heterogeneous acid catalyst in the second stage.

The aim is to propose the novel two-stage transesterification technology and provide solutions in order to reduce the biodiesel production cost.

1.2. Theoretical background and literature review

1.2.1. Biodiesel

Biodiesel is a mixture of mono alkyl esters obtained via the transesterification of different feedstock (vegetable oil, waste oil, animal fat, algae) in the presence of alcohol and catalyst [7]. Biodiesel has similar characteristic to petroleum-diesel and can be used directly or blended with petro-diesel. It can be used without modifying existing engines and discharges less toxic gases, such as sulful dioxide [26,27].

Table 1 Technical properties of biodiesel [29].

Common name	Biodiesel
Common chemical name	Fatty acid (m)ethyl ester
Chemical formular range	C_{14}–C_{24} methyl esters or $C_{15-25}H_{28-48}O_2$
Kinetic viscosity range (mm^2/s, at 40 ^oC)	3.3–5.2
Density range (kg/m^3, at 15 ^oC)	860–894
Boiling point range (^oC)	>180
Flash point range (^oC)	147–177
Distillation range (^oC)	197–327
Vapor pressure (mmHg, at 22 ^oC)	<5
Solubility in water	Insoluble in water
Physical appearance	Light to dark yellow, clear liquid
Odour	Light musty/soap odour
Biodegradability	More biodegradable than petroleum-diesel
Reactivity	Stable, but avoid strong oxidizing agents

Biodiesel can be stored in the same condition as petroleum-diesel. Moreover, biodiesel is safer than petroleum-diesel due to a high flash point (150 ^oC) [28]. The technical properties of biodiesel are shown in Table 1.

1.2.2. Feedstock for biodiesel production

1.2.2.1. Feedstock oil

Many previous studies have presented many kinds of feedstocks for biodiesel production. The specified feedstocks are soybean oil in the U.S., rapeseed oil in Europe, and palm oil in Southeast Asia (Thailand, Indonesia, Malaysia). Animal fats and used cooking oil are also significant feedstock for biodiesel production. Other vegetable oils are potential interest for biodiesel feedstock; including coconut, corn, jatropha, safflower and sunflower. Table 2 summarized potential yields of biodiesel that could be produced from various feedstock oils. Moreover, there is great interest in studying and using algae as biodiesel feedstock [30].

Table 2 Potential biodiesel yield from various feedstocks [30].

Source	Potential annual yield, gallons/acre
Corn	18–20
Cotton	35–45
Soybean	40–55
Mustard	60–140
Camelina	60–65
Safflower	80–85
Sunflower	75–105
Canola	110–145
Rapeseed	110–130
Jatropha	140–200
Coconut	250–300
Palm oil	400–650
Algae	>5000

Although biodiesel is produced via transesterification of triglycerides contain numerous individual fatty acid methyl ester species, a particular fuel is generally dominated by only a few species. A list of fatty acids

(FA) most commonly seen in biodiesel is provided in Table 3. Five typical FA derived from vegetable oils and animal fats include; palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3).

Table 3 Typical fatty acid (FA) groups in biodiesel [30].

Common name	Abbreviation	Molecular Formula	Molecular Weight
Lauric acid	12:0	$C_{12}H_{24}O_2$	200.32
Myristic acid	14:0	$C_{14}H_{28}O_2$	228.38
Myristoleic acid	14:1	$C_{14}H_{26}O_2$	226.26
Palmitic acid	16:0	$C_{16}H_{32}O_2$	256.43
Palmitoleic acid	16:1	$C_{16}H_{30}O_2$	254.42
Stearic acid	18:0	$C_{18}H_{36}O_2$	284.48
Oleic acid	18:1	$C_{18}H_{34}O_2$	282.47
Linoleic acid	18:2	$C_{18}H_{32}O_2$	280.46
Linolenic acid	18:3	$C_{18}H_{30}O_2$	278.44
Arachidic acid	20:0	$C_{20}H_{40}O_2$	312.54
Gondoic acid	20:1	$C_{20}H_{38}O_2$	310.53
Behenic acid	22:0	$C_{22}H_{44}O_2$	340.60
Erucic acid	22:1	$C_{22}H_{42}O_2$	338.58

1.2.2.2. Alcohol

The alcohol materials that can be used in the transesterification process include methanol, ethanol, propanol, butanol, and amyl alcohol. These alcohols are as the acyl acceptors for biodiesel production. Among these alcohols, methanol and ethanol are most frequently used. Methanol is prior used due to its lower price and its physicochemical characterization. Ma and Hanna demonstrated that methanol can react with triglycerides quite quickly in the presence of base catalyst; and the base catalyst is easily dissolved in methanol [31]. However, because of its low boiling temperature, there is a serious explosion risk associated with methanol vapor. Methanol and ethanol are hazardous chemicals so they should be stored and handled carefully [4]. However, ethanol is less toxic and more renewable because it can be easily produced from the fermentation of renewable sources. In contrast, methanol is mostly produced from fossil sources, such as natural gas. The properties of methanol and ethanol are shown in Table 4.

Table 4 Some properties of methanol and ethanol for biodiesel production [31].

Property	Methanol	Ethanol
Molecular formula	CH_3OH	C_2H_5OH
Molecular weight (g/mol)	32.04	46.06
Density (g/cm^3)	0.7918	0.789
Melting point (^oC)	-97	-114.3
Boiling point (^oC)	64.7	78.4
Acidity (pK_a)	15.5	15.9
Viscosity (mPa$\cdot$s at 20 ^oC)	0.59	1.20
Flash point (^oC)	11	13

1.2.2.3. Catalyst

Generally, there are three types of catalysts used for biodiesel production; including base, acid, and enzyme catalyst [4,32,33]. Enzyme catalysts are more attractive recently because it can prevent the saponification; this makes the purification process more simple. However, they are hardly used commercially because of the longer reaction times and higher production cost. In order to overcome this disadvantage, new biocatalysts have recently studied and developed. A remarkable biocatalyst is cell biocatalyst which is immobilized within biomass support particles [34].

Table 5 Merits and demerits at different types of catalysts used in the biodiesel production [4,40].

Type	Name of catalyst	Merits	Demerits
Base			
Homogeneous	NaOH, KOH, CH_3ONa, CH_3OK	High catalytic activity, low cost, favorable kinetics, modest operation conditions, very fast reaction rate	Low FFA requirement, anhydrous conditions, saponification, emulsion formation, more wastewater from purification, non-reusable
Heterogeneous	CaO, $CaTiO_3$, $CaZrO_3$, CaO–CeO_2, $CaMnO_3$, $Ca_2Fe_2O_5$, KOH/Al_2O_3, KOH/NaY, Al_2O_3/KI, ETS-10 zeolite, alumina/silica supported K_2CO_3	Noncorrosive, environmentally benign, recyclable, fewer disposal problems, easily separation, higher selectivity, longer catalyst lifetimes	Low FFA requirement, anhydrous conditions, more wastewater from purification, high molar ratio of alcohol to oil requirement, high reaction temperature and pressure, diffusion limitations, high cost, poisoning of the catalyst when exposed to ambient air
Acid			
Homogeneous	Concentrated sulphuric acid	Intensitive for high FFA and water, catalyze esterification and transesterification simultaneously, avoid soap formation, preferred for low-grade oil, mild reaction conditions, less energy intensive	Equipment corrosion, more waste from neutralization, difficult to recycle, higher reaction temperature, long reaction time, weak catalytic activity
Heterogeneous	ZnO/I_2, ZrO_2/SO_4^{2-}, TiO_2/SO_4^{2-}, carbon-based solid acid catalyst, carbohydrate-derived catalyst, Vanadyl phosphate, niobic acid, sulphated zirconia, Amberlyst-15, Nafion-NR50	Intensitive for high FFA and water, catalyze esterification and transesterification simultaneously, recyclable, preferred for low-grade oil, easy separation of catalyst from product, eco-friendly	Low acid site concentrations, low microporosity, diffusion limitations, high cost, complicated catalyst synthesis procedures, higher reaction temperature, high alcohol to oil molar ratio, longer reaction time
Enzyme	Candida antarctica fraction B lipase, Rhizomucor mieher lipase	Avoid soap formation, non-polluting, easy purification	Expensive, denaturation

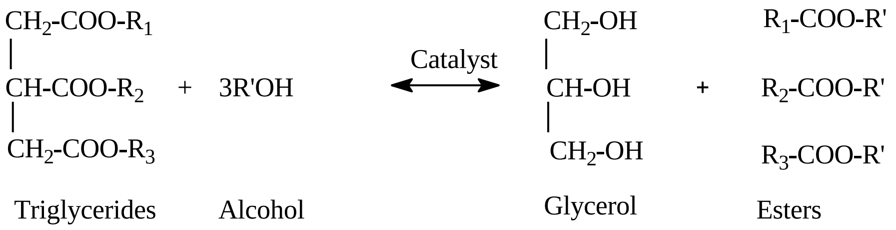
Table 5 summarizes advantage and disadvantage of various catalysts in the biodiesel production. In comparison with enzyme catalyst, the base and acid catalysts are more commonly used in biodiesel production [31]. These catalysts include homogeneous and heterogeneous catalysts. Sodium hydroxide and potassium hydroxide are usually used as homogeneous base catalysts, and base-catalyzed transesterification is most commonly used commercially [35-38]. Using these catalysts is the most economic because the base-catalyzed transesterification is carried out under a smooth condition, and the conversion rate is high. However, the homogeneous base catalyst is strongly hygroscopic and it absorbs water easily from air. It also produces water when dissolved in the alcohol reactant and affect the yield [39]. Therefore, this catalyst should be properly handled.

Some heterogeneous catalysts are solid and it could be rapidly separated by filtration process, and reduces water for biodiesel washing process. In addition, some heterogeneous catalysts can simultaneously catalyze both transesterification and esterification reaction that can avoid the pre-esterification step, thus these catalysts are particularly useful for the high FFA content feedstocks [4]. However, the reaction occurs at a very low rate [40].

1.2.3. Mechanism of homogeneous base and heterogeneous acid catalytic conversion in transesterification reaction

1.2.3.1. Reaction mechanism of homogeneous base catalyzed transesterification

Transesterification or alcoholysis is the process of exchanging the organic group R of an ester with the organic group R′ of an alcohol. These reactions are often catalyzed by the addition of an acid or base catalyst. This process has been widely used to reduce the high viscosity of triglycerides. The general equation of transesterification can be shown in Fig. 1.



$$\begin{array}{l} CH_2\text{-}COO\text{-}R_1 \\ | \\ CH\text{-}COO\text{-}R_2 \\ | \\ CH_2\text{-}COO\text{-}R_3 \end{array} + 3R'OH \xleftrightarrow{\text{Catalyst}} \begin{array}{l} CH_2\text{-}OH \\ | \\ CH\text{-}OH \\ | \\ CH_2\text{-}OH \end{array} + \begin{array}{l} R_1\text{-}COO\text{-}R' \\ R_2\text{-}COO\text{-}R' \\ R_3\text{-}COO\text{-}R' \end{array}$$

Fig. 1 General transesterification reaction equation [7].

Actually, this reaction consists of a sequence of three successive reversible reactions, mono-glycerides (MG) and di-glycerides (DG) are two kinds of intermediate compounds (Fig. 2).

$$\text{1. Triglyceride (TG) + R'OH} \xrightleftharpoons{\text{Catalyst}} \text{Diglyceride (DG) + } R_1COOR'$$

$$\text{2. Diglyceride (DG) + R'OH} \xrightleftharpoons{\text{Catalyst}} \text{Monoglyceride (MG) + } R_2COOR'$$

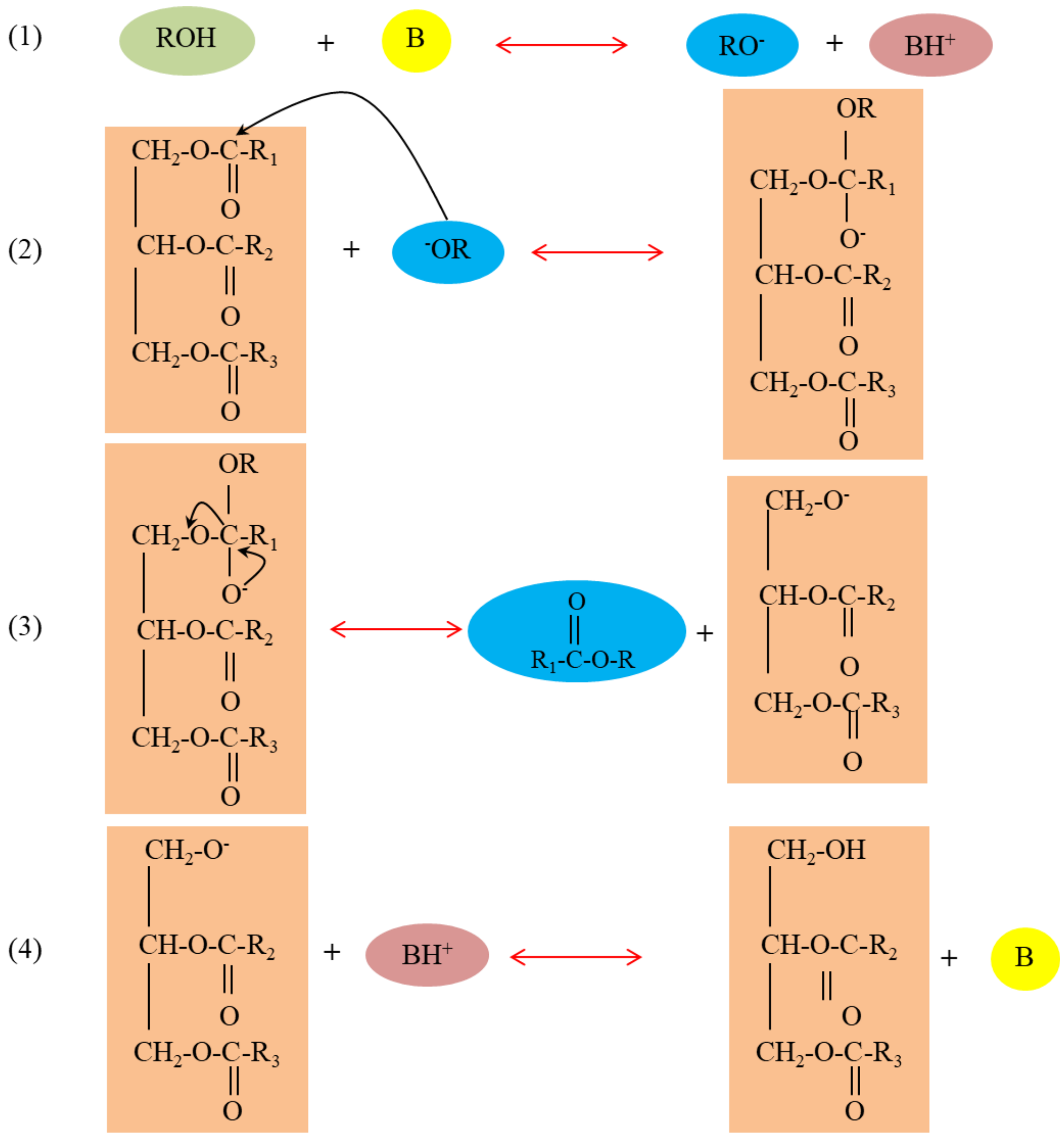
$$\text{3. Monoglyceride (MG) + R'OH} \xrightleftharpoons{\text{Catalyst}} \text{Glycerol (GL) + } R_3COOR'$$

Fig. 2 Mechanism of three successive reversible reactions [7].

Fig. 3 indicates the homogeneous base-catalyzed transesterification mechanism which includes four steps [41]. Firstly, the alkoxide ion is formed and then directly acts as a strong nucleophile. Alkali catalyst has a direct route compare to acid. The main difference between acid and base catalytic activity in transesterification reaction is formation of electrophilic species versus stronger nucleophile formation, respectively [42,43].

1.2.3.2. Reaction mechanism of heterogeneous acid catalyzed transesterification

Recently, new studies for biodiesel research are focusing on heterogeneous acid catalyst. Moreover, it is believed that heterogeneous acid catalyst has the strong potential to replace homogeneous acid catalyst.



B: base catalyst
R_1, R_2, R_3: carbon chain of fatty acid
R: alkyl group of the alcohol

Fig. 3 Reaction mechanism of homogeneous base catalyzed transesterification [41].

Ion-exchange resin is insoluble macroporous polymer which is able to replace with other ions in a solution or reaction. Commonly, sulfonic ion-exchange resins are co-polymers of divinylbenzene (DVB), styrene and sulfonic acid groups [44]. Common types of acidic ion-exchange resin are Amberlyst-15, Amberlyst-35 and Nafion SAC-13. These catalysts are demonstrated to give strong performance in FFA esterification, otherwise, weak in transesterification [45,46].

The transesterification reaction between triglycerides and methanol in the presence of Amberlyst-15 as a heterogeneous acid catalyst is displayed in Fig. 4. The mechanism of the transesterification reaction catalyzed by high acidic cation exchange resin is shown in Fig. 5. The protonation of the carbonyl group leads to the carbocation, and after the nucleophilic attacks the methanol molecule, a tetrahedral intermediate is generated, which forms the FAME and the catalyst. The diglyceride generated in reaction is able to react with methanol, starting another catalytic cycle [47].

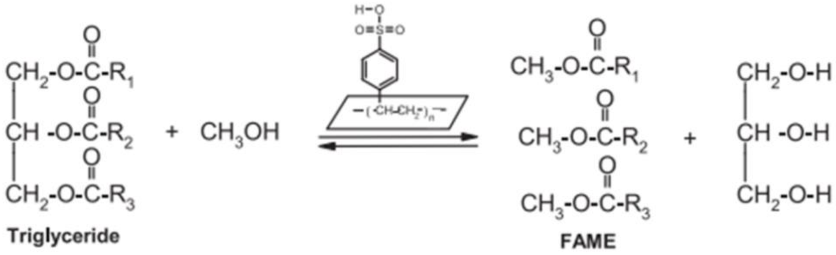


Fig. 4 The transesterification reaction between triglyceides with methanol [47].

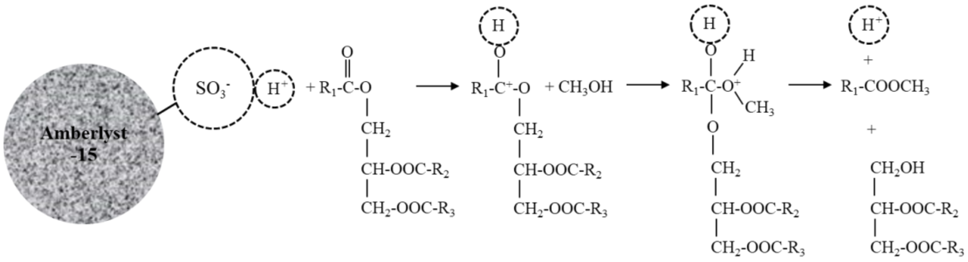


Fig. 5 The mechanisms of the transesterification reaction catalyzed by Amberlyst-15 [47].

1.2.4. Review of two-stage transesterification process in biodiesel production

1.2.4.1. Feedstock oil for the two-stage transesterification process

As presented in “Rational/Problem Statement” section, chemical property of feedstock oil is the most important factor related to the selection of the best suitable two-stage reaction technology. If free fatty acid (FFA) content is more than 2 wt% then the first stage esterification will be followed by the second stage transesterification. The aim of the first stage esterification is to decrease the FFA content in feedstock oils as much as possible in order to reduce the soap formation in the second stage transesterification. Soap formation prevents separation of biodiesel, glycerol and washed water; this is the main reason of biodiesel loss. The two-stage transesterification technology is applied for the low FFA content feedstock oils (less than 2 wt%) to reduce the production cost. More details of this two-stage catalytic conversion have been clearly displayed in **Appendix A**. Review on yield and ester content in two-step transesterification by various researchers are given in Table 6.

1.2.4.2. The two-stage transesterification catalyzed by base catalyst in both stages

Mendow et al. studied an efficient two-stage transesterification process for ethyl esters production using solid sodium methoxide catalyst [16] (Table 6). This process consists of two reaction stages with glycerol separation and an additional part of mixture ethanol and catalyst in each stage. The optimum condition is listed as EtOH/Oil molar ratio of 4.25:1 (2.55:1 for first stage and 1.7:1 for second stage), CH_3ONa content of 1.1 wt% (0.55 wt% for each stage) in the same temperature as well as time (55 ^{o}C and 30 min). Biodiesel with ester content of 99% was attained and meets the required international standards.

In order to extend the ability of using non-edible feedstock for this process, Predojevíc produced biodiesel by two-stage transesterification of WCO using methanol and KOH as base catalyst [23]. Each stage of this process was followed by glycerol separation, purification and drying. The applied two-stage transterification utilized a total molar ratio of methanol to oil of 6:1 (3:1 for each stage), a total catalyst content of KOH to oil of 1 wt% (0.5 wt% for each stage) in the same reaction time (30 min) at 30 ^{o}C and 60 ^{o}C, respectively. The comparison of three purification methods showed similar biodiesel yield after silica gel or acid washing (about 92%) but a lower yield was achieved after the washing process by hot distilled water (about 89%). However, ester content was obtained more than 97% after purification process and absolutely suitable for the minimum acceptable biodiesel purity according to the EN 14103 standard (96.5 min).

Table 6 Two-stage transesterification results on yield, ester content reported by various studies.

Raw material	Heating system	Optimum condition for first stage transesterification	Optimum condition for second stage transesterification	Ester content (wt.%)	Yield of biodiesel (%)	Reference
Microalgae (biomass)	Oil bath	MeOH/Biomass (wt/wt) = 41.59, NaOH/Biomass (wt/wt) = 0.67, 90 °C, 19.33 min	MeOH/Biomass (wt/wt) = 51.3, H_2SO_4/Biomass (wt/wt) = 3.81, 90 °C, 10 min	94.5	ND	[11]
Vegetable oil (Sunflower and linseed oil)	Oil bath	MeOH/Oil molar ratio = 10, KOH/Oil = 1.15 wt%, 60 °C, 60 min	MeOH/Oil molar ratio = 15, H_2SO_4/Oil =15.9 wt%, 60 °C, 60 min	97	85	[12]
WCO	Ultrasonic irradiation	MeOH/Oil molar ratio = 2.5, KOH/Oil = 0.7 wt%, 30-32 °C, 25 min	MeOH/Oil molar ratio = 1.5, KOH/Oil =0.3 wt%, 27-29 °C, 20 min	99	93.8	[14]
Refined palm oil	Oil bath	EtOH/Oil molar ratio = 2.55, CH_3ONa/Oil = 0.55 wt%, 55 °C, 30 min	EtOH/Oil molar ratio = 1.7, CH_3ONa /Oil = 0.55 wt%, 55 °C, 30 min	99	ND	[16]
Vegetable oil (Sunflower and linseed oil) and WCO	Oil bath	MeOH/Oil molar ratio = 10, KOH/Oil = 0.63 wt%, 60 °C, 30 min	MeOH/Oil molar ratio = 5, H_2SO_4/Oil =5.3 wt%, 60 °C, 60 min	97-98	87-93	[22]
WCO (waste sunflower oil)	Oil bath	MeOH/Oil molar ratio = 3, KOH/Oil = 0.5 wt%, 30 °C, 30 min	MeOH/Oil molar ratio = 3, KOH/Oil = 0.5 wt%, 60 °C, 30 min	97-98	89-92	[23]
WCO	Oil bath	EtOH/Oil molar ratio = 12, KOH/Oil = 1 wt%, 78 °C, 120 min	EtOH/Oil molar ratio = 5, KOH/Oil = 0.75 wt%, 78 °C, 120 min	94.5	ND	[24]
Sunflower oil	Oil bath	EtOH/Oil molar ratio = 12, NaOH/Oil = 1 wt%, 80 °C, 150 min	EtOH/Oil molar ratio = 6, NaOH/Oil = 0.75 wt%, 80 °C, 30 min	96.5	ND	[25]

Ultrasonic irradiation assisted technology is considered as a modern method in a two-stage transesterification process in an effort to approach economic efficiency for biodiesel production [14]. The transesterification is carried out with the molar ratio of methanol to WCO of 2.5:1 and 1.5:1, the content of KOH to WCO of 0.7 wt% and 0.3 wt%, time of 25 min and 20 min at ambient temperature (30 ^{o}C) for first stage and second stage, respectively. The ester content of 99% achieved in the short time and low temperature is a significant proof by this technology.

1.2.4.3. The two-stage transesterification catalyzed by base catalyst in first stage and acid catalyst in second stage

The application of base catalyst for both stages has shown advantages such as fast reaction rate with low alcohol/oil molar ratio and high ester content. However, the base catalyst causes saponification and leads to loss of yield. In order to overcome this difficulty, the two-stage transesterification procedure which included base transesterification followed by acid transesterification was indicated clearly in some studies [11,12,22].

Microalgae was considered as an alternative feedstock from biomass used for the two-stage direct transesterification process [11]. This technology has overcome disadvantage of the traditional method of lipid estimation proposed by Bligh and Dyer [48]. This decreases using chloroform and methanol, leads to reduce adverse effects on health and environment [49]. A two-stage direct transesterification method using NaOH in first stage and H_2SO_4 in second step was reported by Kumar et al. [11]. The ester content can be gained up to 94.5% in optimum condition, including methanol to biomass weight ratio 51.59 (wt/wt) and 51.3 (wt/wt), catalyst to biomass weight ratio 0.67 (wt/wt) and 3.81 (wt/wt), reaction time 19.33 min and 10 min at 90 ^{o}C for the first stage and second stage, respectively.

The significant development of this technology was mentioned in two researches of Samios et al. [12,22] which was called under a terminology, Transesterification Double Step Process (TDSP). The process includes continuous homogeneous base–acid catalyst steps and is also proven the effectiveness by high reaction rate, easy separation process as well as high conversion [12]. The ester content can be higher than 97% at 60 ^oC in 60 min for each step and adding 10 and 15 of MeOH/Oil molar ratio, 1.15 wt% of KOH and 15.9 wt% of H_2SO_4 to oil for first stage and second stage, respectively. The improved TDSP process involves to the reduction of reaction conditions (catalyst content in both stages, MeOH/Oil molar ratio in second stage, reaction time in first stage) and the direct adding of MeOH/H_2SO_4 solution without cooling the reaction system between the first and the second step [22].

1.2.5. Novel two-stage transesterification in this present study

Based on the large number of researches mentioned, most present two-stage technology are using base catalyst for the second stage. The advantage of this application is to increase the conversion and lead to improve the ester content. Nevertheless, base catalyst also accelerates the saponification reaction. Soap formation prevents from the separation of biodiesel, glycerol and washed water and is also crucial reason of biodiesel loss. In order to overtake on this unexpected problem, H_2SO_4 is also considered as a homogeneous acid catalyst for second stage. However, using homogeneous acid catalyst causes corrosion on equipment. Therefore, it is recommended that researches on heterogeneous acid catalyst should be carried out extensively to develop this two-stage technology in biodiesel production. Two more advantages of heterogeneous acid catalyst are reusability and stability. These strong points may contribute to decrease the production cost and to strengthen the competition of biodiesel with petroleum diesel.

The two-stage transesterification via the first stage using homogeneous base catalyst and the second stage using heterogeneous acid catalyst is interesting in this study. With regards to this two-stage transesterification, the second stage transesterification using heterogeneous catalyst is the rate-limiting stage. Therefore, the second stage transesterification should have been studied first in order to find the optimum composition of second stage feedstock that supports a mild condition of using solid catalyst and obtains excel quality for commercial biodiesel product (96.5% ester min.). After that, turn to study the first stage transesterification by using homogeneous base catalyst. This will incorporate the advantage of both catalysts in biodiesel production process.

1.3. Objectives

- To study the novel two-stage transesterification catalyzed by homogeneous base catalyst in the first stage and heterogeneous acid catalyst in the second stage.

- Development of new methods in studying biodiesel production by two-stage catalytic conversion; including novel chemical method in determining ester content in biodiesel, evaluating the efficiency as per the total glycerol content in biodiesel, predictive capability evaluation using RSM in modeling and optimization of biodiesel production by two-stage catalytic conversion.

2. EXPERIMENTAL MODEL, PARAMETER AND APPARATUS

2.1. Materials

2.1.1. Raw material

Refined palm oil (RPO) with FFA and water content less than 0.2 wt% and 0.1 wt%, respectively, was purchased from Morakot Industry Public Co. Ltd. (Thailand). Fatty acid methyl ester (FAME) with water content less than 0.1 wt% was obtained from the Specialized R&D Center for Alternative Energy from Palm Oil and Oil Crops, Prince of Songkla University, Thailand.

2.1.2. Chemicals

Methanol (CH_3OH, commercial grade, purity > 99.8 wt%) was obtained from Union Intraco Co. Ltd. (Thailand).

Sodium methoxide (CH_3ONa, 96 wt%) was supplied by Dezhou Long Teng Chemical Co. Ltd. (China).

Amberlyst-15 was purchased from Sigma-Aldrich (USA).

Sodium periodate ($NaIO_4$) was acquired from Fisher Chemical (UK).

Sodium hydroxide (NaOH) was obtained from Merck (Germany).

Phenolphthalein, bromothymol blue and bromophenol blue were provided by Ajax Finechem (Australia).

Citric acid ($C_6H_8O_7$, commercial grade, 95 wt%) was purchased from Weifang Ensign Industry Co. Ltd. (China).

2.2. Equipment and Instrument

A 0.5 L three-necked round bottom flask was used for the first stage transesterification process.

A high pressure apparatus was used for the second stage transesterification process.

3. METHODS

3.1. Study on the first stage transesterification process by homogeneous base catalyst

FAME production from RPO was studied in batch reactor to determine the effects of reaction condition and the most important experimental factors for process.

The procedure of the first stage transesterification process was a sequence of operations, performed approximately in 6 h. The reaction was carried out in a 0.5 L three-necked flask, with magnetic stirring used a magnetic bar and stirring speed of 600 rpm. This batch reactor worked at atmospheric pressure and refluxed by water at 20 ^{o}C to undergo condensation of methanol vapor.

RPO was loaded into the reactor and the temperature was set up to desired value. Once the temperature reached to the required value, the mixture of alcohol and catalyst was added to the reactor. The addition of the alcohol-catalyst mixture was completed within a time range of 3-5 s. The beginning time for the reaction was counted at the moment of all methanol and catalyst entered in the reactor.

After reaction, product mixture was transferred to the separatory funnel and settled for 1 h at room temperature to separate into two phases of methyl ester and glycerol phase. The methyl ester phase was washed by hot water (80 ^{o}C) without and with shaking three times during removal of glycerol, soap and remaining catalyst from methyl ester phase. The methyl ester rich phase was alcohol evaporated by heating at 80 ^{o}C and purified with citric acid 0.03 wt% of RPO (dissolved in water) in order to avoid forming emulsion. The remaining water in product was removed by heating at 110 ^{o}C for 90 min. Finally, the ester content in the product was determined and it becomes feedstock for the second stage transesterification process catalyzed by heterogeneous acid catalyst.

All the experiments were repeated three times in order to determine experimental deviation. Experiments were designed at various conditions; including MeOH/RPO molar ratio (5:1-6:1), catalyst content (0.3-0.7 wt%), reaction time (20–60 min) and reaction temperature (45–65 ^{o}C).

3.2. Study on the second stage transesterification process catalyzed by heterogeneous acid catalyst

The product mixture from the first stage, methanol and Amberlyst-15 were added into 1 L bomb made from stainless steel at high pressure (2,000 psi max) and temperature to synthesize biodiesel in the second stage (as shown in Fig. 6). The operating pressure is from 80 to 100 psi depending on the composition of reaction mixture and the desired temperature. The bomb is also equipped with external electrical heater using split range temperature controller. A 45-mm diameter two-blade turbine stirrer is connected with a motor on the top of the bomb through cap screw with heat-resistant rubber rings for running without leakage at high pressure and temperature. This drive system turns the stirrer at 400 rpm which is appropriate for mixing process and to avoid mechanical damage of the catalyst.

After charging the reaction mass, the reaction mixture is heated to the desired temperature and corresponding pressure in the bomb reactor which automatically maintain by product vapor. Time taken to gain the desired reaction temperature was about 30 min. At the end of reaction, the product mixture was transferred to the separation funnel to recover Amberlyst-15. It was centrifuged to separate glycerol and a small amount of crumbled catalyst during reaction. The purification and heating processes were also requested to guarantee the good quality of biodiesel. Finally, ester content in biodiesel was determined based on the described methods.

Fig. 6 The high pressure apparatus for the second stage transesterification.

All experiments were carried out at 115 °C in order to promote most features of the high pressure reaction apparatus as well as to ensure the working temperature limit of Amberlyst-15 (120 °C max.). Experiments were designed at various conditions; including MeOH/Oil molar ratio (8:1-12:1), catalyst content (4-16 wt%) and reaction time (3-12 h). The schematic diagram for two-stage transesterification is shown in Fig. 7 as in publication attached in **Appendix B**.

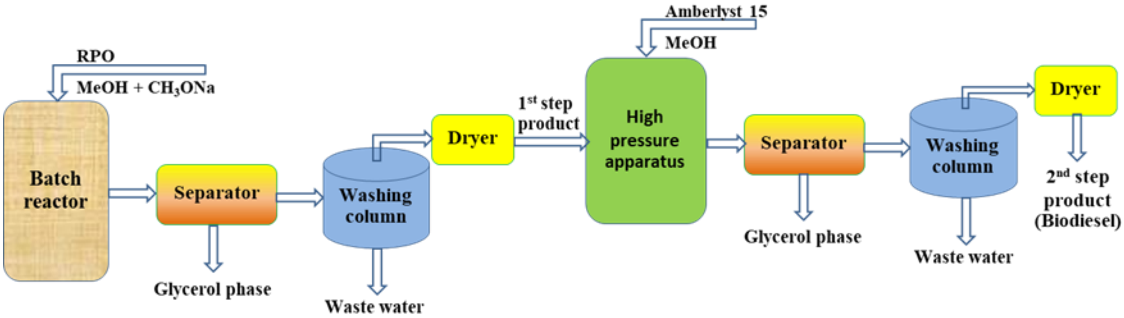


Fig. 7 Schematic diagram of two-stage transesterification process.

3.3. Analytical methods in evaluating the product quality

3.3.1. Determination the water content

The water content in RPO as well as other products can be determined by Karl Fischer Coulometer as per the ASTM D2709 standard.

3.3.2. The analysis of base catalyst and soap content

After reaction time, small samples of the reacting mixture were taken to determine the remaining catalyst and soap content by an acid-base titration method (AOCS Cc17–79). When system containing both catalyst and soap, it is recommended to determine the remaining catalyst content in a first titration step, using HCl 0.1 N solution as reactant, isopropanol as solvent and phenolphthalein as indicator. In a second titration step, the soap content was determined by titration with HCl 0.1 N, using bromophenol blue as indicator.

3.3.3. Determination of the conversion of triglyceride

The conversion of triglyceride was determined by AOCS Official Method Ca 14-56 [50]. According to this method, the conversion of the triglyceride (TG) is defined as:

$$Conversion\ of\ TG = \frac{TG_{(in\ oil)} - TG_{(in\ ester)}}{TG_{(in\ oil)}} \times 100 \tag{1}$$

The TG content in oil and ester is proximately determined as shown in **Appendix C**.

3.3.4. Determination of glycerol in ester phase

Glycerol content in the ester phase is determined by titration method (BS 5711-3: 1979). This test method is based on the cold oxidation of the glycerol by sodium metaperiodate in a strong acidic medium. Formaldehyde and formic acid are produced in this reaction and the latter is used to measure the glycerol content by titration with standard sodium hydroxide solution, to a pH 8.1 ± 0.1. The glycerol content is expressed as a percentage (wt%).

3.3.5. Determination of methanol in ester phase

Methanol content in ester phase can be determined by the evaporation of methanol from ester phase. The ester phase containing excess methanol used for the reaction is placed in an oven at 80 °C for 12 h to evaporate off all the methanol. Methanol content is approximately calculated as per the changing of ester phase weight through this evaporation.

3.3.6. Determination of free and total glycerol in biodiesel

3.3.6.1. Fundamentals of free and total glycerol determination

According to EN 14105 [51], the content of total glycerol (G_T) is calculated as following equation:

$$\%G_T = \%G_F + 0.255(\%MG) + 0.146(\%DG) + 0.103(\%TG) \qquad (2)$$

where, $\%G_F$: weight percentage of free glycerol in biodiesel.

Total glycerol is obtained after all of MG, DG and remaining TG are absolutely transformed into methyl esters and glycerol by transesterification process. After that, glycerol is extracted two times with acidified water and one time with distilled water. Lastly, glycerol content is determined according to standard glycerol titration procedure.

The standard glycerol titration process is based on the oxidation reaction of glycerol by sodium periodate. This reaction produces formic acid when at least two hydroxyl groups are present in the molecule. Primary hydroxyl groups produce formaldehyde by oxidation and secondary hydroxyl groups produces formic acid, as per the following reaction:

$$C_3H_8O_3 + 2NaIO_4 \rightarrow 2HCHO + HCOOH + 2NaIO_3 + H_2O \qquad (3)$$

In order to consume the remaining sodium periodate, ethylene glycol is added when reaction (3) is finished. The reaction is shown in the following reaction:

$$C_2H_4(OH)_2 + NaIO_4 \rightarrow 2HCHO + NaIO_3 + H_2O \qquad (4)$$

3.3.6.2. Free glycerol determination

The free glycerol determination can be done by extracting it from the biodiesel by three consecutive washings. The first and second washing steps are done with 5 wt% HCl and 2.5 wt% HCl, respectively, to avoid the formation of a stable emulsion. The last washing step is done by distilled water. The detailed procedure is described in **Appendix C**.

3.3.6.3. Total glycerol determination

To ensure the accuracy of the method, all of the existence forms of the glycerides (mono-, di- and triglycerides) must be converted into esters. This can be performed by using a large excess of methanol and homogeneous base catalyst. In this method, because the objective is not the production of biodiesel, using big exorbitancy is not a worrying problem. After the finished reaction, the reaction mixture is complemented by HCl 5 wt% solution. This complementarity not only neutralizes the remaining catalyst but also promotes a separating process of glycerol from the ester phase. Also, in order to accelerate the recovery of glycerol, two additional washing steps by HCl 2.5 wt% and distilled water are done, respectively. The detailed procedure is shown clearly in **Appendix D**.

3.3.6.4. Glycerol titration process

In the previous study of Pisarello et al. [56], analyzing the glycerol content in the aqueous phase was carried out based on a complex procedure as described in IRAM 5571 in order to the blank experiment was negligible. However, its demerit is not easy to apply for every experiment. In the procedure of this method, the solution is not boiled prior to the titration. Therefore, the blank experiment is relevant since the carbon dioxide adsorbed from the air during the sample handling is not stripped by boiling process. Therefore, a significant amount of the titrating reactant (NaOH solution) will be used to neutralize the carbonic acid. The detailed process is also seen publications attached in **Appendix C** and **Appendix D**.

3.3.7. Determination of ester content in biodiesel

3.3.7.1. Proximately method for ester content determination (Petty Patent 5060)

As per the Thailand petty patent 5060, total glycerides in biodiesel was determined by transesterification in centrifuge tube (Koehler, ASTM D1796) using microwave irradiation. The residue glycerides in biodiesel is reacted with methanol in the presence of catalyst to produce methyl ester and glycerol. The glycerol content can refer to glycerides content by use the correlation curve. The total glycerides content (wt%) can be proximately converted into ester content by minus with 100 wt%.

3.3.7.2. Ester content analysis using Gas Chromatography (GC)

The fatty acid methyl ester (FAME) content was analyzed following the standard method on B-100 biodiesel specified by the Department of Energy Business, Ministry of Energy, Thailand [53]. This method is based on the EN 14103 standard by the European Standard (EN) and was carried out at Scientific Equipment Center, Prince of Songkla University, Thailand. The methyl esters were quantified directly in GC equipped with flame ionization detector (GC-FID) and column selected for biodiesel (length 30 m, 0.32 mm I.D., film thickness 0.25 mm) with helium as the carrier gas at a flow rate of 1.0 mL/min and split ratio of 50:1. The inlet temperature was kept at 290 ^{o}C and the initial temperature was held at 210 ^{o}C (for 12 min) followed by ramping at a rate of 20 ^{o}C/min till 250 ^{o}C, hold for 8 min. The detector temperature was kept at 300 ^{o}C and the injection volume of 1 ml was used for analysis. Methyl heptadecanoate was used as the standard for GC-FID. FAME content, C_{FAME} (%) was calculated from integration results for a particular determination according to Eq. (5), and the average ester content from duplicate determinations was recorded.

$$C_{FAME} = \frac{(S_A - A_{EI})}{A_{EI}} \times \frac{(C_{EI} \times V_{EI})}{m} \times 100\% \qquad (5)$$

where, S_A is the sum of all methyl ester peak areas from C8 to C24:1, A_{EI} is peak area for methyl heptadecanoate (internal standard), C_{EI} is concentration (mg/ml) of the methyl heptadecanoate solution (10 mg/ml), V_{EI} is volume (ml) of the methyl heptadecanoate solution used (5 ml) and 'm' is precise mass (mg) of the ester sample.

3.3.7.3. Determination of methyl ester content using Nuclear Magnetic Resonance (NMR)

The methyl ester content can be determined by NMR and this method was also carried out at Scientific Equipment Center, Prince of Songkla University, Thailand.

Both ^1H and ^{13}C NMR have been applied for testing the transesterification reaction. In this thesis, ^1H NMR was used in order to determine the methyl ester content in biodiesel. When using ^1H NMR, the protons of the methylene group adjacent to the ester moiety in triacylglycerol (TAG) and the protons in the alcohol moiety of the product methyl esters were used to monitor the yield [54]. The following equation,

$$C = \frac{2A_{ME}}{3A_{\alpha-CH_2}} \times 100 \tag{6}$$

where, C is the conversion of triglycerides to the methyl ester, A_{ME} is the integration value of the protons of the methyl esters, and $A_{\alpha-CH_2}$ is the integration value of the methylene protons. The factors 2 and 3 indicate that the methylene carbon possesses two protons and the methanol-derived carbon has three attached protons.

3.3.7.4. New chemical method in determining of ester content

From the above procedure about the determining of the toal glycerol in biodiesl, we proposed a novel chemical method to determine the ester content. The detailed presentation is shown clearly in publication attached in **Appendix C**.

3.4. Statistical method in evaluating experimental results

3.4.1. Design of experiments

Central Composite Design (CCD) was applied to investigate the influences of the experimental variables on the output variables and to find the optimum conditions for the requested output variables. The CCD incorporates five levels (coded –α, –1, 0, +1, +α) in which axial points (±α) for a factor and 0 for all other factors. In addition, center points coded as 0 were used to estimate pure error. For CCD in the case of 4 independent factors, a list of 30 experiments including 2^4 factorial runs, 8 runs for axial points and 6 runs for center points were carried out. The experimental ester and total glycerol contents were used in the analysis of variance (ANOVA). The performance of RSM model was statistical tested by correlation coefficient (R), coefficient of determination (R^2), adjusted R^2, mean square error (MSE). These parameters are determined using the Eqs. (7) to (10) [55-57]:

$$R = \frac{\sum_{i=1}^{n}(y_{p,i}-y_{p,ave})(y_{a,i}-y_{a,ave})}{\sqrt{\left[\sum_{i=1}^{n}(y_{p,i}-y_{p,ave})^2\right]\left[\sum_{i=1}^{n}(y_{a,i}-y_{a,ave})^2\right]}} \qquad (7)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_{a,i}-y_{p,i})^2}{\sum_{i=1}^{n}(y_{p,i}-y_{a,ave})^2} \qquad (8)$$

$$Adjusted\ R^2 = 1 - \left[(1-R^2)\times\frac{n-1}{n-k-1}\right] \qquad (9)$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_{p.i}-y_{a,i})^2 \qquad (10)$$

where, n is the number of experiments, $y_{p,i}$ is the predicted outputs, $y_{a,i}$ is the experimental results, $y_{a,ave}$ is the average experimental results, $y_{p,ave}$ is the average predicted output and k is the sum of input factors.

3.4.2. Response surface methodology

Response surface methodology (RSM) is one of significant statistical methods used in experimental design, modeling and optimization

[58,59]. This is a modeling method related to one or more responses to the independent factors. It determines the effect of independent factors, including single and in interaction, on the whole process.

The statistical significance of the independent variables, their interactions and the quality of the fitted model are tested via F-value, P-value and ANOVA. ANOVA is also applied to predict the ester content and total glycerol content following the experimental variances.

Contour plots are formed via the multiple regression equation by keeping two independent terms at an average value and varying other two terms. Model gives the optimum conditions for achieving highest-ester content and lowest-total glycerol content from independent experimental factors.

RSM is provided by Essential Experimental Design (EED) software in MS Excel [60]. Additionally, the Minitab software (version 16.2.2) and the Design-Expert® software (version 7.0, Stat-Ease, Minneapolis, USA) were used to check the accuracy of analyzed experimental data.

4. RESULTS AND DISCUSSIONS

4.1. A study of determination the optimum composition of second stage feedstock

As per the previous study about the kinetics of transesterification [62], about 80% conversion (80% ester content, approx.) was obtained after sodium hydroxide-catalyzed transesterification in mild reaction condition. In this study, in order to determine the optimum composition for second stage transesterification, the minimum ester content of 80% was prepared. The test samples were prepared based on percentages of FAME (80-90%) and RPO (10-20%) to obtain the commercial biodiesel (96.5% ester min.) as well as the economic efficiency for the second step transesterification.

All reactions were carried out at MeOH/Oil molar ratio of 10:1, 9 wt% of Amberlyst-15 to oil at 115 ^{o}C in 9 h. The ability to obtain the commercial biodiesel from various feedstock, S1 (80%FAME + 20%RPO), S2 (85%FAME +15%RPO) and S3 (90%FAME + 10%RPO), in the same condition (MeOH/oil molar ratio: 10/1, Amberlyst 15 content: 9 wt%, reaction time: 9 h, reaction temperature: 115 ^{o}C) is shown in Fig. 8.

Commercial biodiesel is not obtained by using feedstock oil with 80% ester. This can be explained based on the low catalytic activity of Amberlyst-15. However, commercial biodiesel can be produced from the Amberlyst-15 catalyzed second step transesterification by using feedstock oil with ester content from 85% to 90%. Processing of feedstock oil with 85% ester content is more challenging than feedstock containing higher ester content. Thus it was chosen as a desired target of the first stage transesterification catalyzed by CH_3ONa catalyst. Moreover, it was also selected to study the Amberlyst-15 catalyzed second step transesterification process to ensure the efficiency and economy of the biodiesel production.

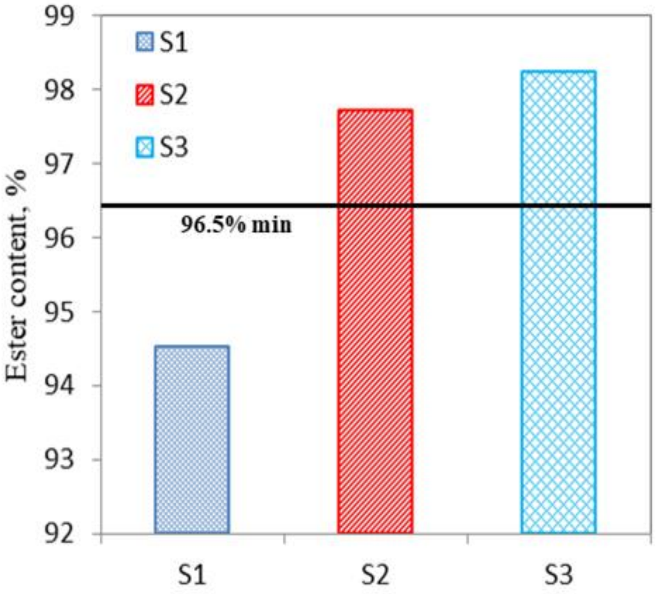


Fig. 8 Experimental results of commercial biodiesel from various feedstock.

4.2. A study of first stage transesterification catalyzed by homogeneous base catalyst in a batch reactor

4.2.1. Design of experiments

The CCD design was used to determine the optimum conditions for the requested ester and total glycerol content. The factorial design incorporates five levels (coded –α, –1, 0, +1, +α) in which axial points (±α) for a factor and 0 for all other factors. In addition, center points were coded as 0 and used to estimate pure errors. The most important factors for the first stage transesterification are molar ratio (X_1), catalyst content (X_2), reaction time (X_3) and reaction temperature (X_4). The experimental limit and coded levels of independent factors are shown in Table 7. A list of 30 experiments including 2^4 factorial runs, 8 runs for axial points and 6 runs for center points was carried out. The ester content (Y_1) and total glycerol content (Y_2) were dependent variables.

Table 7 Limit and coded levels of independent factors for the first stage transesterification.

Factor			Limit and coded level				
Independent variable	Symbol	Dimension	–α	–1	0	+1	+α
Molar ratio	X_1	mol/mol	5.00	5.25	5.50	5.75	6.00
Catalyst content	X_2	wt%	0.30	0.40	0.50	0.60	0.70
Time	X_3	min	20	30	40	50	60
Temperature	X_4	^{o}C	45	50	55	60	65

4.2.2. RSM modeling for the ester content

4.2.2.1. Analysis of variance (ANOVA) and RSM modeling

The relationship between the four independent variables (MeOH/RPO molar ratio, catalyst content, reaction time and reaction temperature) and the ester content are investigated. The ester content for each experimental run and from both RSM and ANN models are listed in Table 8.

Results of ANOVA are summed up in Table 9 in terms of the degree of freedom, the sum and mean of squares, F-value and P-value. The significance of the model, single terms, their squares and interactions is confirmed via their F-value and P-value. P-value less than 0.05 implies significant effects of these parameters on the ester content. More details about ANOVA results and RSM modeling have been presented in publications attached in **Appendix B** and **Appendix E**.

Table 8 The designed independent factors and the ester content from experiment, RSM model.

Run	Independent variables				Ester content (%)	
	X_1	X_2	X_3	X_4	Experiment	RSM
1	5.25	0.40	30	50	83.31	83.42
2	5.75	0.40	30	50	85.18	85.13
3	5.25	0.60	30	50	90.68	90.49
4	5.75	0.60	30	50	91.81	91.44
5	5.25	0.40	50	50	86.03	85.65
6	5.75	0.40	50	50	87.06	87.11
7	5.25	0.60	50	50	92.85	92.89
8	5.75	0.60	50	50	93.45	93.59
9	5.25	0.40	30	60	84.73	84.58
10	5.75	0.40	30	60	87.14	87.08
11	5.25	0.60	30	60	90.43	90.36
12	5.75	0.60	30	60	91.74	92.12
13	5.25	0.40	50	60	87.65	87.99
14	5.75	0.40	50	60	90.05	90.24
15	5.25	0.60	50	60	93.90	93.95
16	5.75	0.60	50	60	95.59	95.45
17	5.00	0.50	40	55	87.53	87.64
18	6.00	0.50	40	55	90.93	90.85
19	5.50	0.30	40	55	84.14	84.10
20	5.50	0.70	40	55	96.31	96.37
21	5.50	0.50	20	55	85.08	85.27
22	5.50	0.50	60	55	91.01	90.84
23	5.50	0.50	40	45	88.52	88.83
24	5.50	0.50	40	65	92.13	91.84
25	5.50	0.50	40	55	92.01	91.93
26	5.50	0.50	40	55	91.46	91.93
27	5.50	0.50	40	55	92.18	91.93
28	5.50	0.50	40	55	92.07	91.93
29	5.50	0.50	40	55	92.10	91.93
30	5.50	0.50	40	55	91.77	91.93
					MSE	0.0443
					R^2	0.9961

Table 9 ANOVA results for the adjusted regression model of the ester content.

Source/Term	Degree of freedom (DF)	Sum of squares (SS)	Mean square (MS)	F-value	P-value	Remarks
Model	14	341.75	24.4107	273.2543	<0.0001	Significant
Linear	4	301.53	75.3825	843.8340	<0.0001	Significant
X_1	1	15.42	15.42	172.6119	<0.0001	Significant
X_2	1	225.95	225.95	2529.2910	<0.0001	Significant
X_3	1	46.54	46.54	520.9701	<0.0001	Significant
X_4	1	13.62	13.62	152.4627	<0.0001	Significant
Square	4	47.41	11.8525	132.6772	<0.0001	Significant
X_1^2	1	12.4	12.4	138.8060	<0.0001	Significant
X_2^2	1	4.92	4.92	55.0746	<0.0001	Significant
X_3^2	1	25.73	25.73	288.0224	<0.0001	Significant
X_4^2	1	4.36	4.36	48.8060	<0.0001	Significant
2-way interaction	6	4.324	0.7207	8.0672	<0.0001	Significant
X_1X_2	1	0.56	0.56	6.2687	0.0247	Significant
X_1X_3	1	0.063	0.063	0.7052	0.4155	Not significant
X_1X_4	1	0.63	0.63	7.0522	0.0177	Significant
X_2X_3	1	0.031	0.031	0.3470	0.5664	Not significant
X_2X_4	1	1.64	1.64	18.3582	0.0006	Significant
X_3X_4	1	1.4	1.4	15.6716	0.0012	Significant
Residual	15	1.34	0.0893			
Lack of fit (LOF)	10	0.97	0.097	1.3472	0.3943	Not significant
Pure error	5	0.36	0.072			
Total	29	343.09				

R^2 = 0.9961, adjusted R^2 = 0.9925, R^2 for prediction = 0.9822

4.2.2.2. Performance assessment of predictive capability of RSM

The performance evaluation of the developed RSM model in prediction of the total glycerol content in biodiesel is evaluated in Table 10. The RSM model has high values of R, R^2, adjuted R^2 demonstrate the authentic suitability of these models [56]. In addition, MSE checks the significance and accuracy of the suggested model [56,57,63]. The lower value of this statistical parameter, better the performance of the suggested model. From Table 10, the very high values of R, R^2, adjusted R^2 and very low value of MSE clearly indicated a high significance of the requested RSM model.

Table 10 Performance evaluation of RSM model.

Parameter	RSM
R	0.9981
R^2	0.9961
Adjusted R^2	0.9925
MSE	0.0443

4.2.2.3. Optimization of ester content by the RSM

Actual ester content obtained under the experimental conditions are between 80% to 97% as in **Appendix B**. In order to evaluate the optimization capability of the RSM model, the ester content of 85% was chosen as a desired target of the first stage transesterification. The optimum conditions for molar ratio, reaction time and temperature are shown as in Table 11. The catalyst content are the most important factor for the first stage transesterification in this present study as per ANOVA results (Table 9).

Table 11 Optimization conditions and model validation (for 85% ester).

Optimum reaction condition	Model
MeOH/RPO molar ratio (by mole)	5.48
Catalyst content (wt%)	0.32
Reaction time (min)	40
Reaction temperature (^oC)	55

4.2.3. RSM modeling for the total glycerol content

4.2.3.1. Analysis of variance (ANOVA) and RSM modeling

The relationship between the four independent variables (MeOH/RPO molar ratio, catalyst content, reaction time and reaction temperature) and the total glycerol content are also investigated. The total glycerol content for each experimental run and from RSM model are listed in Table 12.

The total glycerol content in the final biodiesel product is influenced by four independent variables. The RSM response obtained in Table 12 is relative to these variables using a polynomial regression model equation as **Appendix B**. The initial regression model is shown in Eq. (11):

$$Y_2 = 47.44 - 12.79X_1 - 16.03X_2 - 0.07329X_3 - 0.123X_4 + 1.168X_1^2 + 4.552X_2^2 + 0.00106X_3^3 + 0.00172X_4^2 + 0.825X_1X_2 + 0.00225X_1X_3 - 0.0165X_1X_4 - 0.00438X_2X_3 + 0.06875X_2X_4 - 0.000662X_3X_4 \quad (11)$$

The ANOVA assessments of this model indicate that the model is suitable and can describe very well experimental work, as shown in Table 13. The fit of the designed model is checked due to F-value, P-value, lack of fit error (LOF), R^2, adjusted R^2 and R^2 for prediction [58,59]. The model's F-value of 273.9042 and the very low P-value (<0.0001) indicated that the corresponding model is significant at the 95% confidence level (Table 13). The LOF of 0.4021 (much larger 0.05) implied that LOF is insignificant relative to the pure error [58]. Insignificant LOF is good for the predicted model. Additionally, the large differences between R^2, adjusted R^2 and predicted R^2 also demonstrate the significance of the model [58,59]. These coefficients are very high and close (0.9961, 0.9925 and 0.9822, respectively) to prove the very high significance of the model (Table 13).

Table 12 The designed independent factors and the total glycerol content from experiment, RSM model.

Run	Independent variables				Total glycerol content (%)	
	X_1	X_2	X_3	X_4	Experiment	RSM
1	5.25	0.40	30	50	1.81	1.80
2	5.75	0.40	30	50	1.61	1.61
3	5.25	0.60	30	50	1.01	1.03
4	5.75	0.60	30	50	0.89	0.93
5	5.25	0.40	50	50	1.52	1.56
6	5.75	0.40	50	50	1.40	1.40
7	5.25	0.60	50	50	0.78	0.78
8	5.75	0.60	50	50	0.71	0.70
9	5.25	0.40	30	60	1.66	1.67
10	5.75	0.40	30	60	1.40	1.41
11	5.25	0.60	30	60	1.04	1.04
12	5.75	0.60	30	60	0.90	0.86
13	5.25	0.40	50	60	1.34	1.30
14	5.75	0.40	50	60	1.08	1.06
15	5.25	0.60	50	60	0.66	0.66
16	5.75	0.60	50	60	0.48	0.49
17	5.00	0.50	40	55	1.35	1.34
18	6.00	0.50	40	55	0.99	1.00
19	5.50	0.30	40	55	1.72	1.72
20	5.50	0.70	40	55	0.40	0.39
21	5.50	0.50	20	55	1.62	1.60
22	5.50	0.50	60	55	0.98	1.00
23	5.50	0.50	40	45	1.25	1.21
24	5.50	0.50	40	65	0.85	0.88
25	5.50	0.50	40	55	0.87	0.88
26	5.50	0.50	40	55	0.93	0.88
27	5.50	0.50	40	55	0.85	0.88
28	5.50	0.50	40	55	0.86	0.88
29	5.50	0.50	40	55	0.86	0.88
30	5.50	0.50	40	55	0.89	0.88
					MSE	0.0005
					R^2	0.9961

Furthermore, the effect of each term in the model is also evaluated to estimate how well the significance and its interaction to the total glycerol content. The highly significant effect of terms is concluded based on the F-value and P-value. A P-value less than 0.05 implies significant effects of those variables. From Table 13, with approaching the linear, quadratic and interaction terms, the model terms X_1, X_2, X_3, X_4, X_1^2, X_2^2, X_3^2, X_4^2, X_1X_2, X_1X_4, X_2X_4 and X_3X_4 are observed to be statistically significant. However, the model terms X_1X_3 and X_2X_3 are statistically insignificant due to P-value above 0.05 (Table 13). Moreover, the linear term X_2 (catalyst content) has very low P-value (<0.0001) and very high F-value (2537.3609) among other terms. This data demonstrated that the catalyst content is the most important factor for this first stage transesterification. Due to the described coefficient in Eq. (11), this factor had a negative effect on the total glycerol in biodiesel. The increasing of catalyst content accelerates the speed of the transformation from glycerides (mono-, di, triglycerides) to esters. Therefore, the content of total glycerol in biodiesel significant reduces.

Based on the coded factors, ANOVA data and by eliminating the insignificant model terms, the final simplified model is given in Eq. (12):

$$Y_2 = 47.03 - 12.70X_1 - 16.20X_2 - 0.0631X_3 - 0.123X_4 + 1.168X_1^2 + 4.552X_2^2 + 0.00106X_3^2 + 0.00172X_4^2 + 0.825X_1X_2 - 0.0165X_1X_4 + 0.06875X_2X_4 - 0.000662X_3X_4 \quad (12)$$

The predicted values of the response (total glycerol content) are determined by the aforementioned equation (Eq.12). The positive sign of the coefficients in regression model indicated a synergistic effect whereas the negative sign represents an antagonistic effect on the total glycerol content [68]. From Eq. (12), it is evident that the constant 47.03 is independent of any factors or interaction of the factors, the linear terms (X_1, X_2, X_3, X_4) and some interaction terms (X_1X_4, X_3X_4) have a negative effect on the total glycerol content. It means an increase in these terms will decrease the total glycerol content. In contrast, the square terms (X_1^2, X_2^2, X_3^2, X_4^2) and other interaction terms (X_1X_2, X_2X_4) have a positive influence which denoted that there would be an increase in the total glycerol content with an increase of the magnitude of these parameters.

Table 13 ANOVA results for the adjusted regression model of the total glycerol content.

Source/Term	Degree of freedom (DF)	Sum of squares (SS)	Mean square (MS)	F-value	P-value	Remarks
Model	14	4.024	0.2871	273.9042	<0.0001	Significant
Linear	4	3.55	0.8875	846.5819	<0.0001	Significant
X_1	1	0.18	0.18	171.7011	<0.0001	Significant
X_2	1	2.66	2.66	2537.3609	<0.0001	Significant
X_3	1	0.55	0.55	524.6423	<0.0001	Significant
X_4	1	0.16	0.16	152.6232	<0.0001	Significant
Square	4	0.568	0.142	135.4531	<0.0001	Significant
X_1^2	1	0.15	0.15	143.0843	<0.0001	Significant
X_2^2	1	0.057	0.057	54.3720	<0.0001	Significant
X_3^2	1	0.31	0.31	295.7075	<0.0001	Significant
X_4^2	1	0.051	0.051	48.6486	<0.0001	Significant
2-way interaction	6	0.0514	0.0086	8.1756	<0.0001	Significant
X_1X_2	1	6.806E-003	0.0068	6.4922	0.0223	Significant
X_1X_3	1	5.062E-004	0.0005	0.4829	0.4977	Not significant
X_1X_4	1	6.806E-003	0.0068	6.4922	0.0223	Significant
X_2X_3	1	3.063E-004	0.0003	0.2922	0.5968	Not significant
X_2X_4	1	0.019	0.019	18.1240	0.0007	Significant
X_3X_4	1	0.018	0.018	17.1701	0.0010	Significant
Residual	15	0.01572	0.0011			
Lack of fit (LOF)	10	0.01139	0.0011	1.0866	0.4021	Not significant
Pure error	5	0.00433	0.0009			
Total	29	4.04				

R^2 = 0.9961, adjusted R^2 = 0.9925, R^2 for prediction = 0.9822

4.2.3.2. Performance assessment of predictive capability of RSM

The performance evaluation of the developed RSM models in prediction of the total glycerol content in biodiesel is evaluated in Table 14. The RSM model has high values of R, R^2, adjuted R^2 demonstrate the authentic suitability of these models [56]. In addition, MSE also checks the significance and accuracy of the suggested model [56,57,63]. The lower value of this statistical parameter, better the performance of the suggested model. From Table 14, the very high values of R, R^2, adjusted R^2 and very low value of MSE clearly indicated a high significance of the requested RSM model.

Table 14 Performance evaluation of RSM model.

Parameter	RSM
R	0.9981
R^2	0.9961
Adjusted R^2	0.9925
MSE	0.0005

4.2.3.3. Optimization of total glycerol content by the RSM

Actual total glycerol content obtained under the experimental conditions are between 0.40% to 1.81% as in Table 12. However, by applying the RSM model, this range was extended from 0.31% to 3.26%. The enlarged predictive capability of RSM compared to experimental result is also a good ability in this present study. Moreover, in order to evaluate the optimization capability of the RSM model, the total glycerol content of 0.5% was chosen as a desired target of the first stage transesterification (Table 15). The total glycerol content of 0.5% was obtained under optimum conditions, including 5.49 of MeOH/RPO molar ratio, 0.64 wt% of CH_3ONa catalyst, 40 min and 55 ^oC.

Table 15 Optimization conditions and model validation.

Optimum reaction condition	RSM model (0.5% total glycerol)
MeOH/RPO molar ratio (by mole)	5.49
Catalyst content (wt%)	0.64
Reaction time (min)	40
Reaction temperature (^oC)	55

4.2.4. Interaction effects of reaction conditions on the ester and total glycerol content

4.2.4.1. Interaction effect of molar ratio and catalyst content

The effect of the MeOH/RPO molar ratio and catalyst content on the ester and total glycerol content are investigated with keeping the reaction time and temperature at the medium values, 40 min and 55 ^{o}C, respectively. This influence on the ester content is shown by response surface plots in Fig. 9a. The slope of the contour decides the degree of the interaction of process factors to the ester content. Higher the slope greater the influence can be seen. However, the ester content significantly increases with an increment in the amount of the MeOH/RPO molar ratio at any levels of the catalyst content (from 0.3 to 0.7 wt%). This result is different in comparison with some previous studies [69,70] due to the range of the alcohol/oil molar ratio. Excess alcohol can drive the forward reaction and produces more biodiesel at higher level of molar ratio (9:1–15:1). However, the higher amount of alcohol also makes a good solubility of alcohol in the presence of esters and intermediate compounds (mono- and di-glycerides). Thus, the importance of alcohol solubility in oil is diminished. In the present study, by investigating a suitable molar ratio (5:1–6:1) and applying RSM, it is concluded that the ester content increases with an increase of catalyst content and molar ratio. The similar explanation can also be seen in previous studies [63,71].

Fig. 9b shows the response surface plots of the total glycerol content for the interaction variable of molar ratio and catalyst content. At low levels of molar ratio in this present study (5:1-6:1), the total glycerol content in biodiesel significantly decreases with an increment in the content of catalyst and molar ratio. This result is different from the previous study as in **Appendix D**. When molar ratio is kept at high levels (7:1-9:1), the role of molar ratio in decreasing of the total glycerol in biodiesel is nearly trivial. Moreover, the higher amount of methanol makes the biodiesel separation difficult due to the good solubility of methanol in the presence of methyl esters and intermediate compounds (mono- and di-glycerides). As seen in Fig. 9b, based on the slope of contour, the catalyst content has higher influence than the molar ratio The results demonstrate that the catalyst content is more important than molar ratio for this first stage. Several studies have got similar results as the present study [69,70], therefore validates the finding of this research.

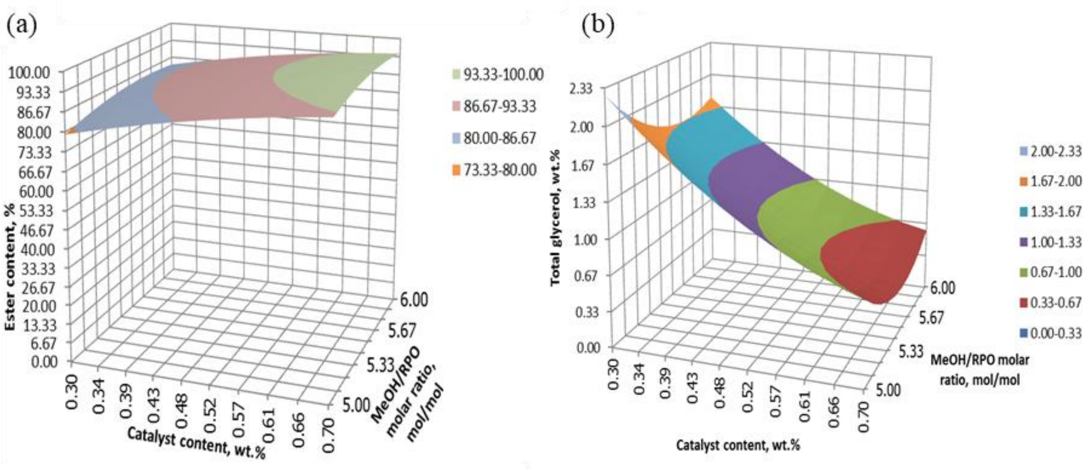


Fig. 9 Response surface plots for interaction effect of molar ratio and catalyst content on; (a) ester content; (b) total glycerol content (at 55 °C for 40 min).

4.2.4.2. Interaction effect of molar ratio and reaction time

In Fig. 10, response surface plots for the interaction variable of molar ratio and reaction time are clearly shown with the constant value of catalyst content and reaction temperature, 0.50 wt% and 55 °C, respectively. This influence on the ester content is shown by response surface plots in Fig. 10a. At low level of reaction time (20-40 min), by comparison the difference about the slope of contour, it is indicated that the reaction time has higher influence than the molar ratio. The methyl esters content increases significantly with increasing reaction time at any MeOH/RPO molar ratio due to higher slope of the contour. However, at longer time (40-60 min), the effect of reaction time on methyl esters content is negligible, suggests that reaction still continues but at a very low rate. The similar explanation is seen in previous results [63,72].

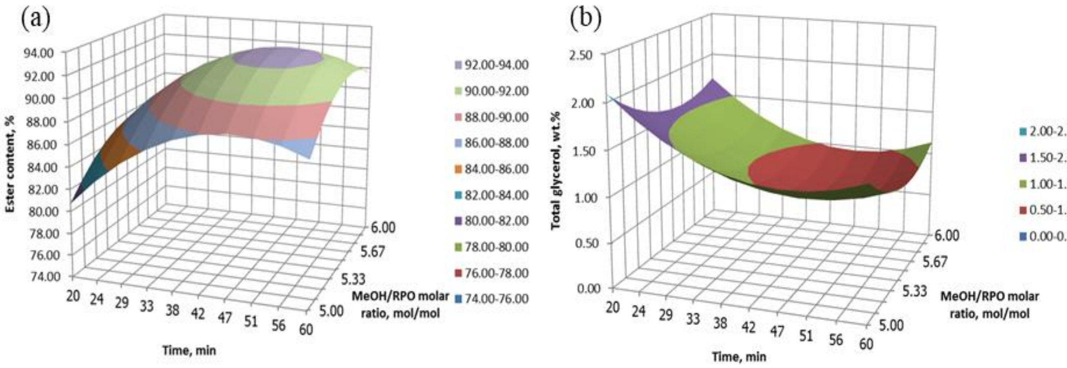


Fig. 10 Response surface plots for interaction effect of molar ratio and reaction time on; (a) ester content; (b) total glycerol content (at 0.50 wt% of CH3ONa catalyst and 55 °C).

4.2.4.3. Interaction effect of molar ratio and reaction temperature

The effect of the MeOH/RPO molar ratio and reaction temperature on the ester and total glycerol content are investigated with keeping the catalyst content and reaction time at the medium values, 0.50 wt% of CH_3ONa and 40 min, respectively.

Fig. 11a shows the response for the interactive factor of molar ratio and temperature. The 3D response surface plots indicate that the methyl esters production increases when methanol concentration increases. Therefore, the maximum ester content is obtained with high molar ratio. This is caused by the stoichiometry of transesterification, which requires a 3:1 molar ratio of alcohol to triglyceride. Because this reaction is reversible, an excess of alcohol is used to drive the reaction near completion (5:1-6:1). At high temperature, higher methyl ester yield is achieved. This significant role is clearly shown when the temperature increases from 45 °C to 60 °C. Several reports have found similar results in biodiesel production [69,70].

The change of total glycerol content as per molar ratio and reaction temperature is shown in Fig. 11b. It indicates that the total glycerol content in biodiesel as a function of molar ratio and reaction temperature. With the chosen temperature limit, the total glycerol content initially decreases with the increase of the molar ratio (5:1–6:1). As compared to previous study as in **Appendix D**, this correlation is not same at higher molar ratio (7:1-9:1). At higher level of molar ratio, the role of molar ratio in decreasing of the total glycerol in biodiesel is nearly trivial. This correlation is similar to the influence of molar ratio and catalyst content.

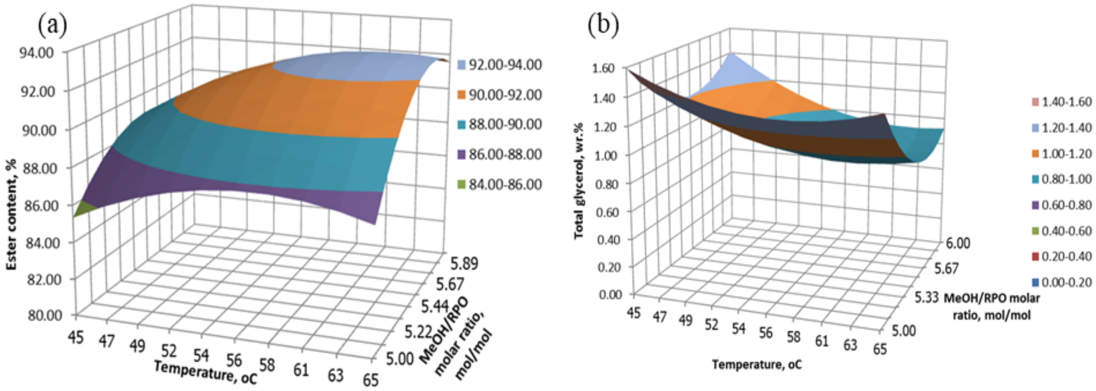


Fig. 11 Response surface plots for interaction effect of molar ratio and reaction temperature on; (a) ester content; (b) total glycerol content (at 0.50 wt% of CH_3ONa catalyst for 40 min).

4.2.4.4. Interaction effect of catalyst content and reaction time

The ANOVA results indicated that the catalyst content is the most important factor in the first stage transesterification. And this is also shown in the interaction effect of catalyst content and reaction time on the ester and total glycerol content. This relationship is carried out by keeping the molar ratio and reaction temperature at the medium values, 5.50 by mole and 55 ^{o}C, respectively (Fig. 12).

Also as per the slope of contour, a higher slope of the contour of catalyst content showed a bigger influence of this factor in comparison with reaction time.

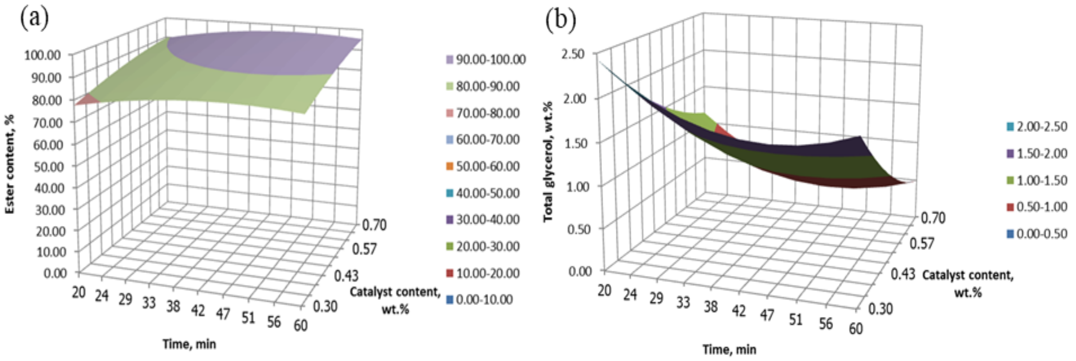


Fig. 12 Response surface plots for interaction effect of catalyst content and reaction time on; (a) ester content; (b) total glycerol content (at 5.50 of MeOH/RPO molar ratio and 55°C).

4.2.4.5. Interaction effect of catalyst content and reaction temperature

The effects of catalyst content and temperature on ester and total glycerol content are investigated with keeping the MeOH/RPO molar ratio and reaction time at the medium values, 5.50 mol/mol and 40 min, respectively (Fig. 13). The higher slope of the contour of catalyst content indicates great influence of this variable in comparison with reaction temperature. It is realized that catalyst loading significantly impacts in ester content in the chosen limit. By using 0.30-0.70 wt% of CH_3ONa (to RPO), ester content clearly increases from 80-95% on the experimental temperature limitation (45–65 °C). In other words, methyl esters content is enhanced by enlargement catalyst concentration at any temperature range. The same conclusion can be seen in some previous results [63].

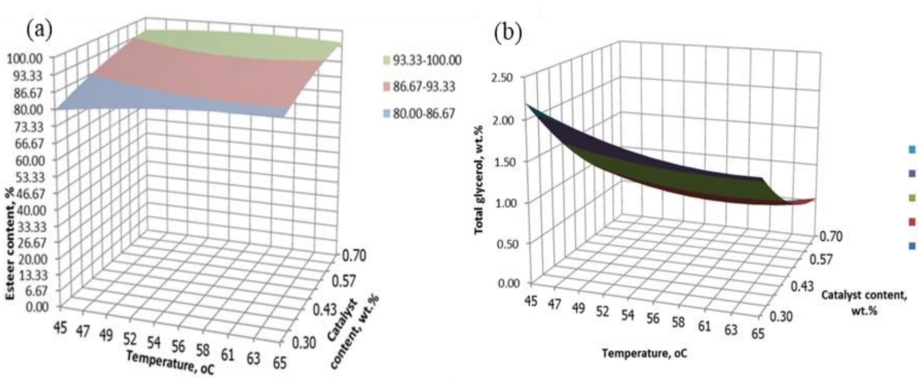


Fig. 13 Response surface plots for interaction effect of catalyst content and reaction temperature on; (a) ester content; (b) total glycerol content (at 5.50 of MeOH/RPO molar ratio for 40 min).

4.2.4.6. Interaction effect of reaction time and temperature

The statistical analysis of the experimental data indicates that time (X_3) and temperature (X_4) are quite important and effective variables in response analysis. They also have significant effects on the ester and total glycerol content in biodiesel. These influences are shown by the response surface plots with the constant value of molar ratio and catalyst content, 5.50 by mole and 0.50 wt%, respectively, as in Fig. 14. By the comparison the difference about the slope of contour, it indicates that the reaction time has a higher influence than the reaction temperature. This conclusion can be seen in the previous study as in **Appendix D**.

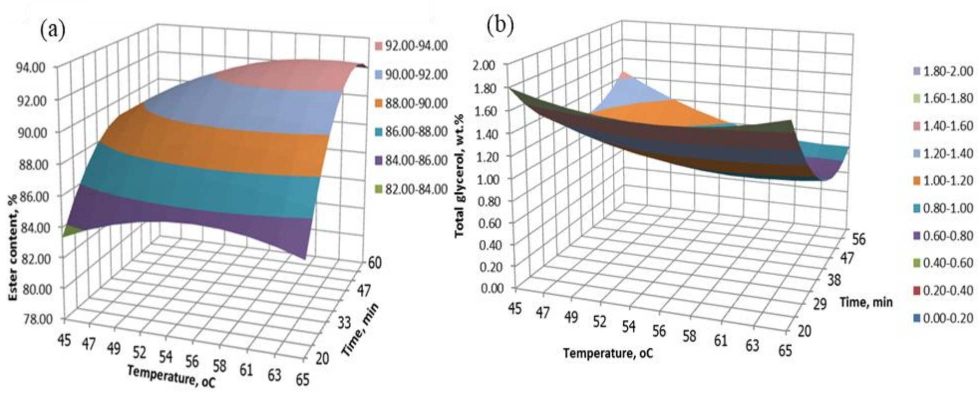


Fig. 14 Response surface plots for interaction effect of reaction time and temperature on; (a) ester content; (b) total glycerol content (at 5.50 of MeOH/RPO molar ratio and 0.50 wt% of CH_3ONa catalyst).

4.3. A study of second stage transesterification catalyzed by heterogeneous acid catalyst in a high pressure apparatus

4.3.1. Effects of reaction variables on the total glycerol and ester content

As above presented sections, the feedstock oil ester content of 85% was used for this study. Factorial design was applied to determine the effects of reaction conditions on the total glycerol and ester content. The variable factors and their values for optimization in the second stage were designed as follows:

- Molar ratio of methanol to oil of 8:1, 10:1 and 12:1.
- Amberlyst-15 catalyst content of 4, 8, 12 and 16 wt%.
- Reaction time of 3, 6, 9 and 12 h.
- Reaction temperature of 115 ^{o}C.

4.3.1.1. Effect of reaction time

In order to investigate the effect of reaction time on the ester content and conversion of triglyceride, the second step transesterification processes were carried out at 115 ^{o}C for 3, 6, 9 and 12 h with 12 wt% of Amberlyst-15 to oil and a MeOH/oil molar ratio of 10. Longer reaction time is required in order to guarantee the conversion of triglyceride due to medium catalytic activity of Amberlyst-15. The conversion of triglyceride is risen up to about 90% by promoting the reaction time from 3 h to 9 h as shown in Fig. 15, contributes in increasing ester content up to 98% in biodiesel. However, the incremental rate of the ester content and conversion of triglyceride reaches stagnation with further increase of reaction time (up to 12 h), suggests that the reaction still continues but at very slow rate.

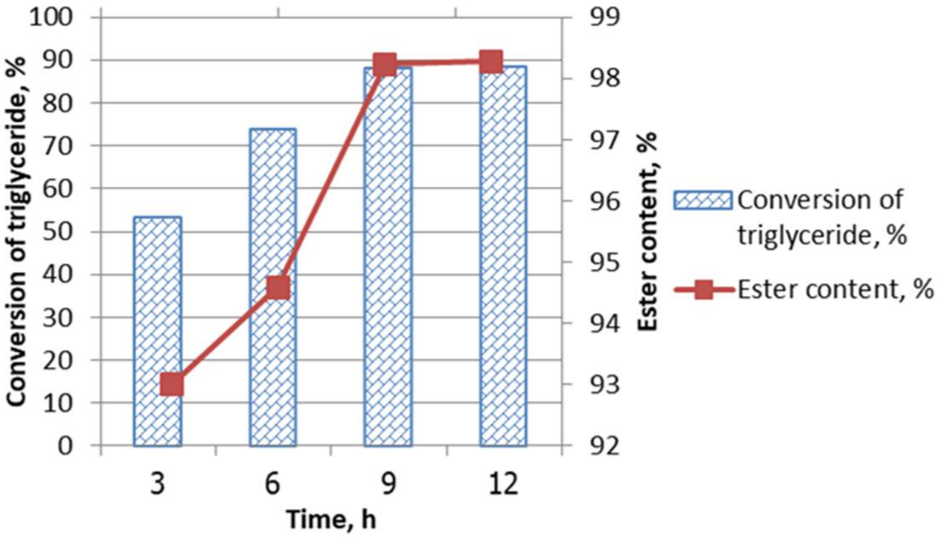


Fig. 15 Effects of reaction time on ester content and conversion of triglyceride.

4.3.1.2. Effect of catalyst content

The effects of catalyst content (4, 8, 12 and 16 wt% of Amberlyst-15 to oil) on the ester content and the conversion of triglyceride are investigated with MeOH/oil molar ratio, reaction time and temperature at 10:1, 9 h and 115 ^{o}C, respectively. The conversion (of triglyceride) and ester content increase with the increase of catalyst content as shown in Fig. 16. The significant increasing of the conversion of triglyceride and ester content between 4 wt% and 12 wt% catalyst content are associated with the increase of the number of acid sites on the surface of the Amberlyst-15 catalyst. However, further increase in Amberlyst-15 content above 12 wt% does not noticeable increase the ester content as well as the conversion of triglyceride. The small concentration of glycerides (< 3.0 wt%) may be a cause of a low reaction rate as same as the effect of reaction time above 9 h.

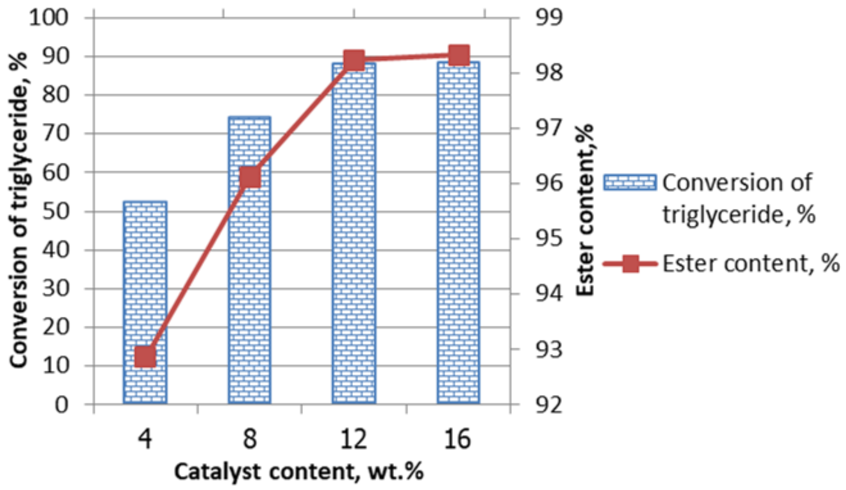


Fig. 16 Effects of Amberlyst-15 catalyst content on ester content and conversion of triglyceride.

4.3.1.3. Effect of methanol/oil molar ratio

The amount of methanol needed for transesterification was calculated based on molar ratio with respect to triglyceride (in oil). The stoichiometry ratio for this reaction requires three moles of methanol per one mole of triglyceride to produce three moles of esters and one mole of glycerol. However, the reaction rate on heterogeneous catalyst is a sequence of elementary reactions, such as the rate of glycerides reacting with active site on catalyst to form reaction intermediates and a later step of contacting to alcohol. The overall reaction rate is determined by the rate of the rate-limiting step. High methanol/oil molar ratio enhances the later step of alcohol-intermediates but gives a lower concentration of glycerides (mole/volume) that makes disadvantages the prior step. Hence, excess quantity of methanol is required to drive the reaction rate, but the optimum amount should be investigated without exaggeration.

The experiments were carried out with the MeOH/oil molar ratio of 8:1, 10:1 and 12:1. These experiments were performed at 115^{o}C temperature with catalyst content of 12 wt% and a reaction time of 9 h. As shown clearly in Fig. 17, the conversion of triglycerides paces significantly as MeOH/oil molar ratio changed from 8:1 to 10:1 and slows down smoothly as molar ratio further changed from 10:1 to 12:1. Highest conversion of triglyceride and ester content of 88% and 98% obtained at the MeOH/oil molar ratio of 10:1 which are higher than the study done by Boz et al. [50].

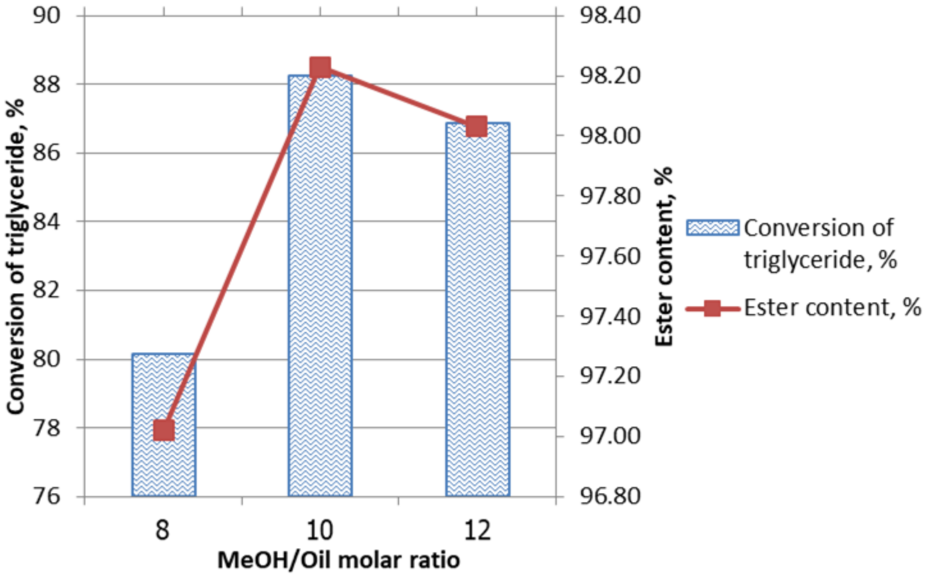


Fig. 17 Effects of methanol/oil molar ratio on ester content and conversion of triglyceride.

The reason can be explained via the good solubility of MeOH in the presence of methyl esters and intermediate compounds (mono- and di-glycerides) on the surface of Amberlyst-15 catalyst. This good solubility enhances the contact between triglycerides with MeOH to Amberlyst-15.

Experimental results show that the high quality methyl ester (98%) is obtained from the feedstock oil (85% ester) in the second step transesterification by using high pressure apparatus. The suitable conditions include the molar ratio of MeOH to oil of 10:1; Amberlyst-15 catalyst content of 12 wt%, reaction time of 9 h and reaction temperature of 115 ^{o}C. Therefore, first stage transesterification using homogeneous base catalyst with the appropriate target 85% of ester content was correctly chosen from this study.

4.3.2. Design of experiments for RSM model

The factorial design was used to determine the optimum conditions for the requested ester and total glycerol content. The factorial design incorporates three levels (-1, 0, +1) in which factorial points for every level. In addition, center points were coded as 0 and used to estimate pure errors. Three independent factors are molar ratio (X_1), catalyst content (X_2) and reaction time (X_3). The experimental limit and coded levels of independent factors are shown in Table 16. A list of 32 experiments including 3^3 factorial runs and 5 runs for center points was carried out. The ester content (Y_1) and total glycerol content (Y_2) were dependent variables.

Table 16 Limit and coded levels of independent factors for the second stage transesterification.

Factor			Limit and coded level		
Independent variable	Symbol	Dimension	-1	0	+1
Molar ratio	X_1	mol/mol	8.00	10.00	12.00
Catalyst content	X_2	wt%	4.00	8.00	12.00
Reaction time	X_3	h	3.00	6.00	9.00

4.3.3. RSM modeling for ester content

4.3.3.1. Analysis of variance (ANOVA) and RSM modeling

The relationship between the three independent variables (MeOH/oil molar ratio, catalyst content and reaction time) and the ester content are investigated. The ester content for each experimental run and from RSM model are listed in Table 17.

Table 17 The coded independent factors and ester content from experiment, RSM model.

Run	Independent variables			Ester content (%)	
	X_1	X_2	X_3	Experiment	RSM
1	12	12	6	94.59	95.15
2	12	12	3	93.00	92.65
3	12	12	9	98.03	98.23
4	12	8	9	95.06	94.27
5	12	4	9	90.70	91.43
6	12	4	3	88.33	88.00
7	12	4	6	89.35	89.42
8	12	8	3	89.26	89.77
9	12	8	6	92.32	91.72
10	8	8	6	92.09	92.94
11	8	8	9	93.99	94.09
12	8	12	6	96.01	95.45
13	8	4	6	92.80	91.54
14	8	8	3	92.60	92.38
15	8	12	9	97.02	97.14
16	8	4	3	91.02	91.52
17	8	12	3	94.30	94.35
18	8	4	9	91.73	92.16
19	10	4	9	92.86	92.72
20	10	4	6	91.62	91.40
21	10	4	3	90.47	90.68
22	10	8	3	92.69	91.99
23	10	8	9	96.12	95.10
24	10	8	6	93.59	93.25
25	10	12	9	98.23	98.60
26	10	12	3	94.09	94.42
27	10	12	6	96.94	96.22
28	10	8	6	92.09	93.25
29	10	8	6	92.62	93.25
30	10	8	6	92.95	93.25
31	10	8	6	93.30	93.25
32	10	8	6	93.06	93.25
				MSE	0.3085
				R^2	0.9462

Results of ANOVA are summed up in Table 18 in terms of the degree of freedom, the sum and mean of squares, F-value and P-value. The significance of the model, single terms, their squares and interactions is confirmed via their F-value and P-value. P-value less than 0.05 implies significant effects of these parameters on the ester content.

Table 18 ANOVA results for the adjusted regression model.

Source/Term	Degree of freedom (DF)	Coefficient	Sum of squares (SS)	Mean square (MS)	F-value	P-value	Remarks
Model	9		173.70	19.300	43.00	<0.0001	Significant
Constant		82.45				<0.0001	Significant
Linear	3					<0.0001	Significant
X_1	1	3.146				0.02273	Significant
X_2	1	-0.796				0.039	Significant
X_3	1	-1.397				0.00863	Significant
Square	3					<0.0001	Significant
X_1^2	1	-0.230				0.001327	Significant
X_2^2	1	0.03507				0.035	Significant
X_3^2	1	0.03290				0.250	Not significant
2-way interaction	3					<0.0001	Significant
X_1X_2	1	0.05687				0.028	Significant
X_1X_3	1	0.11639				0.00155	Significant
X_2X_3	1	0.04458				0.01126	Significant
Residual	22		9.874	0.449			
Lack of fit (LOF)	17		8.482	0.499	1.7931	0.2692	Not significant
Pure error	5		1.391	0.278			
Total	31		183.57				
R^2 = 0.9462, adjusted R^2 = 0.9242, R^2 for prediction = 0.8881							

Based on the coded factors, ANOVA data and by eliminating the insignificant model terms, the final simplified model is given in Eq. (13):

$$Y_1 = 82.45 + 3.146X_1 - 0.796X_2 - 1.397X_3 - 0.23X_1^2 + 0.03507X_2^2 + 0.05687X_1X_2 + 0.11639X_1X_3 + 0.04458X_2X_3 \qquad (13)$$

4.3.3.2. Performance assessment of predictive capability of RSM

As above sections, the capability of the developed RSM model in prediction of the ester content in biodiesel is evaluated in terms of their R, R^2, adjusted R^2 and MSE. These results are shown in Table 19. This model has high values of R, R^2 and adjusted R^2 demonstrate the authentic suitability of these models [56]. The performance evaluation of the developed RSM models in prediction of the total glycerol content in biodiesel is evaluated in Table 19. The RSM model has high values of R, R^2, adjuted R^2 demonstrate the authentic suitability of these models [60]. In addition, MSE checks the significance and accuracy of the suggested model [56,57,63]. The lower value of this statistical parameter, better the performance of the suggested model.

Table 19 Performance evaluation of RSM model.

Parameter	RSM
R	0.9727
R^2	0.9462
Adjusted R^2	0.9242
MSE	0.3085

4.3.3.3. Optimization of ester content by the RSM

By applying MS Excel in evaluating the second stage transesterification, a optimum condition to obtain 98% ester was determined. Moreover, in order to evaluate the optimization capability of the RSM model, the ester content of 98% was also chosen as a desired target of this present study. In comparison with MS Excel, RSM model give an optimum condition similar to MS Excel. Thus, RSM model is appreciable in prediction capability in this present study.

Table 20 Optimization conditions and model validation (for 98% ester).

Optimum reaction condition	MS Excel	RSM model
MeOH/oil molar ratio (by mole)	10.00	10.59
Catalyst content (wt%)	12.00	12.00
Reaction time (h)	9.00	9.00

4.3.4. RSM modeling for the total glycerol content

4.3.4.1. Analysis of variance (ANOVA) and RSM modeling

The relationship between the three independent variables (MeOH/oil molar ratio, catalyst content and reaction time) and the total glycerol content are also investigated. The total glycerol content for each experimental run and from RSM model are listed in Table 21.

Results of ANOVA are summed up in Table 22 in terms of the degree of freedom, the sum and mean of squares, F-value and P-value. The significance of the model, single terms, their squares and interactions is confirmed via their F-value and P-value. P-value less than 0.05 implies significant effects of these parameters on the total glycerol content.

Table 21 The coded independent factors and total glycerol content from experiment, RSM model.

Run	Independent variables			Total glycerol content (%)	
	X_1	X_2	X_3	Experiment	RSM
1	12	12	6	0.59	0.53
2	12	12	3	0.76	0.80
3	12	12	9	0.21	0.19
4	12	8	9	0.54	0.62
5	12	4	9	1.01	0.93
6	12	4	3	1.27	1.30
7	12	4	6	1.16	1.15
8	12	8	3	1.17	1.11
9	12	8	6	0.83	0.90
10	8	8	6	0.86	0.77
11	8	8	9	0.65	0.64
12	8	12	6	0.43	0.49
13	8	4	6	0.78	0.92
14	8	8	3	0.80	0.82
15	8	12	9	0.32	0.31
16	8	4	3	0.97	0.92
17	8	12	3	0.62	0.61
18	8	4	9	0.90	0.85
19	10	4	9	0.78	0.79
20	10	4	6	0.91	0.93
21	10	4	3	1.03	1.01
22	10	8	3	0.79	0.87
23	10	8	9	0.42	0.53
24	10	8	6	0.70	0.73
25	10	12	9	0.19	0.15
26	10	12	3	0.64	0.60
27	10	12	6	0.33	0.41
28	10	8	6	0.86	0.73
29	10	8	6	0.80	0.73
30	10	8	6	0.77	0.73
31	10	8	6	0.73	0.73
32	10	8	6	0.75	0.73
				MSE	0.0037
				R^2	0.9458

Table 22 ANOVA results for the adjusted regression model.

Source/Term	Degree of freedom (DF)	Coefficient	Sum of squares (SS)	Mean square (MS)	F-value	P-value	Remarks
Model	9		2.062	0.229	42.67	<0.0001	Significant
Constant		1.879				0.01622	Significant
Linear	3					<0.0001	Significant
X_1	1	-0.341				0.02393	Significant
X_2	1	0.08840				0.03677	Significant
X_3	1	0.156				0.00758	Significant
Square	3					<0.0001	Significant
X_1^2	1	0.02505				0.00139	Significant
X_2^2	1	-0.00384				0.03531	Significant
X_3^2	1	-0.00368				0.240	Not significant
2-way interaction	3					<0.0001	Significant
X_1X_2	1	-0.00625				0.02736	Significant
X_1X_3	1	-0.01278				0.00150	Significant
X_2X_3	1	-0.00500				0.00961	Significant
Residual	22		0.118	0.00537			
Lack of fit (LOF)	17		0.102	0.00602	1.8938	0.248	Not significant
Pure error	5		0.01588	0.00318			
Total	31		2.181				

R^2 = 0.9458, adjusted R^2 = 0.9236, R^2 for prediction = 0.8880

Based on the coded factors, ANOVA data and by eliminating the insignificant model terms, the final simplified model is given in Eq. (14):

$$Y_2 = 1.879 - 0.341X_1 + 0.0884X_2 + 0.156X_3 + 0.02505X_1^2 - 0.00384X_2^2 - 0.00625X_1X_2 - 0.01278X_1X_3 - 0.005X_2X_3 \quad (14)$$

4.3.4.2. Performance assessment of predictive capability of RSM

As above parts, the capability of the developed RSM model in prediction of the total glycerol content in biodiesel is evaluated in terms of their

R, R^2, adjusted R^2 and MSE. These results are shown in Table 23. This model has high values of R, R^2, adjusted R^2 demonstrate the authentic suitability of these models [60]. The performance evaluation of the developed RSM models in prediction of the total glycerol content in biodiesel is evaluated in Table 23. The RSM model has high values of R, R^2, adjuted R^2 demonstrate the authentic suitability of these models [56]. In addition, MSE also checks the significance and accuracy of the suggested model [56,57,63]. The lower value of this statistical parameter, better the performance of the suggested model.

Table 23 Performance evaluation of RSM model.

Parameter	RSM
R	0.9725
R^2	0.9458
Adjusted R^2	0.9236
MSE	0.0037

4.4. Evaluation of decreasing of homogeneous base catalyst and soap content

4.4.1. Influence of the raw material on the catalyst consumption during the first stage transesterification

Refined palm oil (RPO) with 0.11% of FFA (A=0.11), and crude palm oil with 0.49%, 1.15% and 1.74% of FFA were used as raw material. The reactions were carried out as per the optimum condition of the first stage transesterification (MeOH/Oil molar ratio: 5.48; 0.32 wt% of CH_3ONa; 40 min and 55 ^{o}C).

Depending on FFA content in oil, an extra amount of catalyst has to be added in order to neutralize the FFA. For the oils with FFA 0.11%, 0.49%, 1.15% and 1.74%, the amount of catalyst needed to neutralize FFA were 0.34, 1.54, 3.61 and 5.46 mol% (mol/100 mol oil), respectively. Therefore, the total amount of catalyst loaded in the reaction were 5.63, 6.56, 8.64 and 10.49 mol% with FFA 0.11%, 0.49%, 1.15% and 1.74%, respectively (Fig. 18).

After finish first stage transesterification and overcome the settling process, the soap and catalyst were distributed in biodiesel phase (BP) and glycerol phase (GP). Fig. 18 shows the soap and catalyst distribution in both phases (BP and GP). In the case of RPO (A=0.11), 76% of the initial amount (4.31 mol%) of catalyst was converted to soap, while 24% (1.32 mol%) was in the GP. All the remaining catalyst in the reacting system was concentrated in the GP. This soap was distributed between the two phases, 53% (3.02 mol%) of soap in the GP and 23% (1.29 mol%) in the BP.

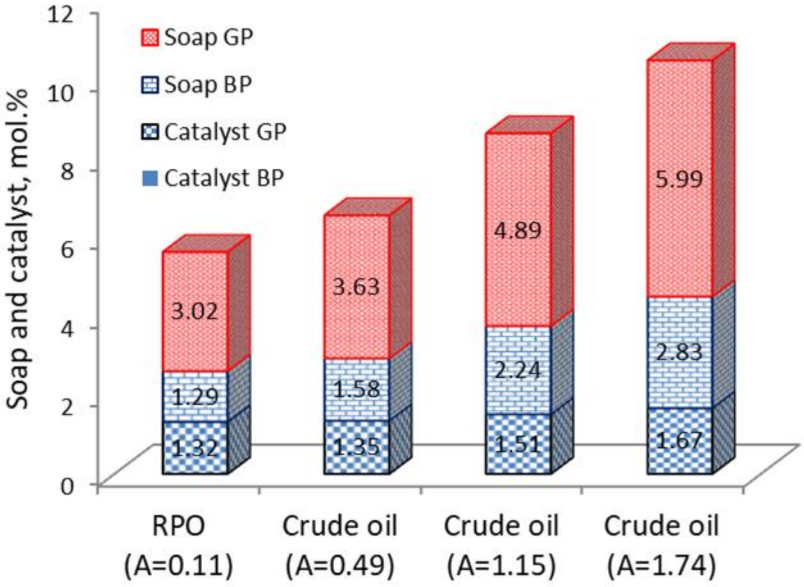


Fig. 18 The effect of raw feedstock on soap and catalyst concentration in biodiesel phase (BP) and glycerol phase (GP) in the first stage transesterification.

In the considering for crude oils, the soap content increases with the increase of FFA content and about 80% soap formed. The soap distribution in the BP was complicated for the purification stages.

4.4.2. Evaluation of decreasing of the soap and catalyst content by applying this novel two-stage transesterification

The most remarkable fact of this thesis is to reduce the soap content in biodiesel in comparison with one-stage reaction using only CH_3ONa as homogeneous base catalyst. One-stage reaction with the required ester content of 98% was carried out at the MeOH/RPO molar ratio of 7.57, catalyst content of 1.20 wt%, reaction temperature of 55 ^oC and reaction time of 50 min as in **Appendix D** and **Appendix F**. Table 24 shows the remaining base catalyst and soap content in biodiesel phase and glycerol phase. The total content of initially catalyst of 17.21 mol% (mol/100 mol of RPO) was distributed in the soap and remaining catalyst when the reaction was carried out in one stage for desire ester content of 98%. The soap was also largely concentrated in the glycerol phase with 7.46 mol%. On the other hand, soap was not produced after the second stage in this two-stage reaction by using Amberlyst-15 as heterogeneous acid catalyst. The total soap content of 1.48 wt% was only produced from the first stage. It is less than two times in comparison with one-stage process (3.21 wt%), approximately. The total content of base catalyst was only for the first stage and less than three times (approximately) compared with the one-stage reaction, 5.63 mol% and 17.21 mol%, respectively. This decrease was also significant finding in comparison with other studies [8,71]. The total soap content of 2.57 wt% was produced via two-stage transesterification of crude soybean oil [8], whereas about 2 wt% of soap content was formed through one-stage transesterification of rapeseed oil [71].

Table 24 Catalyst and soap content in biodiesel phase (BP) and glycerol phase (GP) after reaction for one stage reaction and this present two-stage reaction.

Reaction		Catalyst remains (mol/100 mol RPO)		Soap (mol/100 mol RPO)		Σ(catalyst +soap) (mol/100 mol RPO)	Soap content, (wt% to RPO)	Biodiesel yield (%)
		BP	GP	BP	GP			
One step (98% ester)		0.00	7.89	1.86	7.46	17.21	3.21	96.42
Two- step	1st step (85% ester)	0.00	1.32	1.29	3.02	5.63	1.48	98.13
	2nd step (98% ester)	0.00	0.00	0.00	0.00	0.00	0.00	

From above results, with considering the same target (98% ester), the CH_3ONa catalyst is consumed in the two-step process less than the one-step process, 0.32 wt% and 1.20 wt.%, respectively. By applying the present two-stage transesterification process, CH_3ONa catalyst for the first stage and Amberlyst-15 catalyst for the second stage, there is a soap decreasing from 3.21 wt.% to 1.48 wt.%. As a result, the total biodiesel yield from the two-stage process is higher than from the one-stage process, 98.13% and 96.42%, respectively. This most remarkable result confirms that the present two-step transesterification process is better than the one-step transesterification process.

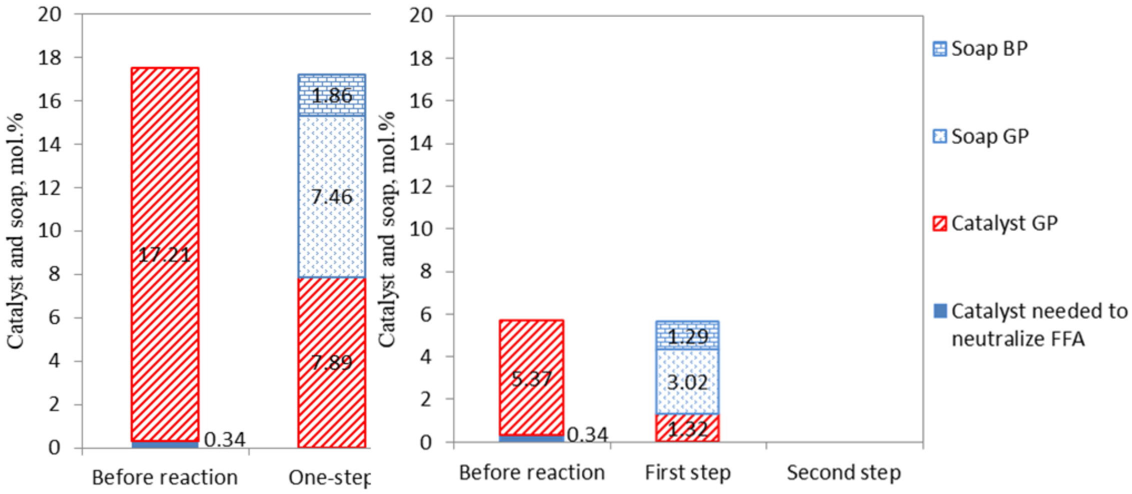


Fig. 19 Soap and catalyst concentration in biodiesel phase (BP) and glycerol phase (GP) after reaction of (a) one-stage transesterification and (b) two-stage transesterification.

Fig. 19 also shows the amount of catalyst initially loaded in the system and the distribution of catalyst after reaction and decantation for one-stage and two-stage transesterification process. The content of catalyst initially indicates the amounts needed to neutralize the FFA and catalyze the reaction. There is an important difference between one-stage process and first stage in two-stage process. In the one-stage process, 46% of catalyst initially (7.89 mol%) was remaining after reaction. The high remaining catalyst content leads to produce more soap, complex washing and yield loss. On the other hand, after the first step of two-stage transesterification, only 23% of catalyst initially (1.32 mol%) was still in reaction product. This contributes to reduce the soap formation and increase the biodiesel yield in the second stage.

4.5. Process development of two-stage transesterification

4.5.1. Fundamentals of process development of two-stage transesterification

In this present study, two-stage transesterification catalyzed by homogeneous base catalyst in first stage and heterogeneous acid catalyst in second stage shows its superiority in biodiesel production (Fig. 7). However, in order to reduce the production cost as much as possible, author also proposes a novel process as per using the ester phase directly for the second stage transesterification (Fig. 20).

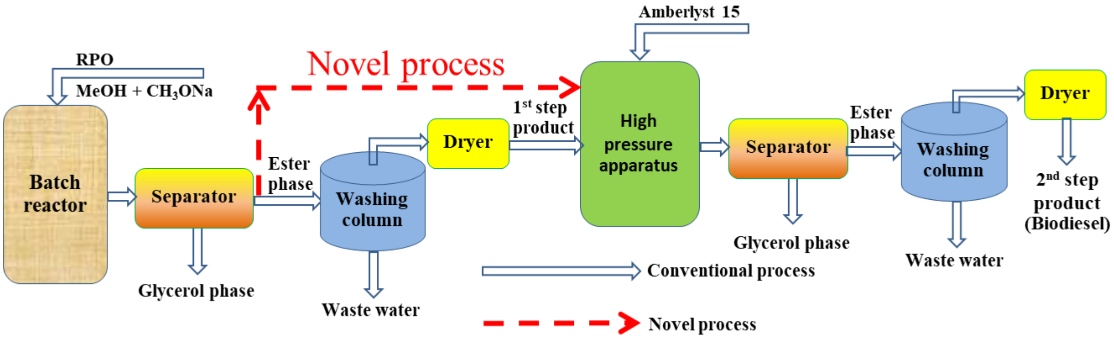


Fig. 20 Flowchart of conventional and novel two-stage transesterification.

After finish the first stage transesterification, the product mixture is transferred to the separator and separated into two phase; ester phase and glycerol phase. The composition of ester phase includes ester, glycerides (mono, di-, triglyceride), alcohol, soap, glycerol, water. In the case of undergoing the washing and drying process, only ester and glycerides are presented in the ester phase as in Fig. 7. However, if we use the remaining alcohol in ester phase then the required alcohol for the second stage will decrease. Therefore, utilization of excess alcohol not only reduces the cost for washing and drying but also decreases the requested alcohol, leads to reduce the production costs.

The process development, presented in this part, focuses on recycling of the MeOH-rich ester phase as alcohol solution for the second stage transesterification. The aim of this attempt is to seek a suitable and lowest-production cost for two-stage transesterification.

4.5.2. Composition determination of ester phase after the first stage transesterification

The first stage transesterification was carried out following to the optimum condition; including 5.48 of MeOH/RPO molar ratio, 0.32 wt% of CH_3ONa/RPO, 40 min and 55 ^oC. The flowchart is shown in Fig. 21 and the composition of ester phase after the first stage transesterification is shown in Table 25.

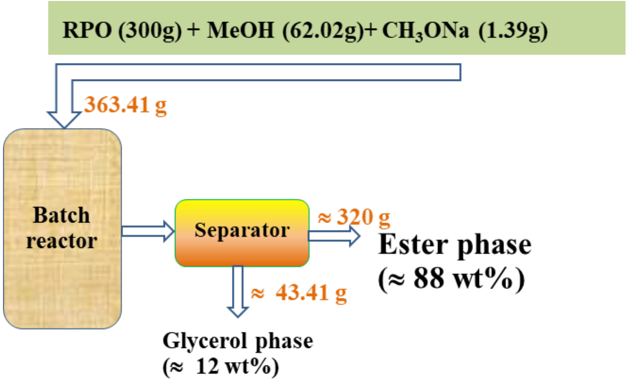


Fig. 21 Flowchart of material balance of the first stage transesterification.

Table 25 shows that the main components in ester phase are ester and glycerides, 78.39 wt% and 13.84 wt%, respectively. Catalyst is not presented in ester phase, while glycerol, soap and water are still small amounts in ester phase. It is a fact that MeOH is contributed in ester phase with a significant content, 6.89 wt%. Therefore, using ester phase directly for the second stage transesterification is a good recommendation for the coming study.

Table 25 Composition of ester phase after the first stage transesterification.

Composition	Weight percentage, %	Analysis method
Ester	78.39	Appendix C
Glycerides (mono-, di-, triglycerides)	13.84	Proximately calculation
MeOH	6.89	Proximately method
Glycerol	0.51	BS 5711-3: 1979
Soap	0.33	AOCS Cc 17-79
Catalyst	0.00	AOCS Cc 17-79
Water	0.04	ASTM D2709

4.5.3. Effect of MeOH utilization in ester phase on the two-stage transesterification

A comparative study between the present and novel process of two-stage transesterification was carried out as per the optimum conditions mentioned in above sections. The effect of MeOH utilization in ester phase on the second stage transesterification of the two-stage transesterifcation process in the present study is shown in Fig. 22.

As clearly seen in Fig. 22, the present process with using 35.09 wt% of fresh MeOH contributes to higher conversion and ester content, 88.23% and 98.23%, respectively. In the present process, after washing and drying process, the composition of ester phase only includes ester and glycerides. On the other hand, in the novel process, without washing and drying for ester phase, ester phase not only includes ester, glycerides and methanol but also contains soap, water and glycerol. These unwanted components inhibit the contact the contact between glycerides with MeOH to Amberlyst-15. Hence, the conversion and ester content in this novel process reduced, 86.41% and 97.96%, respectively. However, the ester content of 97.96% still meets the EN 14103 standard (96.5% min.).

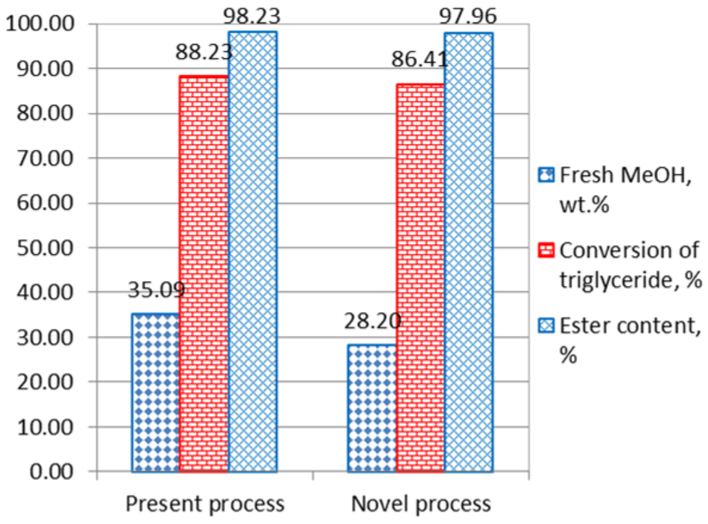


Fig. 22 The effect of MeOH utilization on the conversion and ester content in the second stage transesterification (12 wt% of Amberlyst-15; 9 h; 115 ^{o}C; MeOH/Oil = 10, equivalent 35.09 wt% of MeOH).

A remarkable point of applying the novel process is to reduce the MeOH amount from 35.09 wt% to 28.20 wt%, equivalent to reducing 20 wt% of fresh MeOH. This decreasing along with not applying the washing and drying process lead a good solution for decreasing the production cost.

4.5.4. Reusability of Amberlyst-15 catalyst

In the viewpoint of saving cost, the reusability of Amberlyst-15 was also investigated. In order to determine the reusability of Amberlyst-15, a series of experiments were carried out using the optimum conditions for the second stage transesterification. Following each experiment, the Amberlyst-15 was separated from the reaction mixture by centrifuging, and was washed with methanol to remove every compounds absorbed on the catalyst surface. And then, the Amberlyst-15 was dried at 105 °C for 12 h. Four successive reaction cycles were done at 10 by mole of MeOH/oil, 12 wt% of Amberlyst-15, at 115 °C for 9 h. The ester content following recycling is shown in Fig. 24. There is a negligible decrease of ester content during the second Amberlyst-15 reuse. Subsequent reuses of the Amberlyst-15 catalyst show a smooth decrease of ester content in biodiesel. The third reuse of Amberlyst-15 achieved an ester content of 97.05%, while the fourth reuse obtained 96.72% ester content. In spite of this significant decrease, the ester content is still higher than the EN 14103 standard (96.5% min.), suggesting that no significant loss of catalytic activity of Amberlyst-15 for at least four times reuse. These results are consistent with the investigation of Li et al. and Paterson et al., whereby Amberlyst-15 was recycled 7 times and 4 times for transesterification of yellow horn seed oil and triolein, respectively; and no significant loss of activity was observed [47,73].

In order to evaluate the catalytic durability, a heating treatment was shown as in the previous study [50]. Modified Amberlyst-15 was prepared by heating Amberlyst-15 at 220 °C for 4 h. Some physical properties and hydrogen ion-exchange capacities of Amberlyst-15 and modified Amberlyst-15 were summarized in Table 26. The hydrogen ion-exchange capacity of Amberlyst-15 decreased from 5.1 to 3.4 meq H^+/g after heating treatment at 220 °C for 4 h. However, in the considering the optimum temperature condition for this stage (115 °C for reaction and 105 °C for heat treatment), this drawback may be negligible.

Table 26 Porosity, surface area and hydrogen ion-exchange capacities of Amberlyst-15 under various heat treatment.

Duration of heat treatment (time, temperature)	Hydrogen ion-exchange capacity (meq H^+/g)	Porosity, ε_a	BET surface area (m^2/g)	Reference
0 h, 220 °C	5.1 ± 0.14	0.32 ± 0.01	39.0 ± 1.13	[50]
4 h, 220 °C	3.4 ± 0.23	0.39 ± 0.01	45.0 ± 0.86	[50]
12 h, 105 °C	ND	ND	ND	This present study

ND: not determined.

The surface characteristic of the Amb-15 and Amb-15-1 particles were also tested by SEM as in Fig. 23 [50]. Without heating treatment, the Amberlyst-15 catalyst surfaces were comparatively smooth and largely free of macrocracks (Fig. 23a and b). In contrast, the number of surface macrocracks increases with heating treatment (Fig. 23c and d).

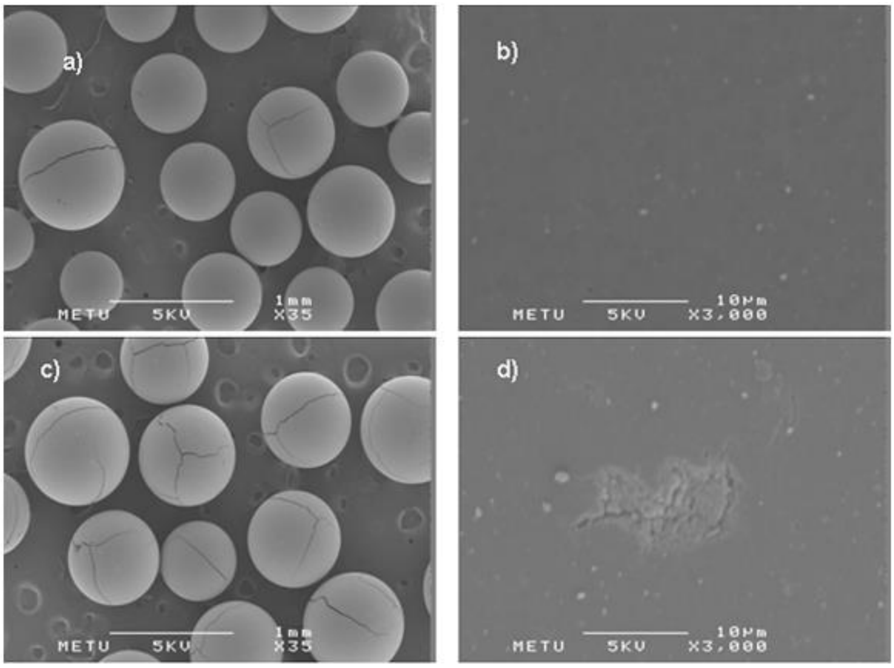


Fig. 23 SEM photographs (a) and (b) fresh Amberlyst-15; (c) and (d) Amberlyst-15 treated at 220 °C for 4 h [50].

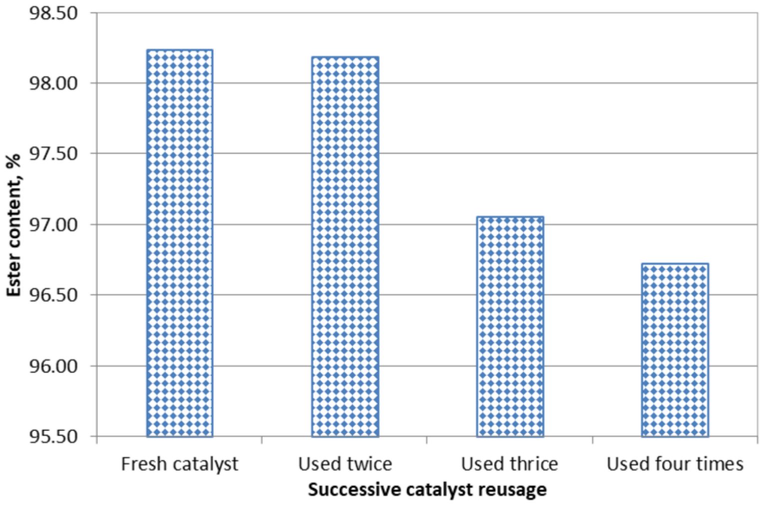


Fig. 24 Reusability of Amberlyst-15 catalyst.

5. CONCLUDING REMARKS

The objective of this research is to study a novel two-stage transesterification for a low-cost biodiesel production. The combination of the homogeneous base catalyst catalyzed first stage transesterification and the heterogeneous acid catalyst catalyzed second stage transesterification is a potential route for biodiesel production. The upgrading process using CH_3ONa in the first stage and Amberlyst-15 in the second stage has been the key of this study.

In order to investigate the experimental results, RSM has been applied as significant statistical method. The two important parameters of biodiesel, the ester content and total glycerol content, are considered as two outputs for these models.

The new chemical method for determining the ester content in biodiesel was demonstrated as an effective method. Moreover, evaluation of two-stage transesterification process as per the determining of the total glycerol content in biodiesel was also a suitable solution in this present study.

A study of determination the optimum composition of second stage feedstock was done in a high pressure apparatus. As a result, commercial biodiesel (96.5% ester min.) was produced from the Amberlyst-15 catalyzed second step transesterification by using feedstock oil with ester content from 85% to 90%. Ester content of 85% was chosen to study the Amberlyst-15 catalyzed second step transesterification process to ensure the efficiency and economy of the biodiesel production. Also, this ester content value was also a desired target for the first stage transesterification.

Following concluding remarks are drawn from two-stage transesterification process catalyzed by homogeneous base catalyst in the first stage and heterogeneous acid catalyst in the second stage:

5.1. The first stage transesterification in batch reactor

In the first stage transesterification, the glycerides conversion significant increased with increasing MeOH/RPO molar ratio, CH_3ONa catalyst content, reaction time and temperature. The high conversion of glycerides contributes to increase the ester content as well as to reduce the total glycerol content in biodiesel.

The quadratic equation for the first stage was established as follows:

$$Y_1 = -331.56 + 116.51X_1 + 149.21X_2 + 0.588X_3 + 1.113X_4 - 10.76X_1^2 - 42.35X_2^2 - 0.00969X_3^2 - 7.45X_1X_2 + 0.159X_1X_4 - 0.64X_2X_4 + 0.00592X_3X_4$$

The ester content of 85% after the first stage transesterification was obtained under optimum condition; including 5.48 molar ratio of MeOH to RPO, CH_3ONa catalyst loading of 0.32 wt%, 40 min and 55 ^{o}C. This value of ester content was demonstrated as a suitable feedstock for the second stage transesterification.

RSM has been applied in order to model and optimize the first stage transesterification. This RSM model demonstrated that the catalyst content is the most important factor for this stage. The increasing of catalyst content accelerates the speed of the transformation from glycerides (mono-, di, triglycerides) to esters. Therefore, the lower content of total glycerol reduces, the higher ester content increases.

5.2. The second stage transesterification in high pressure apparatus

In the second stage transesterification, the glycerides conversion significant increased with increasing MeOH/oil molar ratio (8:1-10:1), Amberlyst-15 catalyst content, reaction time. The high conversion of glycerides also contributes to increase the ester content as well as to reduce the total glycerol content in biodiesel.

RSM has been applied in order to model and optimize the second stage transesterification. The quadratic equation for 2nd stages was established as follows:

$$Y_2 = 82.45 + 3.146X_1 - 0.796X_2 - 1.397X_3 - 0.23X_1^2 + 0.03507X_2^2 + 0.05687X_1X_2 + 0.11639X_1X_3 + 0.04458X_2X_3$$

The ester content of 98% after the second stage transesterification was obtained under optimum condition; including 10 molar ratio of MeOH to oil, Amberlyst-15 catalyst loading of 12 wt%, 115 ^{o}C for 9 h. This value of ester content was fully suitable with EN 14103 standard for commercial biodiesel.

5.3. Decreasing evaluation of homogeneous base catalyst and soap content

As a remarkable point, application of the present two-stage transesterification technology has led to decrease the soap and the total amount of sodium methoxide about two times and three times, respectively, compared to the one stage process.

5.4. Process development of the present two-stage transesterification

A noteworthy aim of applying the novel process, using the ester phase directly for the second stage, is to reduce the MeOH amount from 35.09 wt% to 28.20 wt%, equivalent reducing 20 wt% of fresh MeOH. This decrease along with not applying the washing and drying process lead a good solution for decreasing the production cost.

The catalytic activity of Amberlyst-15 did not deteriorate after reuse at least for four cycles. This is also one advantage more in applying the present two-stage transesterification as described in this thesis.

6. SUGGESTIONS AND FUTURE WORKS

1. Methanol recovery in ester phase after the first stage transesterification should be further studied to reduce the consumption of chemicals for the two-stage transesterification process.
2. The deactivation of heterogeneous catalyst Amberlyst-15 via agglomeration of glycerol on the active site should be farther investigated to clarify the very slow rate of reaction after 80% of glycerides conversion.
3. Reusability of Amberlyst-15 catalyst should be further studied in order to increase the ability of reusing Amberlyst-15 for 2nd stage, reduce the production cost.
4. The laboratory scale of the second stage transesterification using high pressure apparatus should be expanded to utilize its widespread advantages on the industrial scale.

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8. APPENDICES

Appendix A

Journal Paper 1

D.N. Thoai, K. Prasertsit, C. Tongurai, A. Kumar. *Review on biodiesel production by two-step catalytic conversion.* The revised version of this article was submitted to Renewable and Sustainable Energy Reviews (ISI, SCI Impact Factor: 8.05, Initial Date Submitted: 01/05/2017, Review Date Completed: 14/08/2017, 1st Revision Date Submitted: 24/08/2017, 2nd Revision Date Submitted: 19/09/2017).

Review on biodiesel production by two-step catalytic conversion

Dang Nguyen Thoai[1,2], Chakrit Tongurai[1], Kulchanat Prasertsit[1], Anil Kumar[3,4*]

[1]Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla 90112, Thailand
[2]Department of Chemical Engineering, Faculty of Chemistry, Quy Nhon University, Binh Dinh 820000, Vietnam
[3]Department of Mechanical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla 90112, Thailand
[4]Department of Energy (Energy Center), Maulana Azad National Institute of Technology, Bhopal, India
Email address:
[*] Corresponding author: dranilk76@gmail.com (Anil Kumar)

Abstract

Recently, many researchers have done intensive efforts to enhance the biodiesel yield through two-step reaction in the production process. These processes have been applied under various conditions; including the type of feedstock oil, alcohol, catalyst, heating mode and reaction time. Chemical property of feedstock oil is the most important factor related to the selection of the best suitable two-step reaction technology. This review discusses two double-step reaction technologies, the first step esterification followed by the second step transesterification (called E+T technology) and two-step transesterification (denominated T1+T2 technology). The aim of two-step reaction is to optimize the operating cost of production process such as lower alcohol ratio, reduce reaction time and lessen yield loss. For example, if free fatty acid content is more than 2 wt.% then the first step esterification will be followed by the second step transesterification (E+T technology). The target of the first step esterification is to decrease the free fatty acid content in feedstock oils as much as possible in order to reduce the soap formation in the second step transesterification. Soap formation prevents the separation of biodiesel and glycerol phase and needs higher amount of washed water. Especially, it is the main reason of biodiesel loss because of turning ester to soap. The T1+T2 technology should be applied for the low free fatty acid content feedstock oils (less than 2 wt.%) to reduce the production cost. This review highlights the present status and challenges for these technologies, and few recommendations are given for future research in two-step reaction technologies.

Keywords: Biodiesel; Two-step reaction; First step esterification; Second step transesterification; Two-step transesterification.

1. Introduction

Nowadays, the development of human society is essentially dependent on energy resources. In 2015, fossil fuel was still the biggest source of energy (79.34%), whereas, the percentage of nuclear power and renewable energy sources were only 9.91% and 10.75%, respectively [1]. The fossil fuel is a main source of greenhouse gas emissions and leads to the environmental hazards and the global warming [2-4]. Therefore, the most remarkable issue is to produce energy from non-fossil and eco-friendly energy sources. The renewable energy resources include hydroelectric power, geothermal, solar, wind and biomass [1]. These energy resources play noteworthy roles in the future. Biodiesel is one of the most promising liquid fuels with high quality, derived from renewable resources. It is suitable to substitute for petroleum-based diesel without engine modification [5,6]. In comparison with petroleum diesel, biodiesel has proved many outstanding advantages; such as renewability, biodegradability, non-toxic for environment and high safety [7,8].

Biodiesel is a mixture of mono alkyl esters obtained through the transesterification of different feedstock (edible oil, non-edible oil, algae) in the presence of alcohol and catalyst [9]. Some edible oils are palm, sunflower, canola, soybean, coconut oil, used as feedstock for biodiesel production. The main drawback of these feedstock oils is the high price of biodiesel production process compared to the petroleum-based diesel [10]. As a result, non-edible oils are more economic feedstock; including Jatropha oil and waste oils. These feedstock have been encouraged for biodiesel production industry [11,12].

Recently studies have indicated that the algae is also a potential feedstock for biodiesel production [13,14].

Methanol and ethanol are the most popular alcohols in the biodiesel production, however, each has its own pros and cons. Most of biodiesel produced through transesterification of feedstock oils with methanol due to its suitable physicochemical property, low price, gentle reaction condition, higher activity and easy phase separation. However, Reid Vapor Pressure (RVP) of methanol is low due to its low boiling temperature which leads to the explosion risk. One more disadvantage of methanol is higher human toxicity than ethanol [6]. The next suitable trend is to use ethanol because ethanol is produced from renewable resources and is also not as toxic as methanol [15]. However, there is difficult separation of ethyl esters caused by the formation of unexpected emulsions in the product [16,17].

Catalyst plays an important role in biodiesel production. The catalyst is divided into three kinds based on their active site: acid catalyst, base catalyst and enzyme catalyst [18,19]. If the catalyst maintains in the same (liquid) phase status with reactants during reaction process, it is homogeneous catalyst. On the contrary, if the catalyst remains in the different phase status (solid, immiscible liquid) with reactants, it is called heterogeneous catalyst [20,21]. The choice of appropriate catalyst mainly depends on the free fatty acid and water content in the feedstock oils. The base catalyst has been known for strengthening reaction rate, the maximum value of ester content and biodiesel yield can be achieved in mild reaction conditions.

Acid catalyst is chosen if feedstock oils contain the high free fatty acid (FFA) and water content. It catalyzes both esterification and transesterification reaction, but its long reaction time is the biggest drawback [18]. Homogeneous catalysts are commonly used for biodiesel production. However, they involve more complex for separation and purification steps, consume large water for washing process and discharge large waste water as well. One more disadvantage with base homogeneous catalyst is easy occurrence of saponification reaction, especially for high FFA feedstock oils [22,23]. Soap formation is also main reason of yield loss. Although the heterogeneous catalysts convert feedstock into biodiesel slowly but the biodiesel separation process from the reaction mixture is easy. Moreover, the ability of regeneration and reusability of this catalyst is a significant advantage in comparison with the homogeneous catalyst [18]. The enzyme catalyst, for instance lipase enzymes, has been demonstrated to be an effective catalyst for biodiesel production. It overcomes all of the challenges of high FFA feedstock as separates glycerol easily due to no soap formation which produces high quality biodiesel [19].

Some technologies such as one-step reaction [23,24] and two-step reaction [25-29] have been developed to reduce biodiesel production cost from various feedstock. The technology of one-step reaction “transesterification reaction” is suitable for the feedstock oils with a low FFA content. Transesterification reaction will not take place if FFA content in feedstock oils is more than 3 wt.% [30]. Higher consumption of catalyst and alcohol content as compare to the two-step transesterification is another drawback of the one-step reaction [23,31].

The two-step reaction includes; first step esterification and second step transesterification [28,32-36], two-step esterification [37] and two-step transesterification [26,27,29,31,38,39]. Some researchers have used more than two-step esterification [40]. The two-step reaction can be applied to any feedstock oils, especially in case of high FFA content feedstock oils. If the FFA content is more than 2 wt.% then the first step esterification will be followed by the second step transesterification. The aim of the first step is to decrease the FFA content as much as possible and to become an appropriate feedstock oils for the second step transesterification. This two-step reaction has been proven to be the most effective technology for any feedstock oils in biodiesel production process [26-39].

The heating system for the two-step reaction is also a noticeable issue relates to technological conditions and production cost [29,31,41]. Typically, the biodiesel production process operates with a conventional heating system. It often uses oil bath for heating process. The outstanding advantage of the conventional heating is the easy condition about equipment as well as its operation. However, heat transfer is only occurred on the surface of the materials which leads to decreasing the effective heating and consumes higher energy.

The technology of two-step reaction assisted by ultrasonic irradiation efficiently emulsify the immiscible liquids, increases contact area between reactants and contributes to gain in biodiesel yield [25,29,41,42]. Recently, microwave assisted two-step reaction technology has been demonstrated as a promising technology for efficient biodiesel production process [32,34,43]. A microwave assisted reactor significantly reduces the energy consumption and reaction time for the process, especially for the heterogeneous catalysis reaction.

However, the microwave assisted technology cannot implement at industrial scale. It is too difficult to scale up the microwave assisted two-step reaction technology to the industrial scale due to its short penetration of radiation into material [44-46]. A large sealed container has to be used due to the low penetration of microwave irradiation and it causes a huge concern about the security. These disadvantages forbid using this technology at industrial scale.

As mentioned above, the biodiesel production process via two-step reaction has been demonstrated as an effective technology in favor of decreasing the production cost, increasing the competitiveness of biodiesel with petroleum diesel. There is no consolidated source of information on two-step reaction implantation in biodiesel production. Present review paper carefully analyzes, summarizes this technology and several new approaches are also discussed. This review will be very useful for further studies about two-step reaction in biodiesel production.

2. First Step Esterification followed by Second Step Transesterification (E+T Technology)

2.1. Sources of feedstock oil for E+T technology

The E+T technology was applied to most common feedstock oil as crude palm oil [32,43,47], sludge palm oil [48], crude coconut oil [32], Jatropha curcas

L. oil [34,41,49], salmon oil [50] and especially waste oil [28,35-37,51-54]. Waste oil (WO) is one of the potential sources for the production of low-cost biodiesel. It can be obtained from cooking oil, animal fat and yellow or brown grease. The most important WO source is derived from waste cooking oil (WCO). The perfect solution for WCO reuse is to apply E+T technology. The general feature of these feedstock oils is high FFA content. The first step esterification is chosen to convert high FFA in feedstock oil into alkyl esters and this reaction step reduces the FFA content to less than 2 wt.%. The feedstock from the first step esterification is absolutely appropriate for the second step transesterfication.

2.2 E+T technology by conventional heating process

The FFA content plays a prominent role in yield of the first step esterification and it varies from 7 to 30 wt.% depending on the type of feedstock. In order to reduce its content in the feedstock oil, the first step esterification has been done in the conventional heating process. Experimental setup of the conventional heating process "pretreatment of acid feedstock" is shown in Fig. 1. The common pretreatment involves esterification of FFA by alcohol in the presence of acidic catalyst. Methanol is the most appropriate alcohol for pretreatment due to the high solubility. Cai et al. suggested glycerol also a suitable alcohol [28]. Reaction occurs at vacuum pressure (5000 Pa) to decrease the boiling point of glycerol (from 290^{o}C to 210^{o}C). Acid catalysts, such as H_2SO_4 [33,50,54], $Fe_2(SO_4)_3$ [36,55], toluene-4-sulfonic monohydrate acid 'PTSA' [48] and polyferric sulfate 'PFS' [35] are used in this process. H_2SO_4 has proven to be the most appropriate catalyst among these catalysts [33,50,54]. The efficiency of the first step esterification process is affected by MeOH/FFA molar ratio, H_2SO_4/FFA weight percentage, temperature and reaction time.

Stoichiometry of the esterification requires one molecule of methanol to react with one molecule of FFA. However, an excess of the methanol is used to promote the FFA conversion. Therefore, a high molar ratio about 10–30 between methanol and FFA is required for effective reaction. The molar ratio of about 10 to 20 should be used for crude oils (palm oil, coconut oil). The FFA content in crude palm oil decreased significantly from 10 wt.% to less than 1 wt.% by using the molar ratio about 10 [47]. By increasing this molar ratio till 20, the FFA content reduced from 13 wt.% to 0.6 wt.% [33]. A highest molar ratio about 30 is commonly used in the case of WCO [35,36,54,56].

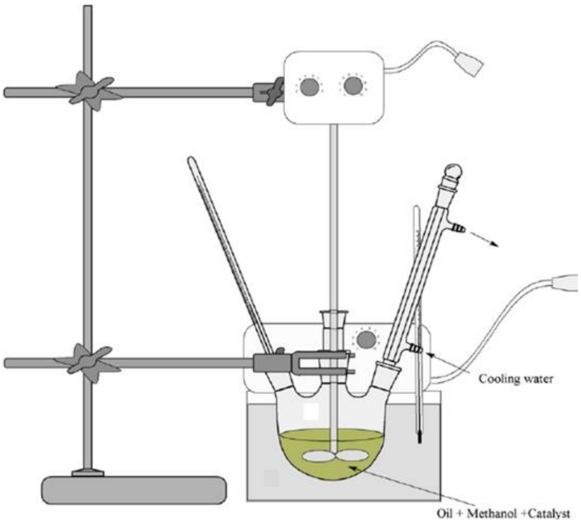

Fig. 1 Experimental setup for the conventional heating process.

Catalyst plays a vital role and its content depends on the type of catalyst as well as other conditions in the reaction (MeOH/FFA molar ratio, time and temperature). H_2SO_4 is the most suitable catalyst for esterification process with content ranging from 5 to 30 wt.% to FFA [33,47,49,50,54,56]. P-toluene sulfonic acid (PTSA) showed the highest catalytic activity in comparison with benzenesulfonic acid and sulfuric acid. FFA content reduced significantly from 22.33 to less than 2 wt.% by using 3.4 wt.% of PTSA to FFA [48]. $Fe_2(SO_4)_3$ is also considered as a suitable catalyst used with the content in a range from 5 to 23 wt.% to FFA in order to decrease FFA content until below 1 wt.% [36,55]. In another study, polyferric sulfate (PFS) produced from ferrous sulfate via three stages (oxidation, hydrolysis, and polymerization) is introduced to catalyze the esterification of FFA of the WCO with methanol [35]. FFA content in WCO could decrease to 1.68 wt.% in the presence of 9 wt.% of PFS to FFA.

Table 1

Summarized E+T technology by conventional heating process.

Raw material	Optimum condition for the first step esterification	Optimum condition for the second step transesterification	Ester content (wt.%)	Yield of biodiesel (%)	Reference
WCO	Glycerol/FFA molar ratio = 1.4, NaOH/FFA = 0.8 wt.%, 210°C, 4 h	MeOH/Oil molar ratio = 6, NaOH/Oil = 0.3 wt.%, 40°C, 1 h	98.6	93.1	[28]
Crude coconut oil	MeOH/FFA molar ratio = 21.8, H_2SO_4/FFA = 11.3 wt.%, 60°C, 60 min	MeOH/Oil molar ratio = 10, KOH/Oil = 1.7 wt.%, 60°C, 60 min	98.4	ND	[33]
WCO	MeOH/FFA molar ratio = 28.8, PFS/FFA = 9 wt.%, 67°C, 4 h	MeOH/Oil molar ratio = 6, KOH/Oil = 1.2 wt.%, 40°C, 1 h	95.08	ND	[35]
WCO	MeOH/FFA molar ratio = 32.9, $Fe_2(SO_4)_3$/FFA = 22.9 wt.%, 100°C, 1 h	MeOH/Oil molar ratio = 9, KOH/Oil = 0.5 wt.%, 100°C, 1 h	96	ND	[36]
Crude palm oil	MeOH/FFA molar ratio = 10, H_2SO_4/FFA = 10 wt.%, 60°C, 11 h	MeOH/Oil molar ratio = 6, NaOH/Oil = 0.7 wt%, 60°C, 35 min	96.5	95	[47]
Sludge palm oil	MeOH/FFA molar ratio = 14.3, PTSA/FFA = 3.4 wt.%, 60°C, 1 h	MeOH/Oil molar ratio = 10, KOH/Oil = 1 wt.%, 60°C, 1 h	96	76.62	[48]
Jatropha curcas L. oil	MeOH/FFA molar ratio = 20, H_2SO_4/FFA = 5 wt.%, 60°C, 60 min	MeOH/Oil molar ratio = 6, CH_3OK/Oil = 0.95 wt.%, 45°C, 30 min	98	ND	[49]
Salmon oil	MeOH/FFA molar ratio = 31.8, H_2SO_4/FFA = 16.7 wt.%, 52°C, 60 min	MeOH/Oil molar ratio = 9, KOH/Oil = 0.5 wt.%, 52°C, 30 min	99	ND	[50]
WCO	MeOH/FFA molar ratio = 26.7, H_2SO_4/FFA = 12.6 wt.%, 60°C, 1 h	MeOH/Oil molar ratio= 5, KOH/Oil = 1.1 wt.%, 60°C, 30 min	98	ND	[54]
WCO	MeOH/FFA molar ratio = 8.4, $Fe_2(SO_4)_3$/FFA = 5.3 wt.%, 95°C, 4 h	MeOH/Oil molar ratio = 6, KOH/Oil = 1 wt.%, 65°C, 1 h	97.02	ND	[55]
WCO	MeOH/FFA molar ratio = 25.9, H_2SO_4/FFA = 37.4 wt.%, 82°C, 3 h	MeOH/Oil molar ratio = 6, KOH/Oil = 1 wt.%, 65°C, 1.5 h	96.66	ND	[56]

ND: not determined

Temperature and time are also important factors which affect the esterification under conventional heating process. Most researches were done at temperature under boiling point of methanol (about 60^{o}C) to avoid unexpected evaporation of methanol in the reactor. Therefore, a long reaction time was requested to ensure the target of this stage.

Esterified feedstock oil will be converted into biodiesel by the second step transesterification process. The main reaction is transesterification reaction between esterified oil and alcohol in the presence of base catalyst (NaOH, KOH, CH3OK). The time for this stage is less than the first step process due to higher catalytic activities of base catalyst as compared to acid catalyst.

The stoichiometry of the transesterification requires three mole of methanol and one mole of triglyceride to get three mole of ester and one mole of glycerol. However, transesterification reaction is reversible, the reaction rate is significantly slow and then rapidly reaches an equilibrium state. Hence, a high MeOH/Oil molar ratio about 5 to 10 is required in order to drive the forward reaction to achieve the highest yield of the product. However, a large amount of methanol interferes with the glycerol separation due to increasing solubility of glycerol in ester phase. The remaining glycerol in the biodiesel enhances the reverse reaction and thus causes loss of biodiesel yield. This can be seen clearly in two studies [28,48]. Hayyan et al. [48] reported that using 10 by mole between MeOH and oil (in the presence of 1 wt.% KOH to oil, 60oC, 1 h), the biodiesel yield and ester content were only 76.62% and 96%, respectively. In a smoother condition (MeOH/Oil molar ratio: 6, 0.3 wt.% of NaOH to oil, 40oC, 1 h), Cai et al. [28] showed that the biodiesel yield and ester content could reach 93.1% and 98.6%, respectively. Therefore, a 6:1 molar ratio of methanol to oil seems to be the best [28,35,47,49,55,56]. Comparisons of esters content and yield of biodiesel of the conventional heated E+T technology by various researchers are shown in Table 1.

2.3. E+T technology by modern heating technology

In conventional heating process, longer reaction time is required. For improvement in this concern issue, novel heating technologies have been studied and developed to create biodiesel with smaller reaction time. Some modern heating technologies can be mentioned such as microwave irradiation [32,34,43], ultrasonic irradiation [41,42] and radio frequency [51].

The E+T technology process by microwave irradiation was applied to make biodiesel from the high FFA feedstock oil. The microwave irradiation can provide strong power and reach reaction temperature in a short time. The FFA content was reduced from 7.5 wt.% to less than 2 wt.% by using 24 of EtOH/FFA molar ratio with 4 wt.% of H_2SO_4/FFA. This esterification process was conducted at 70 W of microwave power in 60 min [32]. A higher reaction condition (EtOH/FFA molar ratio of 54, 5 wt.% of H_2SO_4/FFA, 110 W of microwave power) was done to decrease the reaction time for the first step [34]. FFA content could be decreased from 14 wt.% to less than 1 wt.% in only 35 min in this study. Schematic diagram of microwave irradiation heating technology is given in Fig. 2.

In the second step, despite the fact that transesterification of esterified feedstock oil has been carried out in short time (about 5–12 min) with smoother reaction condition (EtOH/Oil molar ratio: 5–8, KOH/Oil: 1.5–1.7 wt.%), ester content could still be reached more than 97% in comparison with the EN 14103 standard (96.5% min).

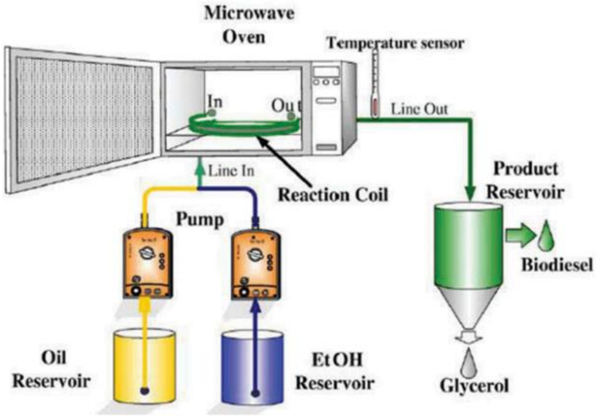

Fig. 2 Schematic diagram of microwave irradiation heating technology [2].

Table 2

Summarized E+T technology by modern heating technology.

Raw material	Heating technology	Optimum condition for the first step esterification	Optimum condition for the second step transesterification	Ester content (wt.%)	Yield of biodiesel (%)	Reference
Crude palm oil	Microwave irradiation	EtOH/FFA molar ratio = 24, H_2SO_4/FFA = 4 wt.%, 70 W, 60 min	EtOH/Oil molar ratio = 4, KOH/Oil = 1.5 wt%, 70 W, 5 min	97.4	80	[32]
Jatropha curcas L. oil	Microwave irradiation	EtOH/FFA molar ratio = 54, H_2SO_4/FFA = 5 wt.%, 110 W, 35 min	5N KOH (in EtOH)/ Oil = 16.3 wt.%, 110 W, 12.21 min	97.29	90.01	[34]
Crude Jatropha curcas L. oil	Ultrasonic irradiation (40 KHz, 400 W)	MeOH/FFA molar ratio = 10, H_2SO_4/FFA = 24 wt.%, 30°C, 20 min	MeOH/Oil molar ratio = 4, KOH/Oil = 1 wt.%, 30°C, 40 min	90	ND	[41]
Jatropha curcas L. oil	Ultrasonic irradiation	MeOH/FFA molar ratio = 60, H_2SO_4/FFA = 1.6 wt.%, 60°C, 1 h	MeOH/Oil molar ratio = 6, NaOH/Oil = 1.4 wt.%, 60°C, 1 h	96.4	ND	[42]
Crude palm oil	Microwave irradiation	EtOH/FFA molar ratio = 26, H_2SO_4/FFA = 16.7 wt.%, 78 W, 90 min	EtOH/Oil molar ratio = 8.5, KOH/Oil = 1.7 wt.%, 78 W, 7 min	97.4	78	[43]
WCO	Radio frequency	MeOH/FFA molar ratio = 20, H_2SO_4/FFA = 8.8 wt.%, 65°C, 8 min	MeOH/Oil molar ratio = 14.2, NaOH/Oil = 0.91 wt.%, 65°C, 5 min	98.8	ND	[51]

ND: not determined

Ultrasonic irradiation energy can also enhance the E+T process in biodiesel production through reducing the reaction time [41,42]. Earlier, FFA was reduced from 5.23 wt.% to 0.6 wt.% at 60°C in 60 min in the presence of 60 of MeOH/FFA molar ratio and 1.6 wt.% of H_2SO_4/FFA at the first step [42]. In the later step, 96.4% of ester content was obtained in a quite lightly reaction condition (MeOH/Oil molar ratio: 6, NaOH/Oil: 1.4 wt.%, 60°C, 60 min). Recently, only 20 min was needed to reduce FFA from 12.5 to less than 2.8 wt.% at ambient temperature (30°C) and other conditions (MeOH/FFA molar ratio: 10, H_2SO_4/FFA: 24 wt.%) [41]. However, only 90% of ester content was gained at 30°C. This low yield of ester can be explained by reaction conditions at the second step (MeOH/Oil molar ratio: 4, KOH/Oil: 1 wt.%, 40 min).

Radio frequency (RF) heating is another promising dielectric heating technology which provides fast heat generation though a direct interaction between a RF electromagnetic field and the object. RF heating technology was applied to create biodiesel from WCO by doing E+T technology [51]. In the first step, FFA content was decreased from 34.1 wt.% to 0.82 wt.% only within 8 min at 65°C by using 20 of MeOH/FFA molar ratio, 8.8 wt.% of H_2SO_4/FFA. In the second step, esterified feedstock oil reacted with MeOH followed a MeOH/Oil molar ratio (14.2) and 0.91 wt.% of NaOH/Oil at 65°C under RF heating for 5 min. Ester content could be achieved to 98.8%. The modern heated E+T technology is summarized in Table 2.

3. Two-Step Transesterification (T1+T2 Technology)

3.1. T1+T2 process catalyzed by base catalyst on both stages

Mendow et al. studied an efficient T1+T2 two-step process for ethyl esters production using solid sodium methoxide catalyst [31] (Table 3). This process consists of two reaction steps with glycerol separation and an additional part of mixture ethanol and catalyst in each of stages. The optimum condition is listed as EtOH/Oil molar ratio of 4.25:1 (2.55:1 for T1 and 1.7:1 for T2), CH_3ONa content of 1.1 wt./% (0.55 wt.% for each step) in the same temperature as well as time (55°C and 30 min). Biodiesel with ester content of 99% was attained and meets the required international standards.

In order to extend the ability of using non-edible feedstock for this process, Predojevíc produced biodiesel by two-step alkali transesterification of WCO using methanol and KOH as base catalyst [39]. Each stage of this process was followed by glycerol separation, purification and drying. The applied two-step transterification utilized a total molar ratio of methanol to oil of 6:1 (3:1 for each step), a total catalyst content of KOH to oil of 1 wt.% (0.5 wt.% for each step) in the same reaction time (30 min) at 30°C and 60°C, respectively. The comparison of three purification methods showed similar biodiesel yield after silica gel or acid washing (about 92%) but a lower yield was achieved after the washing process by hot distilled water (about 89%). However, ester content was obtained more than 97% after purification process and absolutely suitable for the minimum acceptable biodiesel purity according to standard EN 14103 (96.5 min).

Table 3

Summary T1+T2 technology.

Raw material	Heating system	Optimum condition for T1	Optimum condition for T2	Ester content (wt.%)	Yield of biodiesel (%)	Reference
Microalgae (biomass)	Oil bath	MeOH/Biomass (wt./wt.) = 41.59, NaOH/Biomass (wt./wt.) = 0.67, 90°C, 19.33 min	MeOH/Biomass (wt./wt.) = 51.3, H_2SO_4/Biomass (wt./wt.) = 3.81 wt.%, 90°C, 10 min	94.5	ND	[26]
Vegetable oil (Sunflower and linseed oil)	Oil bath	MeOH/Oil molar ratio = 10, KOH/Oil = 1.15 wt.%, 60°C, 60 min	MeOH/Oil molar ratio = 15, H_2SO_4/Oil =15.9 wt.%, 60°C, 60 min	97	85	[27]
WCO	Ultrasonic irradiation	MeOH/Oil molar ratio = 2.5, KOH/Oil = 0.7 wt.%, 30-32°C, 25 min	MeOH/Oil molar ratio = 1.5, KOH/Oil =0.3 wt.%, 27-29°C, 20 min	99	93.8	[29]
Refined palm oil	Oil bath	EtOH/Oil molar ratio = 2.55, CH_3ONa/Oil = 0.55 wt.%, 55°C, 30 min	EtOH/Oil molar ratio = 1.7, CH_3ONa /Oil = 0.55 wt%, 55°C, 30 min	99	ND	[31]
Vegetable oil (Sunflower and linseed oil) and WCO	Oil bath	MeOH/Oil molar ratio = 10, KOH/Oil = 0.63 wt.%, 60°C, 30 min	MeOH/Oil molar ratio = 5, H_2SO_4/Oil =5.3 wt.%, 60°C, 60 min	97-98	87-93	[38]
WCO (waste sunflower oil)	Oil bath	MeOH/Oil molar ratio = 3, KOH/Oil = 0.5 wt.%, 30°C, 30 min	MeOH/Oil molar ratio = 3, KOH/Oil = 0.5 wt.%, 60°C, 30 min	97-98	89-92	[39]
WCO	Oil bath	EtOH/Oil molar ratio = 12, KOH/Oil = 1 wt.%, 78°C, 120 min	EtOH/Oil molar ratio = 5, KOH/Oil = 0.75 wt%, 78°C, 120 min	94.5	ND	[57]
Sunflower oil	Oil bath	EtOH/Oil molar ratio = 12, NaOH/Oil = 1 wt.%, 80°C, 150 min	EtOH/Oil molar ratio = 6, NaOH/Oil = 0.75 wt%, 80°C, 30 min	96.5	ND	[58]

ND: not determined

Ultrasonic irradiation assisted technology is considered as a modern method in a two-step transesterification process in an effort to approach economic efficiency for biodiesel production [29]. The transesterification was carried out with the molar ratio of methanol to WCO of 2.5:1 and 1.5:1, the content of KOH to WCO to 0.7 wt.% and 0.3 wt.%, time of 25 min and 20 min at ambient temperature (30°C) for first step and second step, respectively. The ester content of 99% achieved in the short time and low temperature is a significant proof for this technology.

3.2. T1+T2 process catalyzed by base catalyst in first step and acid catalyst on second step

The applications of base catalyst for both steps in T1+T2 process has shown advantages such as fast reaction rate with low alcohol/oil molar ratio and high ester content. However, the base catalyst causes saponification and leads to loss of yield. In order to overcome this difficulty, T1+T2 two-step transesterification procedure which included base transesterification followed by acid transesterification was indicated clearly in some studies [26,27,38,59]. A schematic diagram of two-step transesterification process (T1+T2) can be seen in Fig. 3.

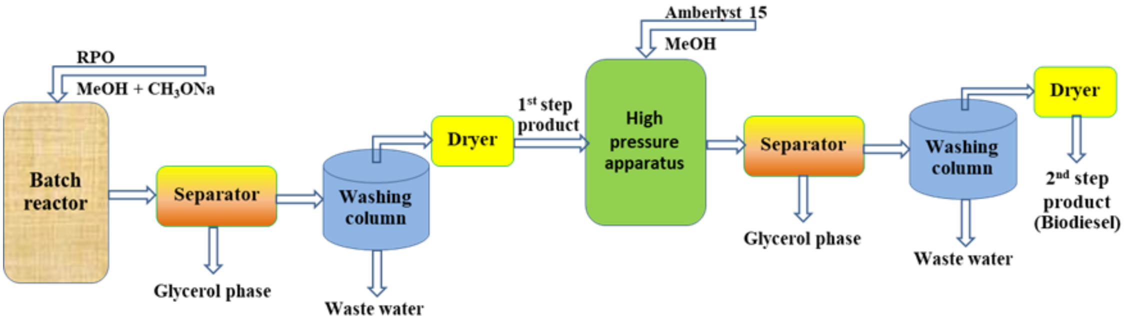

Fig. 3 Schematic diagram of two-step transesterification process [59].

Microalgae was considered as an alternative feedstock from biomass used for T1+T2 two-step direct transesterification process [26]. This novel technology has overcome disadvantage of the traditional method of lipid estimation proposed by Bligh and Dyer [60]. This decreases using chloroform and methanol, leads to reduction of adverse effects on health and environment [61].

A two-step direct transesterification method using NaOH in first step and H_2SO_4 in second step was reported by Kumar et al. [26]. The ester content can be gained up to 94.5% in optimum condition, including methanol to biomass weight ratio 51.59 (w/w) and 51.3 (w/w), catalyst to biomass weight ratio 0.67 (w/w) and 3.81 (w/w), reaction time 19.33 min and 10 min at 90^{o}C in first step and second step, respectively.

The significant development of this technology was mentioned in two researches of Samios et al. [27,38] which was called under a terminology, Transesterification Double Step Process (TDSP). The process includes continuous homogeneous base–acid catalyst steps and also proved the effectiveness by high reaction rate, easy separation process as well as high conversion [27]. The ester content can be higher than 97% at 60^{o}C in 60 min for each step and adding 10 and 15 of MeOH/Oil molar ratio, 1.15 wt.% of KOH and 15.9 wt.% of H_2SO_4 to Oil for first step and second step, respectively. The improved TDSP process involves to the reduction of reaction conditions (catalyst content in both steps, MeOH/Oil molar ratio in second step, reaction time in first step) and direct adding of MeOH/H_2SO_4 solution without cooling the reaction system between first and second step [38].

4. Conclusions and Recommendations

Although a lot of research has been done on biodiesel synthesis, the cost of biodiesel production is still a question because it is somewhat higher than petroleum-diesel and almost cost is from raw material. In order to reduce the production cost, the two-step reaction technology has proven its superiority over one step reaction process in biodiesel production in term of; using various feedstock, decreasing of alcohol and catalyst content, smoother reaction conditions, higher conversion, higher ester content and biodiesel yield. Moreover, future studies should be more focused on making the biodiesel production process more cost-effective either by exploring the novel and cheap feedstock. The potential of using WCO and biomass as cheap and economical feedstock is a promising future for biodiesel production.

Based on the large number of researches mentioned, most present two-step technology is using base catalyst for the second step. The advantage of this application is to increase the conversion and lead to improve the ester content. Nevertheless, base catalyst also accelerates the saponification reaction. Soap formation prevents from the separation of biodiesel, glycerol and washed water and is also crucial reason of biodiesel loss. In order to overtake on this unexpected problem, H_2SO_4 was also considered as a homogeneous acid catalyst for second step in some studies. However, using homogeneous acid catalyst causes corrosion on equipment. Therefore, it is recommended that researches on heterogeneous acid catalyst should be carried out extensively to develop this two-step technology in biodiesel production. Two advantages more of heterogeneous acid catalyst are its reusable and stable. These strong points contribute to decrease the production cost and to strengthen the competition of biodiesel with petroleum diesel.

The two-step transesterification via the first step using homogeneous base catalyst and the second step using heterogeneous acid catalyst may lead to upcoming research interest. With regards to this two-step transesterification, the second step transesterification using heterogeneous catalyst is the rate-limiting step. Therefore, the second step transesterification should be studied first in order to find the optimum composition of second step feedstock that supports a mild condition of using solid catalyst and results in better quality of commercial biodiesel product (96.5% ester min.). After that, study of the first step transesterification using homogeneous base catalyst should be carried out in order to determine the optimum condition for this step. This will incorporate the advantage of both catalysts in biodiesel production process.

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Appendix B

Journal Paper 2

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Research article

A novel two-step transesterification process catalyzed by homogeneous base catalyst in the first step and heterogeneous acid catalyst in the second step

Dang Nguyen Thoai[a,b,*], Chakrit Tongurai[a], Kulchanat Prasertsit[a], Anil Kumar[c,d]

[a] *Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla 90112, Thailand*
[b] *Department of Chemical Engineering, Faculty of Chemistry, Quy Nhon University, Binh Dinh 820000, Vietnam*
[c] *Department of Mechanical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla 90112, Thailand*
[d] *Department of Energy (Energy Center), Maulana Azad National Institute of Technology, Bhopal, India*

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ABSTRACT

The utilization of base catalyst for two-step transesterification prompts soap formation and yield loss in biodiesel production. In order to overcome this difficulty, the two-step transesterification process was performed with homogeneous base catalyst and heterogeneous acid catalyst was performed in the batch reactor and the high-pressure apparatus. As per response surface methodology, optimum condition for the first step is determined. The ester content of 85% is obtained with the molar ratio of methanol to oil of 5.48:1, 0.32 wt% of CH_3ONa catalyst, 40 min and at 55 °C. Final biodiesel production with ester content of 98% is produced from the second step under optimum condition (methanol/oil molar ratio of 10, Amberlyst 15 catalyst content of 12 wt%, 9 h and 115 °C). The soap content was 50% and amount of base catalyst used was 33% as compared to one-step transesterification process.

1. Introduction

Biodiesel is obtained by transesterification of different feedstocks (edible oil, non-edible oil and biomass) in the presence of short chain alcohol (methanol, ethanol) and catalyst (homogeneous, heterogeneous) [1]. Biodiesel has some outstanding advantages; such as renewability, biodegradability, non-toxicity for environment and high safety compared to petroleum diesel [2,3].

In order to reduce the cost of the commercial biodiesel production process, two-step transesterification process was studied and applied in industry [4–9]. Recently, the use of homogeneous base catalyst (NaOH, KOH, CH_3ONa, CH_3OK) has proven its specialty such as high reaction rate, smooth reaction condition. The most astounding effect of homogeneous base catalyst is that the saponification reaction process becomes very easy [10,11]. Large amount of homogeneous base catalyst was consumed in the reaction between free fatty acid (FFA) and esters. In order to ensure the stability of transesterification process, large amount of catalyst was required, in turn accelerating saponification reaction. Excessive soap formation prevents the separation of biodiesel and glycerol, reducing biodiesel yield. Review on yield, ester content and soap formation in two-step transesterification by various researchers are given in Table 1.

It is very difficult to guarantee that all of homogeneous base catalyst will catalyze the reaction. Hence, leads to rigorous catalyst removal process, in turn increases the production cost of biodiesel [15]. To overcome the demerits of homogeneous base catalyst in transesterification step, heterogeneous acid catalyst has proven as a potential choice. Some of its merits are not to cause the saponification reaction, insensitive to FFA and water in the feedstock [10]. The drawbacks of using heterogeneous catalyst include low mass transfer, low solubility of alcohol to oil and strong reaction conditions. However, the composition of feedstock entering the second step transesterification contains low amount of triglycerides (< 20 wt%), high proportion of ester and some of alcohol lessen these drawbacks [6,16].

The advantages and disadvantages of homogeneous and heterogeneous catalysts have been carefully studied and the interest of applying the advantages of both catalysts is a big challenge. Most of the two-step transesterification processes use homogeneous base catalyst for both steps (Table 1) [4,8,9,11–14]. In order to overcome its disadvantage, H_2SO_4 was considered as homogeneous acid catalyst for the second step [6,7]. However, using homogeneous acid catalyst causes corrosion on equipment. The two-step transesterification via the first step using homogeneous base catalyst and the second step using heterogeneous acid catalyst is interest of present research. With regard to this two-step transesterification, the second step transesterification using heterogeneous catalyst is the rate-limiting step. Therefore, the

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[*] Corresponding author at: Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla 90112, Thailand.
E-mail address: dangnguyenthoai@qnu.edu.vn (D.N. Thoai).

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Table 1
Two-step transesterification results on yield, ester content, soap content reported by various studies.

Feedstock	Type of reactor	Catalysis type of two-step transesterification process	Type of alcohol	Transesterification condition alcohol/oil molar ratio, catalyst content (wt%), time (h), temperature (°C)	Yield (wt%)	Ester content (wt%)	Soap content (wt%)	Reference
Refined palm oil	Batch reactor	Homogeneous base catalyst	EtOH	1st step 2.55:1; 0.55%CH_3ONa; 0.5; 55 2nd step 1.7:1; 0.55%CH_3ONa, 0.5; 55	ND ND	ND 99	ND ND	[4]
Sunflower oil and linseed oil	Batch reactor	1st step by homogeneous base catalyst 2nd step by homogeneous acid catalyst	MeOH	1st step 10:1; 1.15%KOH; 1; 60 2nd step 15:1; 15.9%H_2SO_4; 1; 60	ND 85	ND 97	ND ND	[6]
Sunflower oil, linseed oil and WCO	Batch reactor	1st step: homogeneous base catalyst 2nd step: homogeneous acid catalyst	MeOH	1st step 10:1; 0.63%KOH; 0.5; 60 2nd step 5:1; 5.3%H_2SO_4; 1; 60	ND 87–93	ND 97–98	ND ND	[7]
WCO	Batch reactor with ultrasonic irradiation	Homogeneous base catalyst	MeOH	1st step 2.5:1; 0.7%KOH; 0.5; 30 2nd step 1.5:1; 0.3%KOH; 0.5; 30	ND 93.8	81 99	ND ND	[8]
WCO	Batch reactor	Homogeneous base catalyst	MeOH	1st step 3:1; 0.5%KOH; 0.5; 30 2nd step 3:1; 0.5%KOH; 0.5; 60	ND 89–92	ND 97–98	ND ND	[9]
Sunflower oil	Batch reactor	Homogeneous base catalyst	EtOH	1st step 12:1; 1%NaOH; 2.5; 80 2nd step 6:1; 0.75%NaOH; 0.5; 80	ND ND	81.4 96.5	ND ND	[12]
WCO	Batch reactor	Homogeneous base catalyst	EtOH	1st step 12:1; 1%KOH; 2; 78 2nd step 5:1; 0.75%KOH; 2; 78	ND ND	74.2 94.5	ND ND	[13]
Crude soybean oil (1.85%FFA)	Batch reactor	Homogeneous base catalyst	MeOH	1st step 3:1; 0.47%CH_3ONa; 1.5; 60 2nd step 3:1; 0.26%CH_3ONa; 1.5; 60	ND ND	ND ND	2.17 0.39	[11]
Rapeseed oil (0.42%FFA)	Batch reactor	Homogeneous base catalyst	MeOH	One step 6:1; 0.7%KOH; 1.5; 60	ND	ND	2.00	[14]

ND: not determined.

second step transesterification has been studied first in order to find the optimum composition of second step feedstock that supports a mild condition of using solid catalyst and obtain excel quality for commercial biodiesel product (96.5% ester min.). After that, turn to study the first step transesterification by using homogeneous base catalyst.

Sodium methoxide (CH_3ONa) has been demonstrated as the most suitable catalyst for the base-catalyzed methanolysis [4,11], whereas Amberlyst 15 is the best acid heterogeneous catalyst [17–19]. Therefore, CH_3ONa and Amberlyst 15 have been used in the first and the second step transesterification, respectively, in the present research.

2. Materials and methods

2.1. Materials

Food grade refined palm oil (RPO) was purchased from Morakot Industry Public Co. Ltd. (Thailand). The homogeneous base catalyst, CH_3ONa (96 wt%) was supplied by Dezhou Long Teng Chemical Co. Ltd. (China) whereas the heterogeneous acid catalyst, Amberlyst 15 was obtained from Sigma-Aldrich (USA). Methanol (CH_3OH, purity > 99.8 wt%) was acquired from Labscan Asia Co. Ltd. (Thailand). Other important chemicals, sodium periodate ($NaIO_4$) was purchased from Fisher Chemical (UK), HCl was ordered from J.T. Baker (USA), NaOH was obtained from Merck (Germany), bromothymol blue and bromophenol blue were provided by Ajax Finechem (Australia).

2.2. Biodiesel synthesis procedure in the second step transesterification

The mixture of feedstock, methanol and Amberlyst 15 charged into 1 l volume bomb made from stainless steel at the high temperature and pressure to synthesize biodiesel. The bomb has an external electrical heater with split range temperature controller. A 45-mm diameter two-bladed motored turbine stirrer was connected to the top of the bomb through cap screw with heat-resistant rubber rings for operating without leakage at high pressure and temperature. This drive system rotates the stirrer at 400 rpm which is appropriate for mixing and to avoid any mechanical damage of the catalyst. After charging, reaction mixture was heated to the desired temperature and pressure in the bomb reactor. Time taken to gain the desired reaction temperature was about 30 min. At the end of reaction, the product mixture was transferred to the separation funnel to recover Amberlyst 15. It was centrifuged to separate glycerol and crumbled catalyst during reaction. The washing and de-water processes guarantee a good quality of biodiesel. Finally, ester content in biodiesel found in accordance to EN 14103 standard.

All experiments were carried out at 115 °C in order to promote all features of the pressure reaction apparatus as well as to maintain the working temperature limitation of Amberlyst 15 (120 °C max.). Factorial design was used for this stage; including MeOH/Oil molar ratio (8:1, 10:1 and 12:1), catalyst content (4, 8, 12 and 16 wt%) and reaction time (3, 6, 9 and 12 h).

2.3. Procedure of the first step transesterification process

The process is a sequence of operations, performed approximately in 6 h. The transesterification reaction was carried out in a 0.5 l three-necked flask, with magnetic stirring at 600 rpm. It worked at atmospheric pressure and refluxed by water having temperature of 20 °C to condense the methanol vapor. RPO with FFA content of about 0.11 wt% was used as a raw feedstock. The RPO was preheated until the temperature reached to the required limit. After that, the mixture of methanol and catalyst was added and reaction starting time was noted.

After reaction was completed, product was transferred to the separating funnel for 60 min to separate it into two phases namely methyl ester and glycerol phase. The methyl ester phase was washed using hot water (80 °C) without and with shaking thrice during removal of glycerol from methyl ester phase. The washed methyl ester was dried for 90 min at 110 °C. Later, ester content was determined and resulting product became feedstock for the second step transesterification. The schematic diagram for two-step transesterification is shown in Fig. 1.

All the experiments were repeated thrice for error estimation. Experiments were designed at various conditions; including MeOH/RPO molar ratio (5:1–6:1), catalyst content (0.3–0.7 wt%), reaction time (20–60 min) and reaction temperature (45–65 °C).

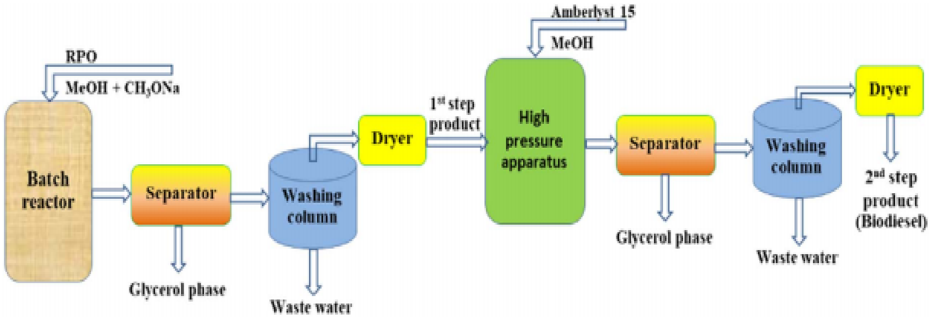

Fig. 1. Schematic diagram of two-step transesterification process.

2.4. Response surface methodology for the first step transesterification

Response surface methodology (RSM) is one of the most significant statistical methods to predict and optimize the biodiesel production. RSM was utilized to design the experiments, model and optimize the ester content as a response. This method uses Essential Experimental Design (EED) software in MS Excel [20]. Additionally, the Design-Expert® software, version 7.0 (Stat-Ease, Minneapolis, USA) was also used to check the accuracy of analyzed experimental data.

Central Composite Design (CCD) was utilized to determine the effect of experimental factors on the ester content as well as the optimum condition for the first step transesterification process. The CCD incorporates five levels (coded –α, − 1, 0, + 1, + α); including factorial points (± 1) for all factors, axial points (± α) for a factor and 0 for all other factors. In addition, center points were coded as 0 and used to estimate pure error. Four important factors were investigated and considered as independent variables are; MeOH/RPO molar ratio (X_1), catalyst content (X_2), time (X_3) and temperature (X_4). The experimental limit and coded levels of independent factors are shown in Table 2. A list of 30 experiments including 2^4 factorial runs, 8 runs for axial points and 6 runs for center points were carried out. The second order polynomial regression model equation is expressed as modeling of the ester content as follows:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2 \qquad (1)$$

where, Y is the predicted ester content (response) for the first step transesterification; β_0, β_i, β_{ii}, β_{ij} are the regression coefficients (β_0 is referred to as the constant term, β_i is a linear term, β_{ii} is a quadratic term and β_{ij} is an interaction term); X_i, X_j are coded independent factors.

2.5. Catalyst and soap analysis for the first step transesterification process

After the completion of reaction, samples of mixture were taken to determine the catalyst and soap content by acid-base titration method (AOCS Cc17–79). If both catalyst and soap traces present in sample, it is recommended to determine the catalyst content in a first titration step, using HCl 0.1 N solution as reactant, isopropanol as solvent and phenolphthalein as indicator. In a second titration step, the soap content should be determined by titration with HCl 0.1 N, using bromophenol blue as indicator.

Table 2
Limit and coded levels of independent factors for the first step transesterification.

Factor			Limit and coded level				
Independent variable	Symbol	Dimension	–α	–1	0	+ 1	+ α
Molar ratio	X_1	mol/mol	5.00	5.25	5.50	5.75	6.00
Catalyst content	X_2	wt%	0.30	0.40	0.50	0.60	0.70
Time	X_3	min	20	30	40	50	60
Temperature	X_4	°C	45	50	55	60	65

2.6. Ester content analysis of the biodiesel using gas chromatography

In order to test the methyl ester content, standard method on B-100 biodiesel was followed as specified by the Department of Energy Business, Ministry of Energy, Thailand. This method is based on EN 14103 standard by the European Standard (EN) and was carried out at Scientific Equipment Center, Prince of Songkla University, Thailand. The methyl esters were quantified directly in Gas Chromatography (GC) equipped with flame ionization detector (GC-FID) and column selected for biodiesel (length 30 m, 0.32 mm I.D., film thickness 0.25 μm) with helium as carrier gas at flow rate of 1.0 ml/min and split ratio of 50:1. The inlet temperature was kept at 290 °C and the initial temperature was hold at 210 °C (for 12 min) followed by ramping at rate of 20 °C/min till 250 °C, hold for 8 min. The detector temperature was kept at 300 °C and the injection volume of 1 μl was used for analysis. Methyl heptadecanoate was used as the standard for GC-FID. FAME content, C_{FAME} (%) was calculated from integration results for a particular determination from Eq. (2), and the average FAME content from duplicate determinations was recorded.

$$C_{FAME} = \frac{(\sum A) - A_{EI}}{A_{EI}} \times \frac{C_{EI} \times V_{EI}}{m} \times 100\% \qquad (2)$$

where, ΣA is the sum of all methyl ester peaks from C8 to C24:1, A_{EI} is peak area for methyl heptadecanoate (internal standard), C_{EI} is concentration (mg/ml) of the methyl heptadecanoate solution (10 mg/ml), V_{EI} is volume (ml) of the methyl heptadecanoate solution used (5 ml) and 'm' is precise mass (mg) of the FAME sample.

2.7. Determination of the conversion of triglyceride

The conversion of triglyceride was determined by AOCS Official Method Ca 14–56 [18]. According to this method, the conversion of the triglyceride (TG) is defined as:

$$\text{Conversion of TG} = \frac{TG_{\text{in oil}} - TG_{\text{in ester}}}{TG_{\text{in oil}}} \times 100\% \qquad (3)$$

3. Results and discussion

3.1. Ester content suitable for the second step transesterification process using heterogeneous catalyst

As per the previous study about the kinetics of transesterification [21], about 80% conversion was obtained after the first step transesterification in mild reaction condition. The test samples were prepared based on percentages of FAME (80–90%) and RPO (10–20%) to obtain the commercial biodiesel (96.5% ester min.) as well as the economic

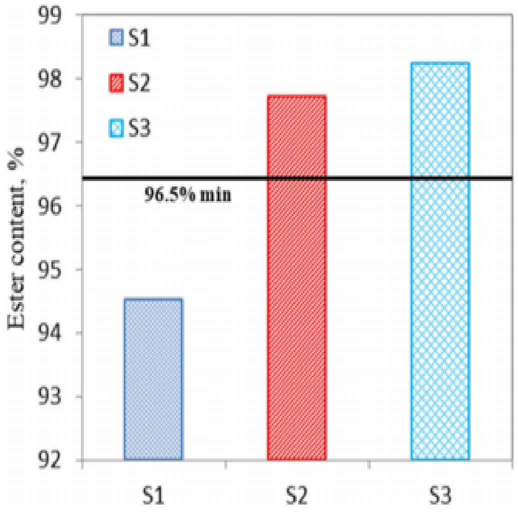

Fig. 2. Experimental results of commercial biodiesel from various feedstock.

efficiency for the second step transesterification. All reactions were carried out at MeOH/Oil molar ratio of 10:1, 9 wt% of Amberlyst 15 to oil at 115 °C in 9 h. The ability to obtain the commercial biodiesel from various feedstock, S1 (80%FAME + 20%RPO), S2 (85%FAME + 15% RPO) and S3 (90%FAME + 10%RPO), in the same condition (MeOH/oil molar ratio: 12/1, Amberlyst 15 content: 9 wt%, reaction time: 9 h, reaction temperature: 115 °C) is shown in Fig. 2. Commercial biodiesel is not obtained by using feedstock oil with 80% ester. This can be explained based on the low catalytic activity of Amberlyst 15. However, commercial biodiesel can be produced from the Amberlyst 15-catalyzed second step transesterification by using feedstock oil with ester content from 85% to 90%. Processing of feedstock oil with 85% ester content is more challenging than feedstock containing higher ester content. Thus it was chosen to study the Amberlyst 15-catalyzed second step transesterification process to ensure the efficiency and economy of the biodiesel production.

3.2. *Effects of reaction variables on ester content in the second step transesterification process*

3.2.1. *Effect of reaction time*

In order to investigate the effect of reaction time on the ester content and conversion of triglyceride, the second step transesterification processes were carried out at 115 °C for 3, 6, 9 and 12 h with 12 wt% of Amberlyst 15 to oil and a MeOH/Oil molar ratio of 10. Longer reaction time is required in order to guarantee the conversion of triglyceride due to medium catalytic activity of Amberlyst 15. The conversion of triglyceride is risen up to about 90% by promoting the reaction time from 3 h to 9 h as shown in Fig. 3, contributes in increasing ester content up to 98% in biodiesel. However, the incremental rate of the ester content and conversion of triglyceride reaches stagnation with further increase of reaction time (up to 12 h), suggests that the reaction still continues but at very slow rate.

3.2.2. *Effect of catalyst content*

The effects of catalyst content (4, 8, 12 and 16 wt% of Amberlyst 15 to Oil) on the ester content and the conversion of triglyceride are investigated with MeOH/Oil molar ratio, reaction time and reaction temperature at 10:1, 115 °C and 9 h, respectively. The conversion (of triglyceride) and ester content increase with the increase of catalyst content as shown in Fig. 4. The significant increase in the conversion of triglyceride and ester content between 4 wt% and 12 wt% catalyst content is associated with the increase of the number of acid sites on the surface of the Amberlyst 15 catalyst. However, further increase in Amberlyst 15 content above 12 wt% does not noticeable increase the ester content as well as the conversion of triglyceride. The small concentration of glycerides (< 3.0 wt%) may be the cause of a low reaction rate as same as the effect of reaction time above 9 h.

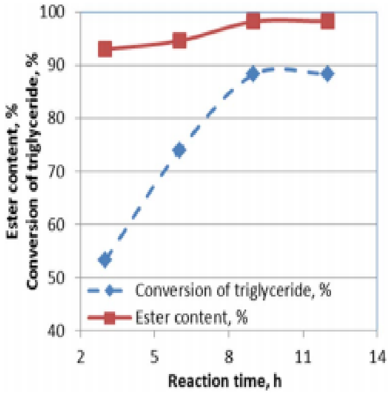

Fig. 3. Effects of reaction time on ester content and conversion of triglyceride.

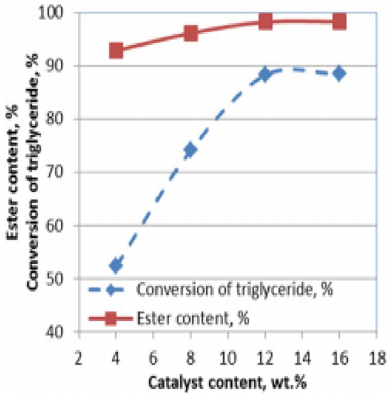

Fig. 4. Effects of Amberlyst 15 catalyst content on ester content and conversion of triglyceride.

3.2.3. *Effect of methanol/oil molar ratio*

The amount of methanol needed for transesterification was calculated based on molar ratio with respect to triglyceride (in oil). The stoichiometry ratio for this reaction requires 3 mol of methanol per 1 mol of triglyceride to produce 3 mol of esters and 1 mol of glycerol. However, the reaction rate on heterogeneous catalyst is a sequence of elementary reactions, such as the rate of glycerides reacting with active site on catalyst to form reaction intermediates and a later step of contacting to alcohol. The overall reaction rate is determined by the rate of the rate-limiting step. High methanol/oil molar ratio enhances the later step of alcohol-intermediates but gives a lower concentration of glycerides (mole/volume) that makes disadvantages the prior step. Hence, excess quantity of methanol is required to drive the reaction rate, but the optimum amount should be investigated without exaggeration.

The experiments were carried out with the MeOH/Oil molar ratio of 8:1, 10:1 and 12:1. These experiments were performed at 115 °C temperature with catalyst content of 12 wt% and a reaction time of 9 h. As shown clearly in Fig. 5, the conversion of triglycerides paces significantly as MeOH/Oil molar ratio changed from 8:1 to 10:1 and slows down smoothly as molar ratio further changed from 10:1 to 12:1. Highest conversion of triglyceride and ester content of 88% and 98% obtained at the MeOH/Oil molar ratio of 10:1 which are higher than the study done by Boz et al. [18].

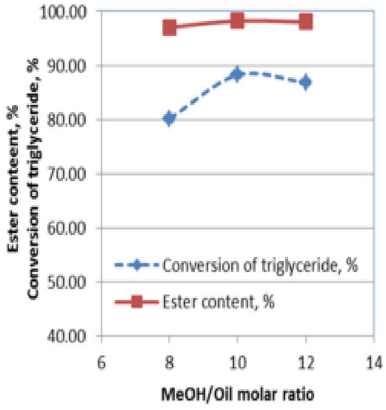

Fig. 5. Effects of methanol/oil molar ratio on ester content and conversion of triglyceride.

The reason can be explained via the good solubility of MeOH in the presence of methyl esters and intermediate compounds (mono- and diglycerides) on the surface of Amberlyst 15 catalyst. This good solubility enhances the contact between triglycerides with MeOH to Amberlyst 15.

Experimental results show that the high quality methyl ester (98%) is obtained from the feedstock oil (85% ester) in the second step transesterification by using high pressure apparatus. The suitable conditions include the molar ratio of MeOH to oil of 10:1; Amberlyst 15 catalyst content of 12 wt%, reaction time of 9 h and reaction temperature of 115 °C. Therefore, first step transesterification using homogeneous base catalyst with the target 85% of ester content is recommended from this study.

3.3. RSM modeling for the first step transesterification

The number of experiments, conditions, results and predicted values for the first step transesterification is shown in Table 3. Results show that the ester content obtained from this stage is affected by four independent variables.

Analysis of variance (ANOVA) is summarized in Table 4. The fit of the designed model is checked from F–value, P–value, lack of fit error (LOF), R^2, adjusted R^2 and R^2 for prediction [22–24]. The model's F-value of 273.92 and the very low *P*-value (< 0.0001) imply that the corresponding model is significant at 95% confidence level. The LOF of 0.3943 (much larger 0.05) indicates that it is insignificant relative to the pure error [22]. Insignificant LOF is good for the predicted model. In addition, in the evaluation of the significance of suggested model, large differences between R^2, adjusted R^2 and predicted R^2 demonstrate the insignificance of the model [23–24]. From the data in Table 4, these coefficients are very high and close (0.9961, 0.9925 and 0.9821, respectively) to prove the significance of the model.

As shown in Table 4, each term is also tested in order to evaluate how well the significance and its interaction to the ester content are. *P*-value < 0.05 implies that the relevant model term is significant. In this case, with considering the linear terms, quadratic and interaction terms, most of terms are significant. However, X_1X_3 and X_2X_3 (interaction terms between reaction time with molar ratio and catalyst content, respectively) are insignificant. The adjusted regression model based on the coded factors and by eliminating the insignificant parameters that have a *P*-value higher than 0.05 is shown in Eq. (4).

$$\begin{aligned} Y = &-331.56 + 116.51X_1 + 149.21X_2 + 0.588X_3 + 1.113X_4 - 10.76X_1^2 \\ &- 42.35X_2^2 - 0.00969X_3^2 - 0.015964X_4^2 - 7.45X_1X_2 + 0.159X_1X_4 \\ &- 0.64X_2X_4 + 0.00592X_3X_4 \end{aligned} \tag{4}$$

The predicted values of response (ester content) are determined by

Table 3
The designed independent factors and experimental results.

Run no.	Independent variables				Ester content (%)		
	X_1 (mol/mol)	X_2 (wt%)	X_3 (min)	X_4 (°C)	Experiment	Prediction	Residual
1	5.25	0.40	30	50	83.31	83.42	− 0.11
2	5.75	0.40	30	50	85.18	85.13	0.05
3	5.25	0.60	30	50	90.68	90.49	0.19
4	5.75	0.60	30	50	91.81	91.44	0.37
5	5.25	0.40	50	50	86.03	85.65	0.38
6	5.75	0.40	50	50	87.06	87.11	− 0.05
7	5.25	0.60	50	50	92.85	92.89	− 0.04
8	5.75	0.60	50	50	93.45	93.59	− 0.14
9	5.25	0.40	30	60	84.73	84.58	0.15
10	5.75	0.40	30	60	87.14	87.08	0.06
11	5.25	0.60	30	60	90.43	90.36	0.07
12	5.75	0.60	30	60	91.74	92.12	− 0.38
13	5.25	0.40	50	60	87.65	87.99	− 0.34
14	5.75	0.40	50	60	90.05	90.24	− 0.19
15	5.25	0.60	50	60	93.90	93.95	− 0.05
16	5.75	0.60	50	60	95.59	95.45	0.14
17	5.00	0.50	40	55	87.53	87.64	− 0.11
18	6.00	0.50	40	55	90.93	90.85	0.08
19	5.50	0.30	40	55	84.14	84.10	0.04
20	5.50	0.70	40	55	96.31	96.37	− 0.06
21	5.50	0.50	20	55	85.08	85.27	− 0.19
22	5.50	0.50	60	55	91.01	90.84	0.17
23	5.50	0.50	40	45	88.52	88.83	− 0.31
24	5.50	0.50	40	65	92.13	91.84	0.29
25	5.50	0.50	40	55	92.01	91.93	0.08
26	5.50	0.50	40	55	91.46	91.93	− 0.47
27	5.50	0.50	40	55	92.18	91.93	0.25
28	5.50	0.50	40	55	92.07	91.93	0.14
29	5.50	0.50	40	55	92.10	91.93	0.17
30	5.50	0.50	40	55	91.77	91.93	− 0.16

Table 4
ANOVA results for the adjusted regression model.

Source/term	Degree of freedom (DF)	Sum of squares (SS)	Mean square (MS)	F-value	P-value	Remark
Model	14	341.75	24.41	273.92	< 0.0001	Significant
Linear	4	301.53	75.38	843.83	< 0.0001	Significant
X_1	1	15.42	15.42	173.08	< 0.0001	Significant
X_2	1	225.95	225.95	2535.45	< 0.0001	Significant
X_3	1	46.54	46.54	522.20	< 0.0001	Significant
X_4	1	13.62	13.62	152.84	< 0.0001	Significant
Square	4	47.41	11.85	132.68	< 0.0001	Significant
X_1^2	1	12.40	12.40	139.11	< 0.0001	Significant
X_2^2	1	4.92	4.92	55.21	< 0.0001	Significant
X_3^2	1	25.73	25.73	288.72	< 0.0001	Significant
X_4^2	1	4.36	4.36	48.89	< 0.0001	Significant
2-Way interaction	6	4.324	0.72	8.07	< 0.0001	Significant
X_1X_2	1	0.56	0.56	6.23	0.0247	Significant
X_1X_3	1	0.063	0.063	0.70	0.4155	Not significant
X_1X_4	1	0.63	0.63	7.09	0.0177	Significant
X_2X_3	1	0.031	0.031	0.34	0.5664	Not significant
X_2X_4	1	1.64	1.64	18.38	0.0006	Significant
X_3X_4	1	1.40	1.40	15.76	0.0012	Significant
Residual	15	1.34	0.0893			
Lack of Fit (LOF)	10	0.97	0.097	1.34	0.3943	Not significant
Pure error	5	0.36	0.072			
Total	29	343.09				

$R^2 = 0.9961$, adjusted $R^2 = 0.9925$, R^2 for prediction = 0.9821.

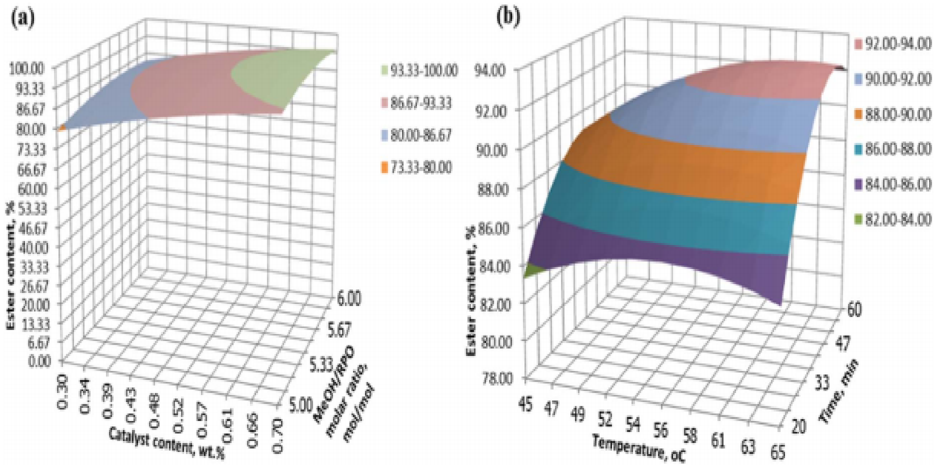

Fig. 6. Response surface plots for interaction effects of; (a) MeOH/RPO molar ratio and catalyst content (time: 40 min and temperature: 55 °C); (b) time and reaction temperature (MeOH/RPO molar ratio: 5.5 and catalyst content: 0.50 wt%).

Table 5
Catalyst and soap content in biodiesel phase (BP) and glycerol phase (GP) after reaction for one step reaction and this present two-step reaction.

Reaction		Catalyst remains (mol/100 mol RPO)		Soap (mol/100 mol RPO)		Σ(cat. + soap) (mol/100 mol RPO)	Soap content, (wt% to RPO)
		BP	GP	BP	GP		
One step (98% ester)		0.00	7.89	1.86	7.46	17.21	3.21
Two- step	1st step (85% ester)	0.00	1.32	1.29	3.02	5.63	1.48
	2nd step (98% ester)	0.00	0.00	0.00	0.00	0.00	0.00

the aforementioned equation (Eq. (4)). The positive sign of the coefficients in regression model indicates a synergistic effect whereas the negative sign represents an antagonistic effect on the ester content [25]. From Eq. (4), it is evident that the constant − 331.56 is independent of any factors or interaction of factors, the linear terms (X_1, X_2, X_3, X_4) and the interaction terms (X_1X_4, X_3X_4) have a positive effect on the ester content. It means increase in these terms will accelerate the ester content. In contrast, the square terms (X_1^2, X_2^2, X_3^2, X_4^2) and other interaction terms (X_1X_2, X_2X_4) have a negative influence which denote that there will be a decrease in ester content with an increase of the magnitude of these parameters.

From the results of ANOVA (Table 4), the catalyst content has a very low *P*-value (< 0.0001) and the highest F-value (2535.45) among other variables. These results reveal that the catalyst content is the most important variable for the first step transesterification. Catalyst content has an active effect on the ester content as already described

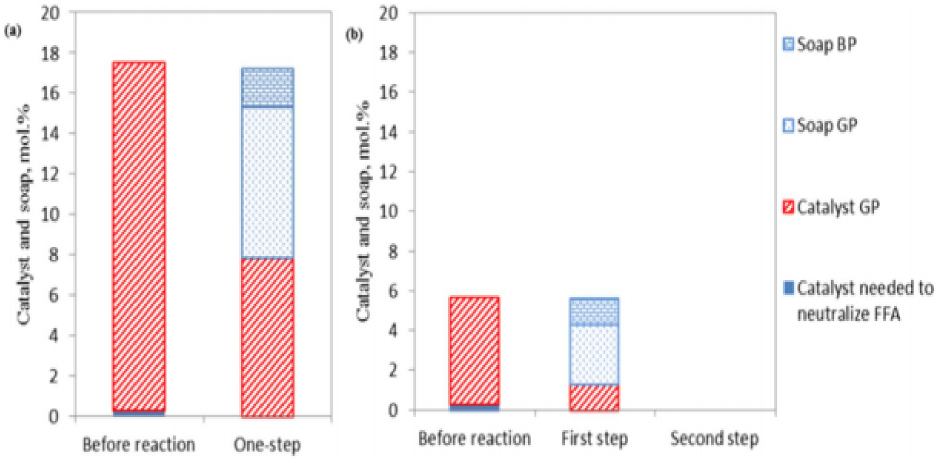

Fig. 7. Distribution of catalyst in the beginning and after reaction/decantation of (a) one-step transesterification and (b) two-step transesterification in this study.

coefficients in Eq. (4). Therefore, the increasing of catalyst content accelerates the speed of the transformation from triglycerides to esters. This conclusion can be seen in some previous studies [22,26,27].

3.3.1. Interaction effects of the parameters

The effect of the MeOH/RPO molar ratio and catalyst content is investigated with keeping the time and reaction temperature at the medium values, 40 min and 55 °C, respectively. This influence is shown by response surface plots in Fig. 6a. The slope of the contour decides the degree of the interaction of process factors to the ester content. Higher the slope greater the influence can be seen. However, the ester content significantly increased with an increment in the amount of the MeOH/RPO molar ratio at any levels of the catalyst content (from 0.3 to 0.7 wt %). This result is different in comparison with some previous studies [22,28] due to the range of the alcohol/oil molar ratio. Excess alcohol can drive the forward reaction and produces more biodiesel at higher level of molar ratio (9:1–15:1). However, the higher amount of alcohol also makes a good solubility of alcohol in the presence of esters and intermediate compounds (mono- and di-glycerides). Thus, the importance of alcohol solubility in oil is diminished. In the present study, by investigating a suitable molar ratio (5:1–6:1) and applying RSM, it is concluded that the ester content increases with an increase of catalyst content and molar ratio. The similar explanation can also be seen in previous studies [29,30].

The effect of time and reaction temperature on the ester content is clearly shown with the constant value of molar ratio and catalyst content, 5.50 by mole and 0.50 wt%, respectively in Fig. 6b. Ester content increases with the progress of reaction and reaches to top and remains at the high ester content for maximum time and reaction temperature, 60 min and 65 °C, respectively. However, based on a higher slope of reaction time in comparison with reaction temperature, the response surface plots shows that the reaction time has more significant influence on the ester content than the reaction temperature as demonstrated by Avramović et al. [29].

3.3.2. Optimization of process factors for the first step transesterification

The aim of the first step is to prepare a suitable feedstock for the second step transesterification. Based on experimental results, ester content in feedstock oil changed from 80% to 95% because of that only reaction condition can be obtained. 85% of ester content is an appropriate target of the first step transesterification process to achieve commercial biodiesel production (96.5% min of ester content) by using Amberlyst 15 in the second step.

Numerical optimization was performed by ER software to determine the optimum conditions for the first step transesterification process. The optimum condition to gain 85% of ester content are: MeOH/RPO molar ratio of 5.48, catalyst content of 0.32 wt%, reaction time of 40 min and reaction temperature of 55 °C.

3.4. Evaluation of decreasing of soap content

The most remarkable fact of this study is to reduce the soap content in biodiesel in comparison with one-step reaction using only CH_3ONa as homogeneous base catalyst. One-step reaction with the required ester content of 98% was carried out at the MeOH/RPO molar ratio of 7.57, catalyst content of 1.20 wt%, reaction temperature of 55 °C and reaction time of 50 min. Table 5 shows the remaining base catalyst and soap content in biodiesel phase and glycerol phase. The total content of initially catalyst of 17.21 mol% (mol/100 mol of RPO) was distributed in the soap and remaining catalyst when the reaction was carried out in one step for desire ester content of 98%. The soap was also largely concentrated in the glycerol phase with 7.46 mol%. On the other hand, soap was not produced after the second step reaction in this two-step reaction study by using Amberlyst 15 as heterogeneous acid catalyst. The total soap content of 1.48 wt% was only produced from the first step. It is less than two times in comparison with one-step process (3.21 wt%), approximately. This decrease was also significant finding in comparison with other studies [11,30]. The total content of base catalyst was only for the first step and less than three times (approximately) compared with the one-step reaction, 5.63 mol% and 17.21 mol%, respectively.

Fig. 7 also shows the amount of catalyst initially loaded in the system and the distribution of catalyst after reaction and decantation for one-step and two-step transesterification process. The content of catalyst initially indicates the amounts needed to neutralize the FFA and catalyze the reaction. There is an important difference between one-step process and first step in two-step process. In the one-step process, 46% of catalyst initially was remaining after reaction. The high remaining catalyst content leads to produce more soap, complex washing and yield loss. On the other hand, after the first step of two-step transesterification, only 23% of catalyst initially was still in reaction product. This contributes to reduce the soap formation and increase the biodiesel yield in the second step.

4. Conclusions

Following concluding remarks are drawn from two-step transesterification process catalyzed by homogeneous base catalyst in the first step and heterogeneous acid catalyst in the second step:

- A novel two-step transesterification process, using sodium methoxide as homogeneous base catalyst in the first step and Amberlyst 15 as heterogeneous acid catalyst in the second step, was found to be effective for biodiesel production.
- The ester content of 85% after the first step was obtained under optimum condition (5.48 M ratio of MeOH to RPO, catalyst loading of 0.32 wt%, 40 min and 55 °C).
- Final biodiesel production with ester content of 98% was produced from the second step under optimum conditions: MeOH/Oil molar ratio of 10, catalyst content of 12 wt%, 9 h and 115 °C.
- As a remarkable point, application of the present two-step transesterification technology has led to decrease the soap and the total amount of sodium methoxide about two times and three times, respectively, compared to the one step process.
- This study can be set as benchmark in reducing the cost for biodiesel production process, therefore, recommended for industrial scale biodiesel production.
- The deactivation of heterogeneous catalyst Amberlyst 15 via agglomeration of glycerol on the active site should be further investigated to clarify the very slow rate of reaction after 80% of glycerides conversion.

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Appendix C

Journal Paper 3

D.N. Thoai, S. Photaworn, A. Kumar, K. Prasertsit, C. Tongurai. *A Novel Chemical Method for Determining Ester Content in Biodiesel.* Energy Procedia 138 (2017) 536–543. DOI: 10.1016/j.egypro.2017.10.156 (Scopus, CiteScore = 1.16, SJR = 0.467, SNIP = 0.586).

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A Novel Chemical Method for Determining Ester Content in Biodiesel

Dang Nguyen Thoai[a,b,*], Songtham Photaworn[a], Anil Kumar[c], Kulchanat Prasertsit[a], Chakrit Tongurai[a]

[a]*Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla, 90112, Thailand*
[b]*Department of Chemical Engineering, Faculty of Chemistry, Quy Nhon University, Binh Dinh, 820000, Vietnam*
[c]*Department of Mechanical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla, 90112, Thailand*

Abstract

Ester content in the biodiesel plays an important role in evaluating the biodiesel quality. Gas chromatography method has proven as the most significant method for determining the ester content till now. Present study proposed a new testing method for investigating the ester content in biodiesel through the content of total glycerol in biodiesel. As per mechanism of transesterification reaction, one mole glycerol is produced from one mole triglyceride in a large excess of alcohol and catalyst. The remaining content of triglycerides can be proximate calculated from the content of total glycerol and then ester content can be converted proximately by subtracting the content of remaining triglycerides from 100 wt.%. This research work introduces a novel procedure for determining the total glycerol and ester content in biodiesel. This method is applied on several samples, and the results are compared with results of gas chromatography method as per the standard EN 14103 guidelines. The results show that the new method gives results comparable to the result from gas chromatography analysis. There is fair agreement between both methods with coefficient of correlation of 0.98. This methodology uses simple laboratory equipment and can also apply for biodiesel from various feedstock oils.

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Keywords: Biodiesel, Chemical method, Ester content, Total glycerol content.

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* Corresponding author. Tel.: +66-955-659940; +84-903-235324.
E-mail address: dangnguyenthoai@qnu.edu.vn

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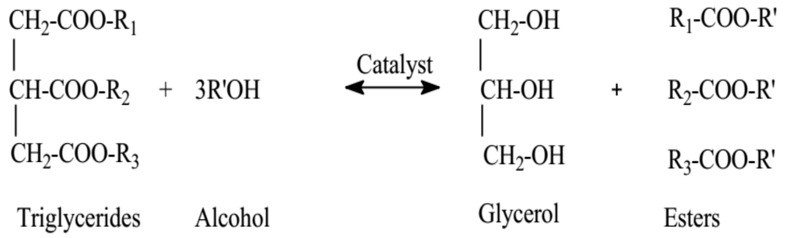
1. Introduction

Recently, biodiesel is well known and widely used as an alternative fuel that is receiving great attention worldwide. Although biodiesel attracts the most attention due to its renewability and biodegradability, it can be also used either pure or to blend with petroleum diesel in the unmodified diesel engine, and it reduces exhaust pollutants [1]. There are many kinds of oils used for biodiesel production, including edible oils (soybean, palm or sunflower oil) and non-edible oils (Jatropha oil, algae or waste oils). These raw oils also relate to the agricultural characteristic of each country and affect to biodiesel quality. The use of very small scale production facilities to produce biodiesel for self-consumption has also spread throughout many countries. Therefore, the quality control of biodiesel is a remarkable concern and must be controlled based on international standards [2].

The quality of biodiesel is specified by the European Standard (EN) and the American Society for Testing and Materials (ASTM). Among the various properties which are examined in order to meet these specifications, total glycerol and ester content are two of the most important standards, since they are related to the remaining amount of intermediate compounds (mono- and di-glycerides) as well as unconverted triglycerides. Wherever the content of total glycerol increases, the ester content in biodiesel reduces. According to ASTM or EN standards, these parameters must be determined by gas chromatography (GC) analysis [3,4].

In determining the total glycerol content, ASTM D6584 and EN 14105, these determinations must be carried out by chromatographic analysis, prepared samples must be fresh and silylated [5-7]. Both methods are suitable only for methyl esters and need to be counseled about quantification problems for ethyl esters. The standard method EN 14103 for determining ester content is also a GC-based method [5,8]. Also as ASTM D6584 and EN 14105, EN 14103 is appropriate for testing the methyl ester content in biodiesel. In short, the GC analysis has a limitation in total glycerol and ester content determining of biodiesel from the numerous feedstock oils. Therefore, the aim of this study is to propose a new chemical method to determine ester content from the content of total glycerol.



$$\begin{array}{lcl} \begin{array}{l} CH_2\text{-}COO\text{-}R_1 \\ | \\ CH\text{-}COO\text{-}R_2 \\ | \\ CH_2\text{-}COO\text{-}R_3 \end{array} + 3R'OH & \xleftrightarrow{\text{Catalyst}} & \begin{array}{l} CH_2\text{-}OH \\ | \\ CH\text{-}OH \\ | \\ CH_2\text{-}OH \end{array} + \begin{array}{l} R_1\text{-}COO\text{-}R' \\ \\ R_2\text{-}COO\text{-}R' \\ \\ R_3\text{-}COO\text{-}R' \end{array} \end{array}$$

Scheme 1 General equation for transesterification of triglycerides.

1. $\text{Triglyceride (TG)} + R'OH \xrightleftharpoons{\text{Catalyst}} \text{Diglyceride (DG)} + R'COOR_1$
2. $\text{Diglyceride (DG)} + R'OH \xrightleftharpoons{\text{Catalyst}} \text{Monoglyceride (MG)} + R'COOR_2$
3. $\text{Monoglyceride (MG)} + R'OH \xrightleftharpoons{\text{Catalyst}} \text{Glycerol (GL)} + R'COOR_3$

Scheme 2 Mechanism of three successive reversible reactions.

The general equation for transesterification of triglycerides is shown in Scheme 1, one mole triglycerides (TG) reacts three moles alcohol to produce three mole esters and one mole glycerol. Actually, this reaction consists of a sequence of three successive reversible reactions, mono-glycerides (MG) and di-glycerides (DG) are two kinds of intermediate compounds (Scheme 2). According to EN 14105 [7], the content of total glycerol (G_T) is calculated as following equation:

$$\%G_T = \%G_F + 0.255(\%MG) + 0.146(\%DG) + 0.103(\%TG) \quad (1)$$

where, $\%G_F$: weight percentage of free glycerol in biodiesel.

However, based on the mechanism of transesterification, one mole glycerol is produced from one mole glyceride in a large excess of alcohol. From this point, the remaining content of triglycerides can be proximate calculated from the content of total glycerol and then ester content can be converted proximately by subtracting the content of remaining triglycerides from 100 wt%. In this present study, the determining process of total glycerol content is followed and developed based on a study of Pisarello et al. [3]. This methodology can apply for any feedstock oils, it is clear and simple for development. The laboratory equipment involved is simple and available in the most of the laboratories and the chemical products involved are common and easy to obtain. The accuracy of this new method is high in comparison with GC-based analysis methods.

2. Materials and Methods

2.1. Fundamentals of determining the total glycerol content in biodiesel

Total glycerol is obtained after all of MG, DG and remaining TG are absolutely transformed into methyl esters and glycerol by transesterification process. After that, glycerol is extracted first with acidified water and then with distilled water. Finally, glycerol is titrated according to standard procedures.

The standard glycerol titration process is based on the oxidation reaction of glycerol by sodium periodate. This reaction leads to the formation of formic acid when more than two hydroxyl groups are present in the molecule. Primary hydroxyl groups react forming formaldehyde upon oxidation and secondary hydroxyl groups lead to formic acid production, according to the following reaction:

$$C_3H_8O_3 + 2NaIO_4 \rightarrow 2HCHO + HCOOH + 2NaIO_3 + H_2O \qquad (2)$$

In order to consume the remains of sodium periodate, ethylene glycol is added when reaction (2) is completed. The reaction is showed in the following reaction:

$$C_2H_4(OH)_2 + NaIO_4 \rightarrow 2HCHO + NaIO_3 + H_2O \qquad (3)$$

2.2. Procedure of total glycerol determination

To ensure the accuracy of the method, all of the existence forms of the glycerides (mono-, di- and triglycerides) must be converted into esters. This is achieved by using a large excess of methanol and catalyst. In this study, because the objective is not the production of biodiesel, using big exorbitancy is not a worrying problem. After the finished reaction, the reaction mixture is complemented by HCl 5 wt% solution. This complementarity not only neutralizes the residue catalyst but also promotes a separating process of glycerol from the ester phase. Also in order to accelerate the recovery of glycerol, two additional washing steps by HCl 2.5 wt% and distilled water are done, respectively. The detailed procedure is shown clearly in Appendix.

2.3. Glycerol titration process

In the previous study of Pisarello et al. [3], analyzing the glycerol content in the aqueous phase was carried out based on a complex procedure in order to the blank experiment was always negligible. However, its demerit is not easy to apply for every lab. In the procedure of this study, the solution is not boiled prior to the titration. Therefore, the blank experiment is more relevant since the carbon dioxide adsorbed from the air during the sample handling is not stripped by boiling the solution, and thus a significant amount of the titrating reactant (NaOH solution) will be used to neutralize the carbonic acid. The detailed process is also seen in Appendix.

2.4. Proximately computation procedure of ester content from the glycerol content

From the titration result, the ester content in the biodiesel was determined using the following relation:

mole of glycerides remains = mole of glycerol; weight of esters = Weight of initially biodiesel – Weight of glycerides remains; Finally, Ester content (%) = (Weight of esters / Weight of initially biodiesel) * 100

2.5. Ester content analysis of the biodiesel using GC

In order to evaluate the accuracy of this method, the fatty acid methyl ester (FAME) content was analyzed following the standard method on B-100 biodiesel specified by the Department of Energy Business, Ministry of

Energy, Thailand [9]. This method is based on EN 14103 standard by the European Standard (EN) and was carried out at Scientific Equipment Center, Prince of Songkla University, Thailand. The methyl esters were quantified directly in GC equipped with flame ionization detector (GC-FID) and column selected for biodiesel (length 30 m, 0.32 mm I.D., film thickness 0.25 mm) with helium as the carrier gas at a flow rate of 1.0 mL/min and split ratio of 50:1. The inlet temperature was kept at 290°C and the initial temperature was hold at 210 °C (in 12 minutes) followed by ramping at a rate of 20°C/min till 250°C, hold for 8 minutes. The detector temperature was kept at 300°C and the injection volume of 1ml was used for analysis. Methyl heptadecanoate was used as the standard for GC-FID. FAME content, C_{FAME} (%) was calculated from integration results for a particular determination according to Eq. (4), and the average FAME content from duplicate determinations was recorded.

$$C_{FAME} = \frac{(S_A - A_{EI})}{A_{EI}} x \frac{(C_{EI} \text{ x } V_{EI})}{m} \text{ x } 100\% \tag{4}$$

where, S_A is the sum of all methyl ester peak areas from C8 to C24:1, A_{EI} is peak area for methyl heptadecanoate (internal standard), C_{EI} is concentration (mg/ml) of the methyl heptadecanoate solution (10 mg/ml), V_{EI} is volume (ml) of the methyl heptadecanoate solution used (5 ml) and ‘m’ is precise mass (mg) of the FAME sample.

3. Results and Discussions

3.1. Results for determining the total glycerol content in biodiesel samples

In order to test the accuracy degree of the procedure of the total glycerol determination, biodiesel (0.19 wt% of total glycerol) from Specialized R&D Center for Alternative Energy from Palm and Oil Crops, Prince of Songkla University is used as a raw feedstock. The pure glycerol (96.63 wt%) was added in biodiesel samples following a random content of glycerol. Test results of total glycerol content were shown in Table 1. The results of analyses by the new chemical method testing and the calculation method were compared each other. As a result, they showed a good relation, the results of total glycerol content tested by the new method, including total glycerol in both of biodiesel and added pure glycerol, are comparable to those of calculation based testing.

Also, to determine the limit of the total glycerol testing method, testing on the samples with the extremely high total glycerol content has been performed. To achieve this task, these simulated samples were prepared based on certain percentages of biodiesel and refined palm oil. Based on the fatty acid composition of RPO in Table 2, the triglycerides content can be proximately calculated as 99.7%. Combined with Eq. (1), the total glycerol content can be proximately calculated as following equation:

$$\%G_T = \frac{(99.7*0.103*\%RPO + 0.19*\%B100)}{100} \tag{5}$$

where $\%G_T$: total glycerol content in simulated sample, %RPO: wt% of RPO in simulated sample, %B100: wt% of biodiesel in simulated sample.

Comparative analysis of results from the new chemical method, and calculation method on the samples made from biodiesel blended with refined palm oil on the different ratio give different error about total glycerol content are shown in Table 3. It is indicated that the difference between two methods is less than 10% for all simulated samples from Sim1 to Sim11 (added RPO from 0% to 100%). The maximum total glycerol content could be determined correctly up to about 10 wt%. It is seen that the limit of this method is larger than the previous study of Pisarello et al. [3], only 5.15 wt% of maximum total glycerol content. That means this new method can be applied for every different samples and without any limit about the total glycerol content to be recorded. However, it is also noteworthy that adding more than 50% of RPO in the simulated sample, the accuracy of this method would not be guaranteed due to the amount of sodium periodate added in the analysis and the weight of aqueous phase used in the titration process. Therefore, in order to warrant this accuracy, 50 ml of sodium periodate solution should be used and weight of aqueous phase should not be larger than 10 g (for more details in Appendix).

Table 1 Comparison results of total glycerol content by the new chemical method and the calculation method.

Sample no.	Sample description	Total glycerol in biodiesel, wt%	Glycerol added, wt%	Total glycerol, wt%		Error, %
				Calculation	New method	
B1	Biodiesel	0.19	0.00	0.19	0.18	5.26
B2	Biodiesel + 0.16 wt% Glycerol	0.19	0.16	0.35	0.33	5.71
B3	Biodiesel + 0.36 wt% Glycerol	0.19	0.36	0.55	0.52	5.45
B4	Biodiesel + 0.52 wt% Glycerol	0.19	0.52	0.71	0.67	5.63
B5	Biodiesel + 0.23 wt% Glycerol	0.19	0.23	0.42	0.41	2.38
B6	Biodiesel + 0.67 wt% Glycerol	0.19	0.67	0.86	0.84	2.33
B7	Biodiesel + 0.40 wt% Glycerol	0.19	0.40	0.59	0.56	5.08
B8	Biodiesel + 0.72 wt% Glycerol	0.19	0.72	0.91	0.89	2.20
B9	Biodiesel + 0.54 wt% Glycerol	0.19	0.54	0.73	0.70	4.11
B10	Biodiesel + 0.44 wt% Glycerol	0.19	0.44	0.63	0.62	1.59

This performance of the new chemical method for very high total glycerol content fulfills completely the initial requirements and the aim of this study.

Table 2 The fatty acid composition of RPO and methyl ester product from GC analysis.

Fatty acid composition	Weight percentage (wt%)	
	RPO	Methyl ester
Lauric acid (C12:0)	0.5	0.36
Myristic acid (C14:0)	1	0.83
Palmitic acid (C16:0)	31.5	36.64
Stearic acid (C18:0)	3	4.19
Oleic acid (C18:1)	49	46.01
Linoleic acid (C18:2)	14	9.45
Linolenic acid (C18:3)	0.3	0.13
Arachidic acid (C20:0)	0.4	0.36
Others (unresolved)	0.3	2.03

Table 3 Total glycerol content in samples analyzed by the new chemical method and the calculation method

Sample no.	Simulated sample description	Total glycerol (G_T), wt.%		Error, %
		New method	Calculation	
Sim1	Biodiesel (100%)	0.18	0.19	5.26
Sim2	Biodiesel (90%) + RPO (10%)	1.14	1.20	5.00
Sim3	Biodiesel (80%) + RPO (20%)	2.09	2.21	5.43
Sim4	Biodiesel (70%) + RPO (30%)	3.03	3.21	5.61
Sim5	Biodiesel (60%) + RPO (40%)	3.91	4.22	7.35
Sim6	Biodiesel (50%) + RPO (50%)	4.81	5.23	8.03
Sim7	Biodiesel (40%) + RPO (60%)	5.92	6.24	5.13
Sim8	Biodiesel (30%) + RPO (70%)	6.99	7.25	3.59
Sim9	Biodiesel (20%) + RPO (80%)	8.74	8.25	5.94
Sim10	Biodiesel (10%) + RPO (90%)	9.76	9.26	5.40
Sim11	RPO (100%)	10.97	10.27	6.82

3.2. Results for determining the ester content in biodiesel samples

In the present study, the performance of two testing methods, including GC analysis and our new method, are studied and compared. As shown in Table 4, these values were quite close together and the difference between two methods was only below 5 %. For most samples, the results from the new method and GC analysis method are comparable. However, as mentioned in previous sections, the new chemical method gives the better precision in case of high-total glycerol-content sample (low-ester content), which is the major advantage of this new method.

Chromatograms of fatty acid components in the sample M1 from the suitable conditions, measuring using GC with heptadecanoic acid (C17:0) methyl ester as an internal standard, are shown in Fig. 1. The GC analysis showed that the main fatty acid components in the sample M1 were lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and arachidic acid (C20:0), with a major distribution by oleic acid. It was found that the methyl ester components were a little different from the composition of RPO as indicated in Table 2. The similar conclusion was also seen clearly in samples from M2 to M5.

Table 4 Comparison results of ester content by the new method and GC analysis method.

Sample no.	Methyl ester content, wt.%		Error, %
	GC analysis	New method	
M1	83.79	85.89	2.51
M2	91.47	94.56	3.38
M3	94.50	98.34	4.06
M4	99.00	99.19	0.19
M5	93.20	96.55	3.59

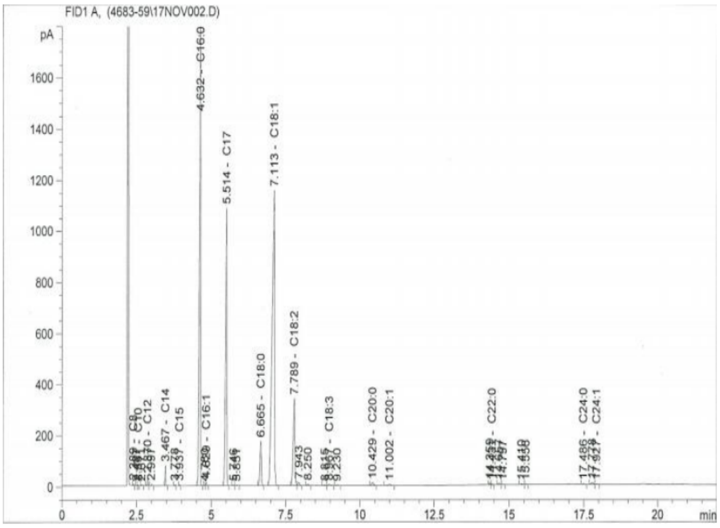


Fig. 1 Chromatogram of methyl ester obtained by GC.

3.3. Comparison between the present method and other analytical methods

A comparison study has been done for methods to determine the ester content between the present chemical method and other analytical methods obtained from the various references is represented in Table 5. It can be clearly seen from the table that the present chemical method is relevant to analytical methods investigated by other researchers. One more good property about this new method is its usage for easily variable feedstock with the quite high accuracy in determining the ester content in biodiesel.

Table 5 The advantages and disadvantages of different methods in determining the ester content in biodiesel.

Method	Advantages	Disadvantages	Reference
GC	- the most widely used method (EN 14103, ASTM 6584)	- apply to methyl esters and not to higher esters (ethyl and isopropyl)	[5,10]
HPLC	- apply to some higher esters (ethyl, isopropyl, 2-butyl and isobutyl esters of soybean oil and tallow	- not popular as GC analysis	[5,11]
TLC/FID	- easy to learn and use - apply for every feedstock	- lower accuracy (largely abandoned, material inconsistencies, sensitivity to humidity, high cost for instrument)	[5,12,13]
NMR (^{1}H NMR and ^{13}C NMR)	- faster and simpler than GC and/or HPLC	- instrumentation and maintenance cost - lower accuracy	[5,12-14]
The chemical method	- quite high accuracy - simple equipment and chemical products	- need to good skill in doing experiments	[Present study]

4. Conclusions

Depending on modern analytical methods leads to some drawbacks for non-funding researchers. The new chemical method applied to determine the ester content is a very significant alternative for the instrumental methods used at the present. The methodology is clear and simple for development, the laboratory equipment involved are also simple and available in the most of the laboratories, the chemical products involved are common and easy to obtain. The accuracy of measurement is quite high in the comparison with the GC analysis method described in EN standard. There is fair agreement between both methods with coefficient of correlation of 0.98. Although a lot of advantages of this novel method have been indicated clearly, the skill of researchers is required in order to ensure the accuracy of the method. Therefore researchers should be more focused on doing the experimental procedure as shown in Appendix.

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Appendix

Procedure for total glycerol determination

1. Use a 500 mL three-necked flask: weigh an exact amount of biodiesel sample between 50 and 100 g.
2. Do transesterification reaction: the reaction should be carried out in a batch reactor with reflux condenser:
    a. put the three-necked flask in an oil bath (60 to 65 °C) on a magnetic stirrer (stirring speed about 800 rpm)
    b. add 1.6 wt% of CH_3ONa and 40 wt.% of CH_3OH (to weighed biodiesel) into the three-necked flask
    c. keep the temperature and the stirring speed during 2 h at 60–65 °C.
3. Extraction of glycerol from biodiesel by washing:
    a. remove the reflux condenser
    b. add a weight of HCL 5 wt%, equal to the amount used in step b of section 2 into the flask, keep the temperature from 60 to 65 °C with strong stirring (800 – 1000 rpm), approximately 20 min
    c. without cooling, be careful in transfer process the whole mixture to the separatory funnel
    d. wait about 5–10 min, separate and collect the aqueous phase into a 250 mL Erlenmeyer flask
    e. return the biodiesel phase into the used flask and repeat the washing using a weight of HCl 2.5 wt%

equivalent to half of that weight in step b

f. wait about 5 min, separate and collect the aqueous phase into the 250 mL Erlenmeyer flask as above

g. repeat step e and f but using distilled water instead of HCl 2.5 wt%

h. rinse the three-necked flask with distilled water (about 20 ml), and add it to the funnel containing the biodiesel phase. Transfer the aqueous phase to the Erlenmeyer flask containing the previous washing phase.

i. discard the biodiesel phase. Rinse the separatory funnel with distilled water (about 20 ml) and collect it in the same Erlenmeyer flask

j. weigh the total amount of aqueous phase exactly (Σm).

4. Analyze the glycerol content in the aqueous phase

a. weight about 10–50 g of aqueous phase (m_1)

b. add 5–6 droplets of bromothymol blue as an indicator. At that time, the colour of the mixture is yellow

c. neutralization by adding NaOH 0.1 mol/L (drop by drop) until the colour turns to blue (using magnetic stirrer for this process, using pH paper to test colour correctly if needed)

d. repeat steps from b to c but using 10–50 g of distilled water (equal weight of the aqueous phase in step a) as a blank sample

e. add 50 ml $NaIO_4$ solution (60g $NaIO_4$, 60 ml H_2SO_4 0.1 mol/L in 1000 mL solution) into both samples (aqueous phase and blank), check and keep it in the dark for 30 min

f. continue to add 10 ml $C_2H_4(OH)_2$ 50% (in H_2O), check and keep it in the dark for 20 min

g. titrate the solution by NaOH 0.125 mol/L (the concentration can be lower than) with bromothymol blue as an indicator (5 – 7 droplets) until the colour changes to blue.

Calculation:

$$\%G_T = \frac{9.209 \times N_{NaOH} \times (V_1 - V_2) \times \sum m}{m_1 \times W}$$

where $\%G_T$: g of glycerol/100 g of biodiesel sample, V_1: volume titrated of NaOH (mL) for aqueous phase, V_2: volume titrated of NaOH (mL) for blank sample, N_{NaOH}: exactly concentration of NaOH solution, Σm: total amount of aqueous phase in step j (section 3), m_1: weight of aqueous phase in step a (section 4), W: weight of biodiesel (g).

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Appendix D

Journal Paper 4

D.N. Thoai, A. Kumar, K. Prasertsit, C. Tongurai. *Evaluation of Biodiesel Production Process by the Determining of the Total Glycerol Content in Biodiesel*. Energy Procedia 138 (2017) 544–551. DOI: 10.1016/j.egypro.2017.10.157 (Scopus, CiteScore = 1.16, SJR = 0.467, SNIP = 0.586).

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Evaluation of Biodiesel Production Process by the Determining of the Total Glycerol Content in Biodiesel

Dang Nguyen Thoai[a,b,*], Anil Kumar[c], Kulchanat Prasertsit[a], Chakrit Tongurai[a]

[a]*Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla, 90112, Thailand*
[b]*Department of Chemical Engineering, Faculty of Chemistry, Quy Nhon University, Binh Dinh, 820000, Vietnam*
[c]*Department of Mechanical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla, 90112, Thailand*

Abstract

Total glycerol content is one of the most important properties among the various properties of biodiesel. It has a close relationship with ester content because both are related to the remaining amount of intermediate compounds (mono- and di-glycerides) as well as the unconverted triglycerides. The total glycerol content increases then the ester content in biodiesel decreases. In this study, the efficiency of the biodiesel production process via the total glycerol content derived from transesterification refined palm oil (RPO) by methanol in the presence of sodium methoxide as a homogeneous base catalyst is estimated. The effects of experimental variables; including methanol/refined palm oil molar ratio (3:1-9:1 by mole), catalyst content (0.50-1.30 wt% CH_3ONa to RPO), reaction temperature (45-65 °C) and reaction time (30-70 min) on the total glycerol content in biodiesel were investigated. A mathematical model was established a correlation based on response surface methodology. It was concluded that the molar ratio and catalyst content are the two most important factors affect the total glycerol content in biodiesel as well as the efficiency of the biodiesel production process.

Keywords: Biodiesel, Refined palm oil, Sodium methoxide, Total glycerol, Ester content.

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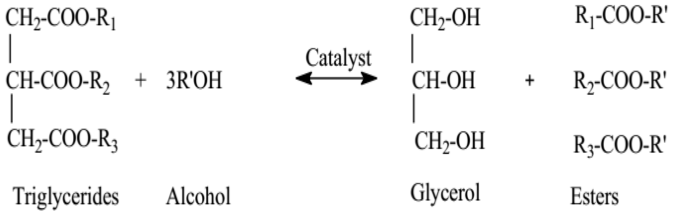
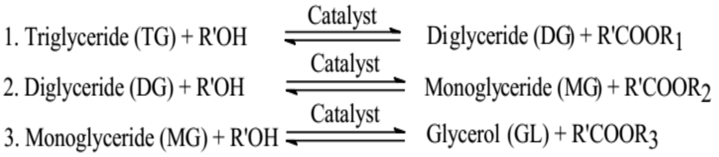
* Corresponding author. Tel.: +66-955-659940; +84-903-235324.
E-mail address: dangnguyenthoai@qnu.edu.vn

1. Introduction

In recently years, the more the world economy increases, the more the requirement about the fuel rises. However, for fossil fuels (coal, petroleum) cause many serious problems for environment, the use of alternative resources such as biofuel is more recommended [1]. Biodiesel, an alternative renewable biofuel, is a mixture of mono alkyl esters of fatty acids which is gained via the transesterification reaction of triglycerides (TG) – a main component in many feedstock oils (edible oil, non-edible oil) – with alcohol (methanol, ethanol) in the presence of a relevant catalyst [2,3]. In comparison with petroleum diesel, biodiesel has many outstanding benefits, including renewability, biodegradability, non-toxic for environment, high safety and acceptable properties for diesel engine [1-3].



Scheme 1 General equation for transesterification of triglycerides.



Scheme 2 Mechanism of three successive reversible reactions.

The general equation for transesterification of triglycerides is shown in Scheme 1, one mole triglycerides (TG) reacts three moles alcohol to produce three mole esters and one mole glycerol. Actually, this reaction consists of a sequence of three successive reversible reactions, mono-glycerides (MG) and di-glycerides (DG) are two kinds of intermediate compounds (Scheme 2). According to EN 14105 [4], the content of total glycerol (GT) is calculated as following equation:

$$\%G_T = \%G_F + 0.255(\%MG) + 0.146(\%DG) + 0.103(\%TG) \tag{1}$$

where, $\%G_F$: weight percentage of free glycerol in biodiesel.

However, based on the mechanism of transesterification, one mole glycerol is produced from one mole glyceride in a large excess of alcohol. From this literature, the remaining content of triglycerides can be proximate calculated from the content of total glycerol and then ester content can be converted proximately by subtracting the content of remaining triglycerides from 100 wt%. Therefore, we can confirm that total glycerol has a close relationship with ester content and both are related to the remaining amount of intermediate compounds (mono- and di-glycerides) as well as unconverted triglycerides. Wherever the content of total glycerol increases, the ester content in biodiesel reduces.

Normally, the quality of biodiesel is specified by the European Standard (EN) and the American Society for Testing and Materials (ASTM). Total glycerol and ester content are the most important properties among the various other properties of the biodiesel which are examined in order to meet standard specifications. As per previous studies efficient evaluation of biodiesel production process is based on ester content [5-9]. Therefore, the aim of this study is to propose a new evaluation method based on total glycerol content.

This investigation has been carried out through transesterification reaction refined palm oil by methanol in the presence of sodium methoxide as a homogeneous base catalyst. Response surface methodology (RSM) has been used in design the experimental work. The main goals of this present study is to analyze the impacts of experimental conditions on the total glycerol content through analysis of variance (ANOVA) and to determine the optimal condition to achieve the minimum total glycerol in biodiesel.

2. Materials and Methods

2.1. Materials

Refined palm oil (RPO) was purchased from Morakot Industry Public Co. Ltd. (Thailand). The homogeneous base catalyst, CH_3ONa (96 wt%) was supplied by Dezhou Long Teng Chemical Co. Ltd. (China). Methanol (CH_3OH, 99.8 wt%) was acquired from Labscan Asia Co. Ltd. (Thailand). Other important chemicals, sodium periodate ($NaIO_4$) was obtained from Fisher Chemical (UK), HCl was from J.T. Baker (USA), NaOH was obtained from Merck (Germany) and bromothymol blue was provided by Ajax Finechem (Australia).

2.2. Methods

2.2.1. The procedure of the transesterification process

The procedure of the transesterification process is a sequence of operations, performed approximately 4h. The transesterification reaction was carried out in a 0.5 L three-necked flask, with magnetic stirring used a magnetic bar and stirring speed of 600 rpm, worked at atmospheric pressure, refluxed by water at 20 ^oC to condense the methanol vapor. RPO with the FFA content about 0.11 wt% was used as a raw feedstock. The feedstock oil was preheated until the temperature reached to the requested limit. And then, the mixture of methanol and catalyst was added. The beginning time for the reaction was counted at the moment all of methanol and catalyst were entered to the reactor. After finish the reaction, the product was transferred to separatory funnel. The settle process was done in 60 min to separate into two phases (methyl ester phase and glycerol phase) and the remaining methyl ester phase was washed by hot water (80 ^oC), for three-three times without and with shaking. The washed methyl ester was dried by the heating in 90 min, at 110 ^oC. Finally, the product was carried out some continuous steps to determine the total glycerol content in biodiesel. This procedure was shown clearly in Fig. 1.

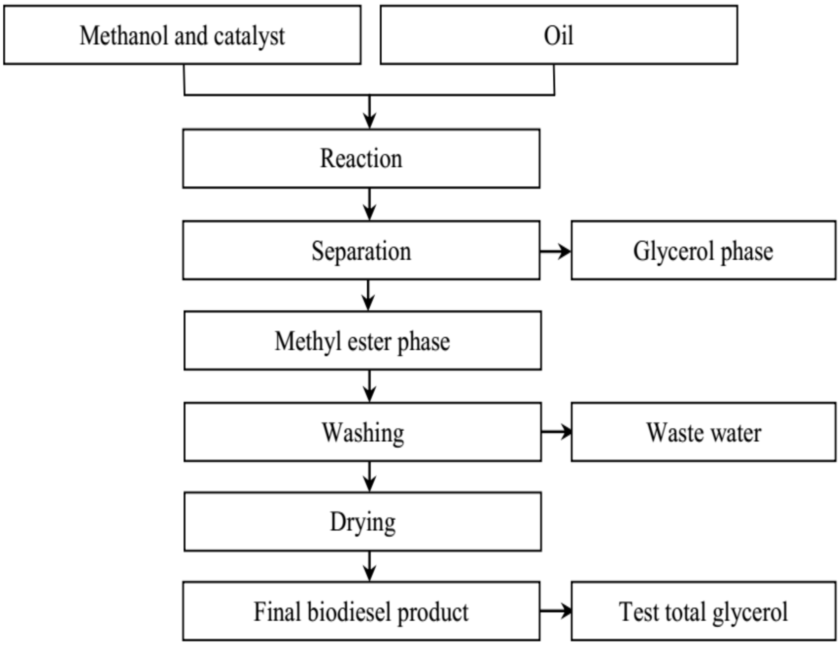


Fig. 1 Experimental flow diagram.

2.2.2. The procedure of total glycerol determination

To ensure the accuracy of the method, all of the existence forms of the glycerides (mono, di- and triglycerides) must be converted into esters. This is achieved using a large excess of methanol and catalyst. In this procedure, because the objective is not the production of biodiesel, using big exorbitancy is not a worrying problem. After the finished reaction, the reaction mixture is complemented by HCl 5 wt% solution. This complementarity not only neutralizes the residue catalyst but also promotes a separating process of glycerol from the ester phase. Also in order to accelerate the recovery of glycerol, two additional washing steps by HCl 2.5 wt% and distilled water are done, respectively. The detailed procedure is clearly shown in our other publication under the title "A Novel Chemical Method for Determining Ester Content in Biodiesel" on the present "Energy Procedia" Journal's volume.

2.2.3. Experimental design based on response surface methodology

Response surface methodology (RSM) is one of the most significant mathematical methods to predict and optimize the biodiesel production. RSM was utilized to design experiments, model and optimize the ester content as a response. This method uses Essential Experimental Design (EED) software in MS Excel [10]. After loading EED, an additional menu option, DOE (Design of Experiment), is become available in the main menu of MS Excel (menu Add-Ins). ER (Essential Regression) software is used for the essential regression (multiple regression and polynomial regression) of experimental data. Additionally, the Design-Expert® software, version 7.0 (Stat-Ease, Minneapolis, USA) was also used to check the accuracy of analyzed experimental data.

A central composite design (CCD) was utilized to determine the effect of experimental factors on the ester content as well as the optimum condition for this study. The CCD incorporates five levels (coded –α, –1, 0, +1, +α) in which factorial points (±1) for all factors, axial points (±α) for a factor and 0 for all other factors. In addition, center points were coded as 0 and used to estimate pure error. The four important factors investigated as independent variables were MeOH/RPO molar ratio (X_1), catalyst content (X_2), temperature (X_3) and time (X_4). The experimental limit and coded levels of independent factors were shown in Table 1. A list of 30 experiments including 16 factorial runs, 8 runs for axial points and 6 runs for center points were carried out. The second order polynomial regression model equation was expressed in modeling the ester content as follows:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2 \tag{2}$$

where, Y is the predicted total glycerol content (response) for this process; β_0, β_i, β_{ii}, β_{ij} are the regression coefficients (β_0 is referred to as the constant term, β_i are linear terms, β_{ii} are quadratic terms and β_{ij} are interaction terms); X_i, X_j are coded independent factors.

Table 1 Limit and coded levels of independent factors for the RSM modeling and optimization.

Factor			Limit and coded level				
Independent variable	Symbol	Dimension	–α	–1	0	+1	+α
Molar ratio	X_1	mol/mol	3.00	4.50	6.00	7.50	9.00
Catalyst content	X_2	wt%	0.50	0.70	0.90	1.10	1.30
Temperature	X_3	°C	45	50	55	60	65
Time	X_4	min	30	40	50	60	70

3. Results and Discussions

3.1. Experimental results

The relationship between the four independent variables (molar ratio, catalyst content, temperature, time) and the total glycerol content is investigated. The total glycerol content for each experimental run, predicted its and residual are listed in Table 2.

The total glycerol content in the final biodiesel product is influenced by four independent variables. The RSM response obtained in Table 2 is relative to these variables using a polynomial regression model equation as Eq. (2). The initial regression model is shown in Eq. (3):

$$Y = 15.36 - 1.818X_1 - 6.971X_2 - 0.109X_3 - 0.04878X_4 + 0.06227X_1^2 + 1.69X_2^2 + 0.000104X_3^2 - 3.6\text{E-}05X_4^2 + 0.265X_1X_2 + 0.00892X_1X_3 + 0.000875X_1X_4 + 0.00437X_2X_3 + 0.01532X_2X_4 + 0.000513X_3X_4 \tag{3}$$

Table 2 The designed independent factors and experimental results.

Run No.	Independent variables				Total glycerol content (%)		
	X_1 (mol/mol)	X_2 (wt%)	X_3 (ºC)	X_4 (min)	Experiment	Prediction	Residual
1	4.50	0.70	50	40	1.79	1.81	-0.02
2	7.50	0.70	50	40	0.60	0.60	0.00
3	4.50	1.10	50	40	1.02	1.05	-0.03
4	7.50	1.10	50	40	0.15	0.15	0.00
5	4.50	0.70	60	40	1.43	1.48	-0.05
6	7.50	0.70	60	40	0.51	0.53	-0.02
7	4.50	1.10	60	40	0.71	0.73	-0.02
8	7.50	1.10	60	40	0.13	0.11	0.02
9	4.50	0.70	50	60	1.55	1.57	-0.02
10	7.50	0.70	50	60	0.40	0.41	-0.01
11	4.50	1.10	50	60	0.92	0.93	-0.01
12	7.50	1.10	50	60	0.14	0.09	0.05
13	4.50	0.70	60	60	1.31	1.34	-0.03
14	7.50	0.70	60	60	0.48	0.45	0.03
15	4.50	1.10	60	60	0.72	0.72	0.00
16	7.50	1.10	60	60	0.13	0.14	-0.01
17	3.00	0.90	55	50	2.08	2.00	0.08
18	9.00	0.90	55	50	0.17	0.22	-0.05
19	6.00	0.50	55	50	1.40	1.35	0.05
20	6.00	1.30	55	50	0.27	0.29	-0.02
21	6.00	0.90	45	50	0.71	0.70	0.01
22	6.00	0.90	65	50	0.44	0.42	0.02
23	6.00	0.90	55	30	0.69	0.64	0.05
24	6.00	0.90	55	70	0.41	0.43	-0.02
25	6.00	0.90	55	50	0.56	0.55	0.01
26	6.00	0.90	55	50	0.51	0.55	-0.04
27	6.00	0.90	55	50	0.52	0.55	-0.03
28	6.00	0.90	55	50	0.57	0.55	0.02
29	6.00	0.90	55	50	0.60	0.55	0.05
30	6.00	0.90	55	50	0.54	0.55	-0.01

3.2. Regression model and statistical analysis for the total glycerol content by RSM

The ANOVA assessments of this model indicate that the model is suitable and can describe very well experimental work, as shown in Table 3. The fit of the designed model is checked due to F-value, P-value, lack of fit error (LOF), R^2, adjusted R^2 and R^2 for prediction [6,11,12]. The model's F-value of 266.01 and the very low P-value (<0.0001) indicated that the corresponding model is significant at the 95% confidence level (Table 3). The LOF of 0.1975 (much larger 0.05) implied that LOF is insignificant relative to the pure error [6]. Insignificant LOF is good for the predicted model. Additionally, the large differences between R^2, adjusted R^2 and predicted R^2 also demonstrate the significance of the model [11,12]. These coefficients are very high and close (0.9960, 0.9922 and 0.9801, respectively) to prove the very high significance of the model (Table 3).

Furthermore, the effect of each term in the model is also evaluated to estimate how well the significance and its interaction to the total glycerol content. The highly significant effect of terms is concluded based on the F-value and P-value. A P-value less than 0.05 implies significant effects of those parameters. From Table 3, with approaching the linear, quadratic and interaction terms, the model terms X_1, X_2, X_3, X_4, X_1^2, X_2^2, X_1X_2, X_1X_3, X_2X_4 and X_3X_4 are observed to be statistically significant. However, the model terms X_3^2, X_4^2, X_1X_4 and X_2X_3 are statistically insignificant. Moreover, the linear term X_1 and X_2 (molar ratio and catalyst content) have very low P-value (<0.0001) and very high F-value (2371.72 and 846.41, respectively) among other terms. These data demonstrated that the molar ratio and catalyst content are the two most important factors for this study. Due to the described coefficient in Eq. (3), these factors had a negative effect on the total glycerol in biodiesel. The increasing of molar ratio and catalyst content accelerates the speed of the transformation from glycerides (mono-, di, triglycerides) to esters. Therefore, the content of total glycerol in biodiesel significant reduces.

Table 3 ANOVA results for the adjusted regression model.

Source/ Term	DF	Sum of squares (SS)	Mean square (MS)	F–value	P–value	Remarks
Model	14	7.53261	0.53804	266.01	<0.0001	Significant
Linear	4	6.69332	1.67333	827.29	<0.0001	Significant
X_1	1	4.79720	4.79720	2371.72	<0.0001	Significant
X_2	1	1.71200	1.71200	846.41	<0.0001	Significant
X_3	1	0.11900	0.11900	58.83	<0.0001	Significant
X_4	1	0.06510	0.06510	32.19	<0.0001	Significant
Square	4	0.63835	0.15959	78.88	<0.0001	Significant
X_1^2	1	0.50721	0.50721	250.76	<0.0001	Significant
X_2^2	1	0.13050	0.13050	64.52	<0.0001	Significant
X_3^2	1	0.00027	0.00027	0.13	0.7659	Not significant
X_4^2	1	0.00036	0.00036	0.18	0.6772	Not significant
2-Way interaction	6	0.20094	0.03349	16.56	<0.0001	Significant
X_1X_2	1	0.10081	0.10081	49.84	<0.0001	Significant
X_1X_3	1	0.07156	0.07156	35.38	<0.0001	Significant
X_1X_4	1	0.00276	0.00276	1.36	0.2613	Not significant
X_2X_3	1	0.00031	0.00031	0.15	0.7027	Not significant
X_2X_4	1	0.01501	0.01501	7.42	0.0157	Significant
X_3X_4	1	0.01051	0.01051	5.20	0.0377	Significant
Residual	15	0.03034	0.00202			
Lack of Fit (LOF)	10	0.02474	0.00247	2.21	0.1975	Not significant
Pure Error	5	0.00560				
Total	29	7.56295				
$R^2 = 0.9960$, adjusted $R^2 = 0.9922$, R^2 for prediction = 0.9801						

Based on the coded factors, ANOVA data and by eliminating the insignificant model terms, the final simplified model is given in Eq. (4):

$$Y = 14.66 - 1.775X_1 - 6.737X_2 - 0.09321X_3 - 0.04718X_4 + 0.06233X_1^2 + 1.693X_2^2 + 0.265X_1X_2 + 0.00892X_1X_3 + 0.01531X_2X_4 + 0.000512X_3X_4 \qquad (4)$$

The predicted values of the response (total glycerol content) are determined by the aforementioned equation (Eq. 4). The positive sign of the coefficients in regression model indicated a synergistic effect whereas the negative sign represents an antagonistic effect on the total glycerol content [13]. From Eq. (4), it is evident that the constant 14.66 is independent of any factors or interaction of the factors, the linear terms (X_1, X_2, X_3, X_4) have a negative effect on the total glycerol content. It means an increase in these terms will decrease the total glycerol content. In contrast, the square terms (X_1^2, X_2^2) and other interaction terms (X_1X_2, X_1X_3, X_2X_4, X_3X_4) have a positive influence which denoted that there would be an increase in the total glycerol content with an increase of the magnitude of these parameters.

3.3. Influence of molar ratio and catalyst content

Fig. 2 shows the response surface and contour plots of the total glycerol content for the interaction variable of molar ration (X_1) and catalyst content (X_2) with the medium value of temperature and reaction time, 55 °C and 50 min, respectively. At low levels of molar ratio, the total glycerol content in biodiesel significantly decreases with an increment in the content of catalyst and molar ratio. Based on the slope of contour, it is seen that the catalyst content has higher influence than the molar ratio. However, when molar ratio is kept at high levels (7:1-9:1), the role of molar ratio in decreasing of the total glycerol in biodiesel is nearly trivial. Moreover, the higher amount of methanol makes the biodiesel separation difficult due to the good solubility of methanol in the presence of methyl esters and intermediate compounds (mono- and di-glycerides). The results demonstrate that the catalyst content is more important than molar ratio for biodiesel production. Several studies have got similar results as the present study [5,6,8], therefore validates the finding of this research.

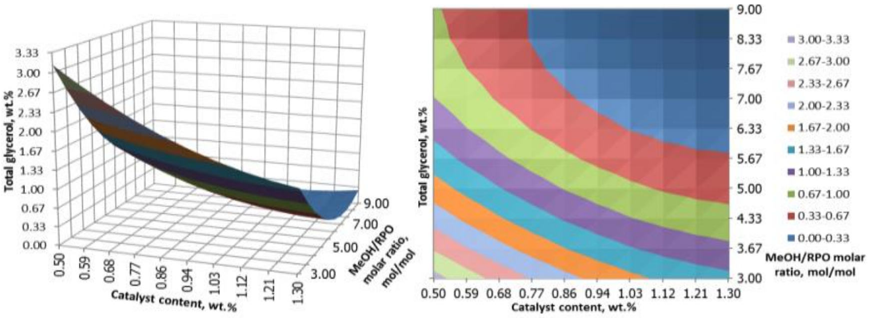


Fig. 2 The effect of molar ratio and catalyst content in the total glycerol content in biodiesel (temperature: 55 °C and reaction time: 50 min).

3.4. Influence of molar ratio and reaction temperature

The effect of molar ratio and reaction temperature on the total glycerol in biodiesel is investigated with the keeping the catalyst content and reaction time at the medium value, 0.90 wt% and 50 min, respectively. Fig. 3 presents the total glycerol content in biodiesel as a function of molar ratio and reaction temperature. With the chosen temperature limit, the total glycerol content initially decreases with the increase of the molar ratio and then keeping an equilibrium stage at higher molar ratios (7:1–9:1). This correlation is similar to the influence of molar ratio and catalyst content.

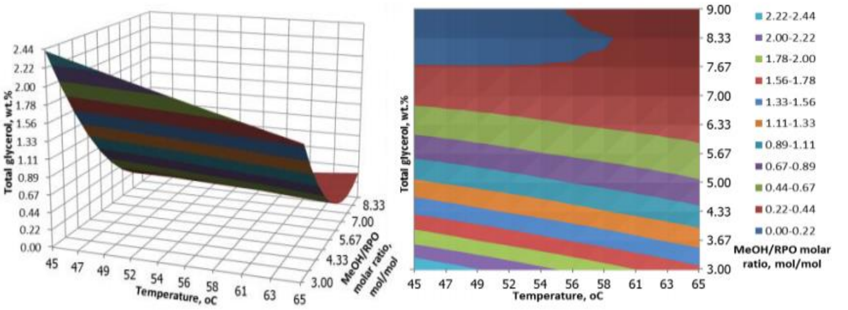


Fig. 3 The effect of molar ratio and reaction temperature on the total glycerol content in biodiesel (catalyst content: 0.90 wt% and reaction time: 50 min).

3.5. Influence of temperature and reaction time

The statistical analysis of the experimental data indicates that temperature (X_3) and time (X_4) are quite important and effective variables in response analysis and they have linear and positive effect on the total glycerol content in biodiesel. This influence is shown by the response surface and contour plots with the constant value of molar ratio and catalyst content, 6 by mole and 0.9 wt%, respectively, as in Fig. 4. By the comparison the difference about the slope of contour, it indicates that the reaction time has a higher influence than the reaction temperature.

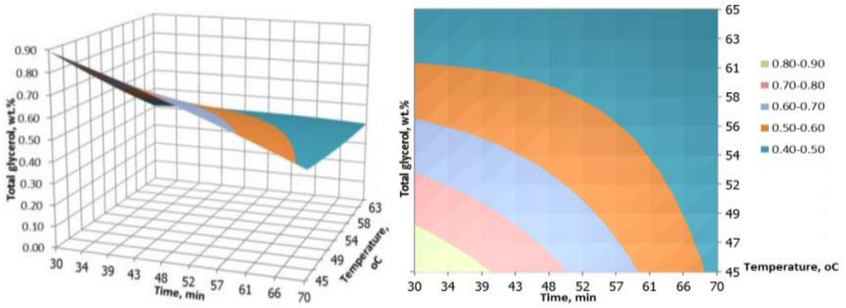


Fig. 4 The effect of temperature and reaction time on the total glycerol content in biodiesel (MeOH/RPO molar ratio: 6 and catalyst content: 0.9 wt%).

3.6. Prediction and confirmation the optimum condition with RSM

Optimization is defined as the process of the finding optimized settings of the regressions in the model to obtain a predefined output or response value [10]. In order to determine the optimum condition for the biodiesel production, numerical optimization is a function in the ER software as well as in the Design-Expert® software, version 7.0 (Stat-Ease, Minneapolis, USA). In order to evaluate the efficiency of the biodiesel production through the total glycerol content, the total glycerol content was influenced by four mentioned factors. Hence, to achieve the maximum desirability, parameters were set in range without a target. The predicted optimal condition for the independent variables are as follows: X_1 = 5.76 by mole, X_2 = 0.88 wt%, X_3 = 55 °C, X_4 = 50 min to achieve the total glycerol content in biodiesel of 0.25 wt% based on EN 14105 standard.

Triplicate experiments were repeated under the predicted optimum conditions in order to check the validity of this prediction. The total glycerol content in biodiesel obtained was 0.26 wt%, approximately. It is close agreement with the value calculated from the model (0.25 wt%).

4. Conclusions

In this study, a novel evaluation method for biodiesel production based on the total glycerol content in biodiesel has been carried out. The methanolysis of RPO in the presence of sodium methoxide is evaluated through the total glycerol content in the final biodiesel product. RSM based on CCD was successful applied to study the effects of the reaction conditions on the total glycerol content. The catalyst content is the most important term among the other variables. RSM model predicts the total glycerol content in biodiesel of 0.25 wt% under the following conditions: MeOH/RPO molar ratio of 5.76 by mole, catalyst content of 0.88 wt%, temperature of 55 °C and reaction time of 50 min. Results of this research will be helpful for further development in energy efficient recovery of biodiesel.

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Appendix E

Conference Paper

D.N. Thoai, C. Tongurai, K. Prasertsit, S. Photaworn. *Optimization of the first step transesterification of refined palm oil using solid sodium methoxide catalyst.* The 23rd Regional Symposium on Chemical Engineering (RSCE2016), Vung Tau, Vietnam, ISBN: 978-604-73-4690-5 (book); 978-604-73-4690-8 (CD disk); Section 3: 137–144. DOI: 10.13140/RG.2.2.17398.19521.

CERTIFICATE OF PARTICIPATION

To

Dang Nguyen Thoai

Has participated in

The 23rd Regional Symposium on Chemical Engineering

As

ORAL PRESENTER

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S03-04

Optimization of the first step transesterification of refined palm oil using solid sodium methoxide catalyst

Dang Nguyen Thoai*, Chakrit Tongurai, Kulchanat Prasertsit, Songtham Photaworn
Department of Chemical Engineering, Faculty of Engineering
Prince of Songkla University
Songkhla 90112, Thailand
*Corresponding author's email: dangnguyenthoai@qnu.edu.vn

Abstract: **The two-step transesterification process in biodiesel production has been studied in the previous studies. However, there has not had any appropriate method to optimize for the first step of transesterification by base homogeneous catalyst. In this novel study, based on response surface methodology (RSM), a mathematical model was established which indicated the impact of independent experimental conditions as well as the relationship between these variables (methanol/refined oil molar ratio, catalyst content, time, temperature) to the methyl esters content. This model was significant by comparison with both experimental value and predicted number of the methyl ester content, and shown strong agreement for all four experimental parameters. The methyl ester content of 80% as the target of this stage was obtained in optimal conditions: methanol/refined oil molar ratio (5.30:1), catalyst content (0.29 wt.% of CH_3ONa to refined palm oil), time (40 min), temperature (57 °C). The results also indicated that catalyst content is the most significant factor for the first step transesterification.**

Keywords: **Biodiesel, two-step transesterification, first step transesterification, sodium methoxide, RSM.**

I. INTRODUCTION

The hotter and hotter development of the world economy requires the higher and higher requirement about the fuel. However, petroleum-based fuel increases greenhouse gas emissions and leads to the environmental hazards and the global warming, the studying and using of nonpetroleum-based fuel as alternative resources are more appreciated [1]. Biodiesel, an alternative renewable fuel, is a mixture of mono alkyl esters of fatty acids which is gained via the transesterification reaction of triglycerides (TG) – main component in many feedstocks (vegetable oils and animal fats) – with methanol, ethanol or other suitable alcohols in the presence of a relevant catalyst [1,2]. In comparison with petroleum diesel, biodiesel has many outstanding advantages, such as renewability, biodegradability, non-toxic for environment, high safety as well as acceptable properties for diesel engine [1–4].

The catalyst plays an important role in transesterification reaction. In both of the recently studies and industry production, the use of base homogenous catalysts has indicated its strong point, especially for feedstock with free fatty acid (FFA) below 2 wt.%, reported to proceed about 4000 times of reaction speed faster than acid homogeneous catalyst [3]. The common base homogeneous catalysts include sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide (CH_3ONa) and potassium methoxide (CH_3OK) [5].

These popular catalysts can catalyze reaction at smooth condition (low temperature, atmospheric pressure) but high conversion can be obtained in a minimum time. In all of these base homogeneous catalysts, CH_3ONa has been demonstrated to be the most effective catalyst [5-7]. Dias et al. [7] reported that the high ester content (97%) could be reached by using 0.6 wt.% of CH_3ONa in a quite slight condition (MeOH/refined oil molar ratio 6:1, reaction temperature 60°C, reaction time 60 min).

However, the most remarkable disadvantage of using base homogeneous catalyst is very easy to occur the saponification reaction, especially for high FFA feedstock [5,8]. The saponification reaction is a significant side-reaction in the transesterification process. This reaction is occurred between FFA and base homogeneous catalyst and consumes the amount of catalyst to produce soaps. That means in order to ensure the stability of transesterification process, a higher amount of catalyst should be added, but this also accretes the saponification reaction. Soap formation inhibits the separation of biodiesel, glycerin and washed water. The soap formation is also the main factor of yield loss.

Moreover, one of the drawbacks of base homogeneous catalyst is the existence of the residual amount of catalyst. If the transesterification process is carried out at conventional conditions (atmospheric pressure, 65 °C of temperature) with the excess of methanol, then it is so difficult to guarantee that alkaline homogeneous catalyst catalyzed the reaction completely. Residual catalyst leads many challenges for catalyst removal technology. Therefore, it increases the production cost and the final product, respectively [9].

In order to overcome the demerits of base homogeneous catalyst, solid acid catalyst was studied and used in experimental scale. One of its merits is able to be insensitive to FFA and water content in the feedstock [5]. This advantage makes acid heterogeneous catalyst to be suitable for various feedstock oils (edible oil, non-edible oil, waste oil) and to be easy to separate residue catalyst due to no soap formation. However, low conversion is the most disadvantage of using this catalyst. Ji et al. [10] shown that in normal experimental condition (MeOH/Oil molar ratio 8:1, 3 wt.% Amberlyst 15 to oil, reaction temperature 60 °C, reaction time 90 min), the conversion was only 25%. In a research later of Nezahat et al. [11], by increasing some experimental conditions ((MeOH/Oil molar ratio 12:1, 3 wt.% Amberlyst 15 to oil, reaction temperature 65 °C, reaction time 9 h), ester content was about 78%. This value of ester content did not respond to the quality of commercial biodiesel seen in standards such as ASTM D-6571 or EN 14214.

The technology of doing two-step reaction in biodiesel production process was shown in some previous studies [8,12,13]. This process can be done by homogeneous catalyst as well as heterogeneous catalyst. The most significant advantage of this technology is to decrease experimental conditions and to gain the efficiency of total transesterification process. According to the study of Noureddini et al. [14] about the kinetics of transesterification of soybean oil, approximately 80% conversion was obtained after transesterification process in conventional experimental conditions. Therefore, 80% of ester content is appropriate target for the first step transesterification by base homogeneous catalyst.

Two-step transesterification via the first step transesterification using homogeneous base catalyst and the second transesterification using heterogeneous catalyst is our interest. In this work, by using RSM as a statistical method, the novel point is to analyze the impact of experimental conditions to the methyl ester content and to determine the optimal condition for the first step transesterification process.

II. MATERIALS AND METHODS

A. Materials

Refined palm oil (RPO) in this project was purchased from Morakot Industry Public Co. Ltd. (Thailand). The base homogeneous catalyst, solid sodium methoxide (CH_3ONa, 96 wt.%), was supplied by Dezhou Long Teng Chemical Co. Ltd. (China) whereas methanol (CH_3OH, 99.8 wt.%) and H_2SO_4 (98 wt.%) were obtained from Labscan Asia Co. Ltd. (Thailand). Other important chemicals, sodium periodate ($NaIO_4$) was acquired from Fisher Chemical (UK), NaOH was obtained from Merck (Germany) and bromothymol blue were provided by Ajax Finechem (Australia).

B. Transesterification reaction procedure and phase separation

Transesterification reaction of RPO with methanol in the presence of CH_3ONa as a base homogeneous catalyst was carried out in a 0.5 L three-necked flask, with magnetic stirring used magnetic bar and stirring speed of 600 rpm, worked at atmospheric pressure, refluxed by water at 20 °C to condense the methanol vapor. RPO with FFA content about 0.1 wt.% (to RPO) was used as a raw feedstock. The feedstock oil was preheated until the temperature reached to the requested value. And then, the mixture of methanol and catalyst was added. The beginning time for the reaction was counted at the moment all of methanol and catalyst have been entered to the reactor. After finished reaction, the reaction product was transferred to separatory funnel and settled within 60 min to separate into two phases, methyl ester phase and glycerol phase. When glycerol phase was removed from the methyl ester phase, the methyl ester phase was washed with hot water, at 70 °C, about three times without stirring and three times with stirring. The washed methyl esters were dried by the heating in 90 min, at 80 °C. The last biodiesel was weighed to determine the methyl esters content.

All the experimental runs were done three times to estimate its errors. Experiments were designed at various conditions such as, methanol/RPO molar ratio (4.8–5.8), catalyst content (0.25–0.50 wt.% of CH_3ONa to RPO), reaction time (20–60 min) and reaction temperature (50–65 °C).

C. Experimental designs

Response surface methodology (RSM) is one of significant methods which more details regression models are used to determine response behavior. This statistical method was utilized for analyzing of the experimental data using EED software in MS Excel [15]. After loading EED, an additional menu option, DOE (Design of Experiment), will become available in the Main Menu of MS Excel (Menu Add-Ins). The Central Composite Design (CCD) was used to find the optimum conditions for the requested methyl esters content. The CCD incorporates five levels (coded $-\alpha$, -1, 0, $+1$, $+\alpha$) in which axial points ($\pm\alpha$) for a factor and 0 for all other factors. In addition, center points were coded as 0 and used to estimate pure error. The most important factors for transesterification including molar ratio (X1), catalyst content (X2), reaction time (X3) and reaction temperature (X4) were chosen as independent variables and the methyl esters content was the dependent variable. The experimental limit and coded levels of independent factors for this study were shown in Table 1. A list of 29 experiments including the 2^4 factorial runs, 8 runs for axial points and 5 runs for center points were carried out.

A smooth interpolating function is usually used, and it generally includes quadratic and interaction effects. The general form of second order polynomial regression model equation is expressed as follows:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2 \qquad (1)$$

where Y is the predicted response (methyl esters content); β_0, β_i, β_{ii}, β_{ij} are the regression coefficients (β_0 is referred to as the constant term, β_i is a linear term, β_{ii} is a quadratic term and β_{ij} is an interaction term); X_i, X_j are coded independent factors.

TABLE 1. LIMIT ANF CODED LEVELS OF INDEPENDENT IN TRANSESTERIFICATION REACTION

Factor			**Limit and coded level**				
Independent variable	**Symbol**	**Dimension**	**–α**	**–1**	**0**	**+1**	**+α**
Molar ratio	X1	mol/mol	4.80	5.05	5.30	5.55	5.80
Catalyst content	X2	wt.%	0.25	0.31	0.38	0.44	0.50
Time	X3	min	20	30	40	50	60
Temperature	X4	°C	50	54	58	61	65

After the completion of the design of experiments and doing 29 experimental runs, ER software is used for the essential regression, including Multiple Regression and Polynomial Regression. In order to use this software, ER is loaded into MS Excel. After that, a new additional menu option, Regress, becomes available in the Main Menu of MS Excel.

D. Approximate analysis of total glyceride and ester content

The ester content determination was carried out according to the Thailand Patty Patent No. 5060 [16] as well as from Pissarello et al. [17]. Tongurai [16] indicates that total glyceride content in biodiesel can be determined from transesterification using microwave radiation technique. Residues glycerides in a biodiesel will react with methanol in the presence of potassium methoxide to produce methyl esters and glycerol. The amount of glycerol can be defined by using a correlation curve. Then the total glyceride content can be converted into ester content by subtracting the value from 100 wt.%. In the other proximate method [17], an exact amount of biodiesel sample will be weighed and the glyceride in biodiesel will react with sodium methoxide solution (in methanol) in a batch reactor. After finished reaction, the amount of glycerin, stand-in of glyceride content, is determined by titrimetric method. Approximate ester content is calculated by subtracting the glyceride content from 100 wt.%.

III. RESULTS AND DISCUSSIONS

A. Regression model and statistical analysis for methyl esters content

It is clear that the methyl ester content obtained from transesterification reaction is affected by four independent variables. In this study, the mathematical relationships between the methyl esters content as a response and four reaction conditions, methanol to RPO molar ratio, catalyst content, time and temperature were estimated. Twenty nine experiments were established and the results were analyzed by using RSM for the methyl esters content as shown in Table 2. By applying multiple regression analysis in Table 2, the full factorial central composite design was used to fit the obtained date to (1). Based on the coded factors and by removing the insignificant model terms, the adjusted regression model was shown in (2):

$$\text{Esters content (\%)} = 19.90 + 184.12X2 - 225.76X2^2 + 0.07437X1X3 - 0.641X2X3 + 1.019X2X4 \qquad (2)$$

The analysis of variance (ANOVA) was summarized in Table 3. The fit of the designed model with the experimental results was tested due to F-value, P-value, R^2 as well as lack of fit (LOF). As shown in Table 3, the F-value (45.40) of suggested model was much higher than F_{crit} (F(0.05,5,23) = 9.87) and the P-value was very low (3.63E-11). These values indicated clearly that the corresponding model was significant at the 95% confidence level. The performance of the model was also based on using the coefficient of multiple determination or correlation coefficient, R^2, which was determined to be 0.908 at 95% of confidence interval. Correlation coefficient, R^2, illustrated that 90.8% of the methyl esters content changing was affected by independent variables including methanol to RPO molar ratio, catalyst content, reaction time and reaction temperature. About 9% of this changing was caused by unidentified factors (random error). In addition, the LOF (0.06436) was higher than 0.05 indicated that the LOF was insignificant relative to the pure error [18]. All things considered this model was quite appropriate to predict the methyl esters content.

As has been shown in Table 3, each term in the adjusted regression model was also checked in order to evaluate how well the significance and its interaction to the methyl esters content. A P-value less than 0.05 implies that the relevant model term is significant. From Table 3, it was noted that after removing insignificant model terms, with approaching the linear, quadratic and combined impacts, linear term of catalyst content (X2), quadratic term of catalyst content ($X2^2$), interaction term between molar ratio and time (X1X3) and interaction terms of catalyst content with time (X2X3) as well as with temperature (X2X4) were significant. Almost these data proved the catalyst content is the most important factor in transesterification process by base homogeneous catalyst. Due to the described coefficients in Eq. (2), catalyst content has an active effect on the methyl esters content. That means the increasing of catalyst content accelerates the speed of the transformation from triglycerides to methyl esters. This conclusion can be seen in some previous studies [19-21]. In the considering for other interaction terms, the combination of catalyst content and temperature (X2X4) was the most significant with the lowest P-value (9.8E-06).

Fig. 1a described the relationship between the predicted values and the experimental values of the methyl esters content. This relationship was nearly linear, that means to be sure about the high significance of the model. One more important to realize the role both R^2 and adjusted R^2 in the evaluation the significance of the suggested model, large differences between R^2 and adjusted R^2 demonstrate the insignificance of the model [22]. From the data in Table 3, this deviation was only 2.25%. Therefore, the suggested model was right description of the process.

TABLE 2. THE CODED INDEPENDENT FACTORS, EXPERIMENTAL RESULTS AND PREDICTED VALUES

Run	Independent variables				Ester content (%)		Residuals
	X1 (mol/mol)	X2 (wt.%)	X3 (min)	X4 (°C)	Experimental value	Predicted value	
1	–1 (5.05)	–1 (0.31)	–1 (30)	–1 (54)	78.35	78.01	0.34
2	+1 (5.55)	–1 (0.31)	–1 (30)	–1 (54)	76.66	77.52	–0.86
3	–1 (5. 05)	+1 (0.44)	–1 (30)	–1 (54)	85.15	85.07	0.08
4	+1 (5. 55)	+1 (0.44)	–1 (30)	–1 (54)	83.88	85.05	–1.17
5	–1 (5. 05)	–1 (0.31)	+1 (50)	–1 (54)	81.21	80.80	0.41
6	+1 (5. 55)	–1 (0.31)	+1 (50)	–1 (54)	82.57	81.92	0.65
7	–1 (5. 05)	+1 (0.44)	+1 (50)	–1 (54)	84.70	86.39	–1.69
8	+1 (5. 55)	+1 (0.44)	+1 (50)	–1 (54)	87.70	87.99	–0.29
9	–1 (5. 05)	–1 (0.31)	–1 (30)	+1 (61)	80.20	80.49	–0.29
10	+1 (5. 55)	–1 (0.31)	–1 (30)	+1 (61)	82.75	81.80	0.95
11	–1 (5. 05)	+1 (0.44)	–1 (30)	+1 (61)	84.95	86.35	–1.40
12	+1 (5. 55)	+1 (0.44)	–1 (30)	+1 (61)	87.15	88.14	–0.99
13	–1 (5. 05)	–1 (0.31)	+1 (50)	+1 (61)	84.11	83.68	0.43
14	+1 (5. 55)	–1 (0.31)	+1 (50)	+1 (61)	85.96	86.61	–0.65
15	–1 (5. 05)	+1 (0.44)	+1 (50)	+1 (61)	88.37	88.08	0.29
16	+1 (5. 55)	+1 (0.44)	+1 (50)	+1 (61)	90.38	91.48	–1.10
17	–α (4.80)	0 (0.38)	0 (40)	0 (58)	84.75	84.49	0.26
18	+α (5.80)	0 (0.38)	0 (40)	0 (58)	88.48	87.41	1.07
19	0 (5.30)	–α (0.25)	0 (40)	0 (58)	74.85	76.00	–1.15
20	0 (5.30)	+α (0.5)	0 (40)	0 (58)	90.41	87.93	2.48
21	0 (5.30)	0 (0.38)	–α (20)	0 (58)	82.76	81.75	1.01
22	0 (5.30)	0 (0.38)	+α (60)	0 (58)	88.19	87.88	0.31
23	0 (5.30)	0 (0.38)	0 (40)	–α (50)	82.24	81.63	0.61
24	0 (5.30)	0 (0.38)	0 (40)	+α (65)	88.32	87.60	0.72
25	0 (5.30)	0 (0.38)	0 (40)	0 (58)	85.07	85.75	–0.68
26	0 (5.30)	0 (0.38)	0 (40)	0 (58)	85.70	85.75	–0.05
27	0 (5.30)	0 (0.38)	0 (40)	0 (58)	85.26	85.75	–0.49
28	0 (5.30)	0 (0.38)	0 (40)	0 (58)	86.42	85.75	0.67
29	0 (5.30)	0 (0.38)	0 (40)	0 (58)	86.31	85.75	0.56

The outlier t plot for all experimental runs was shown in Fig. 1b. The outlier t plot specifies the amplitude of the residuals for each case to determine if any of experimental cases has considerable residuals [23]. Almost the standard residuals should be limited in the interval of ±3.00. As can be seen in Fig. 1b, there were not any value outside the prescribed interval (±3.00). It means that the suggested model is relevant with all the data.

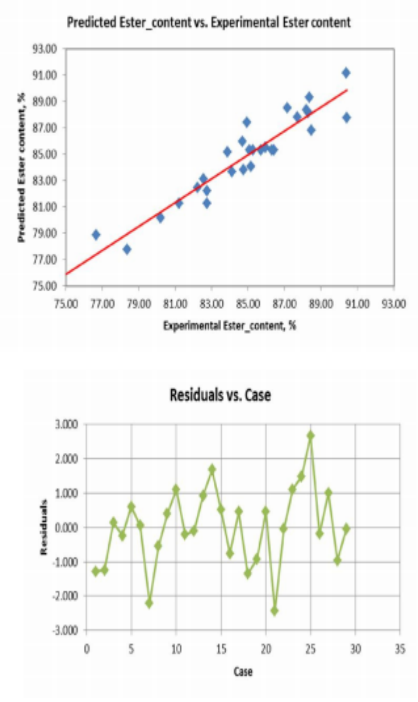

Fig.1 (a) Correlation between experimental and predicted methyl esters content. (b) The outlier t plot for all cases.

TABLE 3. ANOVA RESULTS FOR THE ADJUSTED REGRESSION MODEL

Source/ Term	**Sum of squares (SS)**	**Coefficient**	**Degree of freedom (DF)**	**Mean squares (MS)**	**F value**	**F-Signif/ P value**	**Remarks**
Regression	358.65		5	71.73	45.40	3.63E-11	Significant
β_0		19.90				0.04821	Significant
β_2		184.12	1			0.000896	Significant
β_{22}		–225.76	1			0.00122	Significant
β_{13}		0.07437	1			0.00147	Significant
β_{23}		–0.641	1			0.03945	Significant
β_{24}		1.019	1			9.8E-06	Significant
Residual	36.34		23	1.580	5.0178		
Lack of fit error	34.88		19	1.836		0.06436	Not significant
Pure error	1.46		4	0.366			
Total	394.99		28				
Correlation coefficient: $R^2 = 0.908$ (R^2 adjusted = 0.888)							
$F_{crit} = F(0.05,5,23) = 9.87$							

B. Interaction effects of the factors on methyl esters content

1. Interaction effect of catalyst content and temperature on methyl esters content

The effects of catalyst content and temperature on methyl esters content were investigated with keeping the MeOH/RPO molar ratio and reaction time at the medium values, 5.30 mol/mol and 40 min, respectively. This influence was shown by the response surface and contour plots in Fig. 2. The slope of the contours will decide the degree of the interaction of process factors to methyl esters content. The higher slope of the contour of catalyst content indicated great influence of this variable in comparison with reaction temperature. It was realized that catalyst loading significantly impacted on methyl esters content, especially at the concentration higher than 0.32 wt.%. By using at least 0.32 wt.% of CH_3ONa (to RPO), methyl esters content clearly increased without depending on the experimental temperature limitation (50 – 65 °C). In other words, methyl esters content was enhanced by enlargement catalyst concentration at any temperature range. The same conclusion can be seen in some previous results [19,24]. However, at lower than catalyst loadings (less than 0.32 wt.%), the effect of reaction temperature on methyl esters content was appreciable. The increasing rate of methyl esters content followed the growth limit of reaction temperature, although this effect was slow because of lower slope of the contour.

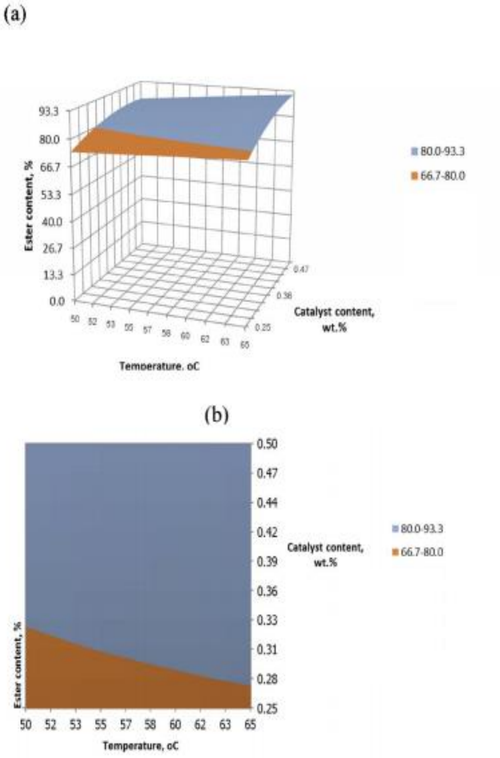

Fig. 2. Response surface and contour plots for interaction effect of catalyst content and reaction temperature (MeOH/RPO molar ratio: 5.3 and reaction time: 40 min).

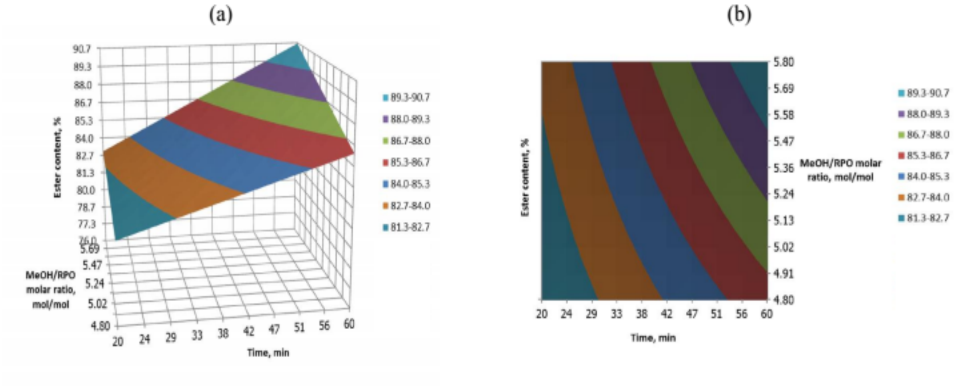

Fig. 3. Response surface and contour plots for interaction effect of molar ratio and reaction time (catalyst content: 0.38 wt.% and reaction temperature: 58 ºC).

2. Interaction effect of molar ratio and reaction time on methyl esters content

In Fig. 3, response surface and contour plots for the interaction variable of molar ratio and reaction time were clearly shown with the constant value of catalyst content and reaction temperature, 0.38 wt.% and 58 ^{o}C, respectively. By comparison the difference about the slope of contour, it was indicated that the reaction time had higher influence than the molar ratio. The methyl esters content increased significantly with increasing reaction time at the same MeOH/RPO molar ratio due to higher slope of the contour. In contrast, the effect of MeOH/RPO molar ratio on methyl esters content was not as much as the reaction time. This can be explained by the good solubility of MeOH in the presence of methyl esters and intermediate compounds (mono_ and di_glycerides). Thus the importance of MeOH solubility ability in RPO is diminished. The similar explanation was seen in some previous results [25,26].

However, with considering the objective of this first stage (80% of esters content), the role of this interaction effect of molar ratio and reaction time on methyl esters content was not as significant as the interaction effect of catalyst content and reaction temperature. As shown in Fig. 3, the methyl esters content was absolutely higher than the target of this stage. One more explanation, from Table 3, interaction term of catalyst content with temperature (X2X4) was much higher than interaction term of molar ratio with time (X1X3), 9.8E-06 and 0.00147, respectively.

C. Optimization of process factors for the first step transesterification process

Optimization is defined as the process of finding optimized settings of the regressions in the model in order to obtain a predefined output or response value [16]. In order to determine the optimum conditions for first stage transesterification process, numerical optimization was performed by the response optimization built in ER software loaded into MS Excel. The optimum conditions to gain 80% of methyl esters content were indicated at the catalyst content of 0.29 wt.%, MeOH/RPO molar ratio of 5.30:1, reaction time of 40 min and reaction temperature of 57 ^{o}C.

IV. CONCLUSIONS

This work has been successful in constituting the feedstock with 80% of methyl esters for next steps in the process of manufacturing commercial biodiesel. This feedstock is very convenient for second step transesterification process by heterogeneous catalyst.

Our study reaffirms the important role of the response surface methodology (RSM) in optimizing the experimental conditions in order to gain the expected content of methyl esters. By the considering the objective of the first step transesterification process, the optimal conditions were indicated, including catalyst content (0.29 wt.%), MeOH/RPO molar ratio (5.30:1), reaction time (40 min) and reaction temperature (57 ^{o}C).

This novel research can be applied for different feedstock oils, such as edible oil, non-edible oil as well as waste cooking oil. Further study is recommended to determine the optimal conditions for the second step transesterification process by heterogeneous catalyst for the higher biodiesel yield and the lower price of commercial biodiesel.

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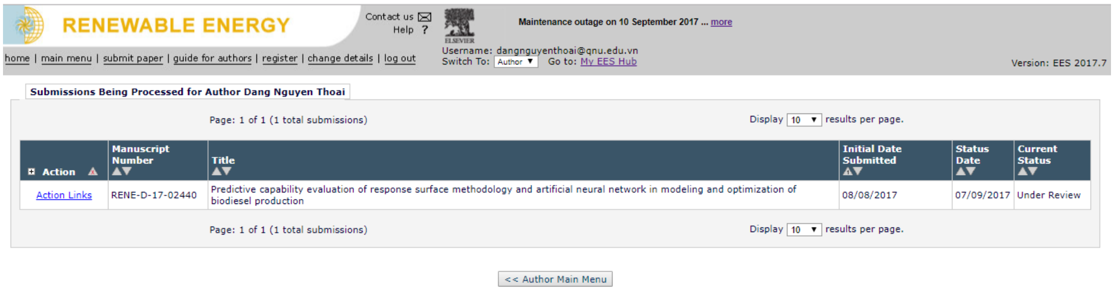
Appendix F

Journal Paper 5

D.N. Thoai, K. Prasertsit, C. Tongurai, A. Kumar. *Predictive capability evaluation of response surface methodology and artificial neural network in modeling and optimization of biodiesel production.* This article is under peer reviewing for publication in Renewable Energy (ISI, SCI Impact Factor: 4.357, Initially Date Submitted: 08/08/2017, Under Review Status Date: 07/09/2017).



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Page: 1 of 1 (1 total submissions)

Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
Action Links	RENE-D-17-02440	Predictive capability evaluation of response surface methodology and artificial neural network in modeling and optimization of biodiesel production	08/08/2017	07/09/2017	Under Review

Page: 1 of 1 (1 total submissions)

Predictive capability evaluation of response surface methodology and artificial neural network in modeling and optimization of biodiesel production

Dang Nguyen Thoai[1,2,*], Chakrit Tongurai[1], Kulchanat Prasertsit[1], Anil Kumar[3,4]

[1]Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla 90112, Thailand
[2]Department of Chemical Engineering, Faculty of Chemistry, Quy Nhon University, Binh Dinh 820000, Vietnam
[3]Department of Mechanical Engineering, Faculty of Engineering, Prince of Songkla University, Songkhla 90112, Thailand
[4]Department of Energy (Energy Center), Maulana Azad National Institute of Technology, Bhopal, India
[*]Corresponding author: dangnguyenthoai@qnu.edu.vn (Dang Nguyen Thoai)

Abstract

In present study, response surface methodology (RSM) and artificial neural network (ANN are applied for biodiesel production via base-catalyzed transesterification. These models are also compared in order to optimize the methyl esters production process from edible oils. Methanol/oil molar ratio (3:1-9:1), sodium methoxide catalyst content (0.50-1.30 wt.%), reaction temperature (45-65°C) and time (30-70 min) were considered during process using Central Composite Design. RSM and ANN models show a high accuracy in terms of coefficient of determination ($R^2 > 0.99$) and mean relative percent deviation (MRPD = 0.22-0.27%). Molar ratio and catalyst content are identified as two most important factors for base-catalyzed methanolysis. A high predicted output of FAME percentage of 98% was determined by the ANN model under optimum conditions; including MeOH/oil molar ratio of 5.88, catalyst content of 0.89 wt.%, reaction temperature of 55°C in 50 min. Therefore, ANN model is a better solution over the RSM model and recommended for optimizing biodiesel production.

Keywords: Biodiesel; Response Surface Methodology; Artificial Neural Network; Transesterification; Sodium methoxide.

1. Introduction

The development of the world economy needs to be depended on energy resources. As per the U.S. Energy Administration report, of 2016, fossil fuels were the biggest source of energy (77.93%), whereas, the nuclear electric power and renewable energy were only 10.01% and 12.06%, respectively [1]. Many environmental complications like global warning, pollution, ozone layer exhaustion are due to fossil fuels ignition [2]. Therefore, the remarkable issue is to produce energy from non-fossil and eco-friendly sources. Biodiesel has proven as a good replacement because of its renewability, biodegradability, non-toxic and high safety [3,4].

The most common method for biodiesel production is vegetable alcoholysis or transesterification [5,6]. In this method, vegetable oil reacts with alcohol in the presence of catalyst and create biodiesel and glycerol [5,7]. Among the catalyzed transesterifcation process, base-catalyzed transesterification has been studied and applied by various researchers due to its high catalytic activity and, high conversion of triglyceride to biodiesel [8,9].

Alcohol/oil molar ratio, catalyst content, time and reaction temperature are factors affect base-catalyzed transesterification process [2,3,8]. Process optimization is an important and remarkable issue and, required to increase the biodiesel production efficiency and to reduce the production cost. The base-catalyzed transesterification process involved in these factors have been surveyed, modeled and optimized by RSM and ANN [8,10-15].

RSM is one of significant statistical methods used in experimental design, modeling and optimization [16,17]. It is relation between one or more responses with independent factors. It determines the effect of independent factors, including single and in interaction, on the whole process. Moreover, this method gives a mathematical relation for predicting the desired output. Thus, biodiesel can be modeled from RSM with minor estimation error in different conditions [6,18]. Several researchers have been used this tool effectively for the efficiency evaluation of biodiesel production from base-catalyzed transesterification. Thoai et al. [8] have applied RSM in the optimization of the first step methanolysis of refined palm oil (RPO) catalyzed by sodium methoxide as homogeneous base catalyst. RSM was also used in based-catalyzed ethanolysis of sunflower oil [12].

ANN is the most popular artificial learning tool with a wide application range. It has been extensively accepted as an alternative technique to represent the complicated input and output relationship of the process [19]. It is able to use for prediction outputs of a new input data, if ring of data are successfully trained, validated and tested by ANN. It has been successfully used for several transesterification processes through based-catalyzed mechanism, including the one-step and two-step process [11-13]. Betiku et al. [11] modeled and optimize the two-step process for biodiesel synthesis from non-edible neem seed oil. The results demonstrated that the model developed from ANN accurately represent the process. In another study, Stamenkovíc et al. showed optimization capability of ANN in base-catalyzed one-step ethanolysis of sunflower oil. RSM and ANN were also considered for developing and comparing their predictive and generalization abilities in the ethanolysis reaction of refined sunflower oil [12].

RSM and ANN have been applied from long time in order to model and optimize the alkyl esters production process from edible oils, however, their results hardly compared. The aim of present study is to combine the central composite design (CCD) with both RSM and ANN performance for palm oil methanolysis process catalyzed by homogeneous base catalyst – sodium methoxide. This might be first effort to study the predictive capability evaluation of RSM and ANN models of the said process.

2. Materials and Methods

2.1. Materials

Refined palm oil (RPO) was bought from Morakot Industry Public Co. Ltd. (Thailand). Methanol (CH_3OH, 99%) and sodium methoxide (CH_3ONa, 96%) was supplied by Labscan Asia Co. Ltd. (Thailand) and Dezhou Long Teng Chemical Co. Ltd. (China), respectively. Sodium hydroxide (NaOH) was obtained from Merck (Germany), while sodium periodate ($NaIO_4$) was acquired from Fisher Chemical (UK) and bromothymol blue was provided by Ajax Finechem (Australia).

2.2. Methods

2.1.1. One-step biodiesel production

The RPO used for this study had the low free fatty acid (FFA) content (0.11%) suitable feedstock oil for one-step biodiesel production. The catalyzed methanolysis reaction was carried out in a 0.5 L three-necked flask, with magnetic stirring of 600 rpm, at atmospheric pressure, and refluxed by water at 20°C to condense the methanol vapor. RPO was preheated until attain the set limit. Later, the mixture of methanol and catalyst was added. The beginning time for the reaction was recorded at the moment all of methanol and catalyst were entered to the reactor. After finish this reaction, the product mixture was poured into separatory funnel to separate into two layers of ester and glycerol. The settling time was around 60 min. Glycerol was taken out of separating funnel, the ester phase was washed by hot water (80°C) for three-three times without and with shaking. The washed methyl ester was dried by the heating at 110°C for 90 min. Finally, the biodiesel product (FAME) was checked for the ester content.

All the experimental runs were performed three times to estimate its errors. Experiments were designed at various conditions; including MeOH/Oil molar ratio of 3/1-9/1, CH_3ONa catalyst content of 0.50-1.30 wt.%, reaction temperature of 45-65°C and reaction time of 30-70 min.

2.1.2. Procedure of the ester content determination in biodiesel

Methyl ester content was analyzed following the standard method on B-100 biodiesel specified by the Department of Energy Business, Ministry of Energy, Thailand [20]. This method is based on European Standard (EN 14103) and was carried out at Scientific Equipment Center, Prince of Songkla University, Thailand. The methyl esters were quantified directly in gas Chromatography (GC) equipped with flame ionization detector (GC-FID). The column selected for biodiesel have length 30 m, 0.32 mm I.D., film thickness 0.25 μm with helium as the carrier gas at a flow rate of 1.0 mL/min and split ratio of 50:1. The inlet temperature was kept at 290°C and the initial temperature was hold at 210°C (for 12 minutes) followed by increasing at a rate of 20°C/min till 250°C, hold for 8 minutes. The detector temperature was kept at 300°C and the injection volume of 1μl was used for analysis. Methyl heptadecanoate was used as the standard for GC-FID. FAME content, C_{FAME} (%) is calculated from integration results for a particular determination according to Eq. (1).

$$C_{FAME} = \frac{(\sum A) - A_{EI}}{A_{EI}} \times \frac{C_{EI} \times V_{EI}}{m} \times 100\% \quad (1)$$

where, ΣA is the sum of all methyl ester peaks from C12 to C24:1, A_{EI} is peak area for methyl heptadecanoate (internal standard), C_{EI} is concentration (mg/ml) of the methyl heptadecanoate solution (10 mg/ml), V_{EI} is volume (ml) of the methyl heptadecanoate solution used (5 ml) and 'm' is exact weight (mg) of the FAME.

2.1.3. Design of experiments

The CCD was applied to investigate the influences of the experimental variables on the FAME content and to find the optimum conditions for the requested FAME content. The CCD incorporates five levels (coded –α, –1, 0, +1, +α) in which axial points (±α) for a factor and 0 for all other factors. In addition, center points coded as 0 were used to estimate pure error. For CCD with 4 independent factors, a list of 30 experiments including 2^4 factorial runs, 8 runs for axial points and 6 runs for center points were carried out. These experimental FAME contents were used in the analysis of variance (ANOVA). The performances of RSM and ANN models were statistical tested by correlation coefficient (R), coefficient of determination (R^2), adjusted R^2, mean square error (MSE), root mean square error (RMSE), mean absolute error (MAE), standard error of prediction (SEP) and mean relative percent deviation (MRPD). These parameters are determined using the Eqs. (2) to (9) [12,21,22]:

$$R = \frac{\sum_{i=1}^{n}(y_{p,i}-y_{p,ave})(y_{a,i}-y_{a,ave})}{\sqrt{\left|\sum_{i=1}^{n}(y_{p,i}-y_{p,ave})^2\right|\left|\sum_{i=1}^{n}(y_{a,i}-y_{a,ave})^2\right|}} \tag{2}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_{a,i}-y_{p,i})^2}{\sum_{i=1}^{n}(y_{p,i}-y_{a,ave})^2} \tag{3}$$

$$Adjusted\, R^2 = 1 - \left[(1 - R^2) \times \frac{n-1}{n-k-1}\right] \tag{4}$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(y_{p,i} - y_{a,i}\right)^2 \tag{5}$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(y_{p,i} - y_{a,i}\right)^2} \tag{6}$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|\left(y_{a,i} - y_{p,i}\right)\right| \tag{7}$$

$$SEP = \frac{RMSE}{y_{a,ave}} \times 100 \tag{8}$$

$$MRPD = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{y_{a,i}-y_{p,i}}{y_{a,i}}\right| \tag{9}$$

where, n is the number of experiments, $y_{p,i}$ is the predicted outputs, $y_{a,i}$ is the experimental results, $y_{a,ave}$ is the average experimental results, $y_{p,ave}$ is the average predicted output and k is the sum of input factors.

2.1.3.1. RSM modeling

The four important factors; molar ratio (X_1), catalyst content (X_2), reaction temperature (X_3) and reaction time (X_4), are investigated as independent variables for modeling and optimization of FAME content (y). Multiple regressions were applied for the second-order polynomial regression model equation in order to find correlation between the response value and the independent variables. Eq. (10) shows the fitted quadratic response model.

$$y = \beta_0 + \sum_{i=1}^{4}\beta_i X_i + \sum_{i=1}^{3}\sum_{j=i+1}^{4}\beta_{ij}X_iX_j + \sum_{i=1}^{4}\beta_{ii}X_i^2 \tag{10}$$

where, y is the predicted response (FAME content); β_0, β_i, β_{ii}, β_{ij} are the regression coefficients (β_0 is referred to as the intercept term, β_i are linear terms, β_{ii} are quadratic terms and β_{ij} are interaction terms); X_i, X_j are coded as independent factors.

The statistical significance of the independent variables, their interactions and the quality of the fitted model are tested via F-value, P-value and ANOVA.

ANOVA is also applied to predict the FAME content following the experimental variances.

Contour plots are formed via the multiple regression equation by keeping two independent terms at an average value and vary other two terms. Model gives the optimum conditions for achieving highest-FAME content from independent experimental factors.

RSM uses Essential Experimental Design (EED) software in MS Excel [23]. After loading EED, an additional menu option, DOE (Design of Experiment), is become available in the main menu of MS Excel (menu Add-Ins). ER (Essential Regression) software is used for multiple regression and polynomial regression of experimental data. Additionally, Minitab software, version 16.2.2 is used to check the accuracy of analyzed experimental data.

2.1.3.2. ANN modeling

A feed forward, back-propagation multi-layer perception (MLP) neural network analysis is carried out through the Levenberg-Marquardt (LM) algorithm for modeling of the process parameters for the base-catalyzed methanolysis reaction. This is implemented using the neural network toolbox of MATLAB 2015a (8.5.0.197613). Training parameters of the ANN are given in Table 1. The MLP network is well known and widely applied feed forward network analysis. The feed forward network is a straight forward network that requires outputs in order to train the model. The ANN operating ability is investigated by MSE. The selected ANN has three layers of neurons such as; an input layer, a hidden layer and an output layer. The hyperbolic tangent sigmoid transfer function (Tansig) and linear transfer function (Purelin) are chosen for input and output layers, respectively. The architecture of the ANN is shown in Fig. 1. The sum of input layer neurons are four, correspond to MeOH/RPO molar ratio (X_1), catalyst content (X_2), temperature (X_3) and reaction time (X_4). The output layer is FAME content. The optimum hidden neurons number is found by a heuristic method. It also examines various numbers of neurons until the MSE of the output data is the lowest value.

Table 1

ANN parameters used for training, modeling and optimization of base-catalyzed methanolysis of RPO.

Property	Value/comment
Algorithm	Levenberg-Marquardt (LM) Back propagation (BP) MSE
Minimized error function	
Learning	Supervised
Input layer	No transfer function is used
Hidden layer	Hyberbolic tangent sigmoid transfer function (TANSIG)
Output layer	Linear transfer function (PURELIN)
Number of best interaction/Epoch	27
Number of input neurons	4
Number of hidden neurons	3
Number of output neurons	1

An effective ANN model can be developed if the design terms and its responses are normalized. The input factors and output value are normalized before training to eliminate the over fitting. The input values and output value are normalized as following equations:

$$x_{i,normalized} = \frac{X_{ij} - X_{i,ave}}{0.5(X_{i,max} - X_{i.min})} \tag{11}$$

$$y_{normalized} = \frac{Y_j - Y_{ave}}{0.5(Y_{max} - Y_{min})} \tag{12}$$

where, $x_{i,normalized}$: normalized input layer of input variable i; X_{ij}: the value of input variable i at experimental run j; $X_{i,ave}$: the average value of input variable i; $X_{i,max}$ and $X_{i,min}$: the maximum and minimum value of input variable i, respectively; $y_{normalized}$: normalized output variable; Y_j: the value of output variable at experimental run j; Y_{ave}: the average value of output variable; Y_{max} and Y_{min}: the maximum and minimum value of output variable, respectively.

The output variable (FAME content) of the ANN model is determined and written as follows:

$$y_{normalized} = f^2(a^2) \tag{13}$$

$$a^2 = \left(\sum_{j=1}^{3} \omega_j^2 \times f^1\left(a_j^1\right)\right) + b^2 \tag{14}$$

$$a_j^1 = \left(\sum_{i=1}^{4} \omega_{ij}^1 \times X_i\right) + b_j^1 \qquad (15)$$

where *i*: the sum of input terms (*i*=*4*); *j*: the sum of optimum neurons (*j*=*3*); a^1 and a^2: the linear combined outputs of the hidden layer and the output layer, respectively; b^1 and b^2: the bias of the hidden layer and the output layer, respectively; f^1 and f^2: the transfer function for the hidden layer and the output layer, respectively.

Finally, the output value is calculated or de-normalized to the original units by equation:

$$y = \left(y_{normalized} \times 0.5 \times (y_{max} - y_{min})\right) + y_{ave} \qquad (16)$$

where *y*: output variable; $y_{normalized}$: normalized output variable; y_{max} and y_{min}: the maximum and minimum experimental output variables, respectively; y_{ave}: the average experimental output variable.

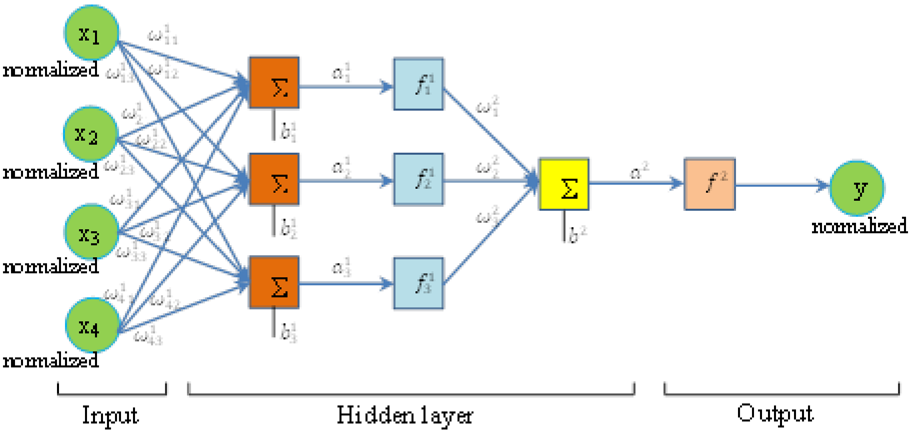

Fig. 1 Structure of single hidden layer network of ANN in this study.

As mentioned above, total 30 experimental runs are requested with CCD design. Data are separated into three parts, including training (70% of total data points), testing (15% of total data points) and validation (15% of total data points) in ANN [22]. In the first, the training data are randomly chosen from the initially data. And the weighted parameters of the interactions are calculated through a chain of repeats to get the minimum number of MSE between the calculated values and experimental FAME content. Subsequently, the testing data are applied to check the trained ANN. Last, the validation data show the prediction of FAME content via the developed ANN modeling.

2.1.4. Evaluation ability of the RSM and ANN models

The developed models using RSM and ANN are investigated for predictive ability for the base-catalyzed methanolysis process. The coefficients of R, R^2, adjusted R^2, MSE, RMSE, MAE, SEP and MRPD were calculated and employed for this purpose.

3. Results and Discussions

3.1. Experimental results

The relationship between the four independent variables (MeOH/RPO molar ratio, catalyst content, reaction temperature and reaction time) and the FAME content are investigated. The FAME content for each experimental run and from both RSM and ANN models are listed in Table 2.

3.2. RSM modeling

3.1.1. Analysis of variance (ANOVA)

Results of ANOVA are summed up in Table 3 in terms of the degree of freedom, the sum and means of squares, F-value and P-value. The significance of the model, single terms, their squares and interactions is confirmed via their F-value and P-value. P-value less than 0.05 implies significant effects of these parameters on the FAME content.

From Table 3, as per the P-value less than 0.05, molar ratio (X_1), catalyst content (X_2), reaction temperature (X_3), reaction time (X_4), square terms of molar ratio and catalyst content (X_1^2, X_2^2), two-way interaction of molar ratio with catalyst content and reaction temperature (X_1X_2, X_1X_3), catalyst content and reaction time (X_2X_4), reaction temperature and time (X_3X_4) have statistically significant effects on the FAME content. However, in other terms, X_3^2, X_4^2, X_1X_4 and X_2X_3 are observed to be insignificant on the FAME content.

Table 2

Designed independent factors and experimental results.

Run	Independent variables/ Input variables				Output variable/ FAME content (%)		
No.	X_1 (mol/mol)	X_2 (wt.%)	X_3 (^oC)	X_4 (min)	Experiment	RSM model	ANN model
1	4.50	0.70	50	40	83.51	83.29	83.29
2	7.50	0.70	50	40	94.48	94.45	94.56
3	4.50	1.10	50	40	90.60	90.32	90.64
4	7.50	1.10	50	40	98.64	98.59	98.02
5	4.50	0.70	60	40	86.87	86.39	86.80
6	7.50	0.70	60	40	95.31	95.11	96.13
7	4.50	1.10	60	40	93.42	93.21	92.99
8	7.50	1.10	60	40	98.83	99.05	98.61
9	4.50	0.70	50	60	85.76	85.55	85.78
10	7.50	0.70	50	60	96.28	96.20	96.27
11	4.50	1.10	50	60	91.56	91.47	91.82
12	7.50	1.10	50	60	98.74	99.23	98.68
13	4.50	0.70	60	60	87.92	87.67	88.05
14	7.50	0.70	60	60	95.59	95.88	95.59
15	4.50	1.10	60	60	93.34	93.38	93.80
16	7.50	1.10	60	60	98.78	98.70	98.80
17	3.00	0.90	55	50	80.84	81.55	81.06
18	9.00	0.90	55	50	98.45	98.03	98.37
19	6.00	0.50	55	50	87.07	87.52	87.02
20	6.00	1.30	55	50	97.53	97.37	97.57
21	6.00	0.90	45	50	93.45	93.54	93.50
22	6.00	0.90	65	50	95.92	96.12	96.23
23	6.00	0.90	55	30	93.61	94.10	94.14
24	6.00	0.90	55	70	96.21	96.01	96.24
25	6.00	0.90	55	50	94.87	94.95	94.82
26	6.00	0.90	55	50	95.32	94.95	94.82
27	6.00	0.90	55	50	95.19	94.95	94.82
28	6.00	0.90	55	50	94.79	94.95	94.82
29	6.00	0.90	55	50	94.47	94.95	94.82
30	6.00	0.90	55	50	95.04	94.95	94.82
					MSE	0.0879	0.0010
					R^2	0.9953	0.9958

Based on the F-value and P-value (Table 3), the important operational variables are molar ratio, catalyst content, temperature and reaction time (F-values of 2318.83, 828.49, 56.45 and 31.41, respectively and P-value <0.0001). Molar ratio and catalyst content have very high F-value as compared to other individual variables. This means that the molar ratio and catalyst content are the two most important factors in the present study. The importance of catalyst content on FAME can be explained by increasing the methoxide anion concentration contributing to increase the FAME formation rate. This result also shows significant role of the MeOH/RPO molar ratio in the enhancement the forward reaction rate. It shifts the reaction equilibrium toward the formation of product at higher concentration of methanol. The present results are similar to previous researches, which demonstrated highest significant effects of molar ratio and catalyst content for base-catalyzed methanolysis [14,24].

Table 3

Results of ANOVA.

Source/ Term	Degree of freedom (DF)	Sum of squares (SS)	Mean square (MS)	F–value	P–value	Remarks
Model	14	639.242	45.660	259.84	<0.0001	Significant
Linear	4	568.491	142.123	808.79	<0.0001	Significant
X_1	1	407.468	407.468	2318.83	<0.0001	Significant
X_2	1	145.583	145.483	828.49	<0.0001	Significant
X_3	1	9.920	9.920	56.45	<0.0001	Significant
X_4	1	5.520	5.520	31.41	<0.0001	Significant
Square	4	54.002	13.500	76.83	<0.0001	Significant
X_1^2	1	45.592	45.592	259.46	<0.0001	Significant
X_2^2	1	10.732	10.732	61.07	<0.0001	Significant
X_3^2	1	0.024	0.024	0.13	0.720	Not significant
X_4^2	1	0.020	0.020	0.11	0.741	Not significant
2-Way interaction	6	16.749	2.792	15.89	<0.0001	Significant
X_1X_2	1	8.309	8.309	47.28	<0.0001	Significant
X_1X_3	1	5.941	5.941	33.81	<0.0001	Significant
X_1X_4	1	0.263	0.263	1.49	0.240	Not significant
X_2X_3	1	0.043	0.043	0.25	0.628	Not significant
X_2X_4	1	1.238	1.238	7.04	0.018	Significant
X_3X_4	1	0.956	0.956	5.44	0.034	Significant
Residual	15	2.636	0.176			
Lack of Fit (LOF)	10	2.171	0.217	2.33	0.181	Not significant
Pure Error	5	0.465	0.093			
Total	29	641.878				

R^2 = 0.9953, adjusted R^2 = 0.9929, R^2 for prediction = 0.9842

3.1.2. Prediction of FAME content by RSM

FAME content in the final biodiesel product is strongly influenced by four operational variables. The RSM response obtained in Table 2 is relative to these variables using a second-order polynomial equation as Eq. (10). The initial regression model is shown in Eq. (17):

$$Y = -42.86 + 16.68X_1 + 64.68X_2 + 1.036X_3 + 0.466X_4 - 0.573X_1^2 - 15.64X_2^2 - 0.00117X_3^2 + 0.000270X_4^2 - 2.402X_1X_2 - 0.08125X_1X_3 - 0.00854X_1X_4 - 0.05187X_2X_3 - 0.139X_2X_4 - 0.00489X_3X_4 \quad (17)$$

The fit of the designed model is checked as per F-value, P-value, lack of fit error (LOF), R^2, adjusted R^2 and R^2 for prediction [8,16,17]. As given in Table 3, the model's F-value 259.84 and the very low P-value (<0.0001) indicates that the corresponding model is noteworthy. The LOF of 0.181 (much higher 0.05) implies that LOF is insignificant relative to the pure error [8]. Insignificant LOF is good for predicted model. Additionally, in the evaluation the significance of the suggested model, large differences between R^2, adjusted R^2 and predicted R^2 also demonstrate the significance of the model [16,17]. These coefficients are very high (0.9953, 0.9929 and 0.9824, respectively) and prove the worth of the model (Table 3).

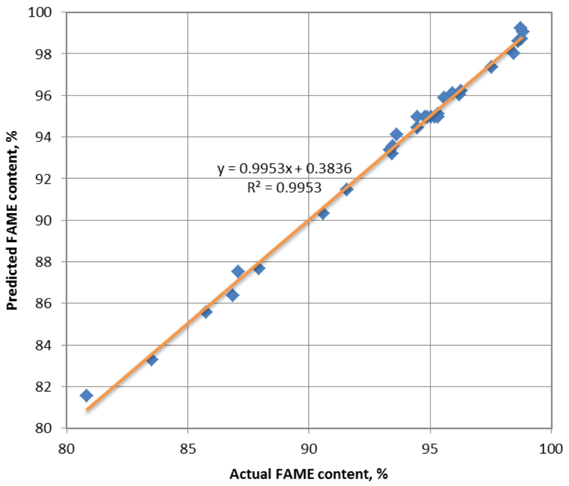

Fig. 2 Comparison of predicted and experimental FAME content.

Correlation is nearly linear and most of experimental points are located on the 45-degree line as depicted in Fig. 2. Therefore, the suggested model is precise description of the process.

Based on the coded factor, ANOVA data and by eliminating the insignificant model terms, the final practical model is given in Eq. (18):

$$Y = -34.86 + 16.25X_1 + 61.82X_2 + 0.86X_3 + 0.442X_4 - 0.573X_1^2 - 15.63X_2^2 - 2.402X_1X_2 - 0.08125X_1X_3 - 0.139X_2X_4 - 0.00489X_3X_4 \qquad (18)$$

3.3. ANN modeling

3.3.1. Development of ANN

The FAME content is known beforehand base on the ANN with LM algorithm includes four input layer neurons and one output. The development of this ANN model also depends on the decisive optimum neuron numbers. The influence of the sum of neurons in the hidden layer is investigated in order to determine the optimum neurons. This process consists of checking a chain of various neurons until the MSE are the lowest value. The number of neurons is varied from 1 to 25. Results for the ANN model are shown in Fig. 3. The optimum sum of neurons for the ANN model is 3 neurons with the minimum MSE of 0.00097664 (Fig. 4). It is also a fact that the initially high MSE reduced rapidly to a smallest value.

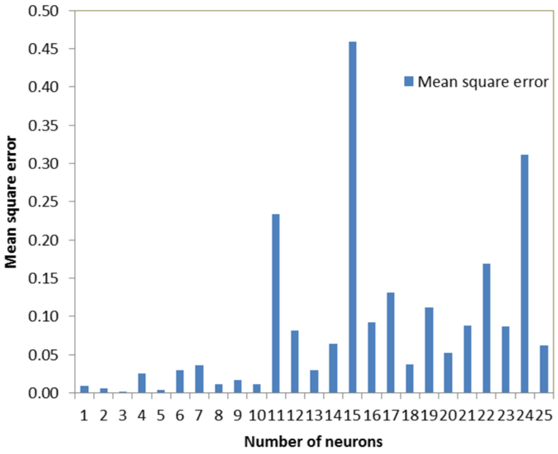

Fig. 3 Validation MSE response for the ANN model.

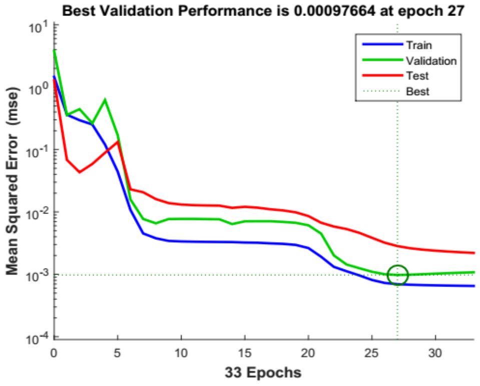

Fig. 4 MSE values for training, validation and testing of the developed ANN model.

3.3.2. Prediction of FAME content by ANN

Fig. 5 compares the predicted and actual FAME content for training (R = 0.99881), validation (R = 0.9986), testing (R = 0.95991) and the overall regression (R = 0.99795) of the developed ANN model as per the 4-3-1 configuration (4 input variables, 3 neurons in hidden layer and 1 output variable). It is clear that most data points are distributed on the 45-degree line. These show a very good mutual relationship between the experimental data and predicted outputs. The results also confirm the developed ANN model is absolutely agreed to predict the output values of the validation and testing data.

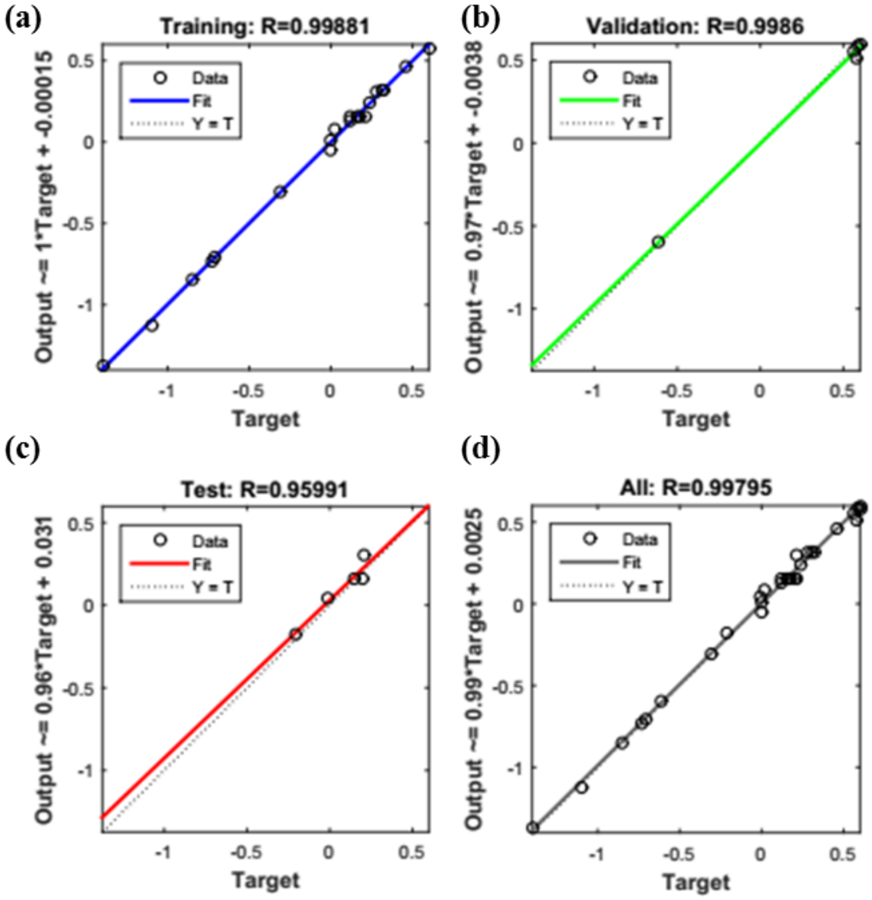

Fig. 5 Comparisons of the predicted and experimental FAME content (output) for training (a), validation (b), testing (c) and the overall regression (d) for 3 neurons.

3.4. Performance assessment of predictive capability of RSM and ANN models

The capability of the developed RSM and ANN models in prediction of the FAME content in biodiesel is evaluated in terms of their R, R^2, adjusted R^2, mean square error (MSE), root mean square error (RMSE), mean absolute error (MAE), standard error of prediction (SEP) and mean relative percent deviation

(MRPD). These results are presented in Table 4. If the value of the R is close to 1 then there is a good correlation between experimental and predicted outputs. The two models have very high values of R^2, demonstrate the authentic suitability of these models [21]. The adjusted R^2 is used in testing over fitting of R^2, and they are also significantly high for the two models, confirm importance of the models. The MSE is tested for both models. Moreover, the RMSE – the square root of the MSE – is also determined for both models. MSE value from ANN model is much lower than compared to RSM model (0.0010 and 0.0879, respectively). The similar difference is also obtained for RMSE, with 0.0313 and 0.2964, respectively. Results verify the ANN model is better than the RSM model (Table 4). MAE, SEP and MRPD check the significance and accuracy of the models [21,22,25]. The lower values of these statistical parameters, better the performance of the model.

Despite of several studies have shown that ANN is better than RSM model in prediction capability [12,21,22,25-27]. Further, these results have not proven the difference between MSE and RSME for the RSM and ANN models. The present study has passed this difficulty and contributes a fully confirmation about the effectiveness of the developed ANN and compared to the RSM model.

Table 4

Performance evaluation of RSM, ANN models.

Parameter	RSM	ANN
R	0.9979	0.9980
R^2	0.9953	0.9958
Adjusted R^2	0.9921	0.9903
MSE	0.0879	0.0010
RMSE	0.2964	0.0313
MAE	0.2448	0.0233
SEP (%)	0.3173	0.0335
MRPD (%)	0.2667	0.2232

3.5. Optimization of FAME content by the RSM and ANN models

Actual FAME content obtained under the experimental conditions are between 80% to 100% (Fig. 2). In order to evaluate the optimization capability of the RSM and ANN models, the FAME content of 96.5% and 98% were chosen as a desired target of base-catalyzed methanolysis. The optimum condition for temperature and reaction time are same for both models with regard to same desired target (Table 5). In a contrary, the optimum molar ratio and catalyst content from these models have a remarkable difference. The molar ratio and catalyst content are the two most important factors for base-catalyzed methanolysis in this present study as per ANOVA results (Table 3). Therefore, the evaluation for the RSM and ANN models is as per these two important factors. The values of molar ratio and catalyst content required for base-catalyzed FAME synthesis by ANN model are lower in comparison with RSM model (Table 5). Thus, ANN model is better in prediction capability as compare to RSM model.

Table 5

Optimization conditions and model validation.

Model	RSM		ANN	
	96.5 % FAME	98% FAME	96.5 % FAME	98% FAME
MeOH/RPO molar ratio	6.15	7.57	5.49	5.88
CH_3ONa content (wt.%)	1.01	1.20	0.87	0.89
Reaction temperature (^oC)	55	55	54.7	54.9
Reaction time (min)	50	50	49.8	50

4. Conclusion

RSM and ANN models were developed and compared for their predictive and generalization abilities in the methanolysis process of palm oil catalyzed by sodium methoxide in the present study. Conclusions are drawn as:

1. The predictive capability of the two models for sodium methozide-catalyzed methanolysis was compared using the same experimental conditions from the CCD.
2. High values of R, R^2, predicted R^2 (> 0.99) clearly indicates high accuracy of both RSM and ANN models.
3. Both models have proven the important role of the molar ratio and catalyst content for base-catalyzed methanolysis.

4. Contrary to the reports that the difference of the predictive capability between RSM and ANN is only based on R^2, adjusted R^2 and R^2 for prediction, this is the first study in evaluating as per MSE, RMSE, MAE, SEP, MRPD.
5. Lower values of the ANN models demonstrated that the ANN model is a better choice compared to the RSM model by paying attention to parameters (MSE, RMSE, MAE, SEP, MRPD).

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VITAE

Name Mr. Dang Nguyen Thoai

Student ID 5710130034

Educational Attainment

Degree	Name of Institution	Year of Graduation
B. Eng. in Petrochemical Engineering	Hanoi University of Science and Technology	2005
M. Eng. in Chemical Engineering	Hanoi University of Science and Technology	2009

Scholarship Awards during Enrolment

1. Thailand’s Education Hub for Southern Region of ASEAN Countries, Prince of Songkla University, 2014 – 2017 (Grant No. TEH-AC 047/2014).
2. PSU.GS. Financial Support for Thesis, Prince of Songkla University, 2016 (Grant No. 950/433).
3. Travel Funding for Conferences from Faculty of Engineering, Prince of Songkla University of Thailand for attending international conference in Vietnam, 2016 (Grant No. 1287.3/2559).

Work – Position and Address

1. Lecturer, Department of Chemical Engineering, Faculty of Chemistry, Quy Nhon University, Vietnam, from September 2005 to December 2014.
2. Teaching Assistant (TA), Department of Chemical Engineering, Faculty of Engineering, Prince of Songkla University, Thailand, from January 2016 until now.

List of Publication and Proceeding (if any)

1. D.N. Thoai, C. Tongurai, K. Prasertsit, S. Photaworn. *Optimization of the first step transesterification of refined palm oil using solid sodium methoxide catalyst*. The 23rd Regional Symposium on Chemical Engineering (RSCE2016), Vung Tau, Vietnam, ISBN: 978-604-73-4690-5 (book); 978-604-73-4690-8 (CD disk); Section 3: 137–144. DOI: 10.13140/RG.2.2.17398.19521.
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