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UNIVERSITI TEKNOLOGI PETRONAS

OPTIMIZATION AND REACTION KINETICS OF *IN-SITU* TRANSESTERIFICATION OF *JATROPHA CURCAS* SEEDS

by

NUNUNG PRABANINGRUM

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OPTIMIZATION AND REACTION KINETICS OF *IN-SITU* TRANSESTERIFICATION OF *JATROPHA CURCAS* SEEDS

by

NUNUNG PRABANINGRUM

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DECLARATION OF THESIS

Title of thesis: OPTIMIZATION AND REACTION KINETICS OF *IN-SITU* TRANSESTERIFICATION OF *JATROPHA CURCAS* SEEDS

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DEDICATION

I have dedicated this thesis to my husband (Agus Arif) and both my daughters (Emira Rahmasari Arif and Kirana Kalammulya Arif).

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ABSTRACT

Minimizing the cost of raw materials and the cost of processing is the key to make biodiesel technology competitive. Use of a non-edible oil such as *Jatropha curcas* oil can be cheaper option; *in-situ* transesterification of oil seeds can reduce the processing costs of oil recovery. *In-situ* alkaline transesterification of *Jatropha curcas* oil seeds with methanol, isopropanol and the mixture of methanol with isopropanol were explored in a stirred tank reactor. Optimum operating conditions were established through response surface methodology for promising options. Inspite of higher solubility of oil in isopropanol, *in-situ* alkaline transesterification of *Jatropha curcas* oil seeds with methanol was observed to be better with a yield of (90.45 ± 0.25)%. Mixing methanol with isopropanol could enhance the yield of methyl-isopropyl ester yield of (94.78 ± 0.29)%. The properties of methyl-isopropyl ester were similar to the properties of methyl ester which were in agreement with the standards of EN 14214 and ASTM D6751.

In-situ transesterification is a catalytic liquid-liquid reaction in the presence of inert solid phase. Analysis of data through shrinking core model indicated reaction rate to be the controlling regime. For *in-situ* methanolysis with the particle size bigger than 425 microns and smaller than 600 microns, the reaction was first order with the rate constant from 5.15×10^{-9} to 8.76×10^{-9} $\mathrm{m\cdot s^{-1}}$ and Arrhenius activation energies of 22.66 $\mathrm{kJ\cdot mol^{-1}}$. For *in-situ* transesterification with mixture of methanol and isopropanol and particle size smaller than 425 microns, the reaction was also first order with the rate constant from 7.72×10^{-9} to 1.49×10^{-8} $\mathrm{m\cdot s^{-1}}$ and Arrhenius activation energies of 20.35 $\mathrm{kJ\cdot mol^{-1}}$. Assuming phase transfer catalysis mechanism to explain the reaction between the immiscible alcohol and oil phases, model equations for reaction rate were developed. The results were compared with the experimental observation.

In-situ methanolysis of *Jatropha curcas* seed in Soxhlet extractor as an unstirred reactor had been investigated. In order to facilitate oil extraction, n-hexane was added in methanol. The optimum reaction parameters were determined and the methyl ester yield of (83.61 ± 0.57)%.

ABSTRAK

Kunci utama untuk menjadikan teknologi biodiesel lebih kompetitif adalah dengan meminimumkan kos bahan mentah dan pemprosesan. Penggunaan minyak yang tidak boleh dimakan seperti minyak *Jatropha curcas* adalah pilihan yang lebih murah dan kos pemprosesan boleh dikurangkan melalui proses transesterifikasi biji minyak secara *in-situ*. Proses transesterifikasi biji minyak *Jatropha curcas* dijalankan secara *in-situ* di dalam reaktor yang mengandungi metanol, isopropanol atau campuran metanol dan isopropanol. Keadaan optimum proses ini dianalisa menggunakan "Response Surface Methodology" (RSM). Walaupun kelarutan minyak dalam isopropanol lebih tinggi, transesterifikasi biji minyak *Jatropha curcas* secara *in-situ* menggunakan metanol memberikan hasil yang lebih baik sebanyak (90.45 ± 0.25)%. Percampuran metanol dan isopropanol boleh meningkatkan hasil metil-isopropil ester sebanyak (94.78 ± 0.29) %. Sifat-sifat metil- isopropil ester hampir sama dengan sifat-sifat metil ester yang berkesesuaian dengan standard EN 14214 dan ASTM D675.

Transesterifikasi secara *in-situ* adalah proses tindakbalas cecair menggunakan pemangkin dengan kehadiran pepejal lengai. Analisis data yang dijalankan menggunakan model teras mengecut menunjukkan bahawa kadar tindakbalas merupakan rejim pengawalan. Metanolisis biji minyak secara *in-situ* dengan saiz zarah di antara 425 dan 600 mikron menunjukkan tindakbalas proses ini mengikut tindakbalas tingkat pertama dengan kadar tetapan tindak balas dari 5.15×10^{-9} sehingga 8.76×10^{-9} $m\cdot s^{-1}$ dan tenaga pengaktifan Arrhenius daripada 22.66 $kJ\cdot mol^{-1}$. Sementara itu, bagi transesterifikasi secara *in-situ* dengan campuran metanol dan isopropanol bagi saiz zarah lebih kecil daripada 425 mikron, tindakbalasnya juga mengikut tindakbalas tingkat pertama dengan kadar tetapan tindakbalas dari 7.72×10^{-9} sehingga 1.49×10^{-8} $m\cdot s^{-1}$ dan tenaga pengaktifan Arrhenius daripada 20.35 $kJ\cdot mol^{-1}$. Model persamaan untuk kadar tindakbalas dibangunkan, dan hasil persamaan ini dibandingkan dengan pemerhatian eksperimen untuk pengesahan. Metanolisis biji minyak *Jatropha curcas* secara *in-situ* di dalam ekstraktor Soxhlet telah dijalankan. Heksana ditambah ke dalam larutan metanol untuk memudahkan proses pengekstrakan minyak. Pada keadaan parameter tindak balas optimum, metil ester yang terhasil adalah (83,61 ± 0.57) %.

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NOMENCLATURE

A_{gi} : Peak area of individual glyceride

A_{is} : Peak area of internal standard

a_o : Slope of calibration function of mono-, di-, and triolein

A_V : Acid value (mg $KOH \cdot g^{-1}$)

b_o : Intercept of calibration function of mono-, di-, and triolein

β_0 : Intercept coefficient

β_i : Linear coefficient

β_{ii} : Quadratic coefficient

β_{ij} : Interaction coefficient

C_{Na} : Sodium hydroxide concentration (wt.%)

Df : Degree of freedom

Dp : Diameter of particle (μm)

G_{Total} : Mass percentage of total glycerin (%)

G_{free} : Mass percentage of free glycerin (%)

G_{bound} : Mass percentage of bound glycerin (%)

G_D : Mass percentage of diglyceride (%)

G_i : Mass percentage of individual glyceride in sample (%)

G_M : Mass percentage of monoglyceride (%)

G_T : Mass percentage of triglyceride (%)

$[H_2O]$: Concentration of water in alcohol phase ($mol \cdot m^{-3}$)

k_1 : Second-order reaction rate constant of formation of $[NaOR]_a$ from $[NaOH]$ ($m^3 \cdot mol^{-1} \cdot s^{-1}$)

k_2 : Second-order reaction rate constant of consumption of $[NaOR]_o$ in oil phase ($m^3 \cdot mol^{-1} \cdot s^{-1}$)

k_3 : Second-order reaction rate constant of consumption of $[NaDG]_a$ in alcohol phase ($m^3 \cdot mol^{-1} \cdot s^{-1}$)

Second-order reaction rate constant of formation of $[NaMG]_a$ from $[NaDG]_a$ in alcohol phase ($m^3 \cdot mol^{-1} \cdot s^{-1}$)

k_4 : Second-order reaction rate constant of consumption of $[NaMG]_a$ in alcohol phase ($m^3 \cdot mol^{-1} \cdot s^{-1}$)

Second-order reaction rate constant of formation of $[NaG]_a$ from $[NaMG]_a$ in alcohol phase ($m^3 \cdot mol^{-1} \cdot s^{-1}$)

k_5 : Second-order reaction rate constant of consumption of $[NaG]_a$ to form [NaOH] in alcohol phase ($m^3 \cdot mol^{-1} \cdot s^{-1}$)

k_{NaOR} : Mass transfer coefficient of NaOR from alcohol phase to oil phase ($m \cdot s^{-1}$)

k_{NaDG} : Mass transfer coefficient of NaDG from oil phase to alcohol phase ($m \cdot s^{-1}$)

k_{app} : First-order apparent rate constant (s^{-1})

M_{NaOR} : Partition coefficient of NaOR complex

M_{NaDG} : Partition coefficient of NaDG complex

M_r : Molecular weight ($g \cdot mol^{-1}$)

N : Normality of standard alkali (N)

[NaOH] : Concentration of sodium hydroxide ($mol \cdot m^{-3}$)

[ROH] : Concentration of alcohol ($mol \cdot m^{-3}$)

$[NaOR]_a$: Concentration of sodium alkoxide in alcohol phase ($mol \cdot m^{-3}$)

$[NaOR]_o$: Concentration of sodium alkoxide in oil phase ($mol \cdot m^{-3}$)

$[NaDG]_a$: Concentration of sodium diglyceride in alcohol phase ($mol \cdot m^{-3}$)

$[NaDG]_o$: Concentration of sodium diglyceride in oil phase ($mol \cdot m^{-3}$)

$[NaMG]_a$: Concentration of sodium monoglyceride in alcohol phase ($mol \cdot m^{-3}$)

$[NaG]_a$: Concentration of sodium glyceride in alcohol phase ($mol \cdot m^{-3}$)

OC : Oil content (wt.%)

R^2 : Determination coefficient

R_V : Ratio of methanol volume to the mixture volume ($mL \cdot mL^{-1}$)

R_{VW} : Ratio of alcohol volume to seed weight ($mL \cdot g^{-1}$)

t : Reaction time (min or h)

T : Reaction temperature (^0C)

$[TG]_o$: Concentration of triglyceride in oil phase ($mol.m^{-3}$)

V_a : Volume of alcohol (m^3)

V_A : Volume of standard alkali used in the titration (mL)

V_B : Volume of standard alkali used in the titrating the blank (mL)

V_o : Volume of oil (m^3)

W_{oil} : *Jatropha curcas* oil weight (g)

W_{seed} : *Jatropha curcas* seed weight (g)

W_{spl} : mass of sample (g)

W_{BD} : weight of biodiesel (g)

x_i and x_j : independent factors

Y : Biodiesel yield (%)

CHAPTER 1

INTRODUCTION

1.1 Background of research

1.1.1 Energy demand

Energy demand is directly related to advances in technology, economic growth, and socio-economic development. Fossil fuels such as coal, petroleum and natural gas contribute for over 83% of the world energy needs. Nuclear power supports to the extent of around 5.9%. Rest is contributed by renewable energy resources such as hydroelectric, agro wastes, solar, wind, *etc*. [1, 2]. The world total primary energy consumption has risen from 6630 million tons of oil equivalent (Mtoe) in 1980 to 12,002 Mtoe in 2010. Based on the International Energy Agency prediction, since 2010 the world total energy consumption will increase 53% in 2030 [3]. BP's annual Statistical Review of World Energy at the end of 2008 stated that the world oil reserves were foreseen at the amount of 1.7×10^{11} tons and it will be exhausted within around 42 years, if the oil consumption increases with the rate of 3% per year. Meanwhile, The World Energy Forum estimated that the petroleum reserves will be depleted in less than another 10 decades [4, 5]. The combustion of fossil fuels generates greenhouse gas and the other pollutant gas emissions, such as oxides of sulfur, nitrogen, carbon and unburnt hydrocarbons. The majority of total anthropogenic greenhouse gas emissions are derived from the combustion of petroleum, coal and natural gas. If fossil fuel consumption continued from 2006 to 2030 without any attempt to reduce the use of these fuels, emissions of greenhouse gases will enhance by 39%. The increase of the accumulation of these gases in atmosphere causes climate change, acid rain and smog that would disrupt the environment [1]. In view of this, utilization of renewable energy sources needs to be explored for sustainable development. Renewable liquid biofuels such as bioethanol and biodiesel from agro- or animal sources are emerging as attractive options to the extent possible to supplement liquid fossil fuels [6-8]. Present work is focused on biodiesel produced from vegetable fatty oils.

1.1.2 Vegetable oils

Vegetable oils are essentially triglycerides with high molecular mass, viscosity and low volatilities. They cannot be used directly in internal combustion engines in place of petro diesel as fuel. Vegetable oils (edible or non-edible or used oils) and animal fats can be converted by transesterification to meet stringent quality specification of ASTM D 6751 or EN 14241 for usage in compression–ignition (diesel) engines. The biodiesel feedstock is selected based on the availability in the region or country. Resources such as soybean oil, canola oil, and palm oil are used to produce biodiesel in United States of America, European countries and Malaysia respectively [3, 6, 9, 10]. Biodiesel is nontoxic, safer, easier to use, handle and store. Biodiesel is degradable up to 90-98% in freshwater or soil environment in comparison with 50% of petro-diesel in 28 days [6, 8-11]. At present, biodiesel production rates are very small in comparison with the demand for petro diesel and hence cannot replace petro diesel. It can be used as an additive to reduce the dependence on petro diesel to the extent possible. Also, biodiesel can substitute mercaptans to improve lubricity of petro diesel. [5-8, 11-13].

Currently, the global biodiesel potential production is 51 Mtoe annually. More than 80% of biodiesel production comes from Malaysia, Indonesia, Argentina, the United States, and Brazil. Most of the feedstock are edible oils and include soybean oil (28%), palm oil (22%), animal fats (20%), coconut oil (11%), rapeseed (5%), sunflower (5%) and olive oils (5%) [14].

1.1.3 Transesterification of Vegetable Oils

Triglycerides in vegetable oils can react with monohydric alcohol in the presence of catalyst to produce fatty acid alkyl ester (biodiesel) and glycerol as a by-product. This reaction is known as transesterification and is a reversible reaction. Excess alcohol is used to shift the equilibrium to products side. The reaction can be catalyzed by alkalis (such as metal hydroxides, alkoxides or carbonates), acids (such as hydrochloric, phosphoric, sulfonic and sulfuric acids) or enzymes (such as lipase). The catalyst can be homogeneous or heterogeneous. Transesterification is also to be carried out in non-catalytic supercritical conditions at high reaction temperature (250 to 400°C) and pressure (35 to 60 MPa) [12, 13, 15, 16]. Alkali-catalyzed transesterification can react faster at moderate temperature with high conversion rate. Presence of high free fatty acids and moisture can lead to saponification. Resultant soap formation can inhibit the reaction and subsequent purification. In comparison, acid-catalyzed reactions and lipase enzyme catalyzed reactions are slower in reaction rate and are not affected by saponification reactions. Similarly, heterogeneous catalyzed reactions are also slower in reaction rates though they offer the advantage of reduced separation steps, recyclable catalyst and continuous operation [6, 7, 9, 13, 17]. Biodiesel production by supercritical alcohol requires high cost equipment and it is not appropriate to be applied in industry scale [10, 12, 18-20].

1.1.4 Challenges

The main challenge of biodiesel use as alternative fuel is the cost of its production. The costs of biodiesel production varied greatly in the range of $0.29 per liter to over $9.00 per liter [14]. If edible oils are utilized as feedstock to produce biodiesel, its cost is adversely affected because of its primary need to food industry [6, 11, 21]. Ma [6] mentioned that the cost of oil feedstock accounts for 60 to 75% of the total biodiesel fuel if the edible oils are used as feedstock. Lim and Teong [1] reported about general expense breakdown for biodiesel production as depicted in Figure 1.1. It can be observed that the highest expense for biodiesel production is for providing oil feedstock. Chemical feedstock and processing prices account for 12% of total production cost.

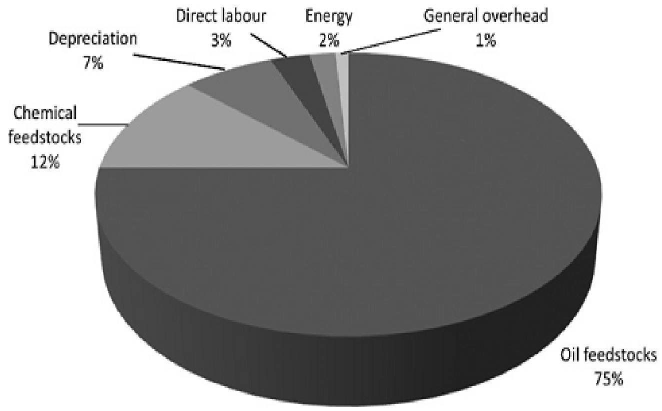


Figure 1.1: General expense breakdown of biodiesel production [1]

Therefore, the exploration of the alternative feedstock which is at low price and available in substantial quantities, instead of refined edible vegetable oils, and alternative biodiesel processing are required to reduce the cost of biodiesel production [22]. Non-edible oils, such as *Jatropha curcas*, *Pongamia pinnata*, *Calophyllum innophyllum*, rubber seed oils, *etc*., and waste cooking oils are other options as biodiesel feedstock which are not consumed by human and do not depend on annual field crops. However, common low-cost oils have high free fatty acid content which is a constraint in the biodiesel preparation. The pre-treatment to remove the free fatty acid before conducting transesterification must be considered, which obviously has consequences in the processing cost.

In the process of transesterification, the vegetable oils must be extracted, purified and refined from the oilseeds before reacting it with catalyzed alcohol. These steps increase the processing cost of biodiesel production. To reduce the cost, vegetable oilseeds can be reacted with catalyzed alcohol directly. This process is known as *in-situ* transesterification or reactive extraction, which was developed by Harington and D'Arcy in 1985 [23]. The steps of oil purification and refining are not necessary anymore, thus the processing time can be reduced as well [24]. Early work on *in-situ* transesterification utilized edible oil seeds, such as soybean flaked [24, 25], rapeseeds [26, 27], sunflower oilseeds [23, 28, 29], rice bran [30, 31], cottonseeds [32], castor seed [33]. Recently, non-edible oil *Jatropha curcas* seeds [34-37] and microalgae [38-40] have been investigated; the reaction times for conversion were high. The homogeneous alkali- or acid-catalyzed methanol or ethanol was used as reagent in this process.

Based on the literature, it is proposed to investigate *in-situ* transesterification of non-edible oilseed to produce fatty acid alkyl ester. *Jatropha curcas* seed is selected as the biodiesel feedstock since it is non-edible oilseed which contains toxic phorbol ester in the oil. Hence, it is not competing as resources with food industry. Moreover, *Jatropha curcas* is cultivated in many countries of South-East Asia, its seed has high oil content and the produced biodiesel has fulfilled the requirement of diesel machine [12, 41].

1.2 Problem Statement

Biodiesel is produced by transesterification of edible oils with excess alcohol in the presence of a catalyst. The raw materials have high cost due to edible oils and have an adverse effect on the demand of food processing industries. In addition, the raw material in transesterification is required to be extracted and purified. Therefore, it is necessary to use non-edible vegetable oils and minimize cost of processing steps. *In-situ* transesterification of non-edible oilseeds can be a viable solution. Due to limited solubility of vegetable oils and methanol, transesterification reaction rates are low and *in-situ* transesterification reactions can be even lower due to the necessity of alcohol to diffuse into the solid matrix to reach oil in the seeds.

It is proposed to investigate sodium hydroxide catalyzed *in-situ* transesterification of non-edible *Jatropha curcas* oilseeds with different alcohols. Reaction rates are expected to be dependent on mutual solubility of oil and alcohols. Information on the solubility of oils in various alcohols is needed. The alcohol has to diffuse through the solid matrix to react with oil phase in the seed; it may experience mass transfer and pore diffusion resistance for the reaction.

In-situ transesterification will be carried out in a stirred-flask as a batch reactor. Effect of different reaction parameters will be studied by response surface methodology to determine the optimum reaction conditions and reaction kinetics by identifying controlling step. It will be of interest to identify mechanism of reaction between two sparingly miscible liquids to explain the reaction kinetics. Investigation on *in-situ* transesterification in Soxhlet extractor as an unstirred reactor is necessary for possible technology transfer to industry.

1.3 Objectives

The objectives of the present work are detailed as follows:

1. To investigate alkaline-catalyzed *in-situ* transesterification of *Jatropha curcas* seeds using methanol, isopropanol, the mixture of methanol and isopropanol in a stirred-flask reactor; to identify the optimum reaction conditions of *in-situ* transesterification of *Jatropha curcas* seeds with methanol and the mixture of methanol and isopropanol using response surface methodology.

2. To identify and to develop the reaction mechanism and kinetics of solid-liquid-liquid *in-situ* transesterification of *Jatropha curcas* seeds.

3. To investigate *in-situ* transesterification of *Jatropha curcas* seeds using methanol and n-hexane in Soxhlet extractor as an unstirred reactor; to identify the optimum reaction parameters using response surface methodology.

1.4 Scope of the Present Research

Information on the solubility of oils in alcohols is scarce. Effect of temperature on the solubility of methanol, ethanol, and isopropanol in vegetable oils (palm oil, sunflower oil, canola oil, corn oil and crude *Jatropha curcas* oil) will be investigated. The effect of solvents such as methanol, ethanol, isopropanol, hexane, mixture of methanol-isopropanol and mixture of methanol and n-hexane on extractability of oil in *Jatropha curcas* seeds will be investigated. Characteristics of *Jatropha curcas* seeds and the extracted oil will be established.

In-situ transesterificaton of *Jatropha curcas* seed will be conducted using isopropanol, methanol, the mixture of methanol and isopropanol catalyzed by sodium hydroxide. The individual effect including the amount of alcohol volume to seed weight, concentration of alkaline catalysts, reaction temperature, the volume ratio of methanol to the mixture on the biodiesel yield will be investigated. The interaction effects among these reaction parameters will be identified using response surface methodology (RSM) and the optimum conditions will be estimated as well.

Soxhlet extractor will be utilized to transesterify *Jatropha curcas* seed in an unstirred reactor using the mixture of methanol and n-hexane catalyzed by sodium hydroxide.

The effects of various reaction parameters on the conversions of triglycerides will be examined at different reaction times. The reaction mechanism and kinetic parameters will be estimated.

1.5 Organization of Thesis

This thesis comprises of six chapters:

- Chapter 1 provides an overview about the background of the research related to biodiesel production by transesterification and *in-situ* transesterification of vegetable oils and oilseeds, problem statement, objectives, and scope of the present research.
- Chapter 2 presents a critical literature review on various biodiesel production processes with an emphasis on *in-situ* transesterification. Reaction mechanisms and kinetics of solid-liquid-liquid reactions are briefly reviewed.
- Chapter 3 elaborates the materials and the methodologies used.
- Chapter 4 deals with the experimental results, the optimization of the process parameters and properties of biodiesel produced.

- Chapter 5 discusses the development of reaction mechanism, kinetic model, and the estimation of reaction rate parameters.

- Chapter 6 summarizes the research conclusions and recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodiesel

Biodiesel is a liquid fuel derived by transesterification from renewable feedstock, such as vegetable oils (edible or non-edible oils), animal fats and waste cooking oils. Biodiesel can be used in diesel engines directly as a fuel, known as B100, or blended in any proportion with petro-diesel, such as B5 (5% biodiesel blended with 95% petro-diesel). Nowadays, many countries, such as United States of America, Brazil, Germany, Italy, UK, France, India, Malaysia, Indonesia, *etc*., are using biodiesel blends commercially [3, 6, 9]. Widely used feedstock includes vegetable oils of soybean, canola (rapeseed), palm and sunflower. Usage of edible oils impacts in competition with food industry. It is preferable to use non-edible oils, waste cooking oils and animal fats as feedstock to produce biodiesel [8, 9, 17, 20, 21, 42].

2.2 Vegetable oils

Main components of vegetable oils or animal fats are fatty acid triglyceride esters.

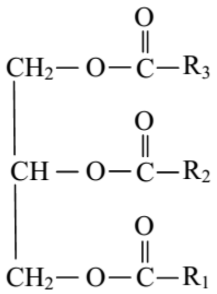


Figure 2.1: Fatty acid Triglyceride ester [17]

The fatty acid components “R-“ can vary in carbon chain length (number of carbon atoms) as well as chemical structure with molecular weights in the range of 650 to 790 and influence the fuel properties of biodiesel [17]. Some of the common fatty acids in vegetable oils are presented in Table 2.1.

Table 2.1: The chemical structure of common fatty acids [17]

Fatty acid	**[no. C atoms: no. double bonds)**	**Chemical structure**
Lauric or dodecanoic acid	C12:0	$CH_3(CH_2)_{10}COOH$
Myristic or tetradecanoic acid	C14:0	$CH_3(CH_2)_{12}COOH$
Palmitic or hexadecanoic acid	(C 16:0)	$CH_3(CH_2)_{14}COOH$
Palmitoleic or 9-cis-hexadecanoic acid	(C-16:1)	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
Stearic or octadecanoic acid	(C 18:0)	$CH_3(CH_2)_{16}COOH$
Oleic or cis-9-octadecenoic acid	(C 18:1)	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleic or cis, cis -9, 12-octadecadienoic acid	(C 18:2)	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
Linolenic or octadecatrienoic acid	(C 18:3)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7$ $COOH$
Arachidic or eicosanoic acid	(C 20:0)	$CH_3(CH_2)_{18}COOH$
Gondoic or cis-11-eicosenoic acid	(C 20:1)	$CH_3(CH_2)_8CH=CH(CH_2)_8COOH$
Behenic or docosanoic acid	(C 22:0)	$CH_3(CH_2)_{20}COOH$
Erucic or *cis*-13-docosenoic acid	(C 22:1)	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$

Fatty acid composition of some commonly used vegetable oils is presented in Table 2.2.

Table 2.2: Fatty acid composition in vegetable oils (wt.%)

Vegetable oil	**12:0**	**14:0**	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**	**20:0**	**20:1**	**22:0**	**22:1**	**16:1**	**No. Ref.**
Cottonseed	0	0.8	27.9	0.7	22.7	47.9	0	0	0	0	0	0	[43]
Palm	1.2	0.1	46.4	0	41.2	11.1	0	0	0	0	0	0	[44]
Sunflower seed	0	0	6.6	5.1	19.6	68.7	0	0	0	0	0	0	[29]
Soybean	0	0	11.5	4.0	24.5	53	7	0	0	0	0	0	[45]
Rapeseed	0	0	1.2	2.5	61.4	22.3	11.5	0	0	0	0	0	[46]
Ricebran	0	0.6	21.5	2.9	38.4	34.4	2.2	0	0	0	0	0	[47]
Jatropha curcas	0	0–0.1	14.1-15.3	3.7–9.8	34.3-45.8	29.0–44.2	0–0.3	0–0.3	0	0–0.2	0	0–1.3	[48]
Pongamia Pinnata	0	1.09	11.7	7.5	51.6	16.6	0	1.35	0	4.45	0	0	[49]
Calo-phyllum inophyllum	0	0	14.8-18.5	6.1-19.2	36.2-53.1	15.8-28.5	0	0	0	0	3.3	0	[50]
Brassica carinata	0	0	2.9		33.9	8.23	1.14	0	10.9	0	42	0	[51]
Rubber seed	0	0	10.2	8.7	24.6	39.6	16.3	0	0	0	0	0	[52]

It can be seen that most of the vegetable oils contain C16 and C18 fatty acid triglycerides. Due to the paramount need of edible oils for world food security, it is necessary to use non-edible oils, waste cooking oils or animal fats for biodiesel production [53, 54].

2.3 Non-edible oils

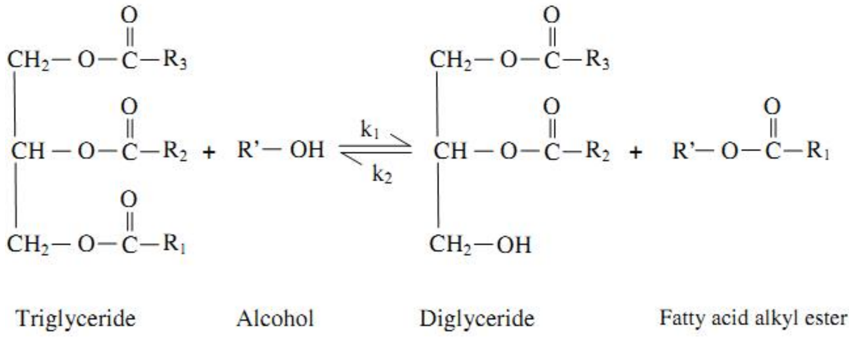
Some potential non-edible oils for biodiesel production are *Jatropha curcas*, *Pongamia pinnata*, *Calophyllum inophyllum, Brassica carinata*, and rubber oilseed, *etc.* Jatropha has higher oil percentage and the yield per hectare of 2.0 to 3.0 tons/ha/year [55]. *Jatropha curcas* is included in *Euphorbiaceae* family which can grow almost everywhere even on gravelly, sandy and saline soils and it is resistance in high drought. Its seed is able to be produced for 50 years with high oil content more than 35%. The plant can be used to prevent or control erosion, to reclaim land, grown as a live fence. *Jatropha curcas* is the most promising non-edible oilseed for biodiesel production based on the oil content of 49% and the composition of its fatty acid which comprises 74.2% oleic and linoleic acid (unsaturated fatty acids) [56]. The free fatty acid and oil content of *Jatropha curcas* depend on the geographical growth soil [41, 53, 54].

2.4 Transesterification

Transesterification is the chemical reaction between fatty acid triglycerides in vegetable oils and short-chain alcohol to produce fatty acid alkyl ester (biodiesel) and glycerol. Short-chain alcohols include methanol, ethanol, propanol, isopropanol, butanol and isobutanol. Selection of alcohol is based on cost, reaction kinetics, and the fuel properties of the obtained biodiesel [15, 17]. To enhance the reaction rate, catalyst is required during the reaction in order that the reaction can be completed in a shorter time with high yield and purity. Alkali, acid, or enzyme catalysts can be employed in transesterification. The free fatty acid and water contents in triglyceride are the important factors to select the catalyst. Most commonly used alkaline catalysts include sodium and potassium hydroxides, carbonates and alkoxides; acid catalysts include sulfuric, organic sulfonic, hydrochloric, phosphoric acids; enzyme catalysts include Lipase, Novozym 435 (*Candida antarctica* immobilized on acrylic resin), *Rhizopus oryzae*, *Pseudomonas cepacea*, and *Mucor miehei* [7, 10, 20]. Acid or enzyme catalysts are preferable for oils with high free fatty acid content.

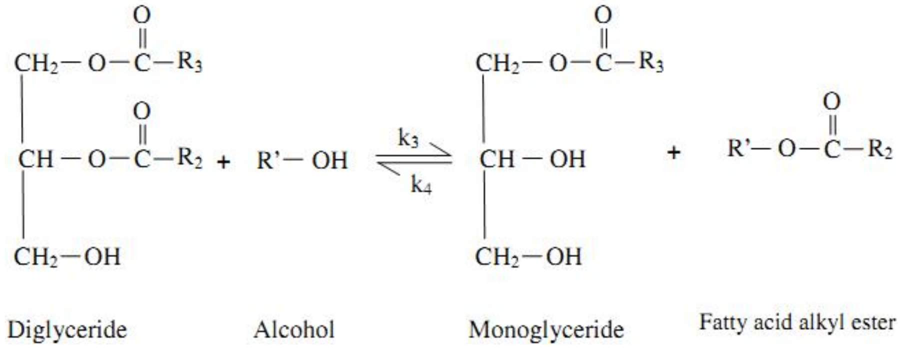
In general, transesterification is composed of a sequence of three consecutive reversible reactions [7, 16, 57].

- In the first step, triglyceride reacts with alcohol to produce diglyceride and fatty acid alkyl ester.



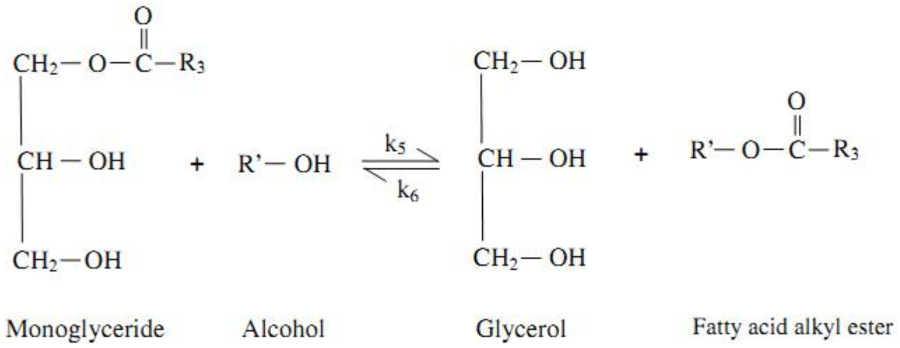
(2.1)

- Diglyceride as an intermediate product is converted into monoglyceride in the presence of alcohol and catalyst.



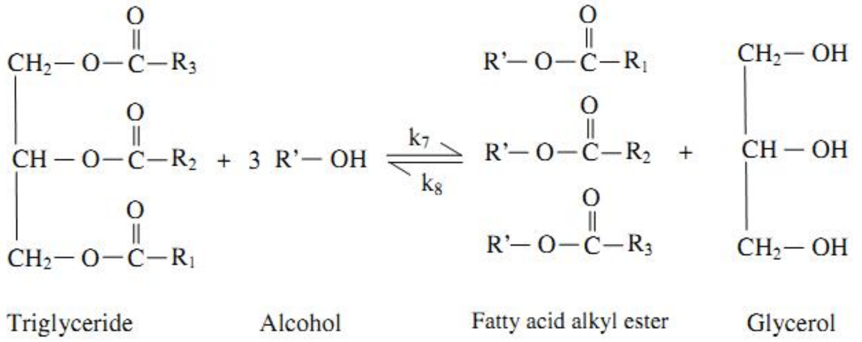
(2.2)

- The last step is the reaction between monoglyceride and alcohol to produce glycerol and fatty acid alkyl ester.



(2.3)

The overall reaction, one mole of triglyceride requires 3 moles of alcohol to produce 3 moles of alkyl ester and a glycerol stoichiometrically.

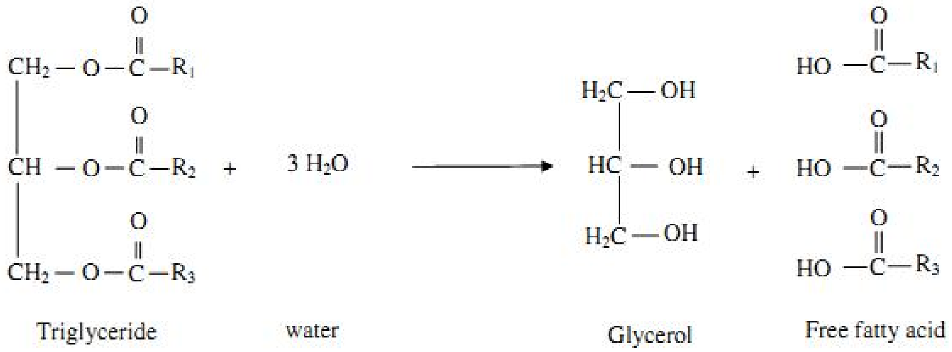


(2.4)

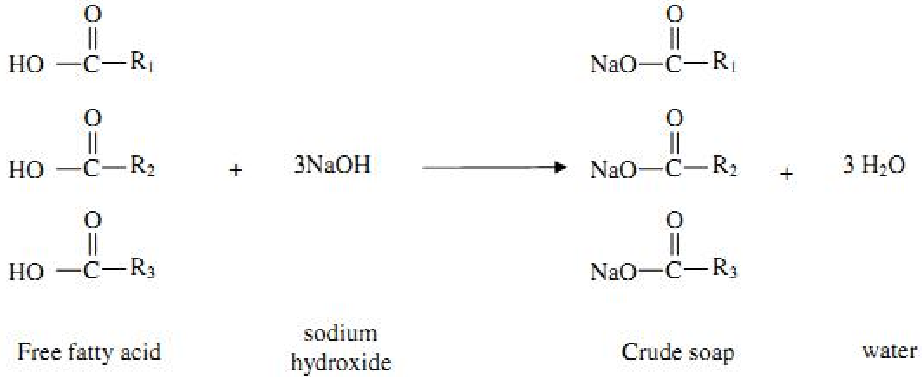
However, due to reversible reactions, excess of alcohol is required to increase biodiesel yield at faster rate and to facilitate biodiesel-rich phase separation from the glycerol-rich phase.

Reaction rates with alkaline-catalyzed transesterification are faster at moderate conditions with lesser catalyst concentration to obtain higher conversion in shorter reaction time than acid and enzyme catalyzed reaction [6, 17, 53]. Some of the relevant literature on alkali catalyzed transesterification of oils is presented in Table A.1. At least molar ratio of oil to alcohol of 1:6 during reaction time over 60 min is required to obtain biodiesel yield above 90% [58-63]. Higher molar ratio of oil to alcohol of 1:25 and addition of co-solvent, such as tetrahydrofuran, could shorten reaction time into 3-7 min using methanol or ethanol at room temperature to obtain the yield of biodiesel higher than 98% [64].

In alkali-catalyzed transesterification, triglyceride and alcohol must be considerably anhydrous. The presence of water in transesterification is able to hydrolyze the triglycerides and some of alkyl esters as well to produce free fatty acid and glycerol or alcohol. Thereafter, saponification occurs, that is free fatty acid reacts with alkaline hydroxide to generate soap and water as presented in reactions (2.9) and (2.10) [7, 16]. In addition, saponification can take place if triglyceride consists of high free fatty acid. Soap formation as side-reactions must be avoided since it consumes the catalyst and reduces the catalyst efficiency. Increasing biodiesel viscosity, gel formation, and problem of glycerol separation are the other negative effects of saponification.



(2.9)



(2.10)

In order to achieve maximum alkyl ester yield, refined vegetable oils should be anhydrous with a free fatty acid content of less than 1% and the alcohol should be moisture free [4, 15]. A comparison of different basic catalysts, CH_3ONa, CH_3OK, NaOH, and KOH was carried out for methanolysis of sunflower oil and cottonseed oil. Transesterification catalyzed by NaOH and KOH lowered biodiesel yields than that catalyzed by CH_3ONa and CH_3OK since hydroxide group reacted with methanol to produce methoxide and water. This water in transesterification caused saponification, resulting soaps which were dissolved in glycerol and affected biodiesel to be dissolved in glycerol as well [15, 65, 66].

Some of the recent literature on acid catalyzed transesterication is presented in Table A.2. Sulfuric acid showed the most effective catalyst to achieve >90 wt.% conversion of triglyceride with methanol [67-73].

The alternative method to produce biodiesel from high free fatty acid feedstock using homogeneous catalysts is by conducting two step transesterification. In this method, high free fatty acid is esterified by acid catalyst in the first step to reduce the free fatty acid content up to reach lower acid value which is suitable to be performed for next step. In this step fatty acid alkyl ester and water were generated from esterification of free fatty acid. The water and impurities should be separated from the vegetable oils or animal fats and fatty acid alkyl ester which are to be raw material for second step. The lower free fatty acid feedstock resulted in first step is transesterified using alkaline catalyst to produce fatty acid alkyl ester (biodiesel). Table A.3 shows some research works of two step transesterification of high free fatty acid of vegetable oils and animal fats. Generally, two step transesterification requires lower molar ratio of alcohol to oil and shorter reaction time than acid transesterification [33, 48, 52, 61, 74-82].

Enzymes can be used as biocatalyst in transesterification of vegetable oils. Enzymatic-catalyzed transesterification can be carried out in moderate process conditions (35-45°C reaction temperature) and simple product separation including simple glycerol recovery, minimal wastewater treatment and without side reactions, as well as more environmentally friendly. Nevertheless, the reaction rate of enzymatic-catalyzed transesterification is relatively lower and the cost of catalyst is more expensive than chemical-catalyzed transesterification, thus it is not commercially developed yet [19, 20, 53, 83-85].

2.5 *In-situ* transesterification

In transesterification process, the vegetable oil must be extracted from the oilseed before reacting it with alcohol to produce biodiesel. The oil extraction can be performed by mechanical extraction, such as expeller pressed oil, or chemical extraction using chemical solvent. The liquid-liquid or solvent extraction is used to remove the oil from the seeds. Non-polar solvent is required to extract oil, such as chloroform, n-hexane, isopropanol, etc. The oil extraction is followed by oil separation from the solvent and purification (degumming, deacidification, dewaxing, dehydration, etc.) from the impurities. These multiple processes increase the cost of oil which account for 75% of the total production costs of biodiesel production when refined oil is used as a feedstock [1, 86]. To reduce the processing time and cost of biodiesel, oil in vegetable oil seeds can be reacted with alcohol directly. This process is known as *in-situ* transesterification where oil extraction and transesterification can be carried out simultaneously.

The concept of *in-situ* transesterification was proposed by Harrington and D'Arcy [23]. They investigated *in-situ* transesterification of sunflower oilseeds and compared with the conventional method of transesterification of pre-extracted sunflower oil with acidified methanol. Higher fatty acid methyl ester yield obtained by *in-situ* than conventional method had been demonstrated. The fatty acid methyl ester composition analyzed by GLC and cloud point of product from *in-situ* transesterification was comparable with the product from conventional method. Table 2.3 shows the previous research works in *in-situ* transesterification of various oilseeds.

Table 2.3: *In-situ* transesterification

Reference	**Feedstock**	**Alcohol**	**alcohol:oil**	**Catalyst**	**amount of catalyst**	**Dp (µm)**	**T (°C)**	**t**	**BD yield (%)**
Shuit, S.H., *et al.*, 2010 [35]	*Jatropha curcas* seeds	Methanol	7.5:1 (mL·g^{-1})	H_2SO_4	15 wt.%	< 0.335 mm	60	24 h	91.02
Kasim, F.H. and Harvey, A.P., 2011 [37]	*Jatropha curcas seeds*	Methanol	400:1 (mol·mol^{-1})	NaOH	0.15 N	< 0.71 mm	60	1 h	86.9%
Hailegiorgis, S.M., et al. 2011 [87]	*Jatropha curcas seeds*	Ethanol	7.5:1 (mL·g^{-1})	CTMAB+ NaOH NaOH/oil	1 mol/mol 0.675 wt.%	500 µm	30	150 min	99.5%
Ginting, M.S.A., et al. 2012 [36]	*Jatropha curcas seeds*	Ethanol	140:20 (mL·g^{-1})	NaOCH3	2.0 wt.%	500 µm	30	2 h	99.98%
Kartika, I.A., et al., 2013 [88]	*Jatropha curcas seeds*	Methanol	6:1 (mL·g^{-1})	KOH	0.075 mol/L		50	5 h	80%
Hailegiorgis, S.M., et al., 2013	*Jatropha curcas seeds*	Methanol	5.92:1 (mL·g^{-1})	BTMAOH + NaOH	1.42:1 (mol/mol)	500 µm	38	1.72 h	89.8%
Harrington, K.J. and D'Arcy-Evans, 1985 [23]	Sunflower oilseeds	Methanol	200:20 (mL·g^{-1})	H_2SO_4	30 (mL)	coarse sand	60	4 h	45.5% (seed basis)
Georgogianni, K.G., et al., 2008 [29]	Sunflower seeds	Methanol 600 rpm	200:20 (mL·g^{-1})	NaOH	2.0 wt.%	coarse sand	60	2 h	97%
		Methanol 24 kHz (us)	200:20 (mL·g^{-1})	NaOH	2.0 wt.%	coarse sand	60	40 min	97%
		Ethanol 600 rpm	266:20 (mL·g^{-1})	NaOH	2.0 wt.%	coarse sand	80	4 h	90%
		Ethanol 24 kHz (us)	266:20 (mL·g^{-1})	NaOH	2.0 wt.%	coarse sand		40 min	98%

Reference	Feedstock	Alcohol	alcohol:oil	Catalyst	amount of catalyst	Dp (μm)	T (°C)	t	BD yield (%)
Zeng, et. al., 2009 [89]	Sunflower seeds	Methanol + DEM	101.39:1 57.85:1 (mol·mol^{-1})	NaOH	0.5:1 (mol·mol^{-1} of oil)	-	20	13 min	97.7%
Ozgül, S. and Türkay, S., 1993 [30]	Rice bran	Methanol	150:50 (mL·g^{-1})	H_2SO_4	2.5 mL	-	-	4 h	85.9%
		Ethanol	150:50 (mL·g^{-1})	H_2SO_4	2.5 mL	-	-	4 h	98.7%
Lei, H., et al., 2010 [31]	Rice bran	Methanol	75 (MeOH) + 150 (ether) : 50 (mL·g^{-1})	H2SO4 NaOH	0.75 g 0.71 g	16 mesh	60	3 h	98.83% 80.47%
Kildiran, G., *et al.*, 1996 [25]	Flaked soybean	methanol	150:50 (mL·g^{-1})	H_2SO_4	6 mL	0.5 – 1 mm	65	3 h	41.5%
		ethanol	150:50 (mL·g^{-1})	H_2SO_4	6 mL	<0.5mm, <1 mm	78	3 h	80.9%
		n-propanol	150:50 (mL·g^{-1})	H_2SO_4	6 mL	<0.5 mm,<1 mm	78	3 h	84.6%
		n-butanol	150:50 (mL·g^{-1})	H_2SO_4	6 mL	<0.5 mm,<1 mm	78	3 h	78%
Haas, M.J., et al., 2004 [24]	Flaked soybean	Methanol	226:1 (mol·mol^{-1})	NaOH	1.6 M	0.28–0.35 mm	60	8 h	84%
		Methanol	543:1 (mol·mol^{-1})	NaOH	2.0 M	0.28– 0.35 mm	23	8 h	84%
Haagenson, D.M, *et al.*, 2010 [26]	Rapeseed	Methanol	275:1 (mol·mol^{-1})	KOH	1.05 M	86.5%(20 mesh) 13.5%(50 mesh)	60	6 h	80%
Zakaria, R. and Harvey, A.P, 2012 [27]	Rapeseed	Methanol	670:1 (mol·mol^{-1})	NaOH	0.1 mol·kg^{-1}	300-500 μm	60	1 h	85%
El-Enin, S.A.A., et al., 2013 [90]	Rapeseed	Methanol	720:1 (mol·mol^{-1})	KOH	0.02 N	coarse sand	65	1 h	90%
Obibuzor, J.U., *et al.*, 2003 [91]	Waste pulp of palm seeds	Methanol	35.16:1 (mol·mol^{-1})	H_2SO_4	15 mL		68	12 h	97.20%

Reference	Feedstock	Alcohol	alcohol:oil	Catalyst	amount of catalyst	Dp (µm)	T (^{0}C)	t	BD yield (%)
		Ethanol	35.16:1 (mol·mol^{-1})	H_2SO_4	15 mL		78	12 h	99.35%
Ehimen, E.A., *et al*., 2010 [38]	Microalgae	Methanol	315:1 (mol·mol^{-1})	H_2SO_4	0.04 mol	powder	60	2 h	92%
Velasquez- Orta	*Chlorella*	Methanol	600:1	NaOH	0.15 mol·L^{-1}	powder	80	75 min	77.6%
S.B., *et al*., 2012 [92]	*vulgaris*	Methanol	600:1	H_2SO_4	0.35 mol·L^{-1}	powder	80	2 h	96.8%
Qian, J., *et al*., 2008 [32]	Cotton seeds	Methanol	135:1 (mol·mol^{-1})	NaOH	0.1 M	0.3– 0.335 mm	40	3 h	98%
Madankar, C.S., *et al*., 2013 [93]	Castor seed	Methanol	250:1 (mol·mol^{-1})	KOH	1 wt.%	-	65	3 h	96.1%
[94]		Ethanol	6.5:1 (mL·g^{-1})	+ NaOH	1.62:1 (mol·mol^{-1})	500 µm	35	1.54 h	99.4%
Sulaiman, S., *et al*., 2013 [95]	solid coconut waste	Methanol	10:1 (mL·g^{-1})	KOH	2.0 wt.%	-	62	7 h	88.5%
Jairurob, P., *et al*. 2013 [96]	after-stripping sterilized palm fruit	Methanol	225:1 (mol·mol^{-1})	KOH	3.85% (w/v)		60	9 h and 36 min	97.25%

DEM: diethoxymethane

CTMAB: cetyltrimethylammonium bromide

BTMAOH: Benzyltrimethylammonium hydroxide

The main factors affecting *in-situ* transesterification including the moisture content, molar ratio of oil to alcohol, catalyst concentration, reaction temperature, reaction time and particle size are discussed in the following section.

2.5.1 Moisture content

The moisture content of oilseed affected the biodiesel yield produced from *in-situ* transesterification, increasing moisture content of the seeds reduced the yield of product since it causes the formation of hydroxide ions, which induce saponification of free fatty acids, triglycerides and esters as shown in reactions (2.9) and (2.10). The resulting soaps cause an increase in viscosity, formation of gels and foams, and made the separation of glycerol difficult [4, 27, 97]

Harrington and D'Arcy [23] investigated the effect of moisture content in sunflower seeds in the process of *in-situ* methanolysis with sulfuric acid as catalyst. They observed that ester yields are generally improved as water is excluded from the transesterification reaction.

Haas, M.J., *et al*. [97] conducted *in-situ* methanolysis of soybean flaked catalyzed by sodium hydroxide. Removing the moisture content in flaked soybean by drying the substrate, a significant reduction in the reagent requirements was achieved. Drying the flaked soybean had eliminated the moisture content from 2.6% to 0% moisture. This condition enhanced the production of fatty acid methyl ester from 97% to 100%. The methanol volume was reduced 60% and sodium hydroxide concentration was decreased 56%.

Zakaria, R. and Harvey, A.P. [27] in direct biodiesel production by *in-situ* methanolysis of rapeseed using sodium hydroxide as alkali catalyst investigated the effect of drying the seed. It was observed that the moisture content more than 6.7 wt.% in the seed substantially decreases the yield of ester.

2.5.2 Type of alcohol

Type of alcohol affects the biodiesel yield since in this process, extraction and transesterification proceed simultaneously. Alcohols with high ability to extract oil from the oilseeds and ability to transesterify it are required to obtain high yield of biodiesel.

Ozgül and Türkay [30] investigated *in-situ* esterification of rice bran with methanol and ethanol in presence of sulfuric acid as acid catalyst. Solubility of oil in ethanol is higher than in methanol and hence the ethyl ester yield was higher than methyl ester. However, purity of methyl ester is better than ethyl ester due to easier separation.

Kildiran, *et al*. [25] investigated *in-situ* alcoholysis of flaked soybean with methanol, ethanol, n-propanol, and n-butanol. The solubility of triglycerides in alcohols was affected by the chain length and molecular weight of alcohol, the longer chain length and the greater molecular weight of alcohols caused the higher triglyceride solubility in alcohols. Among the alcohols, methanol was lacking in soybean oil extraction from the seeds. *In-situ* alcoholysis was influenced by this solubility, hence lower triglyceride conversion into methyl ester, less than 60%, was obtained. Meanwhile, 98% ethanol, n-propanol, and n-butanol had higher solubility of triglyceride, which converted into higher alkyl ester. The highest ester content of 84.6% was obtained by *in-situ* propanolysis. However, alkyl ester produced by *in-situ* ethanolysis, propanolysis, and butanolysis had higher free fatty acid contents than the crude soybean oil in the seeds. The purity of biodiesel could enhance by increasing reaction time and temperature and by decreasing the particle size of soybeans.

Özgül-Yücel and Türkay [98] investigated the effect of various monohydroxy alcohols (methanol, ethanol, n-propanol, 2-propanol, and n-butanol) on *in-situ* esterification and extraction of high free fatty acid rice bran oil catalyzed by sulfuric acid. They found that, during oil extraction, above 90% of free fatty acid content was soluble in alcohol. The molecular weight and chain length of alcohols had no effect on the free fatty acid solubility. However these factors affected on the solubility of neutral oils in alcohol, increasing the molecular weight and chain length of alcohol enhanced this solubility. *In-situ* esterification occurred through dissolution and esterification of free fatty acid to produce monoesters. The presence of this monoester had lessened the solubility of neutral oil components in alcohol. Thus, the highest alkyl ester content could be achieved by *in-situ* esterification of the highest free fatty acid rice bran oil, because the alkyl ester content mostly affected by neutral oil solubility.

2.5.3 Molar ratio of alcohol to oil

Molar ratio of triglyceride to alcohol is one of the important factors which influence the yield of *in-situ* transesterification. Increasing the molar ratio of triglyceride to alcohol enhances the yield of alkyl ester.

Kaul, *et al*. [34] conducted parametric study of jatropha seeds by reactive extraction with methanol and sodium hydroxide as alkali catalyst. They showed that rising seed to solvent ratio (w/w) from 1:2.6 to 1:7.8 along with increasing sodium hydroxide concentration from 0.05 M to 0.1 M with the particle size of more than 2.46 mm augmented the conversion of jatropha oil to biodiesel from 23.8% to 98.82%.

Kasim and Harvey [37] investigated the influence of various parameters on reactive extraction of *Jatropha curcas* L. with methanol catalyzed by sodium hydroxide. They explained that the methanol volume requirement in reactive extraction is very high compared to the conventional process to drive the penetration of alkaline methanol into the seed. Biodiesel was produced if methanol-oil molar ratio was more than 100:1. The yield of biodiesel enhanced from 52% to 86.9% by increasing methanol-oil molar ratio from 200:1 to 600:1. Further rising methanol-oil molar ratio did not significantly increase the yield.

Kartika, *et al*. [88] reported solvent extraction and *in situ* transesterification of jatropha seeds in a single step with methanol and n-hexane as co-solvent catalyzed by potassium hydroxide. They demonstrated that increasing methanol to seed ratio from 2:1 to 6:1 (mL/g) enhanced the yield of biodiesel from 35% to 80%.

Hailegiorgis, *et al*. [94] undertook parametric study and optimization of *in situ* transesterification of *Jatropha curcas* L. assisted by benzyltrimethylammonium hydroxide as a phase transfer catalyst. They demonstrated that increasing the ratio of methanol to *J. curcas* seeds up to 6.5:1 (v/w) has a positive effect on the yield of biodiesel. However, further rising the ratio of methanol to *J. curcas* seeds over 6.5:1

has a negative effect on biodiesel yield. The overloading of methanol volume could dissolve biodiesel in the glycerol phase which reduced the yield.

2.5.4 Catalyst type and concentration

Catalyst concentration can influence the biodiesel yield. *In-situ* transesterification can be alkaline-catalyzed and acid-catalyzed dependence on the free fatty acid and moisture content in the feedstock. Lower catalyst concentration is insufficient to complete the conversion of triglyceride to biodiesel. Increasing catalyst concentration accelerates the reaction that can enhance the conversion and biodiesel yield to achieve maximum result at certain catalyst concentrations. Further addition of catalyst concentration will not speed up the reaction rate and insignificantly enhance the conversion of oil to biodiesel as well as its yield.

Ginting, *et al*. [36] investigated *in-situ* ethanolysis of *Jatropha curcas* catalyzed by sodium hydroxide, potassium hydroxide and sodium methoxide with various concentration of 0.5; 1.0; 1.5; 2.0 and 2.5 wt.% at 70°C for 2 h. Increasing sodium methoxide concentration from 0.5 wt.% to 2.0 wt.% enhanced the biodiesel yield. Further increasing catalyst concentration the ethyl ester was apparently constant. Meanwhile, increasing sodium and potassium hydroxides from 0.5 wt.% to 2.5 wt.% raised the ethyl ester yield. It demonstrated that for reaction completion, the amount of sodium methoxide required was less than that of sodium hydroxide and potassium hydroxide. This is because the sodium methoxide has no significant effect on the triglyceride saponification. Only hydroxide group would promote the triglyceride saponification which resulted in lower biodiesel yield.

Rising sodium hydroxide concentration from 0.1 N to 0.15 N into the reactive extraction of *J. curcas* enhanced the yield of biodiesel from 76.2% to 87.8%. Nevertheless, further addition of sodium hydroxide concentration of 0.2 N decreased the yield of biodiesel into 80.8% since the emulsions started to form caused by saponification and hydrolysis [37].

Hailegiorgis, *et al*. [87] reported that the presence of cetyltrimethylammonium bromide (CTMAB) as phase transfer catalyst could enhance the yield of biodiesel produced by *in situ* ethanolysis of *Jatropha curcas* L. catalyzed by sodium hydroxide.

The highest yield of fatty acid ethyl ester was 89.1% at NaOH concentration of 1.013 wt.% without CTMAB. In the presence of CTMAB with molar ratio of CTMAB/NaOH of 1, the yield of fatty acid ethyl ester enhanced into 98.8% at NaOH concentration of 0.675 wt.%. Thus, the reactions assisted by CTMAB could reduce the concentration of NaOH by 33.3%.

Madankar, *et al*. [93] investigated parametric study of reactive extraction of castor oilseeds for methyl ester production catalyzed by potassium hydroxide at concentrations of 0.5–1.5% of oil and reaction temperature of 65°C with methanol–oil molar ratio of 250:1 and stirring speed of 600 rpm. The lower catalyst concentration, *i.e*. 0.5% of KOH is insufficient to the completion of the reaction after 3 h. At 1% KOH concentration, the yield of the reaction is 96% after 3 h. Potassium hydroxide concentration of 1% is the optimal catalyst concentration giving higher yield of castor oil methyl ester. Further increase in the catalyst concentration decreases the yield due to the high concentrations of alkaline catalyst which prevail the saponification reaction to form soaps.

2.5.5 Reaction temperature

Reaction temperature affects both the rate of diffusion and reaction and the yield of biodiesel. Commonly, an increase in reaction temperature accelerates the diffusion and reaction rate to be faster and enhance the conversion of triglyceride to biodiesel to achieve the maximum conversion and yield in a shorter time.

Hailegiorgis, *et al.* [94] investigated effect of reaction temperature in the range of 25°C to 65°C in parametric study and optimization of *in situ* ethanolysis of *Jatropha curcas* assisted by benzyltrimethylammonium hydroxide as a phase transfer catalyst. As the transesterification reaction between the immiscible phases is controlled by diffusion processes, the effect of temperature is expected to be very slight. An increase in temperature can promote saponification reactions as well; the yield of biodiesel can even decrease with temperature as observed.

Qian, *et al*. [32] performed *in-situ* alkaline transesterification of cottonseed oil for production of biodiesel with methanol and sodium hydroxide as catalyst. They reported that reaction temperature had little influence on the extraction and conversion of cottonseed oil. When increasing the reaction temperature from 30°C to 65°C, the amount of cottonseed oil dissolved in methanol was increased only from 97.7% to 99.7%, and the conversion to methyl ester was almost same. The optimum reaction temperature was 40°C for the *in-situ* alkaline transesterification for 3 hours reaction time.

Siler-Marinkovic and Tomasevic [28] demonstrated that in the process of *in-situ* transesterification of sunflower oilseeds, high yield and good biodiesel quality was achieved in high molar ratio of methanol to seed (200:1 or higher) and a strong sulfuric acid catalyst (100% on oil basis). Increasing reaction temperature (65°C) could reduce reaction time (1 hour) to complete the conversion of methyl ester. The maximum crude methyl ester yield of 98.2% was obtained with the molar ratio of methanol to oil of 300:1, a strong acidic catalyst (100% on oil basis), at reaction temperature of 65°C, during 4 hours reaction time. However, it is possible to obtain a good quality of ester after 4 h of heating at 30°C.

2.5.6 Reaction time

Reaction time is required to complete the transesterification of vegetable oils or animal fats and to achieve the maximum conversion of oil to biodiesel and the yield. Generally, at first the reaction proceeds slowly since of the slow dispersion of alcohol into the oil. After certain time, the reaction occurs faster to achieve the maximum conversion and yield of alkyl ester. Further reaction time will not improve the reaction rate where the conversion and yield are relatively constant. A longer reaction time decreases the conversion and yield of alkyl ester since the reverse reaction, which is the alkyl ester hydrolysis, tends to compete with the forward one which leads the depletion of biodiesel and results in more soap formations [99].

Effect of reaction time was studied by Kasim and Harvey [37] in reactive extraction of *Jatropha curcas* for biodiesel production The methyl ester yield, produced from reactive extraction with molar ratio of alcohol to oil of 400:1, NaOH concentration of 0.1 N, mixing speed of 400 rpm at reaction temperature of 60°C, showed minimal change after 30 min. It is very likely that the reactive extraction itself completed between 20 and 30 min. The reactions less than 20 min did not achieve high yields. This results were in agreement with another work by Kaul, *et al*. [34], who obtained high methyl ester conversions almost up to 30 min and beyond 30 min a constant conversion rate was observed. However, for high free fatty acid of *Jatropha curcas* longer reaction time was required to obtain the maximum methyl ester yield produced from *in-situ* esterification with methanol to seed ratio 7.5 mL/g and 15 wt.% sulfuric acid concentration at 60°C reaction temperature during 24 h reaction time [35].

The extraction and conversion of cottonseed oil was increased with the increase of reaction time. The oil dissolved in methanol was nearly 99% of the total oil, and 98% of this oil was converted into biodiesel within 3 h. Further increase in the reaction time from 3 to 5 h, caused a less effect on the extraction and conversion of cottonseed oil [32].

2.5.7 Particle size

The extraction and conversion of oil rely on the particle size. Particles with smaller size will have larger surface area that favors the extraction of oil out from the seeds.

The extraction and conversion of cottonseed oil were increased by decreasing the particle size of cottonseed flours. Under the optimum reaction conditions including molar ratio of methanol to oil of 135:1 with NaOH concentration of 0.1 mol/L and 40^0C reaction temperature, at the end of 3 h, the amount of cottonseed oil dissolved in methanol was 99.1% of the total oil and the conversion of this cottonseed oil was 98% for particle size between 0.3 and 0.335 mm. However, with further decrease in the particle size, the extraction and conversion of cottonseed oil kept nearly constant [32].

The similar effect was obtained by Shuit, S.H., *et al*. [35] in process of *in-situ* esterification of *Jatropha curcas* with sulfuric acid. The extraction efficiency gradually increased with higher reaction period. Within 8 h of reaction period, almost 50% of the oil content in the seeds has already been extracted out. Initially, both ranges of particle size, 0.355 to 1 mm and less than 0.355 mm, exhibited the same trend for extraction efficiency up to a reaction period of 8 h. Beyond that, for particles ranging from 0.355 to 1 mm, the extraction efficiency was almost constant, while for particles with less than 0.355 mm, the extraction efficiency kept on increasing until it reaches more than 90% at a reaction period of 24 h.

On the contrary, Kaul, S., *et al*. [34] in reactive extraction of *Jatropha curcas* with methanol and sodium hydroxide catalyst investigated the effect of three different size of particles including Dp < 0.85 mm, 0.85<Dp<2.46 mm and Dp>2.46 mm. Increasing particle size enhanced the conversion of oil to methyl ester. The maximum methyl ester conversion of 98% was obtained using seed size more than 2.46 mm at weight ratio of seed to solvent (w/w) 1:7.8 and catalyst concentration 0.1 M.

2.6 Response surface methodology

Optimization of the reaction parameters is very important during conducting experiments. In conventional experiments, optimization is usually performed by varying a single factor while the other factors were fixed at a specific set of conditions. This method requires a large number of experiments and time consuming. Statistical experimental design, such as response surface methodology (RSM), can be used for optimization of reaction parameters in order to avoid the limitations of the classical method [86]. Response surface methodology is a set of methods that includes [100]:

a) Setting up a series (designing a set) of experiments that will result in suitable and reliable measurements of the interest response
b) Establishing a mathematical model that best fits the data compiled from the design selected in (a), by performing suitable tests of hypothesis regarding the model's parameters
c) Determining the optimum condition of the experimental factors that result in maximum or minimum value of the response

A response surface methodology is useful to develop, improve, and optimize processes. In most RSM problems, the true response function is unknown, which is assumed to be continuous in the independent variables within some specified region of interest, and an empirical model is often fit to the data as approximating functions. An empirical model can be linear function of independent variables (first-order model) or polynomial form (second-order model). The purpose of fitting empirical model is to provide a smooth curve that can summarize the data. The method of least square is used to estimate the parameters in the empirical models (linear or polynomial forms) [100]. By applying the RSM, the experimenter can obtain [101]:

- an understanding of how the independent variables affect the selected process response,
- the determination of any interrelationship among the independent variables,
- characterization of the combined effect that all independent variables may have on the process response.

An important aspect of RSM is the design of experiments to develop the model fitting of physical experiments and to select the points where the response should be evaluated. The selection of design of experiments has a large effect on accuracy of the approximation and the cost of constructing the response surface. In planning the experimentation, experimenter requires to select the factors or independent variables to be used in the experiment and choose the range of values and the number of levels of each factor to measure the effects of the factors on the response [102]. The use of coded variables instead of the original independent variables facilitates in experimental design, which are:

- computational ease and increased accuracy in predicting the model coefficients
- improved interpretability of the coefficient estimates in the model

Coding removes the units of measurement of independent variables. A coding formula for defining the coded variable, x_i, is:

$$x_i = \frac{2X_i - \left(X_{iH} + X_{iL}\right)}{X_{iH} - X_{iL}} \qquad i = 1,2,3,.... \tag{2.13}$$

where X_{iL} and X_{iH} are low and high levels of X_i, respectively [100].

In the design of experiments, low-degree polynomials (such as first-order model) are more selected than high-degree polynomials since of the simpler form (fewer numbers of terms). However, if the first-order model is not appropriate to fit the existence of response surface curvature, the first-order model is replaced with higher-order model, such as second-order model. There are two designs of experiment that most used for estimation of the parameters in second-order model, namely central composite design (CCD) and Box-Behnken design (BBD) [100, 103].

A central composite design comprises of

- A full (or fraction of a) 2^k factorial design, where k is the number of independent variables and the factor levels are coded to values of -1 and +1, which is called the factorial portion of the design. It contributes in estimation of linear terms and the interact
- n_0 center points ($n_0 \geq 1$) which provides an internal estimate of error (pure error) and contributes toward the estimation of quadratic terms
- Axial points on the axis of each design variable at a distance of α from the design center, which is called the axial portion of the design. They contribute in estimation of quadratic terms but do not contribute in estimation of interaction terms

The total number of design points is $N = 2^k + 2k + n_0$. The distance of α depends on the number of points in the factorial portion in the design (F), which leads the design to be rotatable central composite design to maintain the consistent and stable variance of the predicted response at points of interest,

$$\alpha = F^{\frac{1}{4}} \tag{2.14}$$

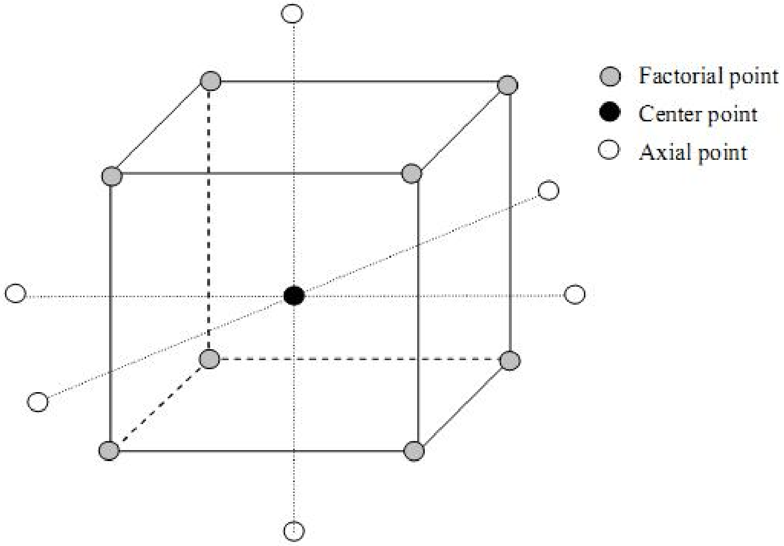


Figure 2.2: Central composite design for k = 3 [103]

Meanwhile, since rotatability is a spherical property, the region of interest is a sphere and the design must include center runs to provide stable variance of predicted response. Commonly, three to five center runs are recommended [100, 102, 103]. Figure 2.2 shows the central composite design for k = 3.

Box-Behnken design is three-level (3^k) incomplete factorial design which is formed by combining 2^k factorials with incomplete block designs for the estimation of the parameters in a second-order model. Box-Behnken design is a spherical or rotatable design, with all points located on a sphere of radius $\sqrt{2}$. Box-Behnken design does not consist of any points at vertices of the cubic region resulted by upper and lower limits for each variable. It is favored if the points on the corners of the cube indicate factor-level combinations that are expensive or impossible to test due to physical process restraints [102, 103]. Figure 2.3 displays Box-Behnken design for k = 3.

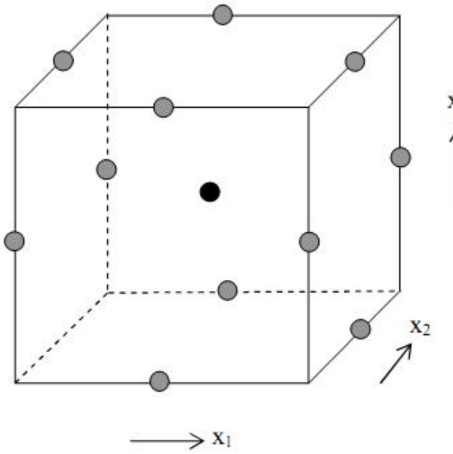


Figure 2.3: Box-Behnken design for k = 3 with a center [103]

A full 2^3 factorial central composite design had been applied in transesterification of rapeseed oil with methanol catalyzed by potassium hydroxide. Tri-, di-, and mono-glyceride concentrations were as response, whereas factors were catalyst concentrations, reaction temperature and time [104]. The same design of experiment was employed in transesterification of *Raphanus sativus* oil, refined and bleached lard, and crude mahua oil. In these researches, the molar ratio of oil to alcohol, catalyst concentration, and reaction temperature became independent variables to estimate the yield of biodiesel [101].

Haas, *et al*. [24] investigated in situ alkaline transesterification of flaked soybeans with methanol catalyzed by sodium hydroxide. Central Composite Response Surface design methods were applied to determine the effects and interactions of the volume of alkaline methanol, the NaOH concentration, and reaction time on the yields of fatty acid methyl ester (FAME), free fatty acid (FFA) content, and unreacted acylglycerol (AG). Best-fit equations correlating the experimental data with the composition of the reactions were constructed using SAS/STAT software. The maximal FAME yield with minimal contaminating FFA and AG was identified by numerical analysis of the equations and examination of the corresponding 3-D surfaces. The reactions were carried out at two different reaction temperatures of 23 ^{0}C and 60 ^{0}C. The highest methyl ester yields with minimal contamination of FFA and AG were predicted at 226:1:1.6 molar ratio of methanol/AG/NaOH during approximately 8-h incubation at 60°C reaction temperature. At room temperature of 23°C higher predicted methyl ester yield than at 60°C was obtained with 543:1:2.0 molar ratio of methanol/AG/NaOH during 8-h incubation. At this optimum condition 95% lipid in flaked soybeans was extracted and 84% lipid was transesterified into FAME.

Optimization of reactive extraction of castor seed to produce biodiesel had been carried out with methanol catalyzed by potassium hydroxide. During reaction, n-hexane (15 v/v % of methanol) was added as co-solvent to enhance oil extraction. A 2^4 full-factorial central composite design was used as a design of experiment with four factors including methanol to oil molar ratio (100:1–350:1), potassium hydroxide concentration (0.5–1.5%), different rotations (100–600 rpm) and reaction temperature (45–65°C) during 3 h reaction time to achieve the maximum yields of methyl ester. A total number of experiments of 30 were conducted and the results were analyzed by multiple regressions to construct a quadratic polynomial model which predicted the methyl ester yield. The predicted optimum conditions, comprising 225:1 methanol to oil molar ratio, 1.0 wt.% potassium hydroxide concentration, reaction temperature 55°C and mixing intensity of 350 rpm, which maximized the methyl ester yield of 88.4% were obtained by employing the second-order model. This predicted methyl ester yield had been verified by conducting experiment at optimum conditions and a very close methyl ester yield of 88.2% to prediction value was obtained [86].

The effects of molar ratio of alcohol to oil, catalyst concentration, reaction temperature and reaction time as well as the interactions between them on the yield of biodiesel had been studied in transesterification of soybean oil [105], waste rapeseed oil [106], pomace oil [107], waste cooking palm oil [108], *Jatropha curcas* oil [109], palm oil [110] using a full 2^4 factorial central composite design to estimate the response of biodiesel yield and parameters of the second-order empirical model.

Two step transesterification of waste frying oil with high free fatty acid had been carried out by utilizing a 3^3 factorial Box-Behnken design. In the first step, sulfuric acid concentration, reaction temperature, and reaction time were factors of the experimental design, whereas molar ratio of methanol to oil, potassium hydroxide concentration, and reaction temperature became factors in the second step. The second-order models were determined by fitting the experimental data to estimate their parameters and the response of fatty acid methyl ester content [111].

2.7 Reaction mechanism and kinetics of *in-situ* transesterification

In-situ transesterification of oil in seeds with alcohol is a complex process. Haas, *et al*. [24] and Qian, *et al*. [32] investigated *in-situ* (reactive extraction) transesterification of flaked soybean and cotton seed and observed that alkaline catalyst could destroy the cell walls and intracellular compartmentalization resulting in cellular solubilisation and subsequent transesterificaton of triglyceride. Zakaria and Harvey [27] investigated progress of *in-situ* methanolysis of rapeseed particles by observing them under a microscope before and after the reaction; the particles were stained with Sudan Black B. They reported that the addition of sodium hydroxide into the solvent does not destroy the cell wall structure and the lipid does not move radially outward into the bulk liquid. There was a reaction front that consumes the lipid similar to a shrinking core process as discussed by Levenspiel [112]. The alcohol in the bulk needs to reach oil in the particle by diffusion to react with oil in the particle. Solid-liquid reactions may be modeled in terms of shrinking core model concept developed for solid-gas non catalytic reactions. Resistance for the reaction include external mass transfer between bulk of liquid and particle surface, diffusion through particle pores to reach the unreacted core and reaction between the alcohol and oil. As alcohol and oil are sparingly soluble, the liquid-liquid reaction can be analyzed by the concept of phase transfer catalysis.

Starks, *et al*. [113] explained phase transfer mechanism as shown in Figure 2.4. Reaction between two immiscible liquids (RY and MX) to form (RX and MY) is made possible due to phase transfer catalyst forming an ion complex Q^+X^- which can react with RY to form RX and ion complex Q^+Y^- which can move to the other phase by mass transfer; the complex (Q^+Y^-) reacts in the other phase to form MY and another ion complex Q^+X^- which can mass transfer back to the first phase to continue the reaction.

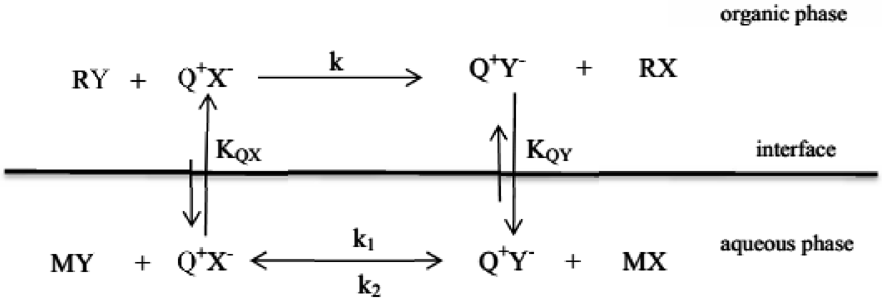


Figure 2.4: Starks extraction mechanism of phase transfer catalyst [113]

Hailegiorgis, *et al*. [114] proposed a reaction mechanism for transesterification of oil with alcohol using phase transfer catalysts.

2.8 Summary

Based on the literature, it is necessary to use non-edible oilseeds as the preferred feedstock for biodiesel. *In-situ* transesterification of non-edible oilseeds can be beneficial to keep the costs under control. It is necessary to identify optimal operating conditions to reduce reaction time. Understanding the reaction mechanism is a key to identify the factors affecting reaction time. Applicability of the information obtained in the laboratory scale units for a packed bed operation need to be investigated to help in design of large scale units. An attempt in these directions is presented in this thesis.

CHAPTER 3

RESEARCH METHODOLOGY

This chapter discusses about the description of the materials, equipment set-up, the experimental approach, and the details of analysis techniques.

3.1 Materials and Chemicals

In this work, the raw material used was *Jatropha curcas* which was obtained from Yogyakarta and Medan, Indonesia. To produce biodiesel some analytical grade chemicals were needed, such as methanol (> 99%), ethanol (>99%, denatured), 2-propanol (>99%), n-hexane (>99%), sodium hydroxide (>99%), and potassium hydroxide (85%), diethyl ether, n-heptane, acetic acid which had been purchased from Merck Sdn. Bhd., Malaysia. The following chemicals were supplied by Sigma-Aldrich Chemical Company, Malaysia: sodium methoxide 30 wt.% solution in methanol, pyridine (99%), glycerin, methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, N-Methyl-N-trimethyl-silyltriflouroacetamide (MSTFA), mono-, di-, tri-olein and tricaprin used for gas chromatography analysis.

3.2 Experimental Approach

The experiment was devided into two sections, the preliminary and main experiments. The preliminary experiments consists of the preparation of *Jatropha curcas* seeds up to the characterization of *Jatropha curcas* oil, that were determination of the acid value, solubility of alcohols in vegetable oil and extractability of oil in *Jatropha curcas* seeds by various alcohols. The main experiment involves *in-situ* transesterification of *Jatropha curcas* seeds, the biodiesel analysis, and reaction mechanism and kinetics studies.

3.2.1 Preliminary experiments

3.2.1.1 Preparation of Jatropha curcas seed

Before using in the process of *in-situ* transesterification, *Jatropha curcas* seeds must be prepared to obtain an appropriate form for experiment. The seeds were dehulled from their shells and dried in the oven at 80°C within 24 hours to remove the water contained inside the seeds. The seeds were ground using the household grinder to certain size and dried again in the oven at 80°C within 1 hour. The seeds were sieved using sieve shaker. The seeds were kept at 60°C in the oven to avoid the moisture absorption from the surrounding humidity level during experiment. Before used in the process of *in-situ* transesterification, the ground seeds were dried in the microwave at the maximum microwave power for 3 minutes in order to minimize the moisture content in the seeds. Microwave heating on the ground seeds could cause the cracking of biomass of seeds and could facilitate the extraction of oil from seeds.

3.2.1.2 Oil Extraction

The *Jatropha curcas* oil was extracted to characterize its properties. The oil extraction was carried out in Soxhlet extractor. *Jatropha curcas* seed was weighed 10, 20, and 30 grams without microwave heating pretreatment and placed in the thimble. Non-polar solvent, n-hexane with the purity more than 99% and the volume of 150 mL was used to extract *Jatropha curcas* oil and placed in round-bottom flask. Figure 3.1 shows the schematic of Soxhlet extraction for extracting *Jatropha curcas* oil.

The extraction time started when the n-hexane temperature reached its boiling point and stopped after 2, 4, and 6 hours to obtain more accurate data. The oil was separated from n-hexane using rotary evaporator and the oil content could be determined as presented in Eq. (3.1).

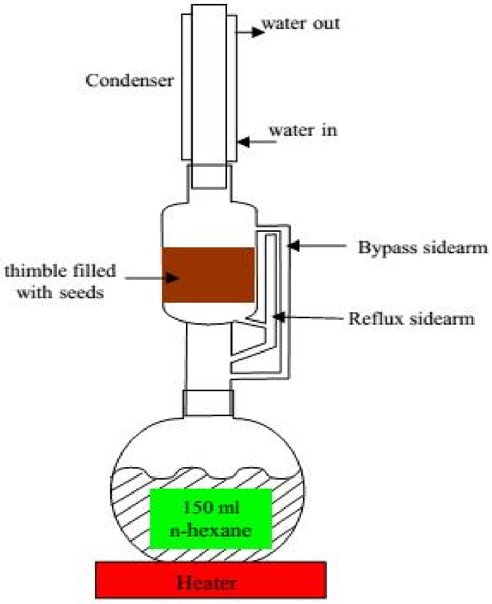


Figure 3.1: The schematic of Soxhlet extraction

$$OC = \frac{W_{oil}}{W_{seed}} \times 100\% \tag{3.1}$$

where,

OC : oil content (%)

W_{oil} : Jatropha curcas oil weight (g)

W_{seed} : Jatropha curcas seed weight (g)

3.2.1.3 Acid Value Determination

Acid value determination was conducted according to Official Methods and Recommended Practices of The American Oil Chemists' Society (AOCS Cd 3d-63), which is defined as the number of milligrams of potassium hydroxide necessary to neutralize the free acids in 1 g sample. Based on AOCS Cd 3d-63 the acid value is determined as,

$$A_V = \frac{(V_A - V_B) \times N \times 56.1}{W_{spl}} \tag{3.2}$$

where,

A_V : Acid value (mg KOH·(g sample)$^{-1}$)

V_A : Volume of standard alkali used in the titration (mL)

V_B : Volume of standard alkali used in the titrating the blank (mL)

N : Normality of standard alkali

W_{spl} : mass of sample (g)

Procedure to determine acid value based on AOCS Cd 3d-63 is as follows. Phenolphthalein indicator solution (1.0% in 2-propanol) of 2 mL was added to 125 mL solvent mixture consisting of equal parts by volume of 2-propanol and toluene in Erlenmeyer flask, and neutralized with standard alkali (potassium hydroxide) to a faint, permanent pink color (B). Ten grams *Jatropha curcas* oil was dissolved in these mixture and titrated with standard alkali to the first permanent pink color (A). The color must persist for 30 s.

3.2.1.4 Solubility of alcohols in vegetable oils

The equipment consists of 50 mL customized glassware equipped with a magnetic stirrer and thermometer, a burette, and a HACH portable turbidimeter Model 2100P.

In order to determine the solubility of methanol, ethanol and isopropanol, 20 mL of oil was poured into 50 mL customized glassware at 25°C and atmospheric pressure. The alcohol was transferred to a burette. Adding of alcohol into the oil was carried out by opening burette valve to take out a few drops of alcohol and the mixture was stirred for 10 minutes at 400 rpm of stirring speed. The mixture was left for 5 minutes. This step was repeated until the mixture was turbid. Figure 3.2 describes the schematic of solubility determination of alcohols in vegetable oil.

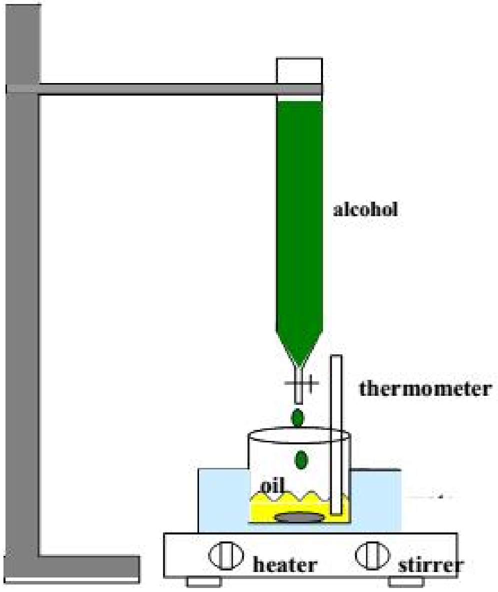


Figure 3.2: The schematic of solubility determination of alcohol

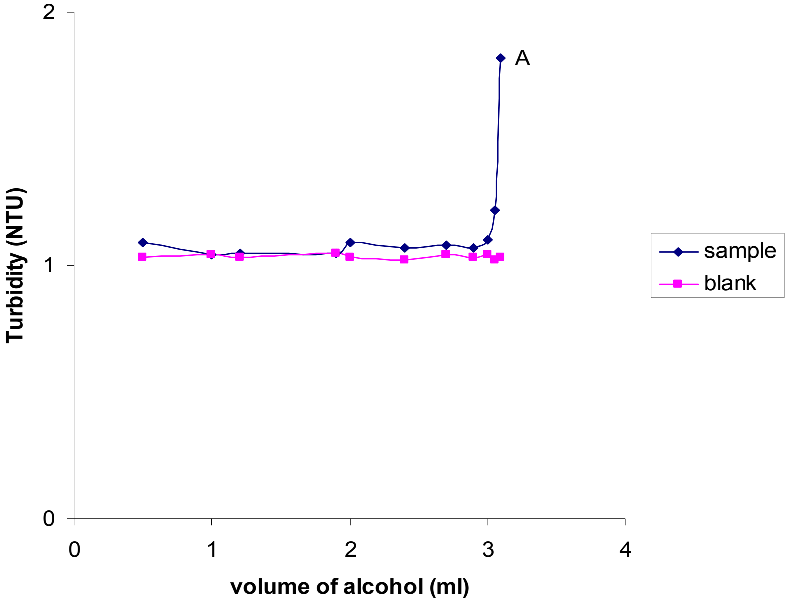


Figure 3.3: Turbidity value of the mixture of oil and alcohol, point A was the endpoint of alcohol solubility in oil

When the turbidity value of the mixture, measured by turbidimeter, exceeded its blank, the adding of alcohol was stopped because the alcohol did not dissolve in the oil anymore and some alcohol droplets appeared on the oil surface. Figure 3.3 shows turbidity value of the mixture of oil and alcohol. Point A was the endpoint of alcohol solubility in oil. The alcohol volume dissolved in the oil could be measured. The same method had been carried out to determine the alcohol solubility in the other types of oil for different temperatures (40°C and 60°C). The solubility determination of methanol at 60°C was carried out using the 50 mL customized and closed glassware equipped with a magnetic stirrer and thermometer to prevent the methanol loss. The experiment was conducted triplicate for every variable.

Table 3.1 presents the properties of vegetable oils, including the molecular weight, the density and the acid value of oils.

Table 3.1: The properties of vegetable oils

Types of oil	**M_r (g·mol^{-1}) [3,7,8,9]**	**Density (g·mL^{-1})**	**Acid value (mg KOH·(g sample)$^{-1}$)**
Palm oil	842.946	0.919	0.27
Corn oil	872.887	0.922	0.85
Sunflower oil	876.386	0.917	0.12
Canola oil	874.191	0.920	1.09
J. carcass oil	868.343	0.899	1.13

3.2.1.5 Extractability of oil in Jatropha curcas seeds by various alcohols

In order to determine the extractability of alcohols to *Jatropha curcas* oil, the soxhlet extractor with the same procedure as discussed in section 2 (oil extraction) was used. However, n-hexane was replaced with alcohols, methanol, ethanol, 2-propanol, the mixture of methanol and n-hexane, the mixture of methanol and 2-propanol as extraction solvents. Twenty grams *Jatropha curcas* seed was placed in the thimble and 150 mL extraction solvent was poured to the round-bottom flask. The extraction was carried out during 2 hours. The oil was separated from the extraction solvent using rotary evaporator and the oil content was determined by Eq. (3.1).

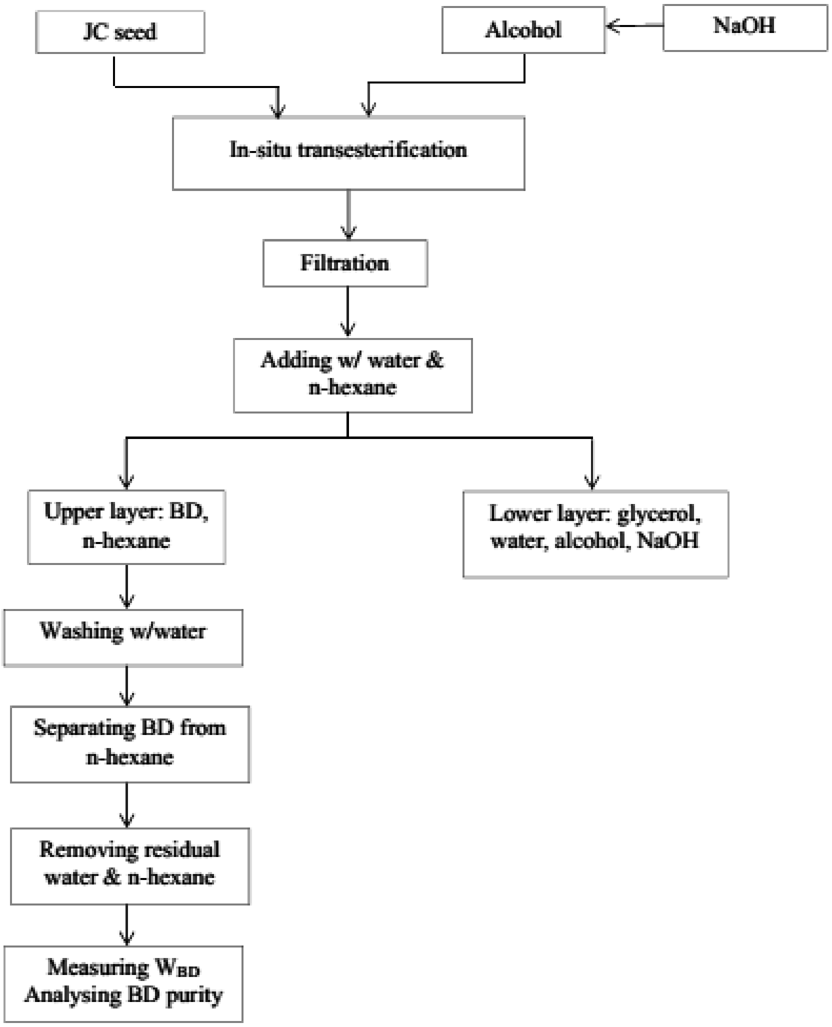


Figure 3.4: Scheme of *in-situ* transesterification procedures

3.2.2 *In-situ* Transesterification of *Jatropha curcas* seeds

Based on the result of solubility and extractability of alcohols to oil, *in-situ* transesterification of *Jatropha curcas* seed in stirred-flask was conducted in three different alcohols catalyzed by sodium hydroxide,

- 2-propanol/isopropanol (IPA)
- methanol (MeOH)

- the mixture of methanol and 2-propanol (MeOH+IPA)

The scheme of *in-situ* transesterification procedures involved in the present research is shown in Figure 3.4.

A certain amount of ground seeds was transferred into a 250 mL two-neck round-bottom flask. Sodium hydroxide as alkaline catalysts was dissolved into the alcohol. After the mixture was heated at the setting temperature, the solution was poured into the flask. The reaction was kept at reaction temperature, certain stirring speed during certain reaction time. Figure 3.5 describes *in-situ* transesterification in two-neck round-bottom flask. At the end of reaction, the mixture was separated by vacuum-filter. The filtrate obtained was transferred into separator funnel, added with water and extracted with n-hexane. Meanwhile, the seeds were disposed and the content of residual oil in seeds was not determined. The solution was left in separator funnel for 24 hours. Two layers were formed; the upper layer which contained biodiesel, n-hexane and a few of impurities was separated from lower layer which contained alcohol, glycerol, catalyst, and water. The upper layer was washed with water and evaporated using rotary evaporator. Biodiesel was placed in the oven at 70°C during 12 hours to remove residual water and n-hexane. The biodiesel was kept in dry bottle at room temperature for further analysis.

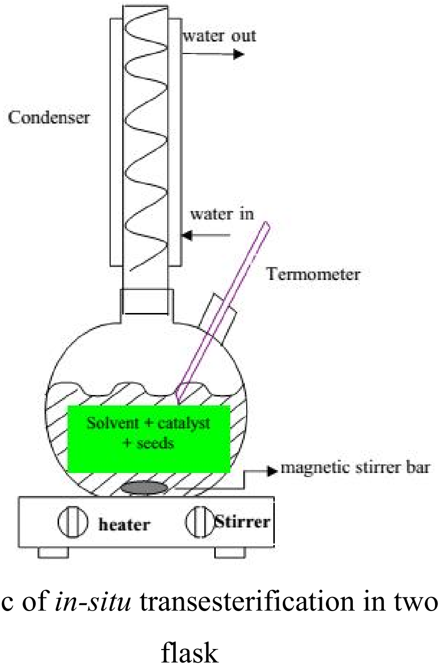


Figure 3.5: The schematic of *in-situ* transesterification in two-neck round-bottom flask

3.2.2.1 In-situ transesterification with isopropanol

The experiments of *in-situ* transesterification with isopropanol were performed by changing one independent variable at a time while holding the other independent variables constant. Three independent variables were investigated which including ratio of isopropanol volume to seed weight, NaOH concentration, and reaction temperature. In order to determine the effect of the ratio of isopropanol volume to seed weight on the biodiesel yield, *in-situ* transesterification of *Jatropha curcas* seeds with the particle size of less than 600 µm was carried out by varying the ratio of isopropanol volume to seed weight into 100:20 (5), 125:20 (6.25), 150:20 (7.5), 175:20 (8.75), 200:20 (10) using the constant of NaOH concentration of 1.0 wt.% at the constant of reaction temperature of 60°C for 2 h. The influences of sodium hydroxide concentration and reaction temperature on biodiesel yield were investigated by varying NaOH concentration of 0.5; 1.0; 1.25; 1.50; 1.75; and 2.0 wt.% with the constant of ratio of isopropanol volume to seed weight of 7.5 (150:20), at different reaction temperatures in the range of 30°C to 70°C during 2 h reaction time. The experiments were performed with the constant of stirring speed of 500 rpm.

3.2.2.2 In-situ transesterification with methanol

The preliminary experiments were done by testing three independent variables which were ratio of methanol volume to seed weight (6.25, 7.5, 8.75), NaOH concentration (1 wt.%, 1.5 wt.%, 2 wt.%), and reaction temperature (40°C, 50°C, 60°C). Meanwhile, variables of stirring speed, particle size, and reaction time were set at the constant values of 500 rpm, less than 600 µm, and 2 h, respectively. The experiments were carried out by changing one independent variable at a time while keeping the other independent variables constant.

Based on the preliminary experiment, the optimum reaction conditions which maximized the yield of biodiesel were determined by designing the experiments with response surface methodology, a central composite design (CCD) which tested three factors included the ratio of methanol volume to seed weight (6.25 - 8.75 $mL \cdot g^{-1}$), sodium hydroxide concentration (1.0 - 2.0 wt.%), and reaction temperature (40 -

60°C). The design of experiments are shown in Table 3.2. Variables of the stirring speed, the particle size, and reaction time were set at the constant values of 500 rpm, less than 600 μm, and 2 h, respectively.

Table 3.2: Test condition of the full 2^3 factorial design of methanol

Test	**Treatment combination**	**Coded factors**			**A**	**B**	**C**
		A	**B**	**C**	V_{MeOH}: W_{seed}	**[NaOH]**	**T (°C)**
1	0	0	0	0	7.50	1.5	50
2	(1)	-1	-1	-1	6.25	1.0	40
3	A	1	-1	-1	8.75	1.0	40
4	B	-1	1	-1	6.25	2.0	40
5	Ab	1	1	-1	8.75	2.0	40
6	C	-1	-1	1	6.25	1.0	60
7	Ac	1	-1	1	8.75	1.0	60
8	Bc	-1	1	1	6.25	2.0	60
9	0	0	0	0	7.50	1.5	50
10	Abc	1	1	1	8.75	2.0	60
11	-	-1.68	0	0	5.40	1.5	50
12	-	1.68	0	0	9.60	1.5	50
13	-	0	-1.68	0	7.50	0.66	50
14	-	0	1.68	0	7.50	2.34	50
15	-	0	0	-1.68	7.50	1.5	33.18
16	-	0	0	1.68	7.50	1.5	66.82
17	0	0	0	0	7.50	1.5	50

The effect of stirring speed on the biodiesel yield was studied by conducting *in-situ* transesterification with methanol at optimum conditions. The stirring speed was varied into 300, 400, 500, and 600 rpm for different reaction time up to 2 h at the optimum conditions of the ratio of methanol volume to seed weight, NaOH concentration, and reaction temperature. The particle size less than 600 μm was set to be constant.

The influences of reaction temperature for different reaction time on the yield of biodiesel were carried out by altering the reaction temperature of 40, 50, 55, and 60°C during reaction time of 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min. The other reaction variables were set to be constant, including the ratio of methanol volume to seed weight, NaOH concentration, 500 rpm stirring speed, less than 600 μm particle sizes.

The effect of particle size on the methyl ester yield was investigated by conducting *in-situ* transesterification for different particle size of Dp < 425 μm, 425 < Dp < 600 μm, 600 < Dp <1180 μm, and 1180 < Dp < 2000 μm during reaction time of 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min at the optimum reaction conditions with 500 rpm stirring speed.

3.2.2.3 In-situ transesterification with the mixture of methanol and isopropanol

In the experiments of the mixture of methanol and isopropanol, design of experiment using response surface methodology, based on a central composite design was used to test four independent variables, which were the ratio of the mixture volume to seed weight (7.5 – 10 $mL\cdot g^{-1}$), NaOH concentration (1.0 - 2.0 wt.%), the ratio of methanol volume to mixture volume (0.3 - 0.7 $mL\cdot mL^{-1}$), and reaction temperature (50°C - 70°C). Variables of stirring speed of 500 rpm, particle size less than 600 μm, and reaction time of 2 h were set to be constant. Thus, a full 2^4 factorial design was applied as presented in Table 3.3.

The influences of reaction temperature for different reaction time on the biodiesel yield were undertaken by altering the reaction temperature of 40, 50, 60, 65, and 70 °C during reaction time of 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min. The other reaction variables were set to be constant, including the ratio of the mixture volume to seed weight, NaOH concentration, 500 rpm stirring speed, particle size less than 600 μm.

Table 3.3: Test condition of 2^4 central composite design of the mixture of methanol and isopropanol

Test	**Treatment combination**	**Coded Factor**				**A**	**B**	**C**	**D**
		A	**B**	**C**	**D**	**V_{mix}: W_{seed}**	**[NaOH] wt.%**	**V_{MeOH} : V_{Mix}**	**T (°C)**
1	(1)	-1	-1	-1	-1	7.5	1.0	0.3	50
2	a	1	-1	-1	-1	10	1.0	0.3	50
3	b	-1	1	-1	-1	7.5	2.0	0.3	50
4	ab	1	1	-1	-1	10	2.0	0.3	50
5	c	-1	-1	1	-1	7.5	1.0	0.7	50
6	ac	1	-1	1	-1	10	1.0	0.7	50
7	bc	-1	1	1	-1	7.5	2.0	0.7	50
8	abc	1	1	1	-1	10	2.0	0.7	50
9	d	-1	-1	-1	1	7.5	1.0	0.3	70
10	ad	1	-1	-1	1	10	1.0	0.3	70
11	bd	-1	1	-1	1	7.5	2.0	0.3	70
12	abd	1	1	-1	1	10	2.0	0.3	70
13	cd	-1	-1	1	1	7.5	1.0	0.7	70
14	acd	1	-1	1	1	10	1.0	0.7	70
15	bcd	-1	1	1	1	7.5	2.0	0.7	70
16	abcd	1	1	1	1	10	2.0	0.7	70
17	0	0	0	0	0	8.75	1.5	0.5	60
18	0	0	0	0	0	8.75	1.5	0.5	60
19	0	0	0	0	0	8.75	1.5	0.5	60
20	-	-2	0	0	0	6.25	1.5	0.5	60
21	-	2	0	0	0	11.25	1.5	0.5	60
22	-	0	-2	0	0	8.75	0.5	0.5	60
23	-	0	2	0	0	8.75	2.5	0.5	60
24	-	0	0	-2	0	8.75	1.5	0.1	60
25	-	0	0	2	0	8.75	1.5	0.9	60
26	-	0	0	0	-2	8.75	1.5	0.5	40
27	-	0	0	0	2	8.75	1.5	0.5	80

The effect of particle size on the methyl ester yield was investigated by conducting *in-situ* transesterification for different particle size of Dp < 425 µm, 425 < Dp < 600 µm, and 600 < Dp <1180 µm during reaction time of 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 75, 90, and 120 min at the optimum reaction conditions with 500 rpm stirring speed.

3.2.2.4 In-situ transesterification with the mixture of methanol and n-hexane in Soxhlet extractor

Besides used to extract an oil and determine the extractability of alcohols to oil, Soxhlet extractor was employed to conduct *in-situ* transesterification of *Jatropha curcas* seed as presented in Figure 3.6.

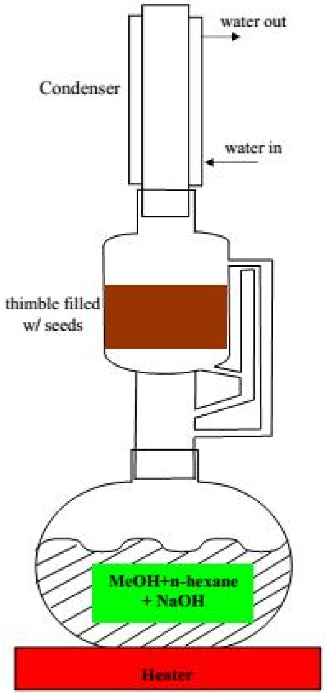


Figure 3.6: The schematic of *in-situ* transesterification in Soxhlet extractor

For this purpose, certain amount of seed was placed in thimble. Sodium hydroxide as alkaline catalyst was dissolved in the alcohol. Methanol was investigated for these *in-situ* transesterification. Due to the low extractability of methanol to *Jatropha curcas* oil, n-hexane was added to enhance the extractability to oil. The volume of mixture of catalyzed methanol and n-hexane was kept constant at 150 mL then was poured in the round-bottom flask. The extraction and transesterification was carried out in one step during 60 min at the temperature above the boiling point of the mixture of methanol and n-hexane. After that, the mixture in round-bottom flask was transfer into vacuum filter to separate the filtrate from the precipitate. The filtrate was placed in the separator funnel, added the water, and the solution was left for at least 3 hours. Two layers was formed, upper layer contained biodiesel, n-hexane, and a few of impurities (catalyst, alcohol, and glycerol) was separated from lower layer which consisted of alcohol, glycerol, and catalyst. Upper layer was washed with the warm water several times to remove the impurities up to the solution was clear. Biodiesel was separated from n-hexane by rotary evaporator and placed in the oven at 70°C during 12 hours to remove residual water and n-hexane. The biodiesel was kept in dry bottle at room temperature for further analysis.

Table 3.4: Test condition of Box Behnken design for the mixture of methanol and n-Hexane

Test	**Coded factors**			**A**	**B**	**C**
	A	**B**	**C**	**V_{mix}: W_{seed}**	**[NaOH]**	**V_{MeOH}:V_{mix}**
1	-1	0	-1	7.5	2.0	0.3
2	+1	0	-1	12.5	2.0	0.3
3	0	-1	+1	10	1.75	0.7
4	-1	-1	0	7.5	1.75	0.5
5	0	+1	+1	10	2.25	0.7
6	0	0	0	10	2.0	0.5
7	0	-1	-1	10	1.75	0.3
8	+1	-1	0	12.5	1.75	0.5
9	0	0	0	10	2.0	0.5
10	-1	+1	0	7.5	2.25	0.5
11	+1	+1	0	12.5	2.25	0.5
12	-1	0	+1	7.5	2.0	0.7
13	+1	0	+1	12.5	2.0	0.7
14	0	0	0	10	2.0	0.5
15	0	+1	-1	10	2.25	0.3

Three level design for the mixture of methanol and n-hexane was applied with factors: ratio of methanol volume (V_{MeOH}) to seed weight (W_{seed}) (7.5 - 12.5 $mL \cdot g^{-1}$), sodium hydroxide concentration (1.75 - 2.25 wt.%) and volume ratio of methanol to mixture (0.3 - 0.7 $mL \cdot mL^{-1}$). Response surface methodology, Box-Behnken design, a mathematical-statistical tool, was used for modeling the biodiesel yield. Tabel 3.4 presents design of this experiment.

3.2.3 Statistical design of experiments for optimization of *in-situ* transesterification

Each test of *in-situ* transesterification was replicated twice. Response surface methodology, a mathematical-statistical tool, was used to determine the biodiesel yield [22]. Second-order polynomials were used to describe the response surface for biodiesel yield.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i<j}^{n} \beta_{ij} x_i x_j \tag{3.3}$$

where Y is predicted response, β_0 is intercept coefficient, β_i is linear coefficient, β_{ii} is quadratic coefficient, β_{ij} is interaction coefficient, x_i and x_j are independent factors. The central point test was replicated three times to produce a good estimation of experimental error. Portable Statgraphics Centurion 15.2.11.0 statistical software (by StatPoint Inc.) was employed to estimate the response of dependent variable and to determine the interaction between factors, the effects, coefficients, standard deviation of coefficients and other parameters of the model. By fitting the experiment data to construct the second-order polynomials using multiple regressions, the response surface graph and contour plot can be obtained to determine the optimum condition of experiments.

3.3 Analysis of biodiesel using gas chromatography

The analysis of biodiesel was conducted to ensure the quality of biodiesel produced based on ASTM D 6584. Gas chromatography, QP 5000 series, Shimadzu Japan, 2100, was utilized to analyze the quality of biodiesel. The GC was equipped with an on-column injection, HT-5, with a diameter of 0.32 mm, a film thickness of 0.1 µm, a length of 30 m and flame ionization detector (FID). The temperature of column was set at initial temperature of 50°C for 1 minute. Then, it was increased to 180°C with the rate of 15°C·min^{-1}, followed by 7°C·min^{-1} up to 230°C. The rate was increased to 30°C·min^{-1} until the temperature of 380 ^{0}C and it was held for 10 minutes. The FID was set at 380°C and helium was used as carrier gas with the flow rate of 3 mL·min^{-1}.

In order to analyze the quality of biodiesel, standard solutions consist of glycerin, monoolein, diolein, triolein, and internal standards (tricaprin) were prepared according to Table 3.5. The components were weighed directly into the volumetric flasks and were diluted with pyridine to mark of the volumetric flasks.

Table 3.5: Stock solution for analyzing biodiesel quality

Compound	**Approximate mass (mg)**	**Volumetric flask size (mL)**	**Concentration (mmol/L)**
Glycerin	25	50	10.86
1-Mono [*cis*-9-octadecenoyl]-racglycerol (monoolein)	50	10	14.02
1,3-Di [*cis*-octadecenoyl]glycerol (diolein)	50	10	8.05
1,2,3-Tri [*cis*-octadecenoyl]glycerol (triolein)	50	10	5.65
1,2,3-Tridecanolylglycerol (tricaprin)- (internal standard 2)	50	10	9.01

Using stock solution, five standard solutions in Table 3.6 were prepared by transferring the specific volumes by microliter syringes to 10 mL septa vials. Each of the five standard solutions was added 100 µl of MSTFA. The vial was closed and shaken. The vial was allowed to stand for 15 to 20 min at room temperature. The solution was added approximately 8 mL n-Heptane to the vial and it was shaken.

Table 3.6: Standard solutions

Standard solution number	**1**	**2**	**3**	**4**	**5**
μl of glycerin stock solution	10	30	50	70	100
μl of monoolein stock solution	20	50	100	150	200
μl of diolein stock solution	10	20	40	70	100
μl of triolein stock solution	10	20	40	70	100
μl of tricaprin stock solution	100	100	100	100	100

The calibration standards were analyzed under the same operating conditions as the sample solutions. The reaction mixture of 1 μl was injected into the cool on-column injection port and the analysis was started. The chromatogram and peak integration were obtained. The calibration curve for each reference component could be prepared as presented in Figure 3.4. Table 3.7 describes the response factor and y-intercept of the calibration functions.

Table 3.7: Response factor and y-intercept of the calibration functions

Standard	**Response factor**	**y-intercept**
Glycerin	0.3003	0.0013
Monoolein	0.7864	0.0203
diolein	0.9838	0.0121
triolein	2.7076	0.0184

The ester, mono-, di-, and triglyceride content of the transesterification product were determined by gas chromatography (GC). The sample of 100 mg was weighed to the nearest 0.1 mg into 10 mL septa vial. The sample was added with 100 μl internal standard and 100 μl MSTFA. After shaking, the sample was allowed to stand for 15 to 20 min at room temperature. The sample was added with 8 mL n-Heptane and the vial was shaken. The reaction mixture of 1 μl was injected into the cool on-column injection port and the analysis was started. The chromatogram and peak integration were obtained. Peaks of chromatogram were identified by comparison of retention time to the standards as listed in Table 3.8.

Table 3.8: Relative retention time of reference standards

Component	**Relative retention time**
Glycerin	0.85
Monoolein	0.83-0.86
Tricaprin (internal standard 2)	1.00
Diglyceride	1.05-1.09
Triglyceride	1.16-1.31

After identifying the peaks, the areas of the peaks were measured and identified as glycerin, monoglyceride, diglyceride, and triglyceride. Using the slope and y-intercept of calibration functions, the mass of each component was calculated as follows.

$$G_i = \left[a_o \times \frac{A_{gi}}{A_{is}} + b_o \right] \times W_{is} \times \frac{100}{W} \tag{3.4}$$

where,

G_i : mass percentage of individual glyceride in sample

A_{gi} : peak area of individual glyceride

A_{is} : peak area of internal standard

W_{is} : weight of internal standard (mg)

W : weight of sample (mg)

a_o : slope of calibration function of mono-, di-, and triolein

b_o : intercept of calibration function of mono-, di-, and triolein

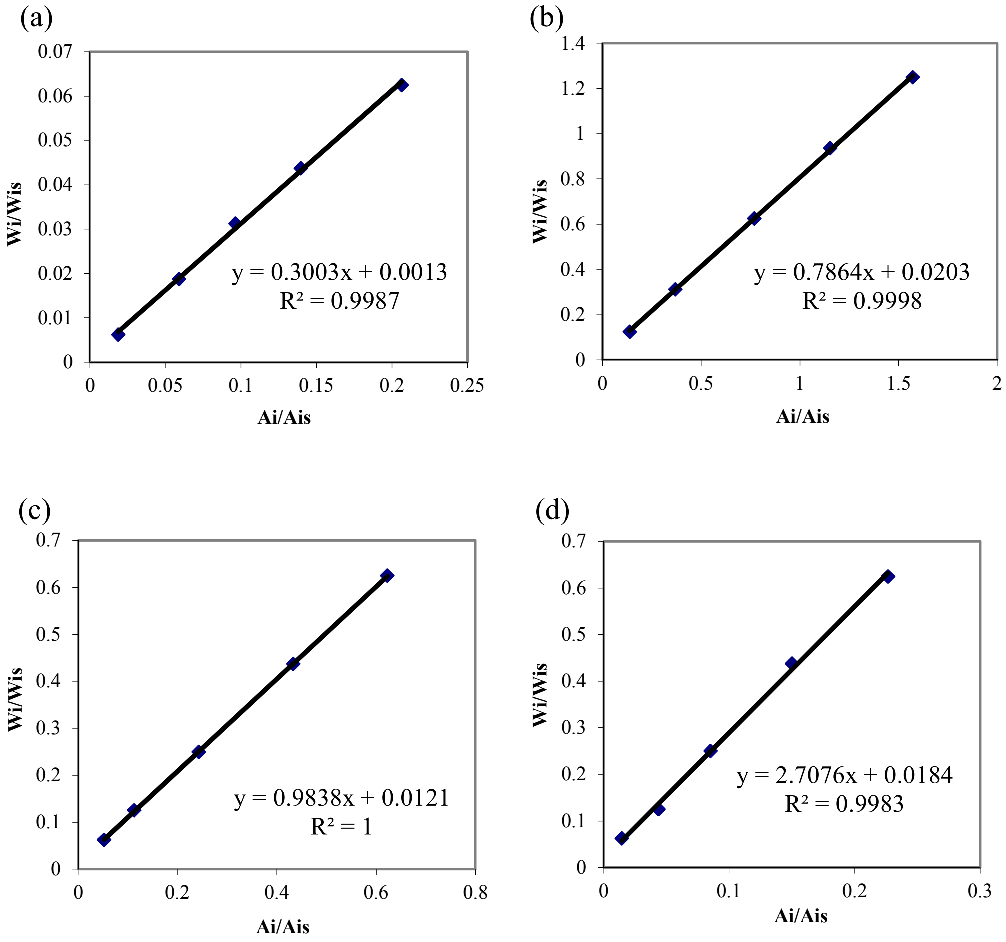


Figure 3.7: Calibration curve for the reference standards of (a) glycerin, (b) monoolein, (c) diolein, (d) triolein

Total glycerin and bound glycerin are defined as,

$$G_{Total} = G_{Free} + G_{Bound} \tag{3.5}$$

$$G_{Bound} = 0.2591 \cdot G_M + 0.1488 \cdot G_D + 0.1044 \cdot G_T \tag{3.6}$$

where,

G_M : mass percentage of monoglyceride

G_D : mass percentage of diglyceride

G_T : mass percentage of triglyceride

The yield of biodiesel was determined as the ratio of actual biodiesel yield to theoretical biodiesel weight and can be expressed as,

$$Y\ (\%) = \frac{W_{BD_{actual}}}{W_{BD_{theoritical}}} \times 100\% \tag{3.7}$$

$$W_{BD_{actual}} = W_{BD_{\exp}} (1 - \frac{G_{Total}}{100}) \tag{3.8}$$

3.4 Analysis of Biodiesel using gas chromatography/mass spectroscopy (GCMS)

The analysis of biodiesel using gas chromatography/mass spectroscopy was conducted to analyse the composition of fatty acids. The sample of 20 mg was accurately weighed and placed in a sample vial. Tricaprin solution (0.5 mg·mL^{-1}) of 100 μL and 20 μL of MSTFA were added to the sample vial. The solution was left to silylate for 15 min after that 0.5 ml of n-heptane was added. The GC was equipped with an on-column injection with a diameter of 0.32 mm, a film thickness of 0.1 μm, a length of 30 m. The temperature of column was set at initial temperature of 50°C for 1 minute. Then, it was heated to 180°C with the rate of 15°C·min^{-1}, followed by 7°C·min^{-1} to 230°C. The rate was increased to 10°C·min^{-1} until the temperature of 370 ^{0}C and it was held for 10 minutes for a total run time of 31.5 min. Helium was used as carrier gas with the flow rate of 1 mL·min^{-1}. The inlet line to the MS was kept at 270°C while the MS source temperature was kept at 250°C. The mass range scanned was 35 to 600 m/z. Peak identification was carried out by comparing mass spectra to the National Institute of Standards and Technology (NIST) 2005 mass spectral library (NIST, Gaithersburg, MD).

CHAPTER 4

RESULTS AND DISCUSSIONS

This chapter comprises the result of the experiments conducted according to previous chapter and discussions. Section 4.1 discusses characterization of *Jatropha curcas* including the properties of *Jatropha curcas* oil, solubility of alcohols in vegetable oils and extractability of *Jatropha curcas* oil by alcohols. *In-situ* transesterification of *Jatropha curcas* seeds with isopropanol is presented in section 4.2. Sections 4.3 and 4.4 demonstrate *in-situ* transesterification of Jatropha *curcas* seeds with methanol and that with the mixture of methanol and isopropanol, respectively. The designs of experiment based on central composite design are employed in these sections to determine the optimum reaction condition. Section 4.5 explains the results of *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and n-hexane in Soxhlet extractor.

4.1 Characterization of *Jatropha curcas*

4.1.1 Properties of *Jatropha curcas* Oil

The properties of oil consist of the oil content, density, acid value, and composition of fatty acid. The oil content was obtained by extracting oil from *Jatropha curcas* seed in Soxhlet extractor and determined using Eq. (3.1). The oil extraction was carried out with different seed weights of 10, 20, and 30 g without microwave heating pretreatment and extraction time of 2, 4, and 6 h to obtain an accurate value. Figure 4.1 depicts *Jatropha curcas* oil contents at various seed weights and extraction time. The effects of seed weight and extraction time on oil content were insignificant. The average oil contents resulted from seed weight of 10 g, 20 g, and 30 g were (49.48 ± 0.69)%, (49.43 ± 0.77)%, and (50.26 ± 0.86)% respectively. The average of all oil contents was (49.72 ± 1.34) %. This oil content was higher than that reported by Islam, *et al*. [115] which obtained the oil content of *Jatropha curcas* in the range of 40.0 to 48.4 wt.%. This oil content discrepancy was influenced by the difference in seed source or plant, cultivation climate, ripening stage, and the harvesting time of the seed.

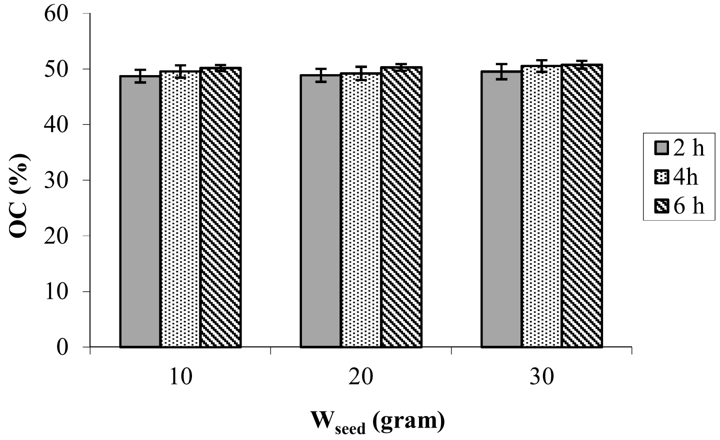


Figure 4.1: *Jatropha curcas* oil content without microwave pretreatment

The density of *Jatropha curcas* oil was measured using picnometer at room temperature three times with the average density of (0.8995 ± 0.0007) g·mL^{-1}. This density was in the range of 0.860 to 0.933 g·mL^{-1} that were the densities of *Jatropha curcas* oil which were obtained by Achten, *et al*. [116].

Acid value of *Jatropha curcas* oil was determined according to AOCS Cd 3d-63, that was (1.13 ± 0.10) mg KOH·g^{-1} which showed low free fatty acid content in *Jatropha curcas* oil as a feedstock in this research, which was 0.57%. This value was lower than the acid values of *Jatropha curcas* oil used by Kaul *et al*. [34], Sahoo and Das [74] which were 3.8 mg KOH·g^{-1}, Corro, *et al* [117] in the amount of 36.10 mg KOH·g^{-1}, Vyas, *et al* [118] and Qian, *et al*. [119] with the value around 10 mg KOH·g^{-1}, Berchmans and Hirata [48], Tiwari, *et al*. [120] that was around 28 mg KOH·g^{-1}. Thus, alkaline catalyst can be employed in the process of *in-situ* transesterification.

Figure 4.2 shows GCMS chromatogram of fatty acid composition of *Jatropha curcas* oil which comprised palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid. This *Jatropha curcas* oil contained 26.99% saturated fatty acids (palmitic and stearic acids), 39.53% mono-unsaturated fatty acids (palmitoleic and oleic acids), and 33.48% poly-unsaturated fatty acid (linoleic acid).

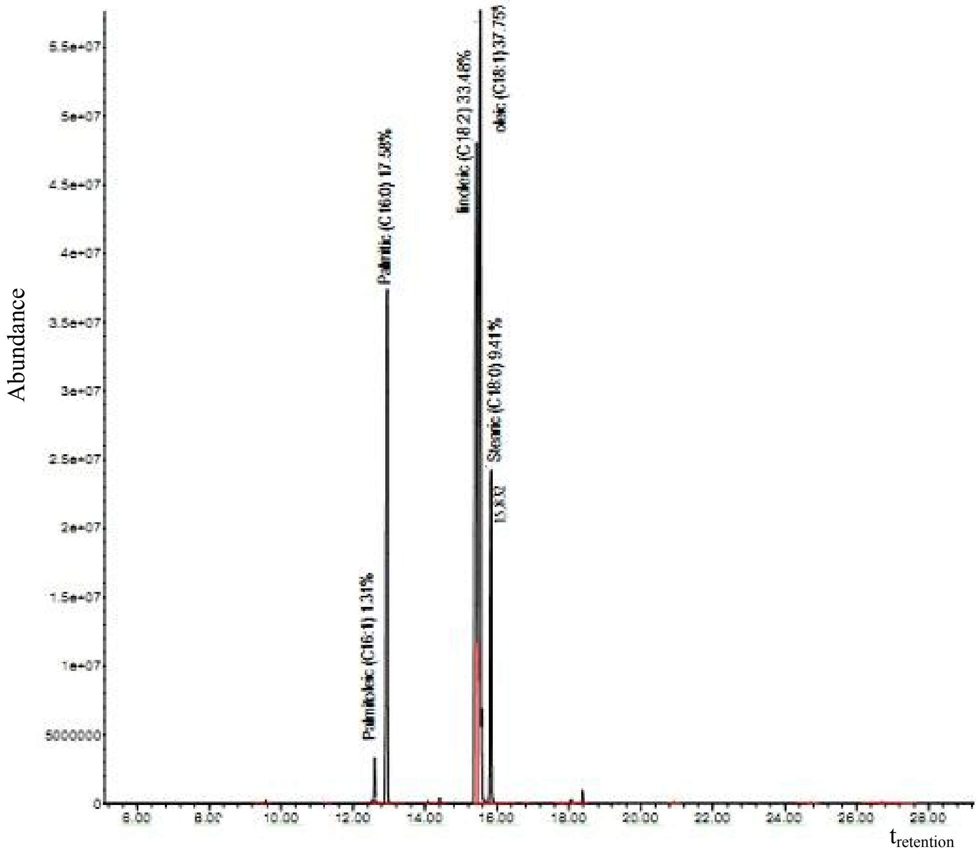


Figure 4.2: GCMS chromatogram of fatty acid composition of *Jatropha curcas* oil

This fatty acid composition was similar to another research conducted by Rathore and Madras [121] and in the range of fatty acid composition obtained by Berchmans and Hirata [48]. The properties of *Jatropha curcas* oil is summarized in Table 4.1.

Table 4.1: The properties of *Jatropha curcas* oil

1. Fatty acid composition :	
Palmitic acid ($C_{16}H_{32}O_2$)	17.58 %
Palmitoleic acid ($C_{16}H_{30}O_2$)	1.31 %
Stearic acid ($C_{18}H_{36}O_2$)	9.41 %
Oleic acid ($C_{18}H_{34}O_2$)	38.22 %
Linoleic acid ($C_{18}H_{32}O_2$)	33.48 %
2. Density ($g{\cdot}mL^{-1}$)	(0.8995 ± 0.0007)
3. Acid value ($mg\ KOH{\cdot}g^{-1}$)	(1.13 ± 0.10)
4. Oil content (wt.%)	(49.72 ± 1.34)

4.1.2 Solubility of Alcohols in Vegetable Oils

In this research, the solubility of methanol, ethanol and isopropanol in palm oil, sunflower seed oil, canola oil, corn oil and *Jatropha curcas* oil was carried out at temperatures 25, 40, 60°C and the results are presented in Figure 4.3.

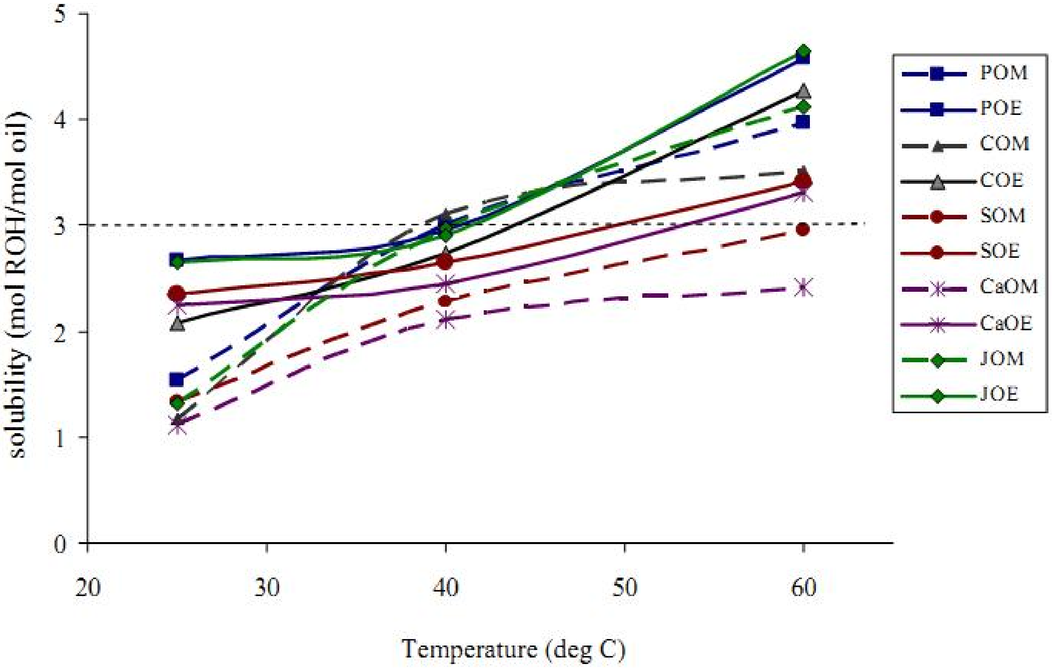


Figure 4.3: The solubility (molar ratio of alcohol to oil) of methanol (---) and ethanol (—) in palm oil (PO), sunflower seed oil (SO), canola oil (CaO), corn oil (CO) and *Jatropha curcas* (JO) oil at temperatures 25, 40, 60°C

It can be observed that the solubility of methanol and ethanol in vegetable oils enhanced with increasing temperature. Factors affected the solubility of a solute in a solvent are the strength of intermolecular force and the speed of molecules. High temperature or heating solution caused the molecules moving easier and faster between solvent and solute molecules. It led the molecules to be more disordered and more highly dispersed in structure. Intermolecular interactions between solute and solvent molecules will be formed and the solubility of a solute in a solvent will increase. However, the isopropanol solubility indicated a different result. Isopropanol was totally soluble in the vegetable oil. In this work, when 20 mL of vegetable oil was added with several drops of isopropanol, these were dissolved directly in the oil for a few minutes of slow stirring speed. The further isopropanol addition up to 40 mL (twice of oil volume) into the oil showed that it was totally dissolved. This isopropanol solubility occurred for all types of oil and temperatures.

The length of carbon chain in alcohol influences in the solubility. The solubility of methanol in vegetable oils was lower than the solubility of ethanol and the solubility ethanol was lower than the solubility of isopropanol in the same oil. In alcohol, the hydroxyl group (-OH) is a polar functional group because it has electronegativity difference between hydrogen and oxygen atoms in a bond. Meanwhile, the bonding of carbon and hydrogen, such as hydrocarbons or the hydrocarbon portion of a molecule with a functional group, is always non-polar. Thus, the longer carbon chains in alcohol, the more non-polar the alcohol [98].

Methanol has only one carbon atom, ethanol has two carbon atoms, and isopropanol has three carbon atoms. Therefore, methanol is more polar than ethanol and isopropanol, and ethanol is more polar than isopropanol. A polar solute can be soluble easily in a polar solvent and vice versa. Because the vegetable oils are non-polar compound, methanol is only slightly soluble in oil, compared to other alcohols. Isopropanol is a non-polar alcohol, in such a way that it can be dissolved easily in vegetable oils.

In transesterification process, the molar ratio of oil to alcohol stoichiometrically is 1:3; however the higher molar ratio is required to drive the reaction toward the product side in order to obtain a high ester conversion in a shorter time. In Figure 4.3, the solubility is defined as molar ratio of alcohol to oil. The solubility of methanol in vegetable oils are in the range of 1.12:1 to 1.53:1 at 25°C, 2.11:1 to 3.10:1 at 40°C, 2.41:1 to 4.12:1 at 60°C. Meanwhile, the solubility of ethanol in vegetable oils in the range of 2.08:1 to 2.67:1 at 25°C, 2.45:1 to 2.96:1 at 40°C, and 3.31:1 to 4.65:1 at 60°C. Based on this solubility, molar ratios of alcohol to vegetables oils over 3:1 were obtain at temperature above 40°C, thus transesterification of palm oil, corn oil, and *Jatropha curcas* oil could be conducted at temperature beyond 40°C if methanol or ethanol was used in this reaction, whereas for sunflower seed oil, the reaction only could be carried out at temperature more than 60°C. For canola oil, the reaction only could be performed using ethanol at temperature more than 60°C, since the solubility of methanol in canola oil at temperature up to 60°C was low, that was 2.41:1 molar ratio of methanol to canola oil. Because isopropanol was totally soluble in vegetable oils and of all temperatures, transesterification using isopropanol could be carried out at lower temperature than using methanol and ethanol. Although isopropanol could be dissolved easily in vegetable oils, using it in transesterification and separation process still required to be proven and compared with the other alcohols performance in biodiesel production.

4.1.3 Extractability of alcohols to *Jatropha curcas* oil

Another important factor in the process of *in-situ* transesterification of vegetable oil than the solubility of alcohol in vegetable oil is the ability of alcohol to extract oil from the vegetable oilseed (the extractability of oil by alcohol). In this study, methanol, ethanol, isopropanol, the mixture of methanol and n-hexane, the mixture of methanol and isopropanol were examined to extract oil from *Jatropha curcas* seeds and the oil contents were compared to theoretical oil content, that was oil content obtained from extraction using n-hexane in Soxhlet equipment. The results of the extractability of *Jatropha curcas* oil by alcohols and n-hexane are presented in Figure 4.4.

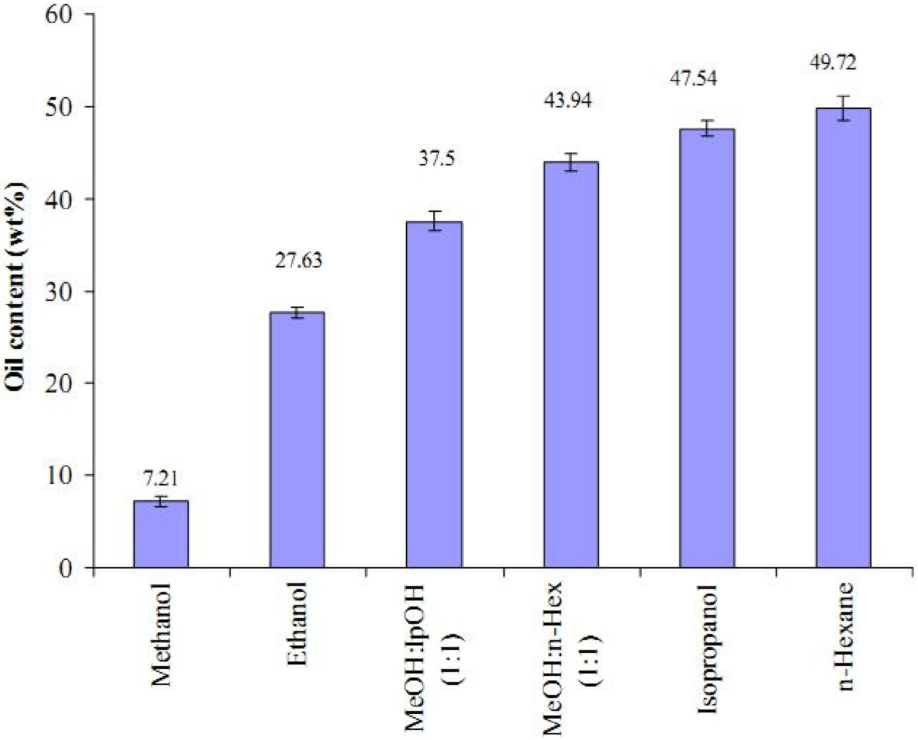


Figure 4.4: *Jatropha curcas* oil content (wt.%) extracted by some solvents

Oil extractability is higher with non-polar solvent compared to polar solvent since vegetable oil is a non-polar compound. N-hexane as a non-polar solvent was the best solvent to extract *Jatropha curcas* oil, the oil content (49.72 ± 1.34) % was achieved and the oil had high quality than oil extracted by the other solvents. Oil extracted by alcohols was turbid and resulted in precipitate. Alcohols with hydroxide group are polar solvent, thus polar compounds in oil such as phospholipids were extracted as well. Phospholipids are amphiphilic due to contain negatively charged phosphate group and other polar groups which can lead a strong emulsion [122]. In addition, during extraction process due to heating, oil came out from the seeds to the surrounding alcohol. Since alcohol is insoluble in oil, the oil-alcohol mixture became turbid.

Methanol is the strongest polar alcohol and shows the lowest oil extracted of (7.21 ± 0.52) %. Mass of oil extracted of 0.8 grams or 8 wt.% oil extracted was reported by Kasim and Harvey [37] when they extracted 10 g *Jatropha curcas* seed with 400:1 molar ratio of methanol to oil, 400 rpm mixing speed at 60°C for 60 min. Zeng, *et. al* [89] used methanol to extract 20 g milled sunflower oilseed using 200 mL methanol in 400 rpm magnetic stirring for 60 min at 25°C and oil extracted of ≤ 0.8621 grams or ≤ 4.31 wt.% oil content was obtained. On the contrary, among short chain alcohols, isopropanol was the highest oil extractability and (47.54 ± 0.85) % oil was extracted from *Jatropha curcas* seed, since isopropanol was less polar than methanol and ethanol.

The poor oil extractability of methanol could be enhanced by mixing methanol with non-polar solvent. The mixture of methanol and isopropanol with 1:1 volume ratio enhanced the oil extracted to be (37.50 ± 1.06) %. Methanol mixed with n-hexane in the same volume proportion considerably increased the oil extractability of (43.94 ± 1.04) %.

In-situ transesterifications of *Jatropha curcas* seeds catalyzed by sodium hydroxide as an alkaline catalyst were carried out in isopropanol, methanol, the mixture of isopropanol and methanol, and the mixture of methanol and n-hexane.

4.2 *In-situ* Transesterification of *Jatropha curcas* seed in isopropanol

Due to the highest solubility in oil and oil extractability of isopropanol, it was examined to conduct *in-situ* transesterification of *Jatropha curcas* seed catalyzed by sodium hydroxide. *In-situ* transesterification of *Jatropha curcas* seeds was carried out by placing 20 g of ground seeds in a three-neck round-bottom flask as a batch reactor and adding with 150 mL of isopropanol which was mixed previously with a certain weight fraction of sodium hydroxide. An excess amount of isopropanol was needed since it must extract oil from the seed and transesterify oil in one step. The experiments were performed by changing one independent variable at a time while holding the other independent variables constant.

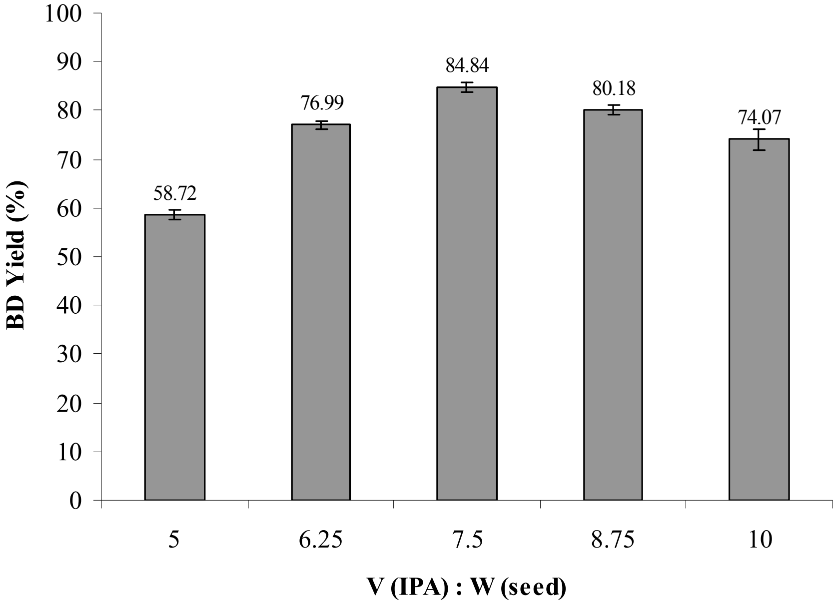


Figure 4.5: Effect of ratio of isopropanol volume to seed weight on biodiesel yield using 1 wt.% NaOH for 2 h reaction time at 60°C and 500 rpm stirring speed

4.2.1 Effect of ratio of isopropanol volume to seed weight on biodiesel yield

The effect of different ratio of isopropanol volume to seed weight (v/w in $mL \cdot g^{-1}$) of 100:20 (5), 125:20 (6.25), 150:20 (7.5), 175:20 (8.75), and 200:20 (10) on biodiesel yield had been studied at 60°C using 1 wt.% NaOH for 2 h as presented in Figure 4.5.

Increasing ratio of isopropanol volume to seed weight from 5 to 7.5 enhanced the conversion of oil to biodiesel from (58.72 ± 1.01)% to (84.84 ± 1.02)%. At ratio 5 and 6.25 the volume of isopropanol was 100 mL and 125 mL. These volumes were not sufficient to extract oil in seeds and to break the linkage of glycerin and fatty acid. Therefore the crude biodiesel yields were not high. Excess isopropanol volume 175 mL and 200 mL reduced the biodiesel yield to (80.18 ± 0.97)% and (74.70 ± 2.20)%, respectively since biodiesel could be dissolved in excess isopropanol, settled down to the lower layer and separated out from biodiesel layer. Additionally, in this experiment sodium hydroxide remained constant in the amount of 1 wt.%, increasing volume of isopropanol diluted the catalyst concentration in reactant which led reducing the conversion of oil to isopropyl ester. The maximum biodiesel yield of (84.84 ± 1.02) % was attained with 7.5 ratio of isopropanol volume to seed weight, 1 wt.% sodium hydroxide concentration at 60°C during 2 h reaction time.

Although isopropanol is the best solvent to extract oil from the ground seeds among short-chain alcohols and it has higher solubility in oils, it is not proper to transesterify oil catalyzed by alkaline catalyst. The main problem of using isopropanol in the process of *in-situ* transesterification of *Jatropha curcas* seed was in the separation process between biodiesel layer and glycerol layer as well as the washing process with water. In preliminary experiment, after *in-situ* transesterification process, the solution was separated from ground seeds and then followed by separation of isopropanol from crude biodiesel using rotary evaporator. However, at the end of this separation only a small amount of crude biodiesel obtained which mixed with impurities to form a gel. Therefore, the separation of biodiesel was carried out in separator funnel. Due to the very high solubility of isopropanol in non-polar compounds, there was only one phase in separator funnel which it should be two phases, biodiesel-rich phase and glycerol-rich phase. Nevertheless, when n-hexane and water in the volume ratio of 3:2 were added, two phases appeared. After overnight settling, the lower layer of glycerol-rich phase was separated out from the upper layer of biodiesel-rich phase followed by washing the upper layer with room temperature water. When water was added into the biodiesel-rich phase, soaps were formed in the interface between biodiesel layer and water layer. It indicated that there was isopropanol catalyzed by sodium hydroxide and contained unreacted triglyceride which was dissolved in biodiesel-rich phase. The presence of water caused saponification that was the base (sodium hydroxide) hydrolysis of triglyceride, isopropanol, water and alkaline catalyst. During biodiesel washing, saponification took place for several times and washing with water was stopped after no soaps formed. The use of warm or hot water in washing step increased the saponification and reduced the yield of biodiesel. A large water consumption and saponification in the biodiesel washing step were the main drawbacks of *in-situ* transesterification of *Jatropha curcas* seed using isopropanol catalyzed by sodium hydroxide.

4.2.2 Effect of sodium hydroxide (NaOH) concentration on biodiesel yield

Figure 4.6 presents biodiesel yield (%) for various NaOH concentration 0.5; 1.0; 1.25; 1.50; 1.75; and 2.0 wt.% with isopropanol/seed (v/w) ratio of 7.5 (150:20), at different reaction temperatures in the range of 30°C to 70°C and reaction time 2 h.

The catalyst percentage was based on the weight of the oil used for *in-situ* transesterification. It was observed that at certain temperature, addition of NaOH concentration from 0.5 wt.% to 1.0 wt.% increased the conversion to isopropyl ester. The concentration of NaOH 0.5 wt.% was not sufficient to convert *Jatropha curcas* oil into isopropyl ester. It can be observed as well from the isopropyl ester content which indicated biodiesel purity as described in Figure 4.7. The isopropyl ester contents at NaOH concentration of 0.5 wt.% were less than that at NaOH concentration of 1 wt.%. Generally, catalyst concentration of 0.5 to 1.0 wt.% of the oil was employed in conventional transesterification. Nevertheless, this range was not sufficient to *in-situ* transesterification since of the physical difference in the reaction phase, such as the presence of solid and the large amount of solvent used [27]. Kaul, *et al*. [34] had carried out reactive extraction of *Jatropha curcas* seed to produce biodiesel using seed/methanol (w/w) ratio of 1:7.8 at 65°C for 1 h. They varied NaOH concentration to 0.05, 0.075, and 0.1 M. It was observed that increasing NaOH concentration raised the conversion of oil to biodiesel. It occurred for all seed sizes. In the present study, due to higher solubility of isopropanol in non-polar substances which led saponification during biodiesel separation as explained previously, increasing concentration of NaOH over 1.0 wt.% decreased the conversions of oil to isopropyl ester and lowered the biodiesel yields. Addition NaOH concentration exceeded 2.0 wt.% (to 2.5 wt.%) caused more soap formed, interfered the separation of isopropyl ester from glycerol, and diminished the conversion of oil to isopropyl ester. The highest conversion to isopropyl ester of (85.32 ± 0.30)% was attained with NaOH concentration of 1.0 wt.% at 70°C for 2 h reaction time.

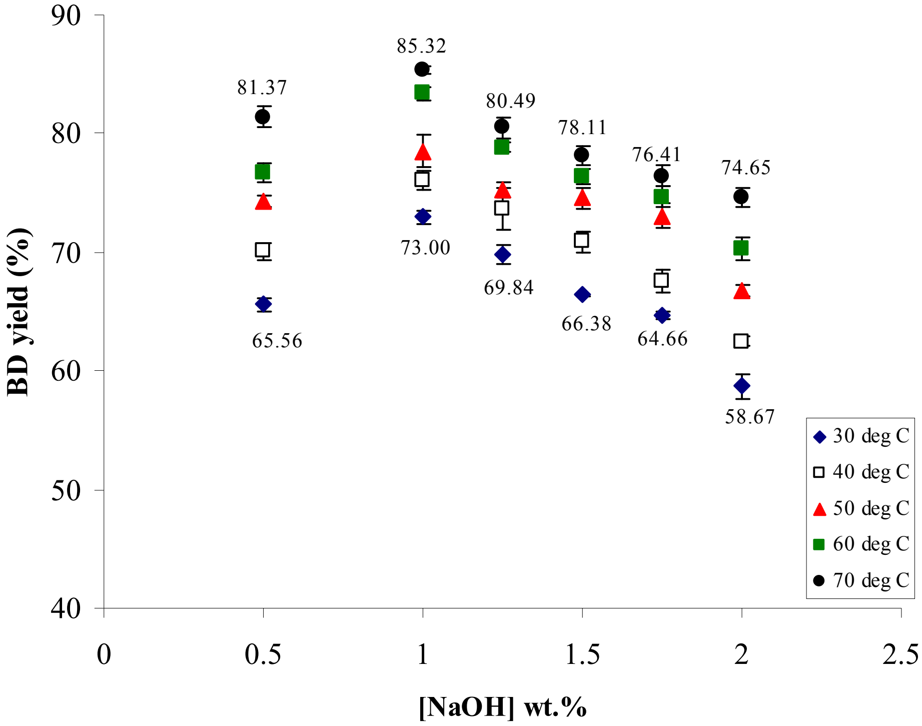


Figure 4.6: Effect of catalyst concentration on biodiesel yield with isopropanol/seed (v/w) ratio of 7.5 for 2 h reaction time at reaction temperatures of 30 - 70°C and stirring speed of 500 rpm

4.2.3 Effect of reaction temperature on biodiesel yield

In-situ transesterification of *Jatropha curcas* seeds with isopropanol was done at various reaction temperatures (30, 40, 50, 60, and 70°C). Temperature can affect the completion of oil extraction and its conversion to biodiesel, as well as the yield of biodiesel.

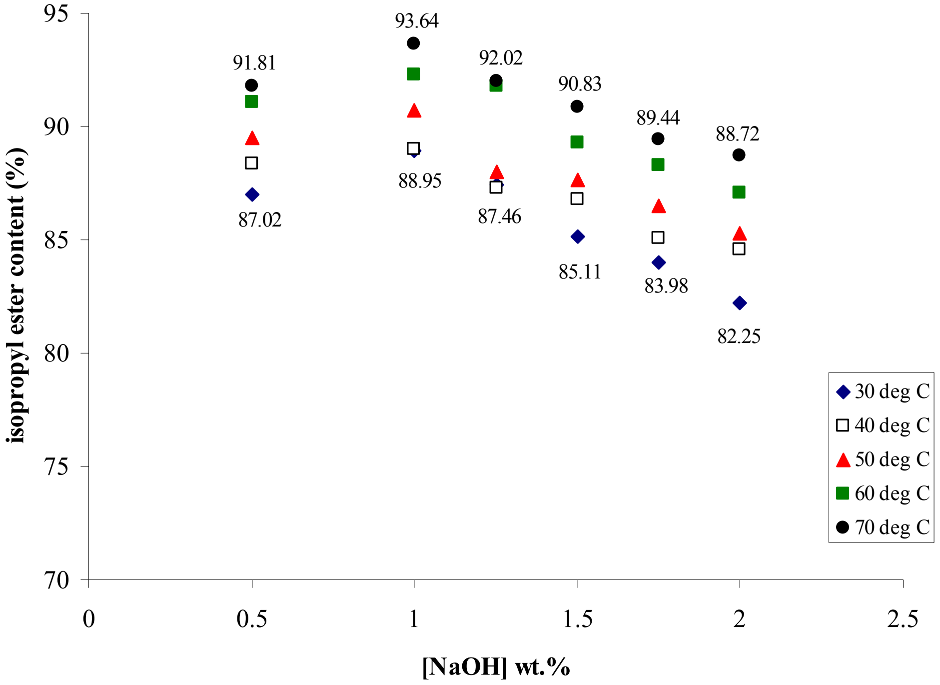


Figure 4.7: Effect of catalyst concentration and reaction temperature on isopropyl ester content at V_{IPA}:W_{seed} of 7.5 for 2 h reaction time and stirring speed of 500 rpm

Figure 4.6 presents the yield of biodiesel at various temperature with isopropanol/seed (v/w) 150:20, different amount of catalyst in the range of 0.5 – 2.0 wt.%, reaction time of 2 h, whereas the influence of reaction temperature on the content of isopropyl ester is depicted in Figure 4.7.

The results show that the yield of biodiesel rose as increasing temperature for all catalyst concentration. The maximum oil extracted during *in-situ* transesterification was (91.11 ± 0.32) % at 70°C and 1.0 wt.% NaOH using 7.5 ratio of isopropanol volume to seed weight and 93.64% of the extracted oil was converted to isopropyl ester (biodiesel). Higher temperature can accelerate the oil extraction from seeds and speed up the conversion of oil to isopropyl ester. However, reaction temperature could not be adjusted at higher temperature exceeding the boiling point of isopropanol, since at this temperature isopropanol could vaporize, many bubbles were formed which could inhibit the reaction. Moreover, saponification by NaOH catalyst would be occurred faster than transesterification. Therefore, the reaction temperature must be selected below boiling point of isopropanol.

Isopropyl ester contents produced in this study were in the range of 82.25% to 93.64% that were less than 96.5% ester content based on EN 14214 standards. Moreover, the density of isopropyl ester at 15°C of 910.4 kg·m^{-3} was slightly higher than the density range of 860–900 kg·m^{-3} according to EN 14214 standards. Its kinematic viscosity at 40°C was 6.75 mm^{2}·s^{-1} that was slightly higher than ASTM D6751 standards for kinematic viscosity at 40°C with the value of 1.9–6.0 mm^{2}·s^{-1}.

4.3 *In-situ* Transesterification of *Jatropha curcas* Seeds with Methanol

Methanol is the shortest-chain alcohol which has the lowest solubility in vegetable oils and lowest extractability of *Jatropha curcas* oil due to the most polar among alcohols. Methanol is the most frequently used to transesterify vegetable oils into biodiesel because it has low cost and biodiesel produced can be separated from glycerol easily.

The preliminary experiment was done to examine that methanol could be used in the process of *in-situ* transesterification of *Jatropha curcas* seed. Kildiran, *et al*. [25] reported that only 20% oil could be extracted from the ground soybean and dissolved in methanol, further only 42% of oil could be converted to methyl ester. Maceration before *in-situ* methanolysis improved the oil extraction to be 40% and enhanced the conversion of the oil to methyl ester of 55%. Ginting, *et al*. [123] reported that *in-situ* methanolysis of *Jatropha curcas* seed only could produce 40.23% methyl ester yield with the ratio of methanol to *Jatropha curcas* seed of 140:10 (mL·g^{-1}), in the presence of 1 wt.% NaOH, with stirring speed of 600 rpm, at 60°C reaction temperature even during 24 h reaction time.

The preliminary experiments were done by testing three independent variables which were ratio of methanol volume to seed weight (6.25, 7.5, 8.75 mL·g^{-1}), NaOH concentration (1 wt.%, 1.5 wt.%, 2 wt.%), and reaction temperature (40°C, 50°C, 60°C). The experiments were carried out by changing one independent variable at a time while keeping the other independent variables constant. The results are displayed in Figure 4.8.

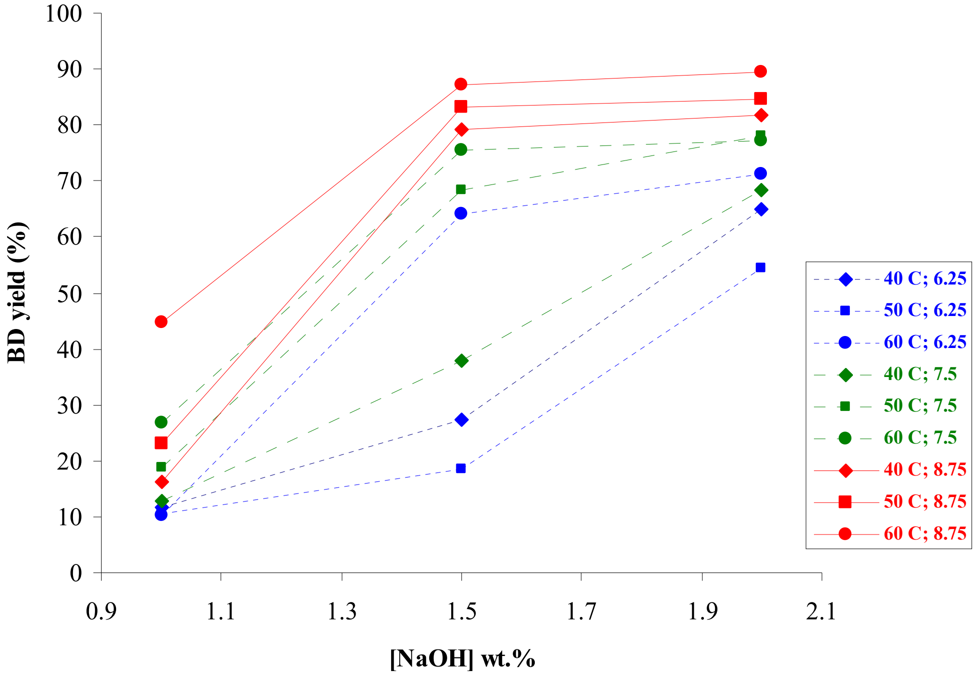


Figure 4.8: Effect of ratio of V_{MeOH}:W_{seed}, NaOH concentration, and reaction temperature on biodiesel yield with 400 rpm stirring speed for 2 h reaction time

It can be observed that increasing ratio of methanol volume to seed weight, NaOH concentration and reaction temperature enhanced biodiesel yield. Without adding the catalyst, 150 mL methanol could extract only a few amount of oil of (7.21 ± 0.52)% from 20 g seeds at boiling point of methanol. By mixing 150 mL of methanol with 1 wt.% NaOH (oil weight based) at 40°C, (12.73 ± 0.16)% oil could be extracted from 20 g seeds and transesterified to methyl ester. Increasing NaOH amount to 2 wt.% enhanced the oil extraction and transesterification to be (68.30 ± 0.49)% at 40°C. The rise in reaction temperature to 60°C augmented the oil extraction and transesterification to be (77.11 ± 0.46)%. The maximum biodiesel yield of (89.50 ± 0.33)% was obtained with 8.75 ratio of methanol volume to seed weight, 2 wt.% NaOH concentrations at 60°C reaction temperature during 2 h reaction time. This yield was higher than the previous research by Ginting, *et al* [123].

4.3.1 Optimization of *In-situ* Transesterification of Jatropha curcas Seeds with Methanol

4.3.1.1 ANOVA and regression analysis

Based on the preliminary experiments, the important parameters influencing the yield of methyl ester were ratio of methanol volume to seed weight, NaOH concentration, and reaction temperature. The optimum condition of experiments which produced the maximum biodiesel yield and the interaction between the independent variables were determined by employing the design of experiment using response surface methodology, central composite design. Low and high level for experimental design were taken from the low and high value of preliminary experiments. The experimental design used in this study was a full 2^3 factorial design, in which three central points (test 1, 9, and 17) and six star points (test 11-16) were added to evaluate experimental errors. Based on Table 3.4 in previous chapter, the experiments were performed. The observed response of factorial design, biodiesel yield, is presented in Table 4.2.

The observed response of the full factorial central composite design was fitted by empirical second-order polynomial model to predict biodiesel yield, as a function of significant variables and showed in Eq. (4.1). Positive sign in front of the terms indicates synergistic effect and negative sign indicates antagonistic effect.

$$\begin{aligned} Y = -654.16 + 104.81 \cdot R_{VW} + 182.29 \cdot C_{Na} + 5.08 \cdot T - 5.98 \cdot R_{VW}^2 - 0.14 \cdot R_{VW} \cdot T - \\ 54.59 \cdot \mathrm{C}_{\mathrm{Na}}^2 + 0.52 \cdot C_{Na} \cdot T - 0.04 \cdot T^2 \end{aligned} \tag{4.1}$$

where,

Y : predicted biodiesel yield (%)

R_{VW} : ratio of methanol volume to seed weight ($mL \cdot g^{-1}$)

C_{Na} : sodium hydroxide concentration (wt.%)

T : reaction temperature (°C)

Table 4.2: The response of factorial design of *in-situ* transesterification of *Jatropha curcas* seeds with methanol

Test	**A** V_{MeOH}: W_{seed} (mL·g^{-1})	**B** **[NaOH] (Wt.%)**	**C** **T** (°C)	**Obsv. value (%)**	**Fitted value (%)**	$\Delta_{obs\text{-}fitted}$ **(%)**
1	7.50	1.5	50	78.34	77.24	1.10
2	6.25	1.0	40	13.29	13.95	-0.66
3	8.75	1.0	40	36.88	37.42	-0.54
4	6.25	2.0	40	54.86	53.31	1.55
5	8.75	2.0	40	75.28	76.78	-1.50
6	6.25	1.0	60	21.09	21.49	-0.40
7	8.75	1.0	60	35.15	37.73	-2.58
8	6.25	2.0	60	70.79	71.28	-0.49
9	7.50	1.5	50	76.48	77.24	-0.76
10	8.75	2.0	60	86.27	87.52	-1.25
11	5.40	1.5	50	33.44	34,14	-0.70
12	9.60	1.5	50	70.31	67.53	2.78
13	7.50	0.66	50	2.94	1.16	1.78
14	7.50	2.34	50	76.42	76.12	0.30
15	7.50	1.5	33	57.32	57.34	-0.02
16	7.50	1.5	67	74.80	72.70	2.10
17	7.50	1.5	50	76.55	77.24	-0.69

Based on a 95% confidence level, the significance of terms was determined by comparing the computed F value to the theoretical $F_{0.05\ (v1,\ v2)}$ value and from the P-value. The fitted model and regression coefficients have significant effect on the response if the computed F value was higher than the theoretical $F_{0.05\ (v1,\ v2)}$ value and the P-value less than 0.05. Table 4.3 describes the analysis of variance for the regression model. As the F-value and P-value of fitted model were 502.75 and ≤0.0001 respectively, the model indicated significant and sufficient for predicting the biodiesel yield. The computed F value of fitted model (502.75) was much higher than the theoretical F value of 3.44. To test the fit of the model, the determination coefficient was evaluated. Figure 4.9 shows the predicted versus observed value for biodiesel yield with the determination coefficient of 0.9971, that was, the second order model explained 99.71% of the variability in biodiesel yield and only 0.29% of the total variations were not explained by the model.

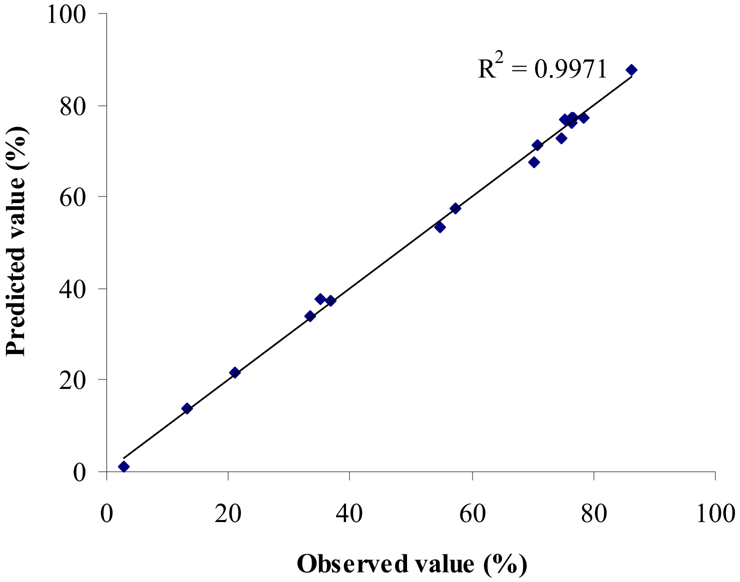


Figure 4.9: Predicted versus observed biodiesel yield

The value of adjusted determination coefficient (adjusted R^2), which was more suitable for comparing model with different numbers of independent variables, was also very high 0.9941.

Each regression coefficients of the model was tested to be significant at confidence level of 95%. The model terms of A, B, C, AA, AC, BB, CC and BC had significant effects on the methyl ester yield because the computed F values for the respective terms were higher than the theoretical $F_{0.05(1,8)}$ value of 5.32 and very low P-values less than 0.05 (5%). According to the F-value, the most significant term in the regression was catalyst concentration. Its positive value shows that increasing catalyst concentration enhanced the methyl ester yield. The quadratic term of catalyst concentration was negative which indicated that the methyl ester yield reached a maximum after certain addition of catalyst concentration. However, further addition of catalyst concentration decreased the yield.

Table 4.3: Analysis of Variance for response surface of quadratic model in Eq. (4.1)

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-Ratio**	**P-Value**
Model	12126.96	8	1515.87	502.75	< 0.0001
A:V_{MeOH}:W_{seed}	1345.54	1	1345.54	334.84	0.0000
B:[NaOH]	6783.43	1	6783.43	1688.04	0.0000
C:Temp	285.002	1	285.002	70.92	0.0000
AA	982.686	1	982.686	244.54	0.0000
AC	26.1726	1	26.1726	6.51	0.0341
BB	2099.88	1	2099.88	522.55	0.0000
BC	54.3403	1	54.3403	13.52	0.0062
CC	210.511	1	210.511	52.39	0.0001
Total error	32.1482	8	4.01852		
Total (corr.)	10985.1	16			

The effect of methanol volume to seed weight ratio was lower than that of catalyst concentration, whereas it was higher than that of reaction temperature. The positive signs of catalyst concentration and reaction temperature parameters demonstrated that increasing in these improved the methyl ester yield. Their quadratic terms were negative which revealed that the methyl ester yield attained a maximum after certain ratio of methanol volume to seed weight at definite reaction temperature. Nonetheless, the yield was diminished at further rising of these parameters. The interaction terms of methanol volume to seed weight ratio and reaction temperature and that of catalyst concentration and reaction temperature had significant effects on the methyl ester yield. Meanwhile, the interaction between ratio of methanol volume to seed weight and NaOH concentration (AB) showed insignificant effect on the response of methyl ester yield due to the F-value of 0.08 and P-value of 0.7799 higher than 0.05. Thus this term (AB) should be excluded from the analysis of variance and it was not appeared in the second-order model of Eq. (4.1). Moreover, by excluding the AB term the determination coefficient and adjusted determination coefficient increased.

4.3.1.2 Effect of the ratio of methanol volume to seed weight and reaction temperature on the yield of methyl ester

The ratio of methanol volume to seed weight is one of the important factors in biodiesel production. *In-situ* transesterification needs excess methanol than conventional transesterification because in this process methanol was employed as solvent extraction and transesterification reagent. Therefore, in this work ratio of methanol volume to seed weight was used instead of molar ratio of methanol to oil. Figures 4.10 and 4.11 depict the effect of interaction between ratio of methanol volume to seed weight and reaction temperature on methyl ester yield at 1.94 wt.% NaOH concentration. As can be seen, increasing ratio of methanol volume to seed weight from 6.25 to 8.08 and reaction temperature from 33°C to 56.98°C enhanced the conversion of oil to methyl ester. At ratio 6.25 the methanol volume was 108 mL. The oil extracted and transesterified into methyl ester was only in a small quantity. Therefore, the biodiesel yields were not high. At low ratio of methanol volume to seed weight, increment reaction temperature enhanced the yield of methyl ester, as a result of positive effect of T term in Eq. (4.1). At moderate to higher ratio of methanol volume to seed weight, rising temperature above 40°C to higher temperature only slightly improved the methyl ester yield. Further increasing ratio of methanol volume to seed weight and higher reaction temperature, the methyl ester yield decreased, as a result of negative interaction term of R_{VW} and T in Eq. (4.1). At this condition, rising methanol volume could dilute the catalyst and oil concentrations in the reaction system. The interactions between them decreased and reduced the oil conversion to methyl ester.

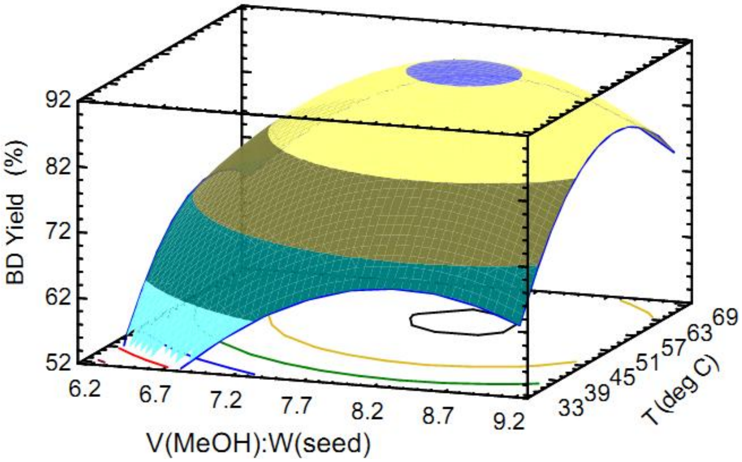


Figure 4.10: Estimated response surface of methyl ester yield at 1.94 wt.% NaOH concentration

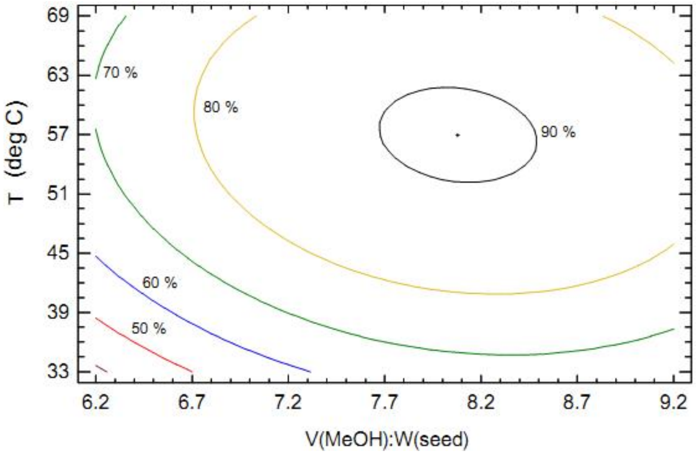


Figure 4.11: Contour of methyl ester yield at 1.94 wt.% NaOH concentration

Wahlen, *et al*. [40] examined the effect of methanol volume on the weight of fatty acid methyl ester produced by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Increasing the volume of methanol did not result in an increase in the yield of FAME. Siler-Marinkovic and Tomasevic [28] reported that the molar ratio was strongly relied on the amount of sulfuric acid and the time of reaction during investigating in situ transesterification of sunflower oilseed. They varied the molar ratio of methanol to oil, sulfuric acid concentration, and reaction time at 64.5°C. A lower molar ratio of methanol to oil of 100:1 was not enough to extract and transesterify the oil. However, with 200:1 molar ratio and 4 h of heating in the presence of 100% sulfuric acid (on oil basis) a good conversion and quality of methyl esters had been obtained. Increasing molar ratio to 300:1 with the same amount of catalyst could achieve the similar conversion and quality of methyl ester in a shorter reaction time of 1 h.

4.3.1.3 Effect of catalyst concentration and reaction temperature on the methyl ester yield

The catalyst concentration affects the biodiesel yield. Figures 4.12 and 4.13 indicate biodiesel yield (%) for various NaOH concentration from 1.0 to 2.5 wt.% and various reaction temperature from 40°C to 65°C. The catalyst percentage was based on the weight of the oil used for *in-situ* transesterification. It was observed that raising NaOH concentration from 1.0 wt.% to 1.94 wt.% and reaction temperature from 33°C to 56.98°C increased the conversion of *Jatropha curcas* oil to biodiesel. Low concentration of NaOH was not sufficient to convert *Jatropha curcas* oil into fatty acid alkyl ester. This result was in accordance with the research by Ginting *et al*. [36]. They reported that in alkaline in situ ethanolysis of *Jatropha curcas*, at concentration of sodium methoxide and sodium hydroxide in the range of 0.5 wt.% to 1.0 wt.% the yield of ethyl ester obtained were around 80 %. Nevertheless, when the catalyst concentration increased to 2 wt.% sodium methoxide concentration or 2.5 wt.% sodium hydroxide concentration, over 99% yield of ethyl ester was achieved with ratio of ethanol volume to seed weight of 7:1 at 70°C for 2 h of reaction time.

However, addition of NaOH more than 1.94 wt.% decreased the conversion of oil to biodiesel because of emulsion formation. Addition NaOH concentration exceeding 2.0 wt.% caused the mixture of reactant more viscous, disturbed the mixing and separation of biodiesel, and lowered the conversion to biodiesel toward around 60% methyl ester yield. Water could be formed due to reaction between methanol and NaOH. The presence of water and NaOH led hydrolysis of triglyceride to form sodium salt of carboxylate (crude soap). Kasim and Harvey [37] reported that in reactive extraction of *Jatropha curcas* using molar ratio of methanol to oil of 400:1 at 60°C for 1 h, increasing NaOH concentration of 0.1 N to 0.15 N enhanced the methyl ester yield from 76.2% to 87.8%. Nevertheless, further increasing to 0.2 N decreased the methyl ester yield to 80.8% due to the formation of soaps.

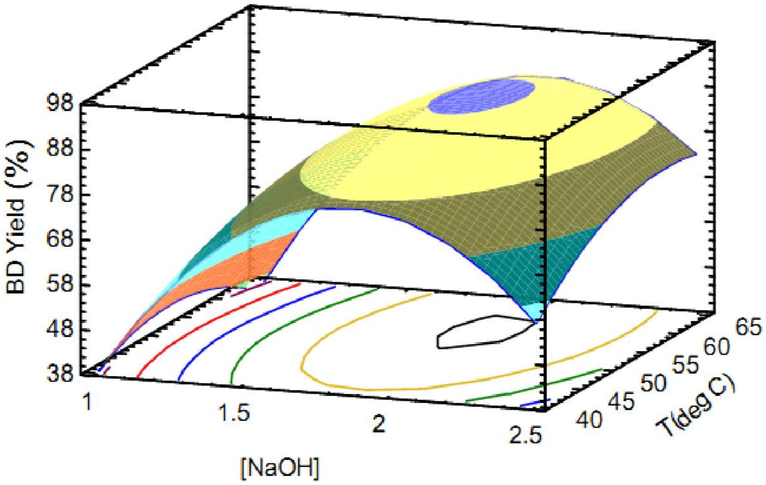


Figure 4.12: Estimated response surface of methyl ester yield at $V_{MeOH} : W_{seed}$ of 8.08 ($mL \cdot g^{-1}$)

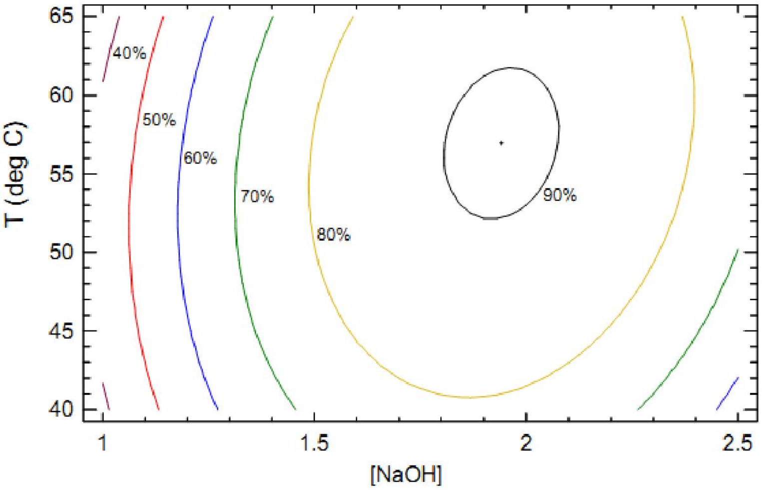


Figure 4.13: Methyl ester yield contour at $V_{MeOH} : W_{seed}$ of 8.08 ($mL \cdot g^{-1}$)

Higher temperature can accelerate the reaction. However, if reaction was carried out at high temperature exceeded the boiling point of methanol; it caused methanol evaporation and its loss. Moreover, saponification by NaOH catalyst occurred faster and reduced the yield of methyl ester. It also can be observed from Figure 4.13, at high NaOH concentration, the increment of temperature beyond 65°C diminished the methyl ester yield.

4.3.1.4 Optimization analysis

Table 4.4 explains the high, low limit experimental region and the optimum factors. The predictions of biodiesel yield obtained from optimization of the experimental design were 90.98%. This value was compared to the methyl ester yield obtained from experiment as shown in Table 4.5. The optimum biodiesel yields obtained from experiment was (90.45 ± 0.25)%. The experiment methyl ester yield showed insignificant difference than predicted yield. This experimental optimum methyl ester yield was in agreement with Jain and Sharma work [124]. They conducted two-step sulfuric acid-sodium hydroxide catalyzed transesterification of *Jatropha curcas* oil with 21.5% free fatty acid content. The reaction time of 6 h was required to achieve reaction completion with the maximum methyl ester yield of 90.1%. This optimum yield of methyl ester was also comparable with research work investigated by Berchmans and Hirata [48]. They carried out two-step sulfuric acid-sodium hydroxide catalyzed methanolysis of *Jatropha curcas* oil due to high free fatty acid of 14.9%. The yield of methyl esters of fatty acids was achieved around 90% in 3 h reaction time.

The methyl ester yield of this work was higher than that produced by Vyas, *et al.* [118] who conducted transesterification of *Jatropha curcas* oil with KNO_3 loaded in Al_2O_3 as catalyst in amount of 6 wt.%. Methanol was used in ratio of 12:1 to the oil. The maximum conversion of oil to methyl ester of 84% was reached with 600 rpm agitation speed at 70°C for 6 h reaction time. Another methanolysis of *Jatropha curcas* oil with solid catalyst of 4 wt.% calcium-based mixed oxide produced the oil conversion more than 80% using 15 molar ratio of methanol/oil at 65°C during 6 h reaction time [125].

Table 4.4: The combination of factor levels which maximize biodiesel yield

Factor	**Low**	**High**	**Optimum**
V_{MeOH}: W_{seed} (mL/g)	5.4	9.6	8.08
[NaOH] (Wt.%)	0.66	2.34	1.94
T (^{0} C)	33	69	56.98

Table 4.5: Optimum condition for methanol

Test	**Factors**			**Response (%)**		**Residual (%)**
	V: W (mL/g)	**[NaOH] (wt.%)**	**T (^{0} C)**	**Obsv**	**Predc**	
1	8.08	1.94	57	90.73	90.98	-0.25
2	8.08	1.94	57	90.27	90.98	-0.71
3	8.08	1.94	57	90.34	90.98	-0.64

In comparison with other previous research works of *in-situ* transesterification with methanol and homogeneous catalysts, the optimum biodiesel yield of this work was higher than other methyl ester yields [24, 27, 94]. It was caused by conducting microwave heating during seeds pretreatment, which led to facilitate the oil extraction. Haas, *et al*. [24] performed *in-situ* transesterification of soy flakes catalyzed by sodium hydroxide. The reaction time of 8 h was required to complete the reaction at 60°C for the molar ratio of methanol/triglyceride/NaOH of 226:1:1.6 and at 23°C for 543:1:2.0 molar ratio of methanol/triglyceride/NaOH. At this condition, 95% removal of oil from the soy flakes and an 84% efficiency of conversion of this solubilized lipid to fatty acid methyl ester or around 79% fatty acid methyl ester yield were obtained. Zakaria and Harvey [27] examined the influences of process parameters on the yield, conversion and reaction rate of *in-situ* transesterification of rapeseed with methanol catalyzed by sodium hydroxide. The ester concentration of (90.3 ± 1.3)% and ester yield of (88.8 ± 0.1)% were accomplished with the molar ratio of methanol to oil of 475, NaOH concentration of 0.1 molal, 300–500 μm particle size, 25 g seed at 60°C for 60 min reaction time. Hailegiorgis, *et al*. [94] reported that in the process of *in-situ* transesterification of *Jatropha curcas* L. with methanol catalyzed by sodium hydroxide, the maximum fatty acid methyl ester of 47.9% was gained after 3.5 h reaction time which much lower compared to the optimum methyl ester yield achieved in this work. To enhance the methyl ester yield, benzyltrimethylammonium hydroxide as a phase transfer catalyst was added in NaOH to catalyze methanol before the reaction was initiated. The methyl ester yield increased considerably to be (89 ± 0.7)% which was slightly lower than (90.45 ± 0.25)% obtained from this work.

4.3.2 Effect of stirring speed

The continuous stirring and adequate reaction time were required in the process of *in-situ* transesterification where oil extraction and transesterification occurred simultaneously. The oil extraction could be improved by increasing the stirring speed; moreover the continuous stirring could facilitate the initial reaction and mass transfer between triglyceride and reagent in order to complete the reaction.

The effect of stirring speed on biodiesel yield was conducted up to 2 h reaction time at stirring speed of 300, 400, 500, and 600 rpm. Other parameters were set at optimum condition, ratio of methanol volume to seed weight of 8.08 : 1 ($mL \cdot g^{-1}$), NaOH concentration of 1.94 wt.%, particle size less than 600 μm, and reaction temperature of 57°C. The biodiesel yields at various stirring speed are presented in Figure 4.14.

Increasing stirring speed raised the extraction of oil from seeds. The biodiesel yields increased considerably within 10 minutes reaction time and these yields were almost the same for all stirring speed. Further reaction time showed that increasing stirring speed up to 500 rpm enhanced slightly the yield of biodiesel up to 90 min reaction time where the maximum yield was achieved in the range of 87.24% to 90.38%. Increasing stirring speed could enhance the homogeneity of *Jatropha curcas* seeds and the reagent. However biodiesel yield was decreased by additional stirring speed over 500 rpm. At higher stirring speed (600 rpm), there was unstable condition since some of methanol evaporated and there was insufficient contact between seeds and the methoxide solution [126]. Hence, oil extraction, oil transesterification and the yield of biodiesel decreased.

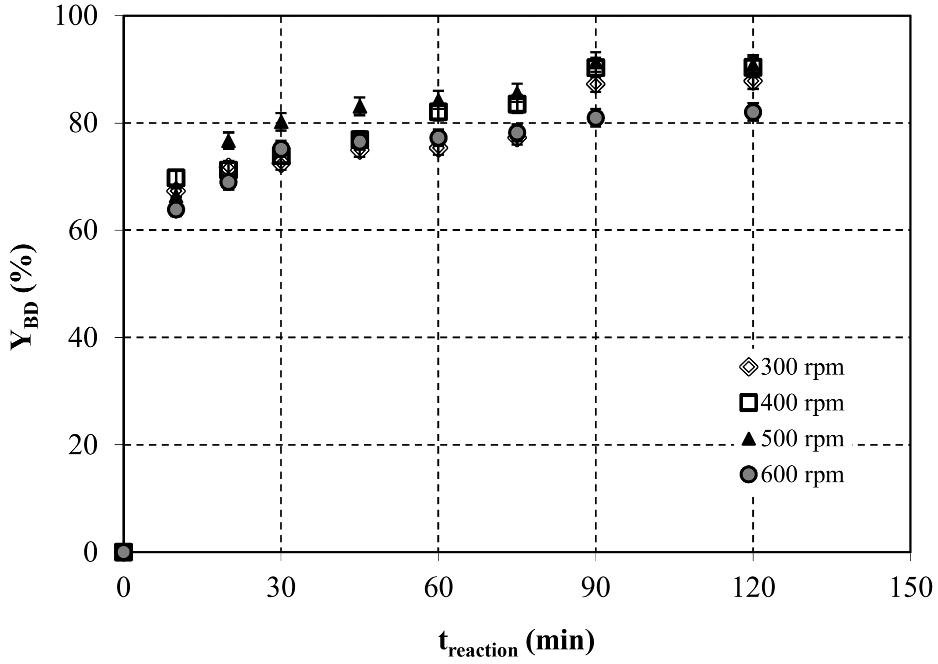


Figure 4.14: Methyl ester yield for different stirring speed and reaction time with ratio of methanol volume to seed weight of 8.08:1, NaOH concentration of 1.94 wt.%, < 600 μm particle size at 57°C

The yields were almost constant after 90 min reaction time, which described that the reaction was completed. Different stirring speeds had no effect on the reaction completion. Zeng, *et al.* [89] investigated that the agitation speed only had a few effect on the biodiesel yield and its purity. In their research the agitation speeds of 150, 300, 450, and 600 rpm were employed and the agitation speed of 150 rpm was selected to carry out the reaction. Kasim and Harvey [37] explained that increasing mixing speed from 100 rpm to 300 rpm improved the biodiesel yield significantly from 37.2% to 94.8% as seed distribution was more uniform at higher mixing speed. The yield at 400 rpm stirring speed only increased very slightly. Ginting, *et al.* [123] reported that at higher stirrer speed, more conversion of oil to methyl ester was obtained since increasing the stirrer speed, more oil could be extracted and promoted the homogenization of the reactants. In their work, the influence of the stirrer speed on the in situ methanolysis of *Jatropha curcas* was carried out by varying the stirrer speed from 100, 200, 400, and 600 rpm with the ratio of methanol to the seed of 7 (v/w), NaOH concentration of 1 wt.% at 60°C for 24 h. The stirrer speed of 600 rpm resulted in the maximum weight of biodiesel. In this condition, mass transfer occurred because of different concentration which enhanced the reaction rate.

4.3.3 Effect of reaction time and reaction temperature

The effect of reaction time on biodiesel yield was conducted up to 180 min at various reaction temperatures of 30, 40, 50, and 60°C. Other parameters were set at optimum condition, ratio of methanol volume to seed weight of 8.08 ($mL \cdot g^{-1}$), catalyst concentration of 1.94 wt.%, stirring speed of 500 rpm and particle size of less than 600 μm. The biodiesel yields at various reaction times and temperature are presented in Figure 4.15.

At 30 ^{0}C, reaction proceeded slowly. The methyl ester yield of (70.31 ± 1.36)% was obtained after 180 min reaction time. The biodiesel yield increased with the increment of reaction time. At the first 15 min, over 60% of the oil was extracted from the seed and converted to methyl ester at reaction temperature above 40°C, as reported by other works [34, 37]. The methyl ester yield improved significantly up to 60 min which indicated that the oil extraction rate and oil conversion rate increased at this reaction period. During the early stage of the reaction, the oil extraction rate and the oil conversion rate were very high at all reaction temperature which revealed the reduction of mass transfer effect. Further reaction time, the oil extraction rate and the oil conversion rate decreased tremendously. It represented that the reaction changed to equilibrium state condition and the methyl ester yield slightly increased. The prolonged reaction time decreased insignificantly the methyl ester yield since it might be saponification and reverse reaction occurred.

Rising reaction temperature from 30°C to 60°C enhanced methyl ester yields. The oil extraction and oil conversion can be accelerated by increasing reaction temperature to reach reaction completion in a shorter time. At 30°C, the reaction was completed after 180 min reaction time. The increment of reaction temperature to 40°C-50°C shortened the reaction completion to 120 min. At 60°C reaction temperature, there was insignificantly difference between the methyl ester yields after

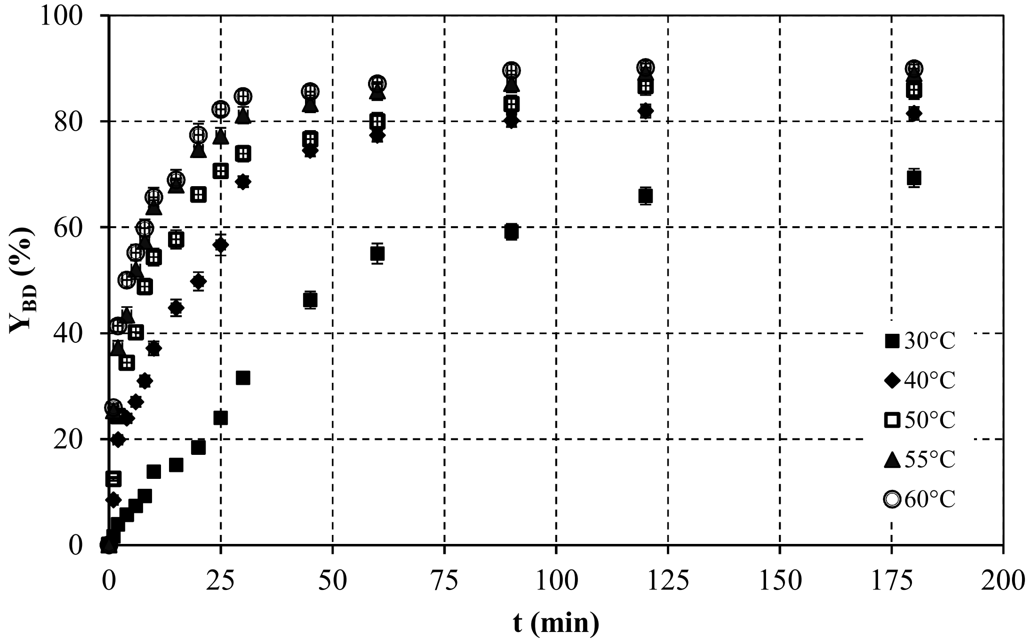


Figure 4.15: Methyl ester yield for different reaction time and temperature with ratio of methanol volume to seed weight of 8.08:1, NaOH concentration of 1.94 wt.%, less than 600 μm particle size

90 min reaction time and that after 120 min. It revealed that the reaction equilibrium was obtained after 90 min reaction time. However the methyl ester yield and reaction completion reduced at higher reaction temperature due to boiling point of methanol exceeded. At this temperature, the reaction tended to speed up the saponification of the triglycerides by the alkaline catalyst before completion of the transesterification [127]. The maximum methyl ester yield of 90.17% was obtained after 120 min reaction time at 60°C.

Figure 4.16 shows the effect of reaction time on intermediate product of *in-situ* transesterification, mono- and di-glyceride concentration at reaction temperature of 40, 50, and 60°C. In biodiesel production, mono-, di-, and triglyceride content were bound glycerin content whereas glycerol was free glycerin which was the impurities of biodiesel. The presence of the impurities must be at a very low content.

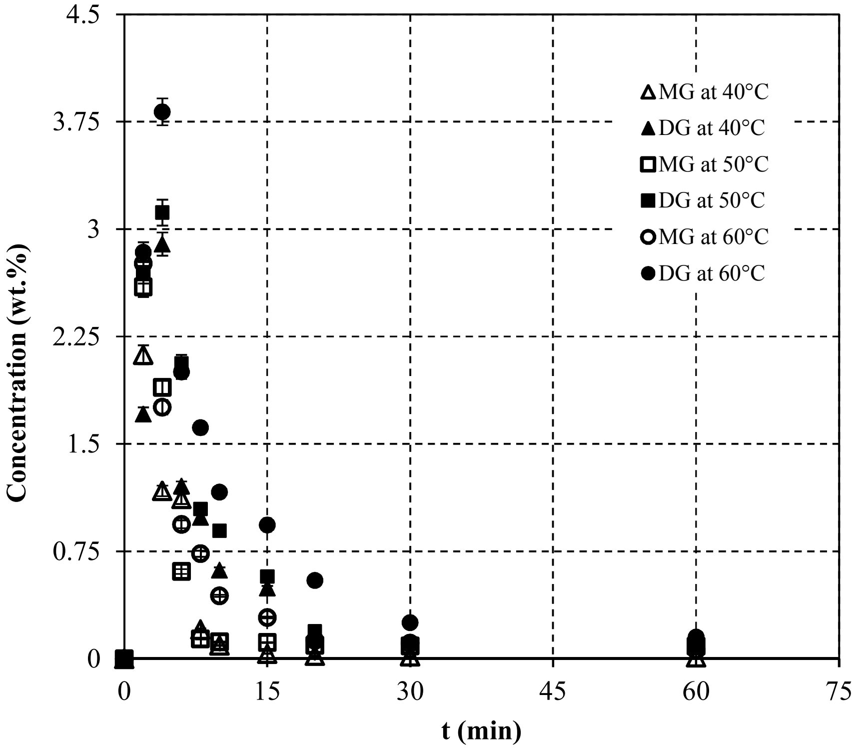


Figure 4.16: The effect of reaction time on concentration of mono- and di-glyceride at 40, 50, and 60°C reaction temperature

During the first 4 min reaction time, the conversion of triglyceride to diglyceride and followed by conversion of diglyceride to monoglyceride enhanced with increase in reaction time indicated by augmenting sharply the concentrations of mono- and di-glyceride. The maximum monoglyceride concentration of less than 2.8 wt.% was reached for 2 min reaction time at 60°C, while the highest diglyceride concentration of 3.82 wt.% was obtained after 4 min reaction time at 60°C. Thereafter the concentrations of mono- and di-glyceride decreased as they were converted into methyl ester and glycerol. At this condition, their concentrations were almost constant toward equilibrium state after 60 min reaction time.

These results were in conformity with other research works. In methanolysis of palm oil, the highest concentrations of monoglycerides and diglycerides, about 10 wt.%, were observed during the first 2 min of reaction at 50°C; then, their values decreased and were approximately constant [128]. Darnoko and Cheryan [129] reported that the maximum monoglyceride concentration of 6 wt.% and diglyceride concentration of 5.6 wt.% were achieved after 4 min reaction time when they carried out transesterification of palm oil with methanol at 50°C. In kinetic study of hydroxide-catalyzed methanolysis of *Jatropha curcas*–waste food oil mixture for biodiesel production, during the first 2 min of reaction time, the triglyceride concentration decreased sharply as the sudden increase of methyl ester concentration. The highest concentrations of diglyceride and monoglyceride observed were visible at the first 3 min of reaction time. Their levels then decreased when reaction time increased [130]. The maximum of about 6 wt.% of mono-, di- and triglycerides in total in the ester phase, was observed very early in the reaction (1 min reaction time) during *in-situ* transesterification of rapeseed with methanol at 60°C [27].

Reaction temperature provided significant effect on the concentration of mono- and di-glyceride. The increment of reaction temperature raised the intermediate products. It can be observed that rising temperature from 40°C to 50°C, the maximum concentration of diglyceride enhanced slightly from 2.89 wt.% to 3.12 wt.%. It occurred also with increase in reaction temperature from 50°C to 60°C, its maximum concentration raised insignificantly to 3.82 wt.%. There was a small increase in the concentration of monoglyceride by enhancing reaction temperature from 40°C to 60°C, that were in the range of 2.13 to 2.76 wt.%. It demonstrated that the purity of biodiesel decreased by increasing reaction temperature. It was in accordance with other works by Ginting, *et al*. [36, 123]. They reported that the highest conversion of *Jatropha curcas* oil to ethyl ester, revealed the highest biodiesel purity, was achieved at 30°C and rising reaction temperature diminished the purity of ethyl ester.

4.3.4 Effect of particle size

In the process of *in-situ* transesterification, the yield of biodiesel depends on the efficiency of extraction and the yield of transesterification [32, 35]. The efficiency of solvent extraction process is affected by several factors, such as the type of solvent used, the extraction temperature, particle size, and solvent to oilseed ratio [131].

Particle size is an important factor in *in-situ* transesterification since it can be overcome the mass transfer limitation during reaction. Decreasing the size of particle could enhance the contact surface area. The effect of particle size on biodiesel yield was conducted at various reaction time up to 240 minutes with the particle sizes of $D_P<0.425$ mm, $0.425<D_P<0.600$ mm, $0.600<D_P<1.18$ mm, and $1.18<D_P<2$ mm. Other parameters were set at optimum condition, ratio of methanol volume to seed weight of 8.08 ($mL\cdot g^{-1}$), catalyst concentration of 1.94 wt.%, stirring speed of 500 rpm, and reaction temperature of 57°C. The biodiesel yields at various particle sizes are presented in Figure 4.17.

It was observed that decreasing particle size from $1.18<D_P<2$ mm up to $0.425<D_P<0.600$ mm enhanced the biodiesel yield at all reaction time. Particle size reduction not only increases the specific area (surface area to volume ratio) of oilseed but also ruptures its cell walls. In small particles which have large specific areas, more oil is available on the surface than in inner, unbroken cells. Therefore, the yield of extracted oil is higher from the smaller particles than the larger particles as there is apparently no diffusion through undamaged cell walls [132].

However the yield was diminished at smaller particle size than 0.425 mm since at this condition, seeds agglomerated to become bigger size and reduced the contact surface area of particles and inhibited the oil extraction. Moreover, due to high polarity of methanol, the smallest particle size of seeds did not only extract the oil but also extract polar compounds such as phospholipids. It can disturb the reaction and reduce the conversion of oil to methyl ester.

At initial reaction time (10 minutes) biodiesel yield of the biggest particle size of $1.18<D_P<2$ mm was 22.12%. It increased appreciably to 51.55% at particle size of $0.600<D_P<1.18$ mm and reached the maximum yield of 63.89% at $0.425<D_P<0.600$ mm particle size.

Reducing particle size of $D_P<0.425$ mm, the yield decreased significantly into 36.55%. Particle size affected the reaction completion. At $0.425<D_P<0.600$ mm particle size, the yield was almost constant after 60 min reaction time. It showed that the reaction was completed and the maximum yield of 90.97% was obtained.

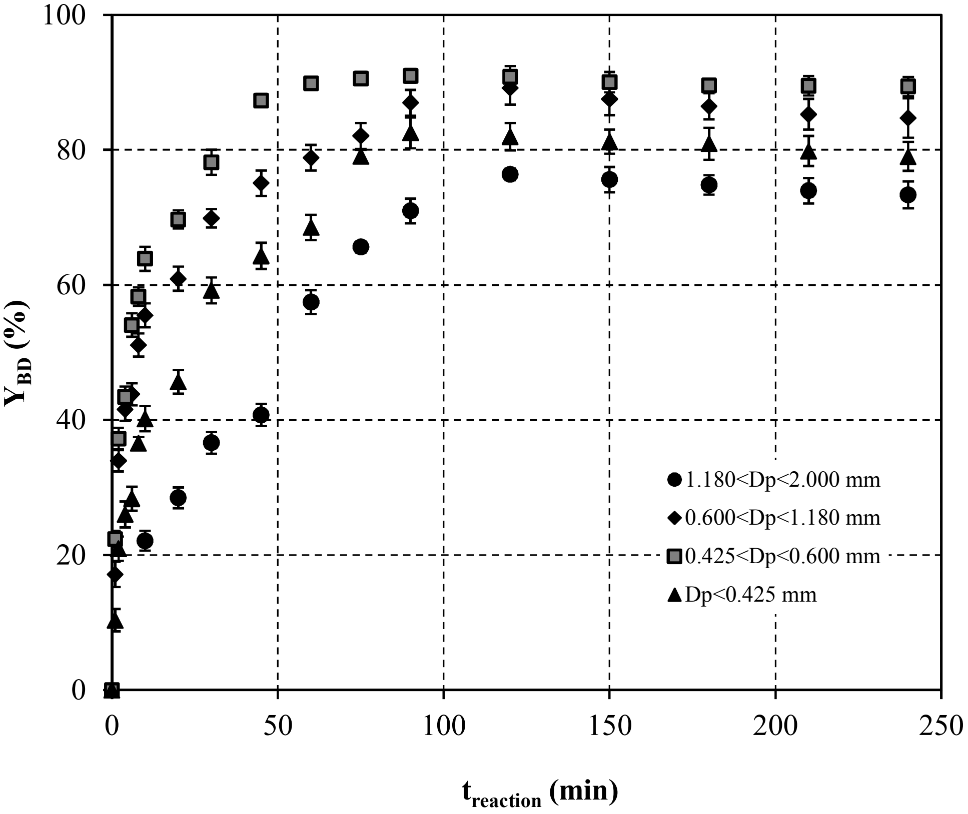


Figure 4.17: Methyl ester yield for different particle size with ratio of methanol volume to seed weight of 8.08:1, NaOH concentration of 1.94 wt.% at 57°C

Meanwhile for the other particle sizes, the reactions were accomplished at longer reaction time that was 90 minutes. At this reaction time, the biodiesel yield of $0.600<D_P<1.18$ mm particle size differed insignificantly from particle size of $0.425<D_P<0.600$ mm, and only these particle sizes produced biodiesel yields more than 85%.

On the contrary of this study, Kaul, *et al.* [34] obtained that the biggest seed size more than 2.46 mm produced the maximum conversion into biodiesel of approximately 98% within 60 minutes reaction time. Kasim and Harvey [37] evaluated the effect of particle sizes of <0.5 mm, 0.5-0.71 mm, 0.71-1 mm, 1-2 mm, and 2-4 mm on the biodiesel yields. They mentioned that the highest yield of 86.1% was achieved at the smallest particle size (<0.5 mm) within 60 minutes reaction time, followed by particle size of 0.5-0.71 mm with the yield of 83.7%. The other particle sizes produced lowered biodiesel yield, less than 80%. Zakaria and Harvey [27] reported that using particle size of 300-500 μm could attain the maximum yield of methyl ester around 1 h. Nonetheless, increasing the particle size to 1000-1400 μm prolonged the reaction completion to be more than 3 h.

4.3.5 The properties of fatty acid methyl ester

In comparison with fatty acid isopropyl ester produced by *in-situ* transesterification of *Jatropha curcas* with isopropanol, the yield of fatty acid methyl ester was higher and its quality much better since methanol was more polar than isopropanol, thus its biodiesel could be separated and washed easier from the by-product and impurities with lower saponification and lower water consumption. The drawback of methanol was the lower ability to extract *Jatropha curcas* oil from the seeds. The properties of methyl ester produced at optimum condition including the ratio of methanol to seed weight of 8.08, NaOH concentration of 1.94 wt.%, $0.425<D_P<0.600$ mm particle size, 500 rpm stirring speed at 57°C for 120 min are presented in Table 4.6. The properties were in the range of standard requirements of EN 14214 and ASTM D6751.

4.4 *In-situ* Transesterification of *Jatropha curcas* Seeds with Methanol and Isopropanol

Based on the extractability of alcohols to *Jatropha curcas* oil, low capacity of methanol in extracting oil can be improved by adding isopropanol. By adding isopropanol in the volume ratio of 1:1 to methanol, its extractability to be 5.4 times larger than without the addition of isopropanol. *In-situ* transesterification of *Jatropha curcas* seeds with isopropanol produced lower biodiesel yield of (85.32 ± 0.30)% and poorer its properties than that with methanol which provided (90.45 ± 0.25)% biodiesel yield and better properties. Therefore, *in-situ* transesterification *Jatropha curcas* seeds were conducted using the mixture of methanol and isopropanol in order to enhance the biodiesel yield by maintaining the better properties of biodiesel produced. Four independent variables which were tested included the ratio of the mixture volume to seed weight, NaOH concentration, and volume ratio of methanol to the mixture of methanol and isopropanol, and reaction temperature.

Table 4.6: The properties of fatty acid methyl ester

Parameter	Unit	Value	Method	Limits
Density at 15°C	kg/m^3	880	ASTM D-4052	860-900
Kinematic viscosity at 40°C	mm^2/s	4.99	ASTM D-445	1.9-6.0
Acid value	mg KOH/g	0.1940	ASTM D-664	0.8 max
Ester content	% mass	99.80	EN 14103	96.5 min
Total glycerin	% mass	0.198	ASTM D-6584	0.240 max
Free glycerin	% mass	0.00082	ASTM D-6584	0.020 max
Flash point (closed cup)	°C	176	ASTM D-93	130 min
Cloud point	°C	8	ASTM D-2500	Report to customer
Pour point	°C	-3	ASTM D-97	-15 to 10

Meanwhile, variables which were kept constant during the reaction comprised stirring speed of 500 rpm (based on the previous experiment with methanol), the particle size of less than 600 μm, the seed weight of 20 g, and reaction time of 120 min. To determine the optimum condition of this process with a minimum number of experimental runs, response surface methodology, a central composite design was employed.

4.4.1 Optimization of *in-situ* transesterification of *Jatropha curcas* seed with the mixture of methanol and isopropanol

4.4.1.1 ANOVA and regression analysis

The relation between the response of methyl-isopropyl ester yield and four independent variables of reaction (the ratio of mixture volume to seed weight, NaOH concentration, and Volume ratio of methanol to the mixture of methanol and isopropanol, and reaction temperature) were estimated using response surface methodology. Twenty seven experimental runs were conducted according to the full 2^4 central composite design and the results of each test are presented in Table 4.7.

The yields of methyl-isopropyl ester varied in the range of 29.10% to 89.05%. The minimum yield of 29.10 was obtained with 8.75 ratio of the mixture volume to seed weight, 0.5 wt.% NaOH concentrations, and 0.5 volume ratio of methanol to the mixture at 60°C. Meanwhile, the maximum yield of 89.05% was reached at 10 ratio of mixture volume/seed weight, 2 wt.% NaOH concentration, 0.3 ratio of methanol volume to the mixture volume at 70°C. Multiple regression was used to construct the empirical second-order polynomial model based on the observed response of the full factorial central composite design to predict biodiesel yield, as a function of significant variables and was depicted in Eq. (4.2) (with positive sign in front of the terms indicates synergistic effect and negative sign indicates antagonistic effect):

$$\begin{aligned} Y = {} & -325.1 + 42.77R_{VW} + 36.33C_{NaOH} + 346.27R_V + 2.15T - 2.14R_{VW}^2 + 3.93R_{VW}C_{NaOH} \\ & -28.32R_{VW}R_V + 0.13R_{VW}T - 35.15C_{NaOH}^2 + 30.69C_{NaOH}R_V + 0.69C_{NaOH}T - 188.61R_V^2 \\ & + 1.07R_VT - 0.04T^2 \end{aligned} \tag{4.2}$$

where,

Y	:	predicted biodiesel yield (%)
R_{VW}	:	ratio of methanol and isopropanol volume to seed weight ($mL\cdot g^{-1}$)
C_{NaOH}	:	sodium hydroxide concentration (wt.%)
R_V	:	volume ratio of methanol to the mixture of methanol and isopropanol
T	:	reaction temperature (°C)

Table 4.7: The difference between the observed and fitted value ($\Delta_{obs\text{-}fitted}$) of biodiesel yield (%) for the mixture of methanol and isopropanol

Test	**A**	**B**	**C**	**D**	**observed value (%)**	**Fitted value (%)**	**$\Delta_{obs\text{-}fitted}$ (%)**
1	8.75	1.5	0.5	60	85.35	84.97	0.38
2	6.25	1.5	0.5	60	62.33	59.71	2.62
3	10	2	0.3	50	78.16	77.18	0.98
4	10	2	0.7	50	74.54	72.89	1.65
5	7.5	1	0.7	70	50.43	52.59	-2.16
6	11.25	1.5	0.5	60	80.12	83.54	-3.42
7	7.5	1	0.3	50	45.40	45.15	0.25
8	8.75	1.5	0.1	60	44.78	46.79	-2.01
9	8.75	1.5	0.5	60	84.68	84.97	-0.29
10	7.5	2	0.7	50	72.98	73.41	-0.43
11	7.5	2	0.3	70	48.75	50.38	-1.63
12	7.5	1	0.7	50	54.39	56.91	-2.52
13	10	2	0.7	70	87.38	88.81	-1.43
14	10	2	0.3	70	89.05	84.55	4.50
15	10	1	0.7	50	47.02	46.57	0.45
16	10	1	0.3	50	63.84	63.14	0.71
17	10	1	0.7	70	50.15	48.62	1.53
18	8.75	1.5	0.5	60	84.89	84.97	-0.08
19	8.75	0.5	0.5	60	29.10	27.62	1.48
20	8.75	1.5	0.5	80	70.21	70.64	-0.43
21	10	1	0.3	70	55.88	56.63	-0.75
22	7.5	2	0.3	50	49.83	49.38	0.45
23	7.5	1	0.3	70	32.62	32.29	0.33
24	8.75	1.5	0.5	40	67.22	67.59	-0.37
25	7.5	2	0.7	70	84.24	82.96	1.28
26	8.75	2.5	0.5	60	69.75	72.03	-2.28
27	8.75	1.5	0.9	60	64.01	62.80	1.21

Notes: **A** = V_{Mix}: W_{seed} (mL/g), **B** = [NaOH] (wt.%), **C** = V_{MeOH}:V_{mix} (mL/mL), and **D** = T (°C)

The empirical second-order polynomial model was tested against the empirical data to determine the level of significance and the level of the fit according to test procedure of analysis of variance. The confidence level of 95% was selected to determine the significance level of the empirical model and each term in it. Table 4.8 presents the analysis of variance for the regression model. The computed F-value of 143.24 was larger than the theoritical F-value of 2.64 and the P-value of fitted model of < 0.0001 was lower than 0.05, which demonstrated that the empirical model had high significance and could be dependable to predict the yield of methyl-isopropyl ester.

Each regression coefficients of the second-order polynomial model was tested at confidence level of 95% to determine its significance. The empirical model terms of A, B, C, AA, AB, AC, AD, BB, BC, BD, CC, CD, and DD had significant effects on the methyl-isopropyl ester yield because the computed F-values for the respective terms were higher than the theoretical $F_{0.05(1,12)}$ value of 4.75 and P-values lower than 0.05 (5%). According to the F-value, the term of NaOH concentration was the most significant variable to the yield of methyl-isopropyl ester, consequently the quadratic term of NaOH concentration also had high significant effect to methyl-isopropyl ester yield. The linear term of ratio of the mixture volume to seed weight, the interaction term between ratio of V_{mixure} to W_{seed} and the ratio of V_{MeOH} to $V_{mixture}$, the quadratic term of the ratio of V_{MeOH} to $V_{mixture}$ had large effect on the methyl-isopropyl ester yield since their F-values were very high and their P-values were very low.

Meanwhile, the reaction temperature (D) term indicated insignificant effect on the response of methyl-isopropyl ester yield as the F-value of 2.17 was lower than the theoretical $F_{0.05(1,12)}$ value of 4.75 and P-value of 0.1668 was higher than 0.05. This reaction temperature term should be excluded from the analysis of variance and it should not be appeared in the second-order model of Eq. (4.2). However, this term was not excluded from the analysis of variance and from the empirical model since the interaction between reaction temperature (D) and other variables as well as the quadratic term of reaction temperature had larger the computed F-values than the theoretical one and lower P-values than 0.05 which demonstrated that these terms had significant effects on methyl-isopropyl ester yield. Consequently, by including the D term the adjusted coefficient of multiple determinations was slightly lower than the coefficient of multiple determinations.

Table 4.8: Analysis of Variance for the empirical second-order polynomial model in Eq. (4.2)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	8930.00	14	637.86	143.24	< 0.0001
A: V_{mix} : W_{seed}	851.565	1	851.565	131.89	0.0000
B:[NaOH]	2959.26	1	2959.26	458.32	0.0000
C: V_{MeOH}:V_{mix}	384.48	1	384.48	59.55	0.0000
D:Temp	13.9843	1	13.9843	2.17	0.1668
AA	237.541	1	237.541	36.79	0.0001
AB	96.4324	1	96.4324	14.94	0.0022
AC	802.022	1	802.022	124.22	0.0000
AD	40.5132	1	40.5132	6.27	0.0277
BB	1647.13	1	1647.13	255.10	0.0000
BC	150.676	1	150.676	23.34	0.0004
BD	192.377	1	192.377	29.79	0.0001
CC	1214.24	1	1214.24	188.06	0.0000
CD	73.1025	1	73.1025	11.32	0.0056
DD	335.28	1	335.28	51.93	0.0000
Total error	77.4803	12	6.45669		
Total (corr.)	7814.54	26			

In order to test the fit of the model, the determination coefficient was evaluated. Figure 4.18 shows the predicted versus observed value for methyl-isopropyl ester yield with high coefficient of multiple determination of 0.99. The empirical second-order polynomial model could explain 99% of the variability in biodiesel yield and only 1% of the total variations were not explained by this model. The value of adjusted coefficient of multiple determinations (adjusted R^2), which was more suitable for comparing model with different numbers of independent variables, was also high of 0.9785. A high R^2 and adj-R^2 reveals that the empirical model can be applied to predict the response of biodiesel yield with high precision.

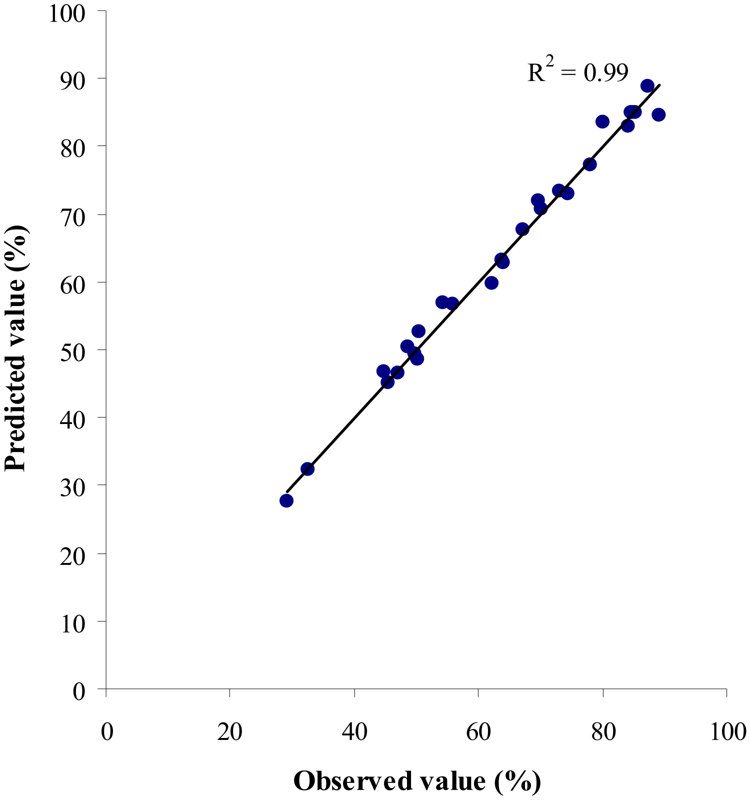


Figure 4.18: Predicted versus observed methyl-isopropyl yield

4.4.1.2 Effect of the ratio of methanol and isopropanol volume to seed weight (V(MeOH+IPA)/Wseed) on the yield of methyl-isopropyl ester

Discussion the effect of the mixture volume to seed weight ratio on the methyl-isopropyl yield must involve the influences of other independent variables, namely NaOH concentration, volume ratio of methanol to the mixture of methanol and isopropanol, and reaction temperature, since the interactions between the mixture volume to seed weight ratio and these variables had significant influences on the yield of methyl-isopropyl ester.

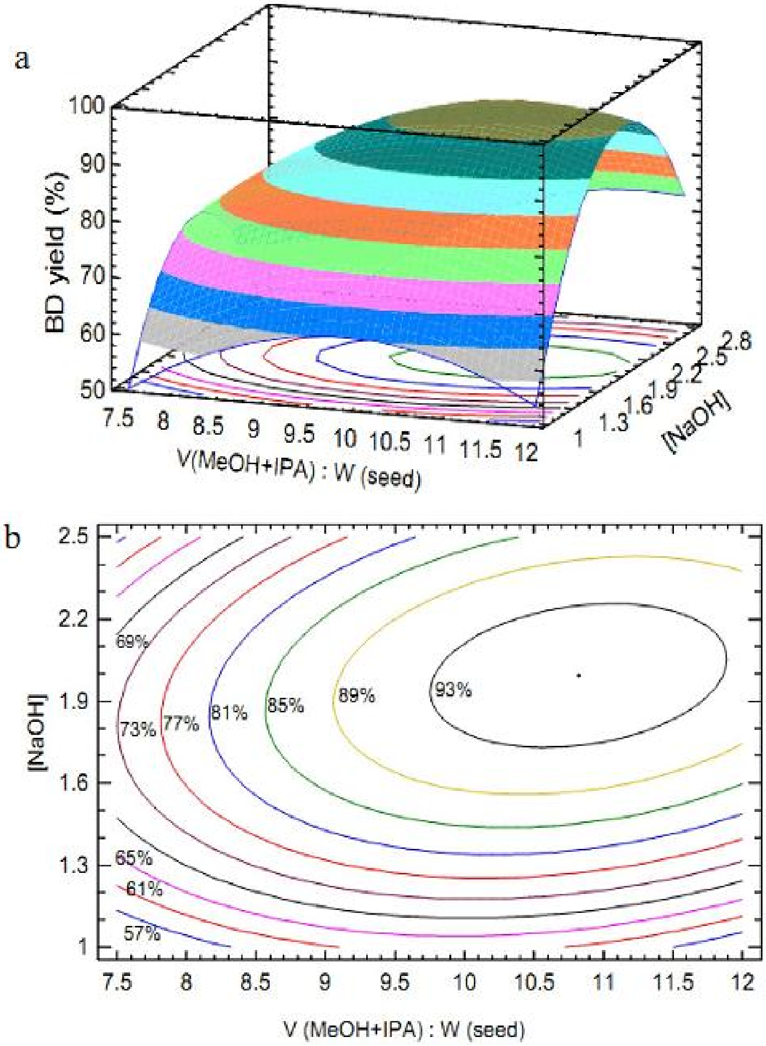


Figure 4.19: The estimated response surface (a) and contour plot (b) of methyl-isopropyl ester yield with the constant of $V_{MeOH}/V_{(MeOH+IPA)} = 0.46$ at 68°C

Figure 4.19 shows the estimated response surfaces and contours of methyl-isopropyl yield at 68°C and the constant of methanol volume to the mixture volume ratio of 0.46. In *in-situ* transesterification with methanol, the interaction term of V_{MeOH}/W_{seed} ratio and NaOH concentration had insignificant effect on methyl ester yield since the computed F-value was lower than the theoretical one and the P-value was more than 0.05, thus it was not included in the empirical model of methanol of

Eq. (4.1). Meanwhile, in this process, the interaction term of $V_{(MeOH+IPA)}/W_{seed}$ ratio had significant influence on the methyl-isopropyl ester yield, according to the computed F-value of 14.94, which was higher than the theoretical F-value of 2.17 and the P-value of 0.0022 was lower than 0.05.

Increasing the ratio of $V_{(MeOH+IPA)}/W_{seed}$ from 7.5 to 10.8 and rising NaOH concentration from 1 wt.% to 1.99 wt.% enhanced the methyl-isopropyl ester yield, in line with positive interaction term of R_{VW} and C_{NaOH} in Eq. (4.2).

However, further augmenting in $V_{(MeOH+IPA)}/W_{seed}$ ratio and NaOH concentration diminished slightly the biodiesel yield. In this condition, the formation of soap was observed during separation process of the ester-rich phase from the glycerol-rich phase, although the formation of soap in this process was not as much as in the previous process of *in-situ* transesterification with isopropanol.

Increasing $V_{(MeOH+IPA)}/W_{seed}$ ratio augmented the mixture volume of methanol and isopropanol, since the seed weight was kept constant. It was observed during the biodiesel separation that higher the mixture volume enhanced the volume of glycerol-rich phase or lower layer and decreased the volume of biodiesel-rich phase or upper layer. To examine it, the lower layer was drawn out and placed in another separator funnel. It was added with water and shaken several minutes, thereafter two layers were formed which showed that some part of biodiesel-rich phase was dissolved in the glycerol-rich phase. It was conformed with another research work conducted by Zakaria and Harvey [27]. They added n-hexane instead of water to observe the dissolution of biodiesel in glycerol layer at high molar ratio of methanol to oil. It can be inferred that higher ratio of $V_{(MeOH+IPA)}/W_{seed}$ decreased the yield of biodiesel due to some part of biodiesel was dissolved in glycerol-rich layer and separated out from its layer. Besides that, higher volume of methanol and isopropanol could dilute NaOH catalyst and the oil which lessened the interaction between them during transesterification. Consequently, the oil conversion to methyl-isopropyl ester decreased.

Figure 4.20 depicts the estimated response surface of biodiesel yield and its contour of the interaction between $V_{(MeOH+IPA)}/W_{seed}$ ratio and $V_{MeOH}/V_{(MeOH+IPA)}$ ratio.

The interaction between the ratio of $V_{(MeOH+IPA)}/W_{seed}$ and $V_{MeOH}/V_{(MeOH+IPA)}$ ratio provided a very significant effect on the methyl-isopropyl ester yield due to its larger F-value of 124.22 than other interaction terms.

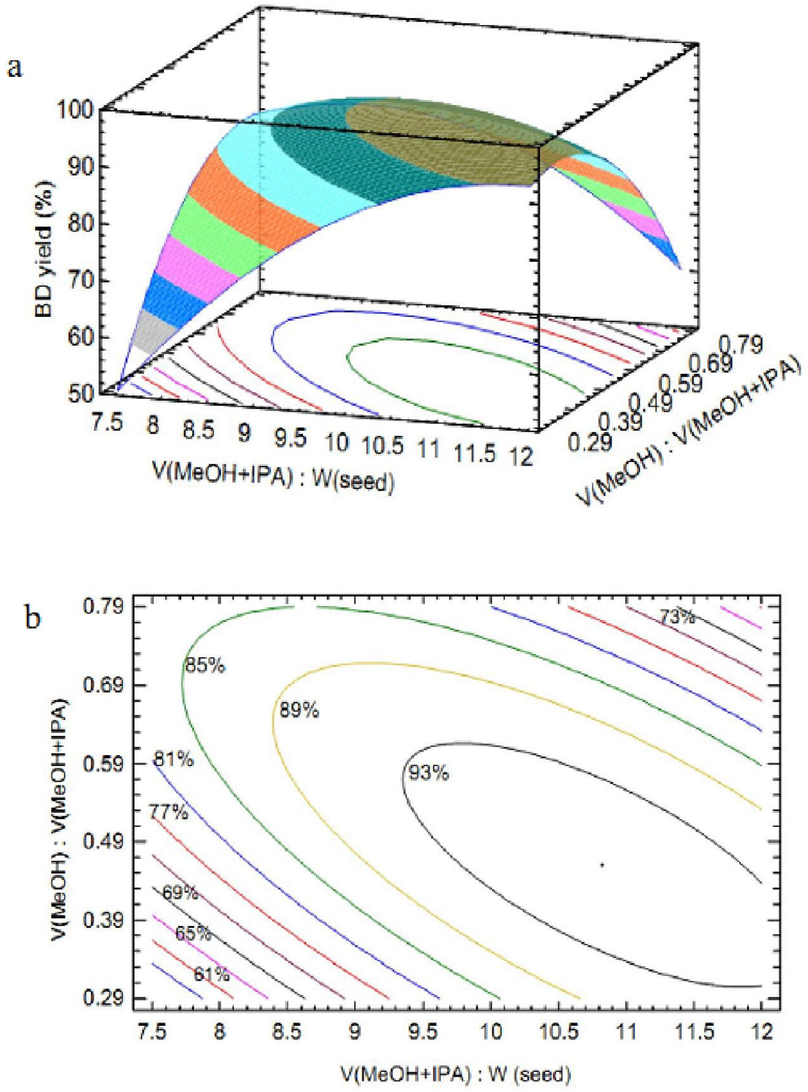


Figure 4.20: The estimated response surface (a) and contour plot (b) of methyl-isopropyl ester yield with the constant of [NaOH] = 1.99 wt.% at 68°C

Rising the ratio of $V_{(MeOH+IPA)}/W_{seed}$ from 7.5 to 10.8 and enhancing the ratio of $V_{MeOH}/V_{(MeOH+IPA)}$ from 0.3 to 0.46 increased the extraction of oil and the methyl-isopropyl ester yield. Subsequently, raising the ratio of $V_{(MeOH+IPA)}/W_{seed}$ above 10.8 along with increase in the ratio of $V_{MeOH}/V_{(MeOH+IPA)}$ decreased the yield of methyl-isopropyl ester, as resulted the negative interaction term of R_{VW} and R_V in Eq. (4.2). Increasing $V_{MeOH}/V_{(MeOH+IPA)}$ ratio revealed rising the volume of methanol and decreasing isopropanol in the mixture. Due to the lowest ability of methanol to extract oil, the higher $V_{(MeOH+IPA)}/W_{seed}$ ratio along with the higher $V_{MeOH}/V_{(MeOH+IPA)}$ reduced the extracted oil from the seed and the conversion of oil to methyl-isopropyl ester as well as the yield. Moreover, diluting effect of catalyst and the extracted oil due to increase in $V_{(MeOH+IPA)}/W_{seed}$ ratio contributed to diminish the oil conversion to the yield of ester. In order to obtain high ester yield, one variable should be set at low value while another variable adjusted at high value.

Figure 4.21 presents the estimated response surface of biodiesel yield and its contour of the interaction between $V_{(MeOH+IPA)}/W_{seed}$ ratio and reaction temperature. This interaction was the term which had the least influence on the yield of methyl-isopropyl ester, due to the lowest of the computed F-value of 6.27 than other terms.

Raising the ratio of $V_{(MeOH+IPA)}/W_{seed}$ from 7.5 to 10.8 along with the increment of reaction temperature from 50°C to 68°C elevated the methyl-isopropyl ester yield. Further increasing $V_{(MeOH+IPA)}/W_{seed}$ ratio beyond 10.8 and reaction temperature above 68°C lowered insignificantly its yield. At low $V_{(MeOH+IPA)}/W_{seed}$ ratio, increasing reaction temperature up to 68°C only enhanced slightly the yield of biodiesel. Next rising reaction temperature reduced inconsiderable the ester yield, corresponding to the small positive interaction term of R_{VW} and T in Eq. (4.2). Over 12 of $V_{(MeOH+IPA)}/W_{seed}$ ratio, the increment of reaction temperature did not affect the conversion of oil to methyl-isopropyl ester.

It was in accordance with the analysis of variance where the linear term of reaction temperature had lower computed F-value than theoretical one which revealed the reaction temperature had insignificant effect on the response of methyl-isopropyl ester yield. The minor enhancement of oil conversion to ester which was gained from increasing the temperature in the range of 30°C to 65°C were reported by other research works [24, 27, 32].

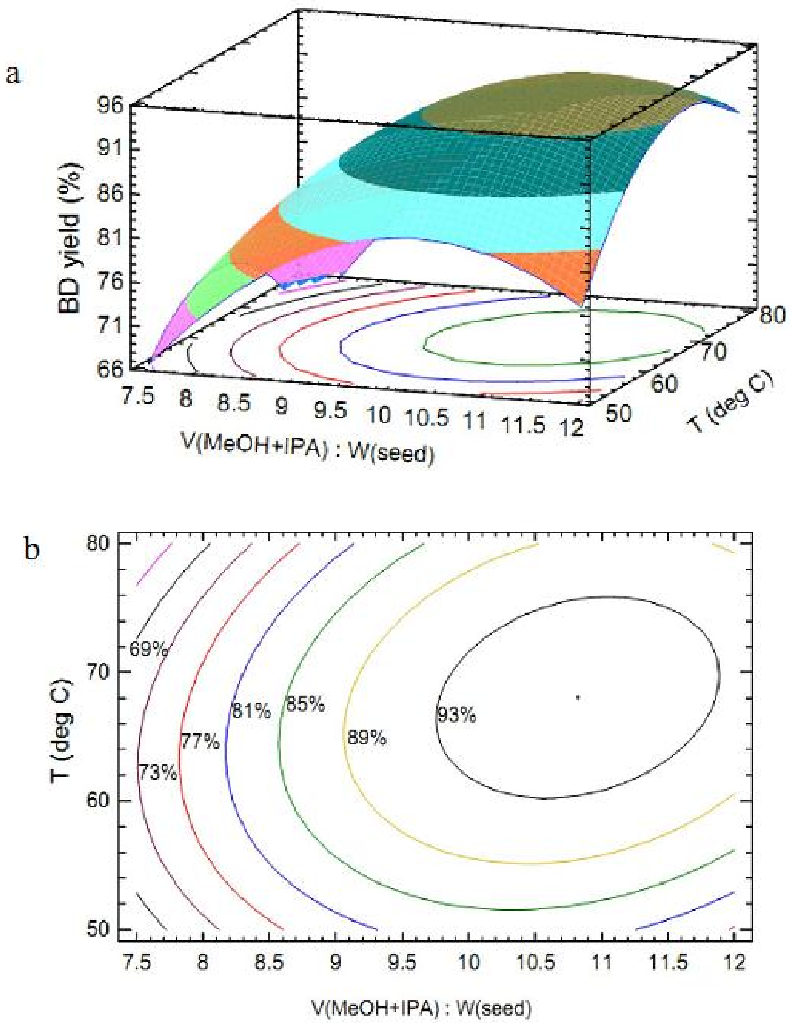


Figure 4.21: The estimated response surface (a) and contour plot (b) of methyl-isopropyl ester yield with the constant of [NaOH] = 1.99 wt.% and $V_{MeOH}/V_{(MeOH+IPA)}$=0.46 effect of NaOH

4.4.1.3 Effect of sodium hydroxide concentration on the yield of methyl-isopropyl ester

The effect of NaOH concentration on the methyl-isopropyl ester yield was associated with its interaction with $V_{MeOH}/V_{(MeOH+IPA)}$ ratio and reaction temperature besides with $V_{(MeOH+IPA)}/W_{seed}$ ratio which has discussed previously. Figure 4.22 displays the interactive effect of NaOH concentration and $V_{MeOH}/V_{(MeOH+IPA)}$ ratio.

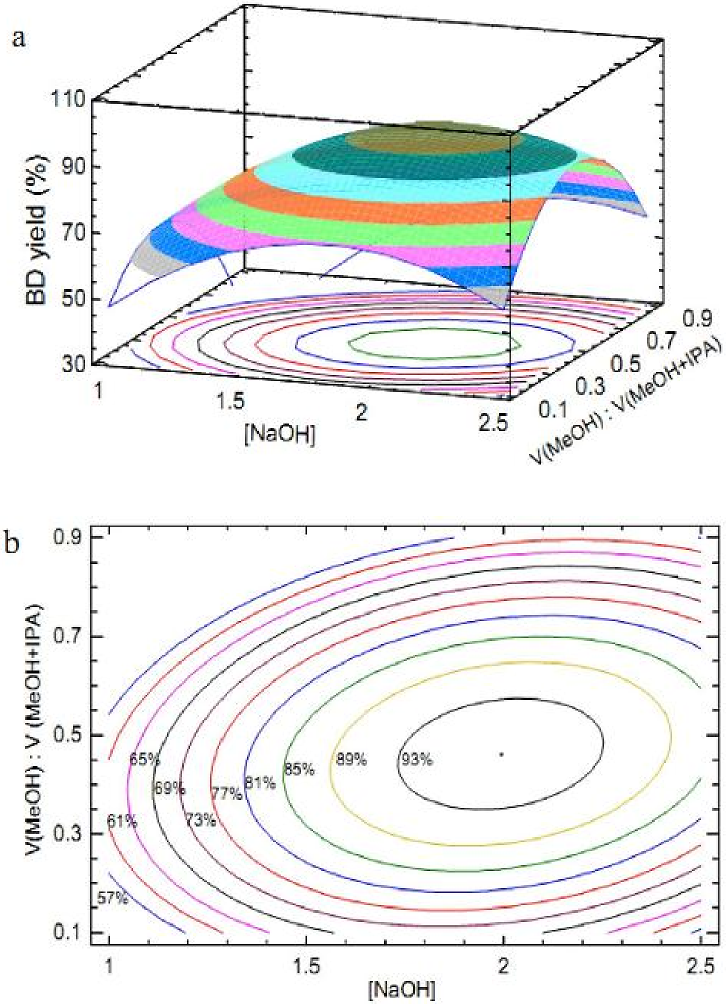


Figure 4.22: The estimated response surface (a) and contour plot (b) of methyl-isopropyl ester yield with the constant of $V_{(MeOH+IPA)}/W_{seed}$ = 10.8 at 68°**C**

Increase in NaOH concentration from 1 wt.% to 1.99 wt.% associated with rising $V_{MeOH}/V_{(MeOH+IPA)}$ ratio from 0.1 to 0.46 enhanced significantly the extraction of oil from the seeds and its conversion to methyl-isopropyl ester, which in line with the positive interaction term of C_{NaOH} and R_V in second-order polynomial model. Kasim and Harvey [37] stated that the presence of NaOH in the alcohol significantly enhanced the extraction efficiency of *Jatropha curcas* oil, almost all of the triglycerides in the seeds were removed.

Nevertheless, excess NaOH concentration addition followed by higher $V_{MeOH}/V_{(MeOH+IPA)}$ ratio lowered the oil extraction as well as its conversion to methyl-isopropyl ester. Higher $V_{MeOH}/V_{(MeOH+IPA)}$ ratio showed the volume of methanol increased, inversely, the isopropanol volume reduced which affected in the oil extracted from the seeds to be lessened.

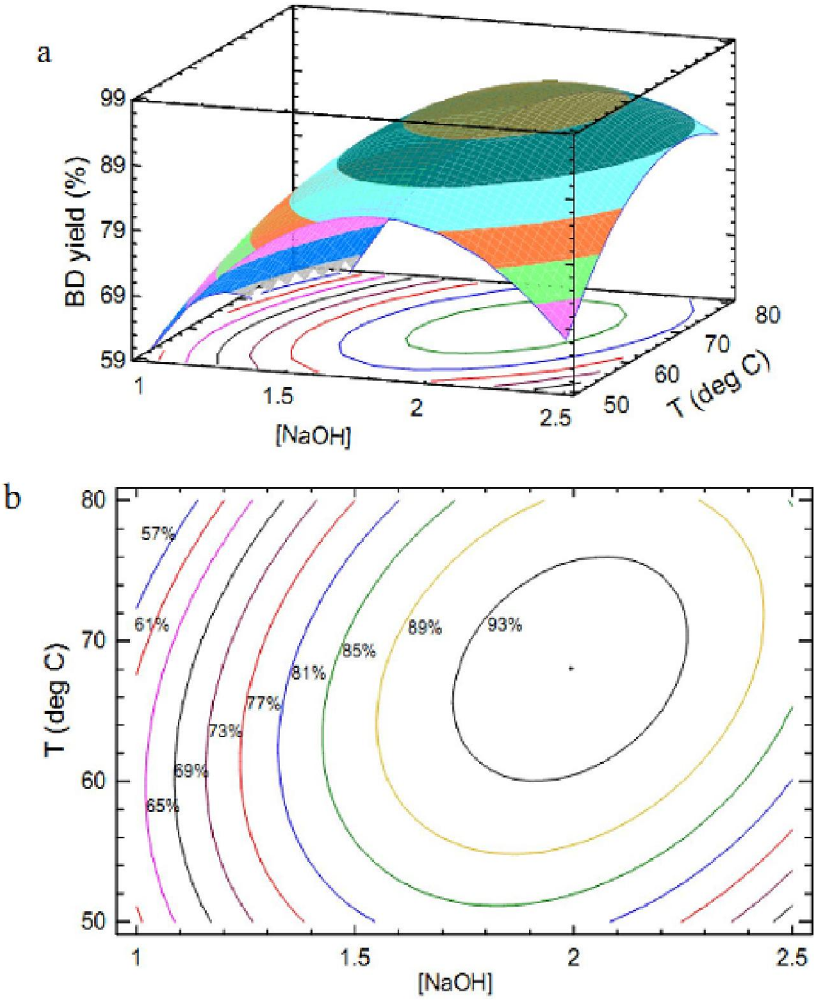


Figure 4.23: The estimated response surface (a) and contour plot (b) of methyl-isopropyl ester yield with the constant of $V_{(MeOH+IPA)}/W_{seed}$ = 10.8 and $V_{MeOH}/V_{(MeOH+IPA)}$=0.46

Figure 4.23 describes the effect of interaction between NaOH concentration and reaction temperature on biodiesel yield. It can be observed that raising NaOH concentration from 1 wt.% to 1.99 wt.% as well as the increment of reaction temperature from 50°C to 68°C augmented greatly the oil extraction and its conversion to methyl-isopropyl ester. It indicated that sufficient NaOH concentration had to be added in reaction to complete the conversion of oil to ester. At lower adding of NaOH concentration, elevating reaction temperature improved indifferently the yield of methyl-isopropyl ester. On the contrary, at higher NaOH concentration than 1.99 wt.%, rising reaction temperature over 68°C decreased inconsiderable the ester yield, complying the minor effect of reaction temperature on the yield of ester.

Higher NaOH amount addition in the reaction led the formation of soaps. When NaOH reacted with methanol and isopropanol, the water was formed besides the alkoxide. Water could hydrolyse triglycerides in the presence of NaOH to produce sodium soaps. The soap inhibits separation of the methyl-isopropyl esters and glycerol. During wash step with water, soaps caused the emulsion formation. The formation of soaps and its separation from biodiesel in *in-situ* transesterification with the mixture of methanol and isopropanol was less than that with isopropanol. However, it was slightly higher than that with methanol. The amount of water consumption in wash step was in the range between its consumption in the process with methanol and that in the process with isopropanol. The formation of soaps increased when the reaction carried out at higher reaction temperature and higher NaOH concentration. In this condition, hydrolysis of triglyceride was accelerated.

4.4.1.4 Optimization analysis

The optimum conditions of four independent variables, the ratio of $V_{(MeOH+IPA)}/W_{seed}$, NaOH concentration, the ratio of $V_{MeOH}/V_{(MeOH+IPA)}$, and reaction temperature, which maximized the methyl-isopropyl yield were attained by numerical optimization using Statgraph Centurion 15.2.11.0 software (by StatPoint Inc.). Table 4.9 shows the high and low limit experimental region as well as the optimum factors. The optimum conditions obtained by design of experiment included 10.82 $V_{(MeOH+IPA)}/W_{seed}$ ratio, 1.99 wt.% NaOH concentration, 0.46 volume ratio of methanol to the mixture of methanol and isopropanol at 68°C reaction temperature. At these optimum conditions, the predicted maximum methyl-isopropyl yield obtained was 95.31%.

Table 4.9: The combination of factor levels which maximized methyl-isopropyl yield

Factor	**Low**	**High**	**Optimum**
$V_{(MeOH+IPA)}$: W_{seed} ratio (mL/g)	6.25	11.25	10.82
[NaOH] wt.%	0.5	2.5	1.99
V_{MeOH} : $V_{(MeOH+IPA)}$ ratio (mL/mL)	0.1	0.9	0.46
T (^{0}C)	40.0	80.0	68

In order to verify the predicted maximum methyl-isopropyl yield, *in-situ* transeterification of *Jatropha curcas* seeds was performed at optimum conditions triplicate. Table 4.10 presents the comparison of the predicted and experimental (observed) maximum methyl-isopropyl yield.

Table 4.10: The comparison of the predicted and observed maximum methyl-isopropyl yield

Test	Factors				Response (%)		Residual
	V_{Mix}:W_{seed} (mL/g)	C_{NaOH} (wt.%)	V_{MeOH}: V_{Mix} (mL/mL)	T (^{0}C)	Obsv.	Predc	$\Delta_{obs\text{-}predc}$
1	10.82	1.99	0.46	68	95.02	95.31	-0.29
2	10.82	1.99	0.46	68	94.87	95.31	-0.44
3	10.82	1.99	0.46	68	94.46	95.31	-0.85

The maximum biodiesel yields obtained from experiment was (94.78 ± 0.29)% indicated that there was insignificantly difference between the predicted maximum methyl-isopropyl yield and the experimental one. This optimum ester yield was slightly higher than the maximum biodiesel yield achieved by Lee, *et al.* [133] who explored transesterification of *Jatropha curcas* oil using CaO–MgO mixed oxide catalyst. They determined the optimum conditions of reaction by employing response surface methodology in conjunction with the central composite design, which included methanol/oil molar ratio of 38.67, reaction time of 3.44 h, catalyst amount of 3.70 wt.% at 115.87°C reaction temperature, to obtain 93.55% biodiesel yield, less 2% than the predicted one. Sahoo and Das [74] examined the biodiesel production from *Jatropha curcas* oil which compared with karanja and polanga oils. They applied triple stage transesterification to transesterify the high free fatty acid oils. Ortho-phosphoric acid, sulfuric acid, and potassium hydroxide were used to convert the oils into fatty acid methyl esters. The conversion of *Jatropha curcas* oil to biodiesel of 93% was produced which had 2% difference from the biodiesel yield of this work.

However, the biodiesel production from *Jatropha curcas* by *in-situ* transesterification which had been done were more economical than the previous transesterification performed by Lee, *et al*. as well as by Sahoo and Das since in the process of *in-situ* transesterification, the step of pre-extracted oil and oil refining were not necessary. The *Jatropha curcas* seeds were subjected as a feedstock and reacted directly with methanol-isopropanol catalyzed by sodium hydroxide, a low-cost catalyst, to achieve the higher yield of biodiesel. Thus the cost of process and chemicals could be reduced.

In optimization of biodiesel production from edible and non-edible vegetable oils, a two-step transesterification of *Jatropha curcas* oil, which had initial acid value of 28 mg KOH/g, was undertaken. Two hours reaction time for each step or 4 h total reaction time was required to complete the oil conversion to biodiesel and 90-95% methyl ester yield was attained which was comparable to the methyl ester yield obtained in this research work [78].

Lei, *et al.* [31] reported that in biodiesel production from low quality rice bran through *in-situ* extraction, esterification and transesterification process, the rate of esterification and the transesterification rate reached 98.83% and 80.47%. , using 75 mL of absolute methanol, 150 mL of petroleum ether, 0.75 g of concentrated sulfuric acid and 0.71 g of sodium hydroxyl. The methyl ester yield of 95.16%, which was similar to the biodiesel yield of this work, was calculated based on the esterification and transesterification rates as well as the free fatty acid and triglyceride contents of the extracted rice bran oil.

The optimum methyl-isopropyl ester yield of (94.78 ± 0.29)% was higher than the optimum methyl ester yield of (90.45 ± 0.25)% which revealed that the addition of isopropanol to methanol could improve the *Jatropha curcas* oil conversion to biodiesel and its yield. This was in accordance with the solubility and extractability of alcohols to the *Jatropha curcas* oil. Methanol was only slightly soluble in the oil, whereas isopropanol could be dissolved easily in the oil. The oil extractability was higher with non-polar solvent compared to polar solvent due to vegetable oil was non-polar. Isopropanol was a short-chain alcohol with the highest oil extractability (47.54 ± 0.85)%, because it was less polarity than methanol. Therefore, the addition of isopropanol in methanol could increase the extracted oil followed by oil conversion to biodiesel. However, *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and isopropanol required more solvent volume than that with methanol. In this study, the optimum variable of alcohol volume to seed weight ratio for methanol was 8.08 that was 162 mL methanol volume to 20 g seed weight, while for the mixture of methanol and isopropanol the ratio was 10.82, which was 216 mL the mixture volume to 20 g seed weight. The mixture volume of 216 mL comprised methanol volume of 99.36 mL and isopropanol volume of 116.64 mL.

The volume of isopropanol was higher than methanol to extract *Jatropha curcas* oil from seeds, and then the extracted oil was mainly transesterified by methanol to be converted to biodiesel. There was a small portion of extracted oil which was transesterified by isopropanol to be biodiesel, with the isopropyl ester yield of 6.17%.

Figure 4.24 depicts the result of gas chromatography-mass spectroscopy (GCMS) of methyl-isopropyl ester at optimum condition. Higher peaks were methyl ester peaks indicated that methyl ester contributed the major composition of biodiesel of 93.83%, whereas isopropyl ester only composed 6.17% of total biodiesel.

The amount of catalyst required was higher for *in-situ* transesterification with the mixture of methanol and isopropanol, that was 1.99 wt.%, than that with methanol of 1.94 wt.%, since the solvent volume also higher in the process with the mixture of methanol and isopropanol. The boiling point of methanol is 64.7°C and boiling point of isopropanol is 82.5°C, then the boiling point of the mixture of methanol and isopropanol lies between those values. Generally, transesterification is conducted at below boiling point of the solvent. Therefore the optimum reaction temperature of *in-situ* transesterification with the mixture of methanol and isopropanol (68°C) was higher than that with methanol (57°C).

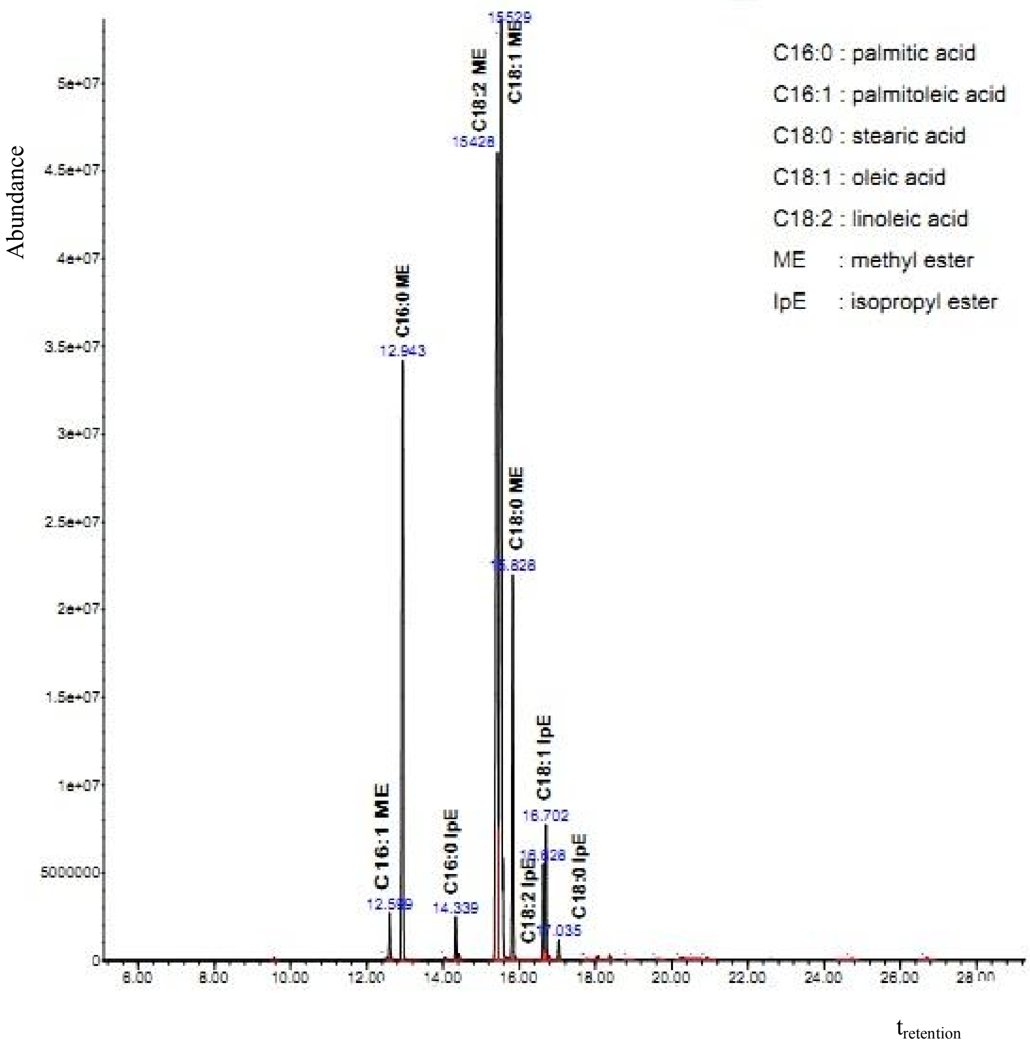


Figure 4.24: GCMS chromatogram of methyl-isopropyl ester produced at optimum condition

4.4.2 Effect of reaction temperature

Figure 4.25 depicts the effect of reaction temperature of 40, 50, 60, 65, and 70°C on the yield of methyl-isopropyl ester for various reaction times at the optimum reaction parameters. The reaction temperature influenced the rate of extraction and transesterification. At the first 30 min of reaction time, the biodiesel yield enhanced by increasing reaction time for all reaction temperature. Further increasing reaction

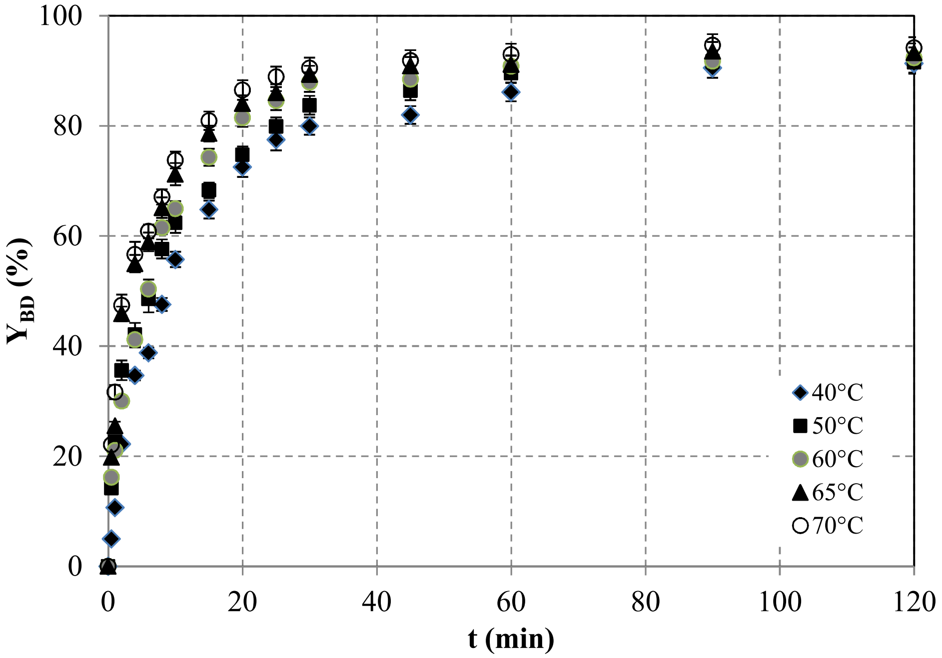


Figure 4.25: Effect of reaction temperature on the yield of methyl-isopropyl ester at various reaction time

time, the yield of biodiesel improved slightly and more than 60 min the biodiesel yield was almost constant. At this condition, the reaction temperature did not affect on enhancing the biodiesel yield, the amount of oil extracted was similar at all reaction temperature and transesterification was almost completed. This result was in conformity with other works [24, 27, 32, 37]. The insignificant effect of reaction temperature occurred at the first 4 min reaction time as well. At this reaction period, the oil extraction occurred through removal of oil by washing/dilution and the alcoholysis showed a lag time before significant amount of biodiesel resulted [134]. Hence, the effect of reaction temperature on extraction rate and on alcoholysis (transeterification) rate could be neglected.

The reaction temperature influenced the methyl-isopropyl ester yield at the reaction time between 4 min and before 60 min. At this condition, the oil extraction which was controlled by diffusion of the oil from capillary channel and molecular diffusion occurred faster at higher reaction temperature. The solubility of alcohol in an oil and rate of alcoholysis increased by increment of reaction temperature and enhance the conversion of triglyceride to biodiesel to achieve the maximum conversion in shorter time [27, 37].

The methyl-isopropanol ester yields were higher than the methyl ester yields for the same reaction time at temperature of 40, 50, 60°C. The oil conversion to methyl-isopropyl ester was completed in shorter time than oil conversion to methyl ester. It indicated that the addition of isopropanol to methanol could be facilitated the oil extraction to be converted into biodiesel.

4.4.3 Effect of particle size

Effect of particle size was determine at the optimum reaction conditions, 10.82 $V_{(MeOH+IPA)}/W_{seed}$ ratio, 1.99 wt.% NaOH concentration, 0.46 volume ratio of methanol to the mixture of methanol and isopropanol at 68 °C reaction temperature and different reaction time. Drying seeds were ground and sieved to obtain three different size of particles, which were 0.600 mm < D_P < 1.800 mm, 0.425 < D_P < 0.600 mm, and D_P <0.425 mm.

Figure 4.26 presents effect of different particle size on the yield of methyl-isopropyl ester at various reaction times. It was different from *in-situ* transesterification of *Jatropha curcas* seeds with methanol which demonstrated diminishing particle size from 1.18<D_P<2 mm up to 0.425<D_P<0.600 mm enhanced the biodiesel yield at all reaction time. Nevertheless, the yield was reduced when the smaller particle size than 0.425 mm was used. In the process of *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and isopropanol, reducing the particle size from 0.600<D_P<1.180 mm to D_P<0.425 mm increased the yield of biodiesel. The yield of biodiesel produced from particle size of 0.425<D_P<0.600 mm was insignificantly different from one of D_P<0.425 mm. Kasim and Harvey [37] evaluated the effect of particle sizes of <0.5 mm, 0.5-0.71 mm, 0.71-1 mm, 1-2 mm, and 2-4 mm on the biodiesel yields. They reported that the highest yield of 86.1% was achieved at the smallest particle size (<0.5 mm) within 60 minutes reaction time, which was slightly different with the yield of 83.7% produced from particle size of 0.5-0.71 mm. The other particle sizes produced lower the biodiesel yield, less than 80%.

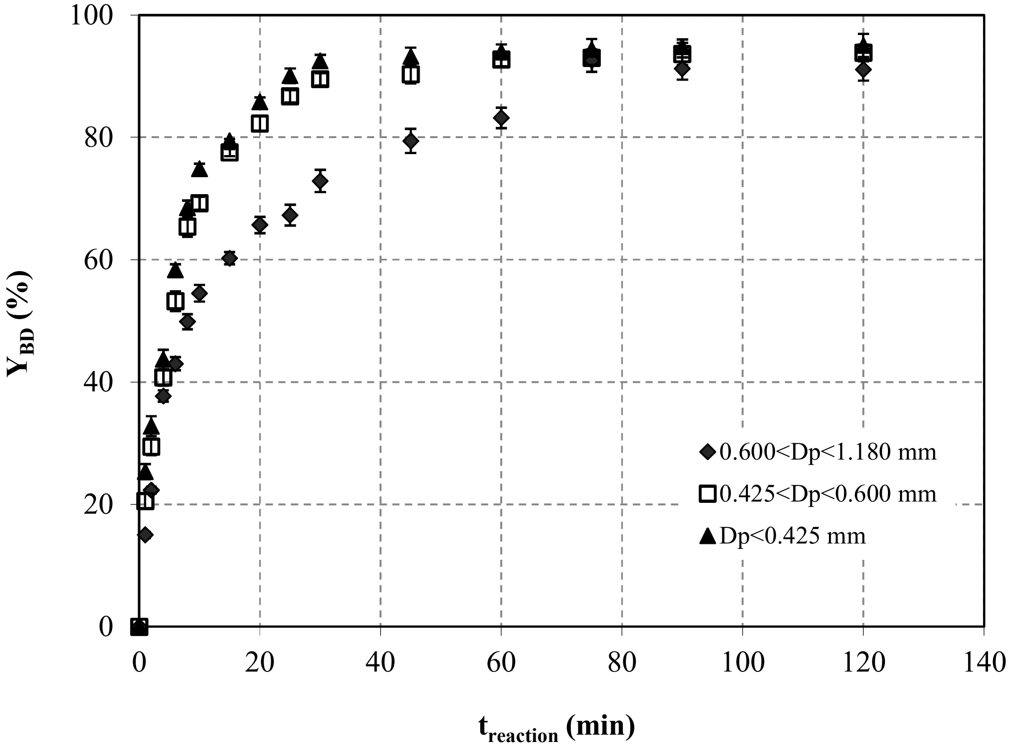


Figure 4.26: Effect of different particle size on the yield of methyl-isopropyl ester at optimum reaction conditions

At the first 30 min for smaller particle size of 0.425 < D_p < 0.600 mm, and D_p <0.425 mm, the yield of methyl-isopropyl ester enhanced sharply indicated that the oil extraction and transesterification proceeded in fast reaction rate and reached the reaction completion in this time. Meanwhile, for particle size of 0.600 mm < D_p < 1.800 mm, the yield improved by increasing reaction time and achieved its completion after 75 min. At the first 4 min and after 75 min of reaction time, the oil extraction and oil conversion rate were almost the same for all particle size indicated by the similar yield of biodiesel, which meant at this reaction period, the seed particle size had no influence on the oil extraction and its conversion to biodiesel. Shuit, *et al.* [35] investigated that the different particle size affected the extraction efficiency and fatty acid methyl ester yield. During 8 h reaction time, the different particle sizes in the range of 0.335 to 1 mm and less than 0.335 mm did not influence the extraction efficiency and fatty acid methyl ester yield which showed increasing gradually. Prolong reaction time more than 8 h, the extraction efficiency and methyl ester yield resulted from particle size less than 0.335 mm enhanced and reached the maximum extraction efficiency of 90% and the maximum methyl ester yield of 99.8% after 24 h reaction period. Meanwhile, the extraction efficiency and methyl ester yield resulted from bigger particle size in the range of 0.335 to 1 mm gave almost constant up to 24 h reaction time.

The highest methyl-isopropyl ester yield of (95.03 ± 1.90)% was achieved at the optimum reaction parameters with the particle size less than 0.425 mm after 2 h reaction time. Generally, the yields of methyl-isopropyl ester were higher than the yield of methyl ester at the same particle size. Moreover, the equilibrium condition of *in-situ* transesterification with the mixture of methanol and isopropanol was reached in a shorter time than one with methanol.

4.4.4 The properties of fatty acid methyl-isopropyl ester

In comparison with fatty acid isopropyl ester produced by *in-situ* transesterification of *Jatropha curcas* with isopropanol, the yield of fatty acid methyl-isopropyl ester was higher and better in its quality. However, the properties of fatty acid methyl-isopropyl ester were similar to the properties of fatty acid methyl ester. The properties of methyl-isopropyl ester produced at optimum condition including the ratio of the mixture volume to seed weight of 10.82, NaOH concentration of 1.99 wt.%, Dp < 0.425 mm particle size, 500 rpm stirring speed at 68°C for 120 min are presented in Table 4.11. The properties met with the standard requirements of EN 14214 and ASTM D6751.

Table 4.11: The properties of fatty acid methyl-isopropyl ester

Parameter	**Unit**	**Value**	**Method**	**Limits**
Density at 15°C	$kg\cdot m^{-3}$	880	ASTM D-4052	860-900
Kinematic viscosity at 40°C	$mm^2\cdot s^{-1}$	5.06	ASTM D-445	1.9-6.0
Acid value	$mg\ KOH\cdot g^{-1}$	0.1851	ASTM D-664	0.8 max
Ester content	% mass	99.33	EN 14103	96.5 min

Parameter	Unit	Value	Method	Limits
Total glycerin	% mass	0.186	ASTM D-6584	0.240 max
Free glycerin	% mass	0.0022	ASTM D-6584	0.020 max
Flash point (closed cup)	°C	177	ASTM D-93	130 min
Cloud point	°C	9	ASTM D-2500	Report to customer
Pour point	°C	-2	ASTM D-97	-15 to 10

4.5 *In-situ* Transesterification of Jatropha curcas seed in Soxhlet Reactor

In-situ alkaline transesterification proceeds through dissolution and alcoholysis of oil successively. The overall reaction rate is determined by the extraction and transesterification rate [32]. The ability of methanol to extract *Jatropha curcas* oil from its seeds was very low and its solubility was low in oil, n-hexane was mixed in methanol to improve the oil extraction and to speed up the reaction. Qian, *et al*. [32] added petroleum ether as a co-solvent to accelerate *in-situ* alkaline methanolysis of cottonseed. Meanwhile, Zeng, *et al*. [89] used diethoxymethane to enhance *in-situ* transesterification of sunflower oilseed. The high oil conversion was achieved in a very short reaction time. The co-solvent of n-hexane was utilized by Shuit, *et al*. [35] to improve the yield of methyl ester in reactive extraction and *in-situ* esterification of *Jatropha curcas* L. seeds.

In this study, *in-situ* transesterification of *Jatropha curcas* seeds was performed in Soxhlet extractor without agitation, as a packed bed reactor. Since methanol had the lowest extractability to *Jatropha curcas* oil, the n-hexane was added to enhance the ability to extract its oil from the seeds. The seeds were placed in thimble, while the mixture of methanol and n-hexane catalyzed by sodium hydroxide was poured in the round-bottom flask as a reactor. The heating mantle was used to heat the solvent and vaporized it. The solvent vapour was cooled by condenser and condensate dripped down into the thimble contained seeds in a chamber housing solid. Non-volatile compound in seeds was dissolved in the solvent. When chamber was full with solvent condensate, chamber was emptied and solvent flew down to the flask. In the flask, the solvent was heated and vaporized again, and then it was cooled by condenser. This process took place several times until certain reaction time. Three reaction parameters, including the ratio of the mixture volume to seed weight (R_{VW}), sodium hydroxide concentration (C_{NaOH}), and the volume ratio of methanol to the mixture of methanol to n-hexane (R_V), were tested to obtain the optimum condition which maximized the methyl ester yield. Response surface methodology with Box-Behnken design was employed to determine the empirical models, to optimize the reaction factors, and to predict the maximum of yield.

4.5.1 Optimization of ***In-situ*** transesterification of Jatropha curcas seeds with the mixture of methanol and n-hexane in Soxhlet Reactor

4.5.1.1 Analysis of variance and regression analysis

The experimental data of three parameters and the response yield are presented in Table 4.12. Each result was expressed as arithmetic mean of duplicate. Experimental results of this analysis were used to develop a second-order polynomial equation which demonstrated the relation between the methyl ester yield, ratio of the mixture volume to seed weight, sodium hydroxide concentration, and volume ratio of methanol to the mixture of methanol and n-hexane. Hence, the data was fitted to the second-order polynomial model and the model parameters were determined by multiple regression analysis as presented in Eq. (4.3).

$$\begin{aligned} Y = & -579.41 - 5.67R_{VW} + 551.25C_{NaOH} + 608.61R_V - 2.62R_{VW}^2 + 28.36R_{VW} \cdot C_{NaOH} \\ & - 8.70R_{VW} \cdot R_V - 211.68C_{NaOH}^2 + 40.9C_{NaOH} \cdot R_V - 585R_V^2 \end{aligned} \tag{4.3}$$

where,

Y : predicted biodiesel yield (%)

R_{VW} : ratio of methanol and n-hexane volume to seed weight ($mL \cdot g^{-1}$)

C_{NaOH} : sodium hydroxide concentration (wt.%)

R_V : volume ratio of methanol to mixture of methanol and n-hexane ($mL \cdot mL^{-1}$)

Positive sign in front of the terms indicates synergistic effect which increases the yield of methyl ester and negative sign indicates antagonistic effect.

Table 4.12: The response of factorial design for the mixture of methanol and n- Hexane

Test	**A**	**B**	**C**	**Observed value (%)**	**Predicted value (%)**	**$\Delta_{obs-predc}$ (%)**
1.	7.5	2.0	0.3	46.89	46.54	0.35
2.	12.5	2.0	0.7	24.67	25.02	-0.35
3.	7.5	2.25	0.5	47.93	49.19	-1.26
4.	12.5	1.75	0.5	17.21	15.97	1.24
5.	10	1.75	0.7	41.89	42.79	-0.9
6.	7.5	1.75	0.5	81.27	80.33	0.94
7.	10	2.25	0.7	52.48	51.19	1.29
8.	10	2.0	0.5	79.89	79.9	-0.01
9.	10	2.0	0.5	78.93	79.9	-0.97
10.	7.5	2.0	0.7	62.58	62.62	-0.04
11.	10	2.0	0.5	80.88	79.9	0.98
12.	12.5	2.25	0.5	54.78	55.72	-0.94
13.	10	2.25	0.3	40.62	39.72	0.9
14.	12.5	2.0	0.3	26.37	26.33	0.04
15.	10	1.75	0.3	38.21	39.5	-1.29

Notes: **A** = V_{Mix}: W_{seed} ($mL \cdot g^{-1}$), **B** = [NaOH] (wt.%), and **C** = V_{MeOH}:V_{mix} ($mL \cdot mL^{-1}$)

Table 4.13 presents the analysis of variance for the regression model, which was conducted to analyze the significance and goodness of the second-order polynomial model.

Table 4.13: ANOVA for the regression model of Eq. (4.3)

Source	**Sum of squares**	**DF**	**Mean square**	**F-value**	**P-value**
Model	7822.36	9	869.15	467.37	< 0.0001
A: V_{mix}:W_{seed}	1671.58	1	1671.58	696.17	0.0000
B: [NaOH]	37.1091	1	37.1091	15.46	0.0111
C: V_{MeOH} : V_{mix}	109.003	1	109.003	45.40	0.0011
AA	993.386	1	993.386	413.72	0.0000
AB	1257.06	1	1257.06	523.53	0.0000
AC	75.603	1	75.603	31.49	0.0025
BB	646.275	1	646.275	269.16	0.0000
BC	16.7281	1	16.7281	6.97	0.0460
CC	2021.76	1	2021.76	842.02	0.0000
Total error	12.0055	5	2.40109		
Total (corr.)	6395.64	14			

Based on a 95% confidence level, the model F-value and P-value were 467.37 and < 0.0001 respectively, indicated that the model was significant and sufficient for predicting the biodiesel yield. Each regression coefficients of the model was tested to be significant at confidence level of 95%. P-value less than 0.05 showed that the regression coefficient was statistically significant. All model terms of A, B, C, AA, AB, AC, BB, BC, and CC had significant effect on the biodiesel yield as the p-values were less than 0.05 and the computed F-values were higher than the theoretical F-values. The linear and quadratic regression coefficients of the mixture volume to seed weight ratio and volume ratio of methanol to the mixture of methanol and n-hexane (A, AA, C and CC) had very high significance effects on the response since the computed F-values were higher than theoretical F-values and the p-values were lower than 0.05. The linear regression coefficient of sodium hydroxide concentration had computed F-value not very high, however, its quadratic term and its interaction term with ratio of the mixture volume to seed weight indicated highly significance effects on the response of methyl ester yield.

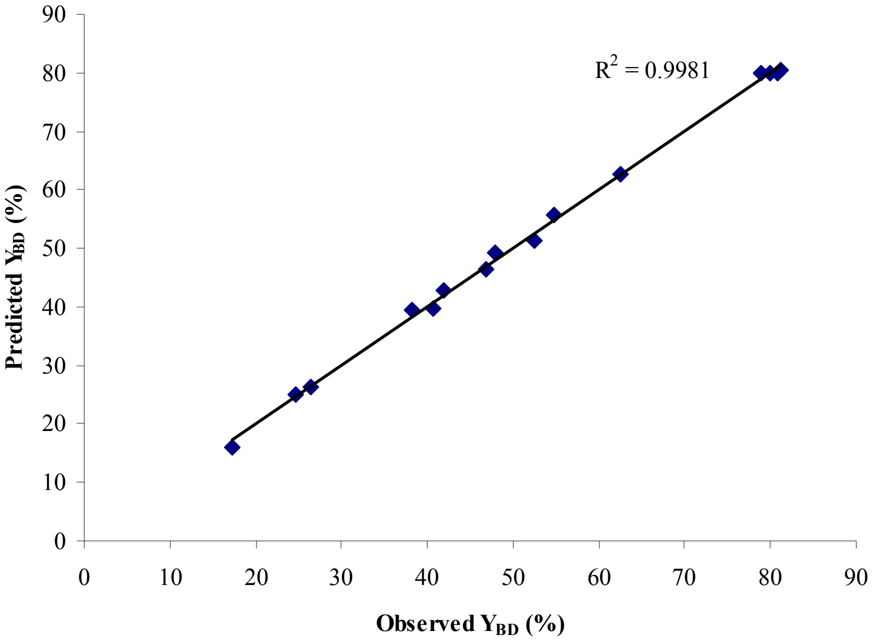


Figure 4.27: Predicted versus observed biodiesel yield

Figure 4.27 shows observed values versus the predicted values of biodiesel yield using the second-order model equation developed. The regression model equation provided a very accurate description of the experimental data, revealing that it was successful in capturing the correlation between the three process parameters to the methyl ester yield [135]. The determination coefficient of 0.9981 showed that the variability in biodiesel yield of 99.81% could be explained by the second-order polynomial model and only 0.19% of the total biodiesel yield variations were not explained by the independent variables. The adjusted determination coefficient (adjusted R^2) was 99.47%.

4.5.1.2 Effects of parameters

The interaction effect between ratio of the mixture volume to seed weight ($V_{(MeOH+n\text{-}hexane)}$/ W_{seed}) and sodium hydroxide concentration [NaOH] is presented in Figure 4.28. This term had substantial effect on the methyl ester yield due to its large F-value of 523.53.

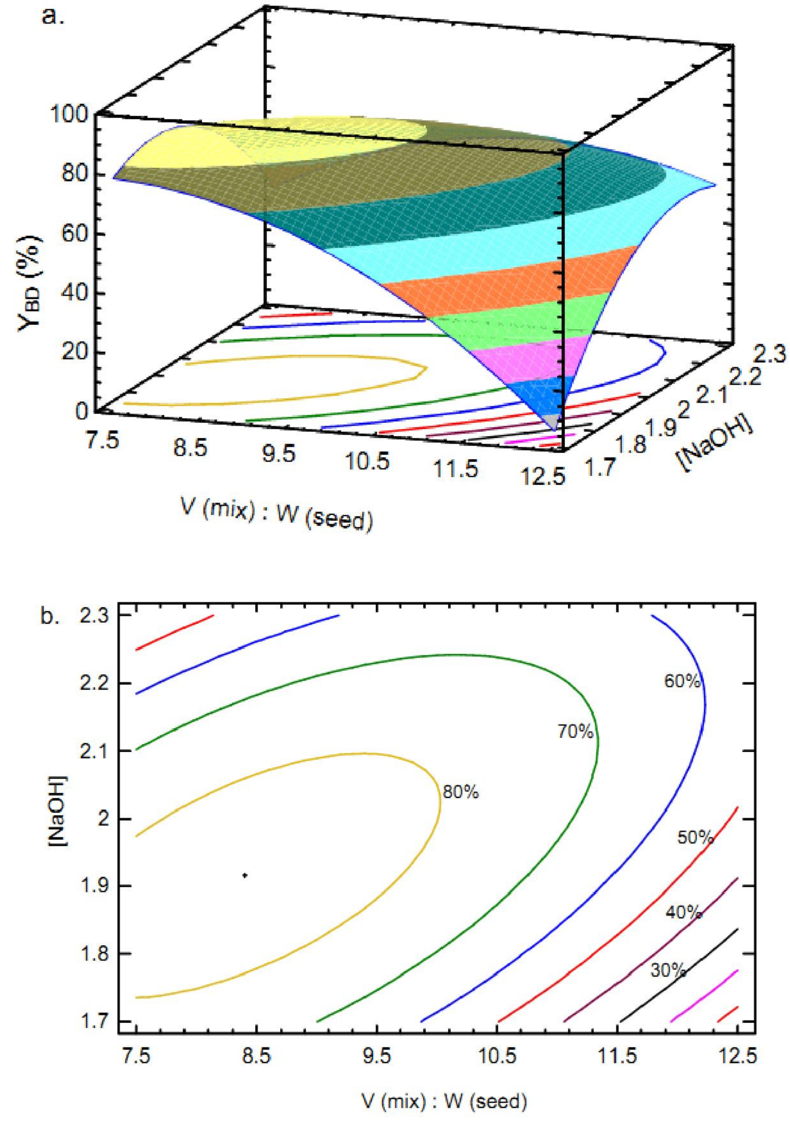


Figure 4.28: The estimated response surface (a) and contour plot (b) of methyl ester yield at constant ratio of $V_{MeOH}/V_{(MeOH+n\text{-}hexane)}$ of 0.52

At constant ratio of $V_{MeOH}/V_{(MeOH+n\text{-}hexane)}$ of 0.52, raising ratio of $V_{(MeOH+n\text{-}hexane)}/W_{seed}$ from 7.5 to 8.4 along with increasing [NaOH] from 1.75 wt.% to 1.92

wt.% enhanced the conversion of oil to biodiesel from 80.91% to 84.67%, in conformity with positive interaction term of R_{VW} and C_{NaOH} in Eq. (4.3). In this work, to obtain various ratio of $V_{(MeOH+n\text{-}hexane)}/W_{seed}$, the seed weight was varied while the mixture volume was kept constant at 150 mL. At ratio of 7.5, the mixture volume was 150 mL and the seed weight was 20 g. This volume could extract the part of *Jatropha curcas* oil in 20 g seeds and convert it into methyl ester. Hence, the maximum biodiesel yield was not reached. At ratio of 12.45, the mixture volume of 150 mL was adequate to extract the most of oil in 12 g seeds. In this condition, the highest biodiesel yield of 84.67% was achieved.

Nevertheless, further increasing ratio of $V_{(MeOH+IPA)}/W_{seed}$ and [NaOH] concentration significantly decreased the methyl ester yield. *In-situ* transesterification was proceeded in the Soxhlet extractor at higher reaction temperature than its boiling point, and then rising [NaOH] concentration led the formation of soaps easier which lowered the oil conversion to methyl ester.

Figure 4.29 describes the interaction effect between the mixture volume to seed weight ratio ($V_{(MeOH+IPA)}/W_{seed}$) and the methanol volume to the mixture volume ratio ($V_{MeOH}/V_{(MeOH+IPA)}$) on the methyl ester yield at the constant of [NaOH] concentration of 1.92 wt.%. This term (AC) had significant influence on the methyl ester yield as the computed F-value was 31.49 and P-value of 0.0008 was less than 0.05.

Raising the ratio of $V_{(MeOH+n\text{-}hexane)}/W_{seed}$ from 7.5 to 8.4 and enhancing the ratio of $V_{MeOH}/V_{(MeOH+n\text{-}hexane)}$ from 0.3 to 0.52 gained the extraction of oil and the conversion of the oil to methyl ester. Then, the increase in ratio of $V_{(MeOH+n\text{-}hexane)}/W_{seed}$ above 8.4 and the raise in ratio of $V_{MeOH}/V_{(MeOH+n\text{-}hexane)}$ decreased the yield of methyl ester, as resulted the negative interaction term of R_{VW} and R_V in Eq. (4.3). The increment of volume ratio demonstrated increasing methanol volume and decreasing n-hexane volume. Methanol, as the most polar alcohol, could extract not only *Jatropha curcas* oil from the seeds in a very small quantity but also the seed polar compound such as phospholipid, whereas n-hexane was a much better solvent. Therefore, decreasing n-hexane volume reduced the extracted oil from the seed and lowered the oil conversion to biodiesel. In addition, raising the ratio of $V_{(MeOH+n\text{-}hexane)}/W_{seed}$ attributed to the dilution of NaOH catalyst and the oil extracted from seeds which decreased their interaction in reaction system, thus the oil conversion to methyl ester was diminished.

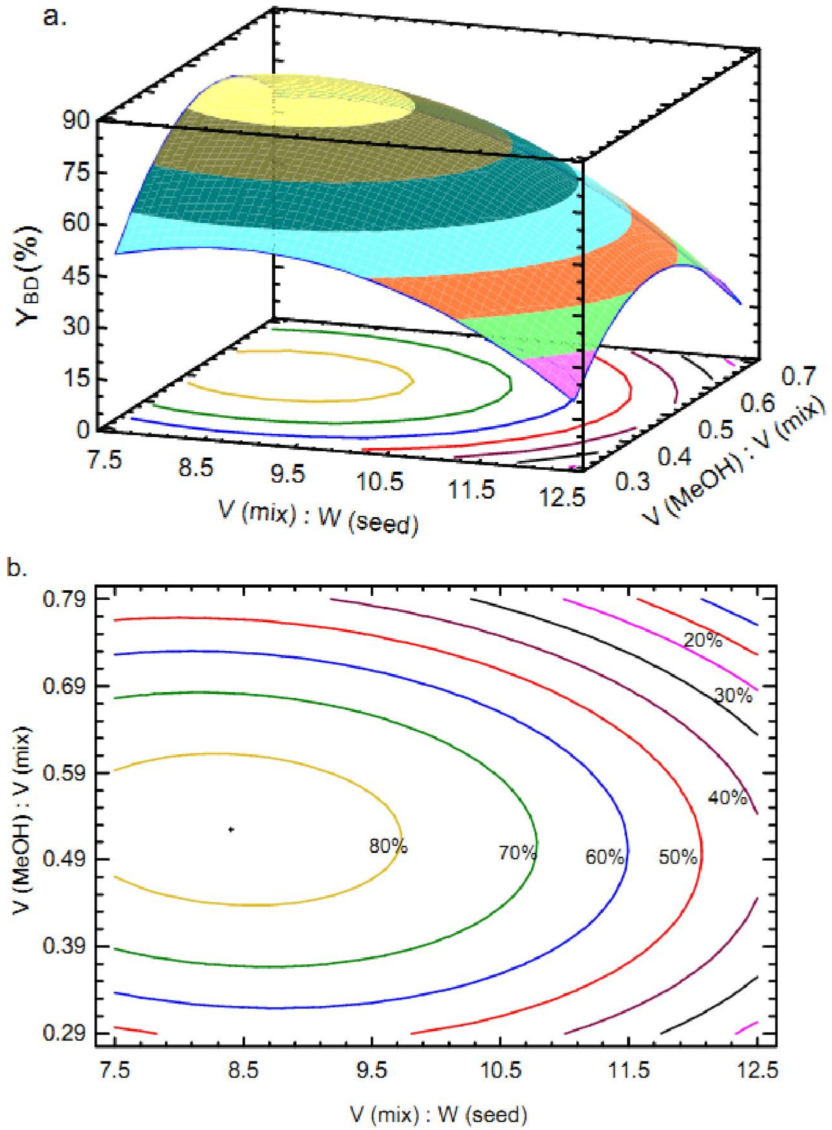


Figure 4.29: The estimated response surface (a) and contour plot (b) of methyl ester yield at the constant of [NaOH] of 1.92 wt.%

Figure 4.30 depicts the interactive effect between sodium hydroxide concentration and the volume ratio of methanol to the mixture of methanol and n-hexane. This term (BC) had the least significance effect on the methyl ester yield due to the lowest of computed F-value of 6.97 than other interaction terms and its P-value was 0.0077.

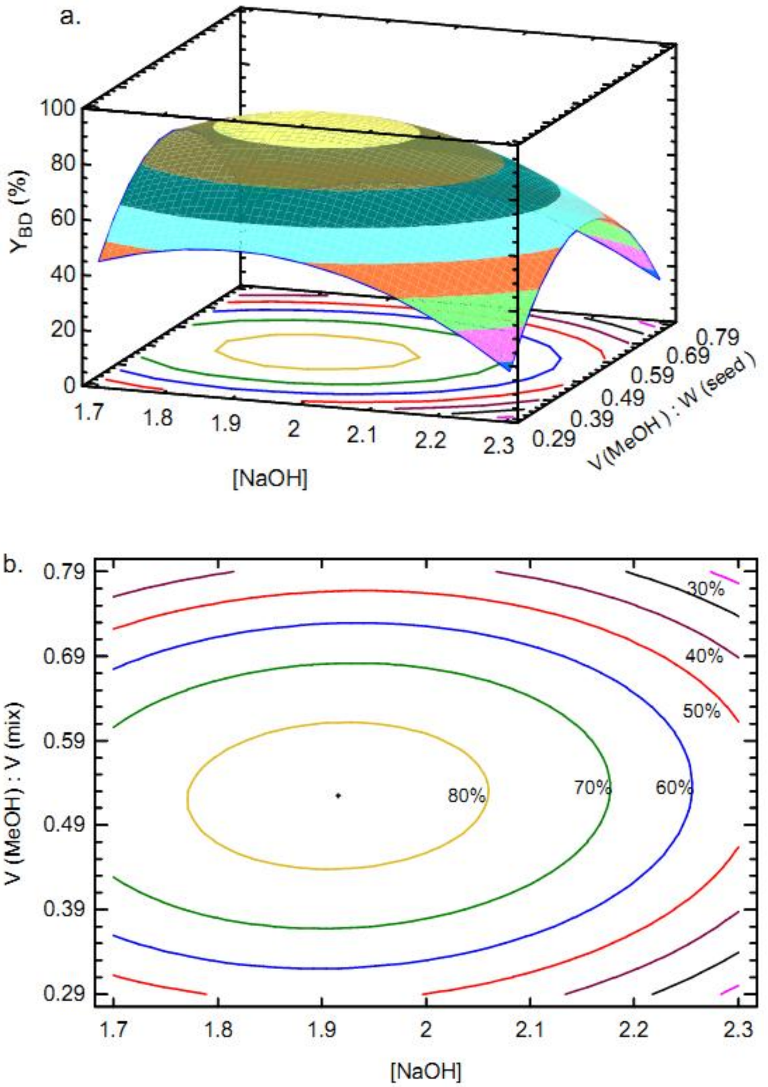


Figure 4.30: The estimated response surface (a) and contour plot (b) of methyl ester yield at the constant of $V_{(MeOH+IPA)}/W_{seed}$ of 8.4

Enhancing NaOH concentration from 1.75 wt.% to 1.92 wt.% associated with rising $V_{MeOH}/V_{(MeOH+n\text{-}hexane)}$ ratio from 0.3 to 0.52 augmented significantly the extraction of oil from seeds and its conversion to methyl ester, which in accordance with the positive interaction term of C_{NaOH} and R_V in second-order polynomial model. The presence of NaOH catalyst in the mixture of methanol and n-hexane significantly enhanced the extraction efficiency of *Jatropha curcas* oil, almost all of the triglycerides in the seeds were removed [37].

However, excess NaOH concentration addition followed by higher $V_{MeOH}/V_{(MeOH+IPA)}$ ratio was able to decrease the oil extraction as well as its conversion to methyl-isopropyl ester. Higher $V_{MeOH}/V_{(MeOH+IPA)}$ ratio showed the volume of methanol increased, inversely, the n-hexane volume reduced which affected the quantity of oil extracted from the seeds.

4.5.1.3 Optimization analysis

Table 4.14 and Table 4.15 explain the high, low limit experimental region and the optimum factors resulted from optimization analysis of experimental design by the Statgraph Centurion XV.

Table 4.14: The combination of factor levels which maximize the methyl ester yield

Factor	**Low**	**High**	**Optimum**
V_{Mix} : W_{seed} (mL/g)	7.5	12.5	8.4
[NaOH] (wt.%)	1.75	2.25	1.92
V_{MeOH}:V_{mix}	0.3	0.7	0.52

Table 4.15: Optimum condition of the mixture of methanol and n-Hexane

Test	**Factors**			**Response (%)**		**Residual (%)**
	V: W (mL/g)	**[NaOH] (wt.%)**	**V_M:V_{mix} (mL/mL)**	**Obsv**	**Predc**	
1	8.4	1.92	0.52	83.75	84.67	-0.92
2	8.4	1.92	0.52	84.09	84.67	-0.58
3	8.4	1.92	0.52	82.98	84.67	-1.69

The predictions of biodiesel yield obtained from numerical optimization of design of the experiment were 84.67%. The experiment was conducted at the optimum reaction condition to test the predictions of biodiesel yield. The average optimum biodiesel yield obtained from experiment was (83.61 ± 0.57)% which was comparable to the predicted one, with the average residual of -1.06%.

4.5.2 Effect of reaction time

The effect of reaction time on biodiesel yield was carried out up to 4 hours at reaction temperature 50°C, 55°C and 60°C. Other parameters were set at optimum condition, ratio of the mixture volume to seed weight of 8.4, catalyst concentration of 1.92 wt.%, volume ratio of methanol to the mixture of 0.52. The biodiesel yields at various reaction times are presented in Figure 4.31.

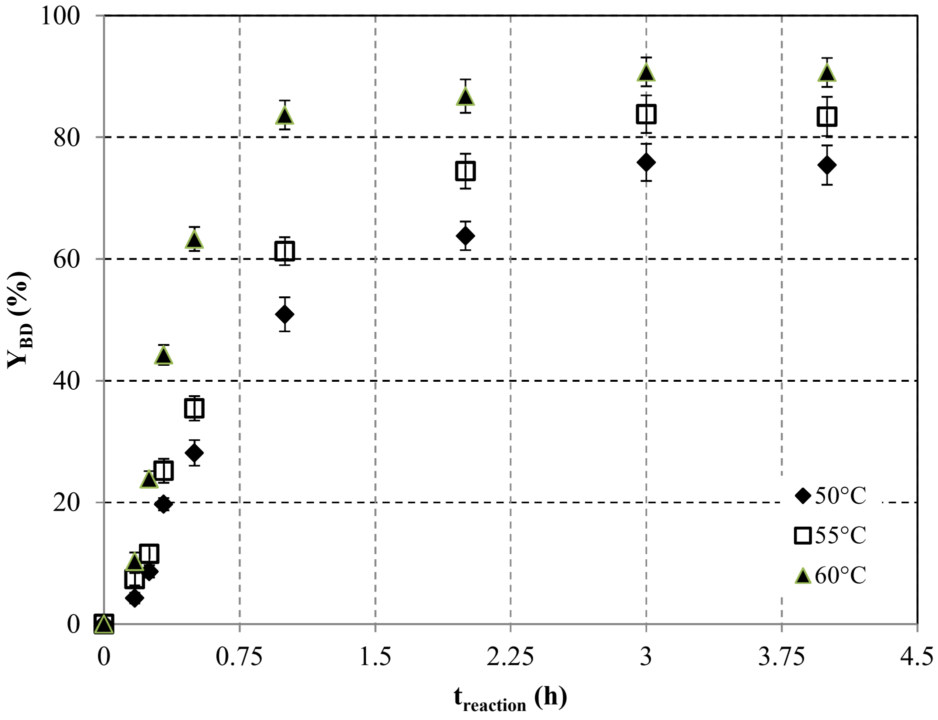


Figure 4.31: Biodiesel yield for different reaction time at 50°C, 55°C and 60°C

It was observed that the biodiesel yield increased with increase in reaction time. The methyl ester yield enhanced significantly at the first hour, after that increasing reaction time, raised slightly the methyl ester yield and reached the maximum yield at 3 hours. The maximum biodiesel yields were obtained after 3 hours reaction time 75.89% at 50°C, 83.78% at 55°C and 90.74% at 60°C, where the maximum oil extraction and transesterification into biodiesel occurred. The methyl ester yield insignificantly decreased at longer reaction time. It might be due to the occuring saponification. In addition, methanol could extract more polar substances from the seeds. Reaction temperature affected the biodiesel yield after reaction time of 0.5 hours, increasing temperature from 50°C to 60°C, the methyl ester yield enhanced significantly. However, raising reaction temperature did not influence the reaction completion. The equilibrium stages were reached after 3 h reaction time at 50°C, 55°C, and 60°C.

In comparison to *in-situ* transesterification with methanol and with the mixture of methanol and isopropanol, the biodiesel yield produced from *in-situ* transesterification with the mixture of methanol and n-hexane in Soxhlet extractor was lower than the methyl ester yields produced from other solvents. *In-situ* transesterification in Soxhlet extractor was carried out without agitation, thus the mass transfer limitation was higher than others which inhibited the oil extraction and reaction and led in reducing the methyl ester yield, the reaction completion was longer than others as well.

4.5.3 Effect of particle size

Effect of particle size was determine at the optimum reaction conditions, $V_{(MeOH+IPA)}/W_{seed}$ ratio of 8.4, NaOH concentration of 1.92 wt.%, volume ratio of methanol to the mixture of methanol and n-hexane of 0.52 at 60°C reaction temperature for different reaction times. Drying seeds were ground and sieved to obtain three different sizes of particle, which were 0.600 mm $< D_P <$ 1.800 mm, 0.425 $< D_P <$ 0.600 mm, and $D_P <$0.425 mm.

Figure 4.32 depicts the effect of particle size on the yield of methyl ester at various reaction times. It was observed that *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and n-hexane demonstrated similar trend to that with the mixture of methanol and isopropanol. Decreasing particle size from $1.18<D_P<2$ mm to $D_P <0.425$ mm enhanced the biodiesel yield at all reaction time. The yield of biodiesel produced from particle size of $0.425<D_P<0.600$ mm was insignificantly different from one of $D_P<0.425$ mm after reaction time of 3 h. Reducing particle size could shorten the reaction time to reach the reaction completion. Decreasing particle size into $D_P < 0.425$ mm reduced reaction time to reach the equilibrium stage from 3 h to 2 h at 60°C.

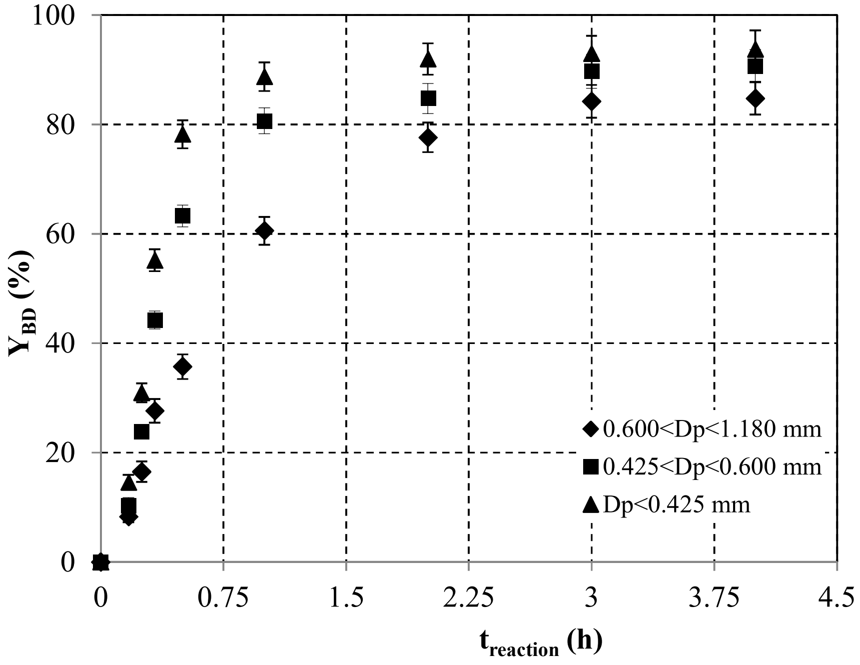


Figure 4.32: Biodiesel yield for different sizes of particle at 60°C

4.6 Summary

Jatropha curcas seeds with the size range of Dp < 0.425 mm to Dp < 1.180 mm, oil content of (49.72 ± 1.34)%, oil density of (0.8995 ± 0.0007) g·mL^{-3}, and acid value of (1.13 ± 0.10) mg KOH·g, were used to investigate *in-situ* transesterification of *Jatropha curcas* seeds catalyzed by sodium hydroxide with isopropanol, methanol, the mixture of methanol and isopropanol, the mixture of methanol and n-hexane.

The maximum yield of isopropyl ester of (85.32 ± 0.30)% was obtained with the ratio of isopropanol volume to seed weight of 7.5 (mL·g^{-1}) and sodium hydroxide concentration of 1.0 wt.% at 70°C for 2 h reaction time. This yield was not high since isopropanol was less polarity than methanol which led saponification during biodiesel separation from the impurities of glycerol, isopropanol, NaOH, and water.

Response surface methodology was employed to determine the optimum conditions of *in-situ* transesterification of *Jatropha curcas* seeds with methanol, the mixture of methanol and isopropanol, the mixture of methanol and n-hexane. For *in-situ* transesterification of *Jatropha curcas* seeds with methanol, NaOH concentration, methanol volume to seed weight ratio, and reaction temperature are the important factors affecting methyl ester yield. Increasing values of these factors up to a certain level enhanced the yield of methyl ester. However, further increasing these factors decreased methyl ester yield. For *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and isopropanol, NaOH concentration had the most significant effect on the methyl-isopropyl ester yield, followed by the ratio of mixture volume to seed weight and the volume ratio of methanol to the mixture of methanol and isopropanol. Elevating these parameters into certain values gained the yield of methyl-isopropyl ester. Next increasing these parameters, lower yield of methyl-isopropyl ester was obtained. For *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and n-hexane, mixture volume to seed weight ratio, volume ratio of methanol to the mixture and NaOH concentration had significant effects on methyl ester yield. Raising these independent variables increased the yield up to certain values. The methyl ester yield diminished when these variables rose subsequently. The optimum conditions and maximum yields obtained are as follows.

Table 4.16: Optimum condition and maximum yield of *in-situ* transesterification of *Jatropha curcas* seeds

Factor	**Methanol**	**Methanol-isopropanol**	**Methanol-n-hexane**
$V_{alcohol}$:W_{seed} (mL·g^{-1})	8.08	10.82	8.4
[NaOH] (wt.%)	1.94	1.99	1.92
V_{MeOH}:$V_{mixture}$ (mL·mL^{-1})	-	0.46	0.52
T (°C)	57	68	-
T (h)	2	2	1
Maximum yield (%)	(90.45 ± 0.25)	(94.78 ± 0.29)	(83.61 ± 0.57)

Decreasing particle size could reduce the reaction time to reach the equilibrium condition,

- For methanol, at the particle size of $0.425 < Dp < 0.600$ mm the equilibrium condition decreased from 90 min to 60 min reaction time.
- For the mixture of methanol and isopropanol, at the particle size of $Dp < 0.425$ mm the reaction time to achieve equilibrium state was reduced from 60 min to 30 min.

CHAPTER 5

REACTION MECHANISM AND KINETICS STUDIES

In-situ transesterification of *Jatropha curcas* seed is a complex reaction involving reaction between a liquid and another immiscible liquid embedded in an inert solid matrix. The concept of Shrinking Core Model for in-situ transesterification is presented in section 5.1 and is utilized in Section 5.2 to evaluate the importance of solid matrix. Section 5.3 explores to explain the Liquid-Liquid catalytic transesterification reaction through the mechanism of phase transfer catalysis.

5.1 Shrinking Core Model for Liquid-Liquid-Solid non Catalytic Reaction

Shrinking Core Model [112] was developed to explain the reactions of the kind

$$\begin{array}{ccccc} \text{A} & + & \text{B} & \rightarrow & \text{Products} \\ \text{Gas} & + & \text{Solid reactant in an Inert solid matrix} & \rightarrow & \text{Gaseous Products} \end{array} \qquad (5.1)$$

Reaction proceeds by

- mass transfer of A from bulk gas to particle surface by external mass transfer
- reaction of A with solid B at the interface
- diffusion of A through pores in the particle inert matrix to reach B as it gets consumed by reaction and recedes into the depth of particle.

Conversion of B can be related to the radius of the unconverted core of radius r_c in a particle of radius R

$$X = 1 - \frac{\text{volume of unreacted core}}{\text{total volume of particle}} = 1 - \left(\frac{r_c}{R}\right)^3 \qquad (5.2)$$

For each of these steps, the progress of the reaction in terms of conversion X were obtained as

For external mass transfer control

$$\frac{t}{\tau} = X_b \qquad \text{with} \quad \frac{1}{\tau} = \frac{3bk_g C_{Ag}}{\rho_B R}$$

For reaction rate control

$$\frac{t}{\tau} = 1 - (1 - X_B)^{1/3} = \frac{t}{\tau} \qquad \text{with} \quad \frac{1}{\tau} = \frac{3bk'' C_{Ag}}{\rho_B R} \tag{5.3}$$

For pore diffusion control

$$\frac{t}{\tau} = 1 - 3(1 - X_B)^{2/3} + 2(1 - X_B) = \frac{t}{\tau} \qquad \text{with} \quad \frac{1}{\tau} = \frac{6bD_e C_{Ag}}{\rho_B R^2}$$

The parameter "τ" can be recognized as the time required for total conversion. Slowest of these three steps is expected to control the reaction. Increasing bulk fluid velocity/ increased stirring/ promoting turbulence can improve external mass transfer to minimize its importance. Reducing particle size can minimize the resistance due to pore resistance. Thus, observed rate of reaction will be controlled by true reaction kinetics for data collected at sufficiently high stirring speed using smaller particle size. Strong effect of temperature indicates importance of true reaction kinetics due to exponential dependence of reaction rate constant on temperature through Arrhenius equation.

By plotting (1-X) versus the ratio of reaction time to the time for complete conversion of a particle (t/τ) and by comparing those curves to curves in Figure 5.1, the rate-controlling step could be recognized. If the curve is linear, then diffusion through liquid film controls the reaction. However, if the curve is non-linear, the reaction mechanism can be controlled by internal diffusion or chemical reaction. However, the difference of curves between internal diffusion and chemical reaction as controlling steps is insignificant.

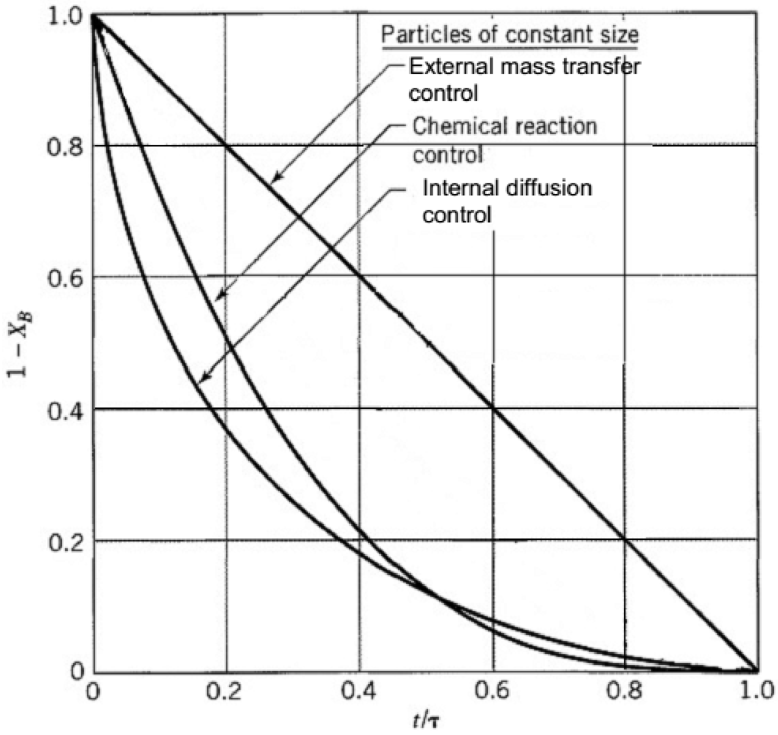


Figure 5.1: The progressive conversion of spherical solid [112]

This technique needs to be adopted to *in-situ* transesterification of oil in the seed with alcohol in the bulk.

Alcohol + Oil in Seed → Products

Liquid + Liquid reactant in an Inert solid matrix → Liquid Products

In-situ transesterification reaction proceeds by

- mass transfer of alcohol from bulk liquid to particle surface by external mass transfer
- react at the interface of alcohol and oil in solid matrix
- diffusion of alcohol through pores in the particle inert matrix to reach oil as it gets consumed by reaction and recedes into the depth of particle.

Slowest of these three steps is expected to control the reaction. External mass transfer is improved by increasing stirring speed.

5.2 Evaluation of *in-situ* transesterification through Shrinking Core Model

Effect of particle size and reaction temperature on the progress of the *in-situ* transesterification with methanol alone as well as with a mixture of methanol and isopropanol are explored to identify controlling steps. The loss of biodiesel during separation process was too small, less than 0.1%, and it could be neglected. Hence, the biodiesel yields obtained at various reaction temperatures, particle sizes and reaction times were converted into fractional conversions of oil (X). By plotting (1-X) versus (t/τ), the ratio of reaction time "t" to the time "τ" for complete conversion of a particle, and comparing those curves to curves in Figure 5.1, the rate-controlling step could be identified. If the curve is linear, then diffusion through liquid film controls the reaction. However, if the curve is non-linear, the reaction mechanism can be controlled by internal diffusion or chemical reaction. However, the difference in the curves for internal diffusion and chemical reaction as controlling steps is not easy to distinguish. Graphs of $[1-3(1-X)^{2/3}+2(1-X)]$ versus reaction time for internal diffusion control and $[1-(1-X)^{1/3}]$ versus reaction time for chemical reaction control may be plotted; data which fit as straight line through the origin point of (0,0) with high linearity coefficient will be the rate-controlling step.

5.2.1 Effect of reaction temperature

The effect of reaction temperature on the reaction mechanism and kinetics of *in-situ* transesterification of *Jatropha curcas* seeds were determined using the data presented in section 4.3.3 for methanol and section 4.4.2 for the mixture of methanol and isopropanol.

5.2.1.1 In-situ transesterification with methanol

Figure 5.2 presents the progress reaction of spherical particle of *Jatropha curcas* seeds with surrounding methanol at different reaction temperature. There were two regimes in this graph. The first regime was irreversible regime, which occurred between 0 min to 30 min reaction time or t/τ of 0.25. At this regime, increasing reaction time enhanced the oil extraction and oil conversion, the biodiesel yield augmented as well. The second regime was equilibrium regime which was achieved after 45 min (or t/τ of 0.375) to 120 min (or t/τ of 1.0) reaction time. At the equilibrium regime, the oil extraction and oil conversion were completed. Increasing reaction time did not affect the biodiesel yield. According to Figure 5.1, the mechanism of *in-situ* transesterification of *Jatropha curcas* seeds with methanol was not controlled by external mass transfer since the curves in Figure 5.2 were not linear. Hence, the reaction mechanism might be internal diffusion control or chemical reaction control.

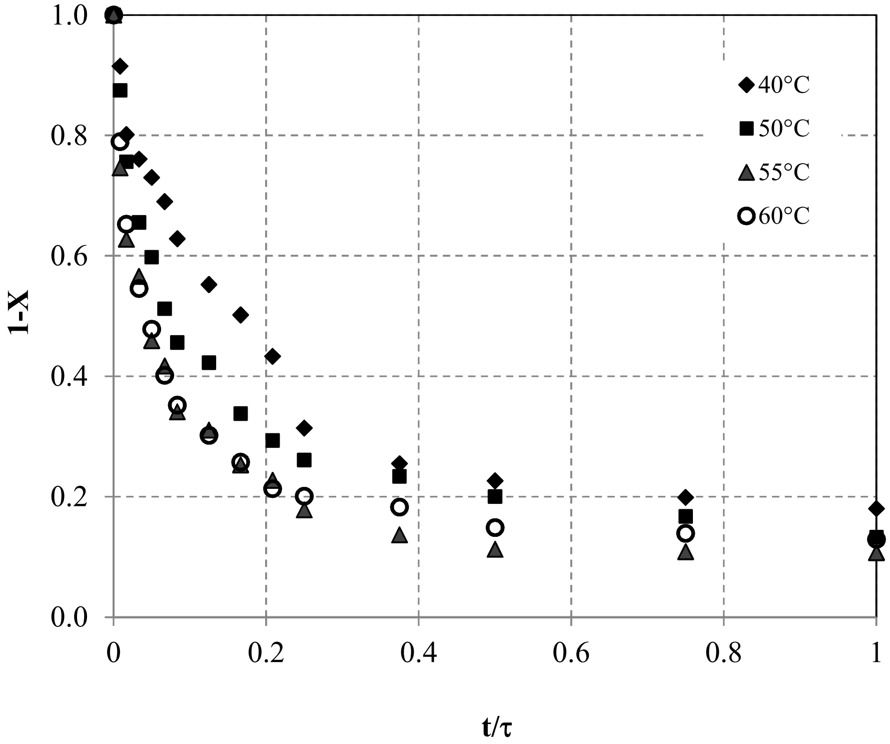


Figure 5.2: Progress reaction of spherical particle of *Jatropha curcas* seeds with surrounding methanol

Next determination of reaction mechanism was carried out using the values of biodiesel fractional conversion as plotted in Figure 5.3 and Figure 5.4, respectively.

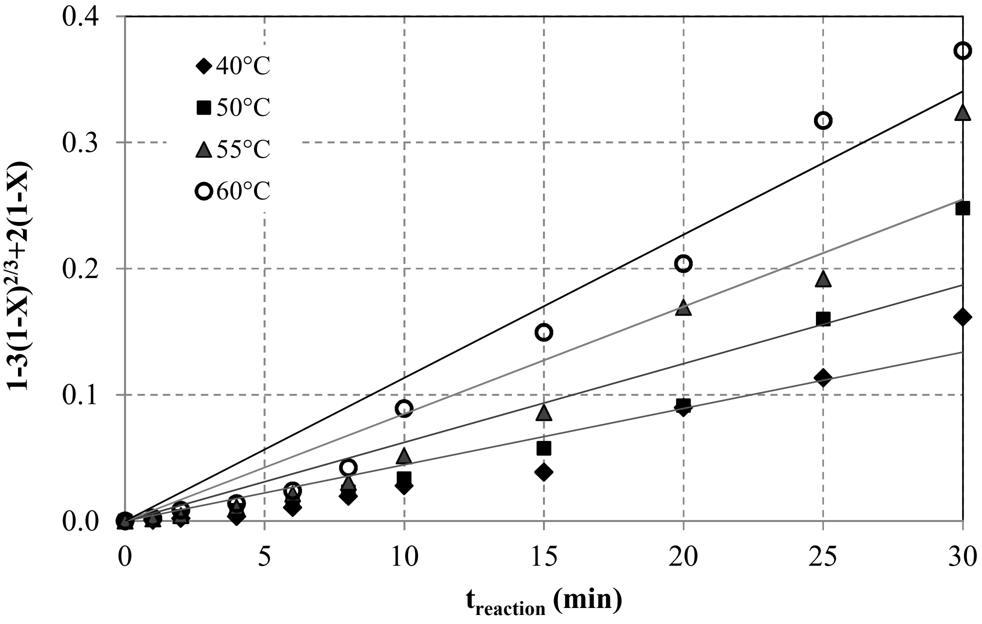


Figure 5.3: Plot of [1-3(1-X)$^{2/3}$+2(1-X)] versus reaction time at different reaction temperature for methanol during 30 min reaction time or t/τ = 0.25

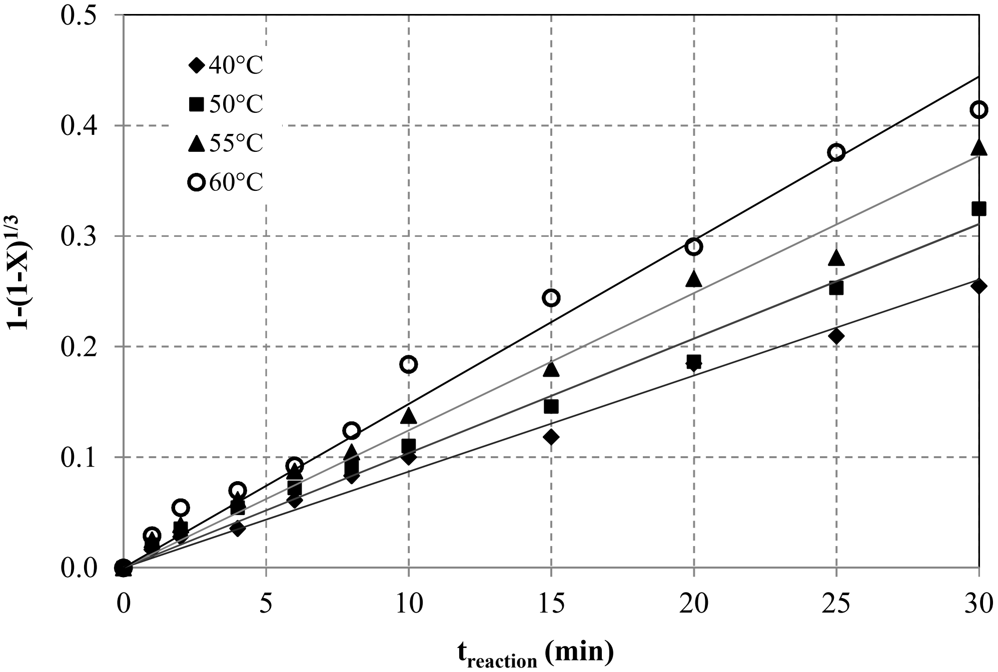


Figure 5.4: Plot of [1- (1-X)$^{1/3}$] versus reaction time at different reaction temperature for methanol during 30 min reaction time or t/τ = 0.25

Table 5.1 shows the comparison of slope ($1/\tau$) and correlation coefficient (R^2) of linear curves of internal diffusion control and chemical reaction control. The linear curves of chemical reaction control had higher correlation coefficients than that of internal diffusion control. Hence, it can be concluded that the mechanism of *in-situ* transesterification of *Jatropha curcas* seeds with methanol was chemical reaction control.

Table 5.1: Slope ($1/\tau$) and regression coefficient (R^2) of linear curves at different temperature

T	**Internal diffusion control**		**Chemical reaction control**	
(^{0}C)	**Slope ($1/\tau$)**	**R^2**	**Slope ($1/\tau$)**	**R^2**
40	0.0045	0.9123	0.0087	0.9864
50	0.0062	0.8654	0.0104	0.9861
55	0.0085	0.8998	0.0124	0.9860
60	0.0113	0.8479	0.0148	0.9817

The first-order rate constant for the surface reaction (k”) was determined using the values of slope according to the Eq. (5.2),

$$slope = \frac{1}{\tau} = \frac{bk''C_{Al}}{\rho_B R}$$
$$k'' = \frac{\left(\frac{1}{\tau}\right)\rho_B R}{bC_{Al}} \qquad (5.2)$$

Table 5.2 presents the values of the first-order rate constant for the surface reaction (k”) at various reaction temperatures. The activation energy (E_a) for *in-situ* methanolysis of *Jatropha curcas* seeds could be calculated using the Arrhenius equation,

$$k = k_0 e^{-\frac{E_a}{RT}} \qquad (5.3)$$

$$\ln(k) = \ln(k_0) - \frac{E_a}{RT} \qquad (5.4)$$

Table 5.2: The first-order rate constant for the surface reaction for methanol

T (^{0}C)	**1/τ**	**k" (m.s^{-1})**
40	0.0087	$5.1479.10^{-9}$
50	0.0104	$6.1538.10^{-9}$
55	0.0124	$7.3372.10^{-9}$
60	0.0148	$8.7573.10^{-9}$

Using the data in Table 5.2, ln (k") could be plotted against 1/T to obtain the linear curve with the slope of E_a/R and intercept of ln (k_0) or pre-exponential factor of 1.813. Figure 5.5 shows a good linearity between ln (k") and 1/T. Using the slope of 2726, the activation energy (E_a) could be determined which was 22.66 kJ·mol^{-1}. This activation energy was less than the activation energy for transesterification of oil with base catalyst which was in the range of 33.6 – 84 kJ·mol^{-1} [136].

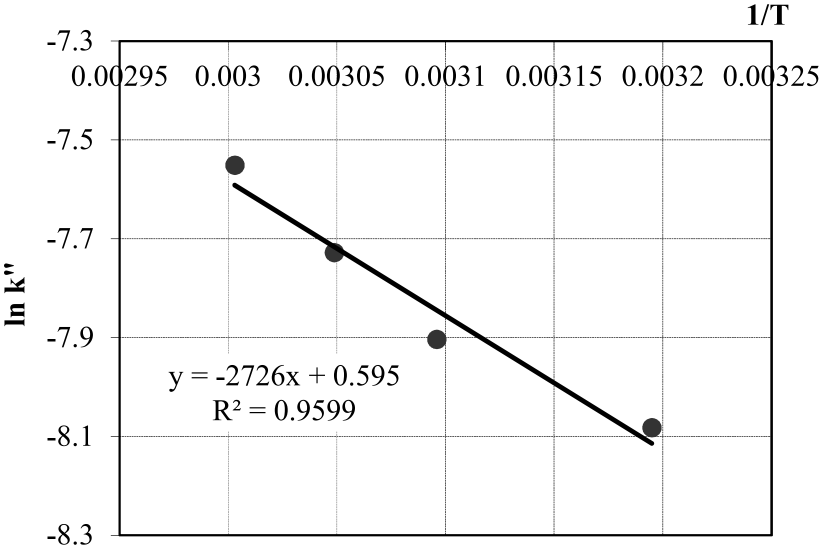


Figure 5.5: Reaction rate constant versus reaction temperature to determine activation energy

The empirical equation of biodiesel yield could be derived as a function of reaction temperature and reaction time according to the shrinking core model with the reaction mechanism of chemical reaction control, as presented in Eq. (5.6).

$$1-(1-X_B)^{\frac{1}{3}} = \frac{b.C_{Al}t}{\rho_B.R}k_0 e^{-\frac{Ea}{RT}} \tag{5.5}$$

$$Y = \left[1-\left(1-49.822\cdot t\cdot e^{-\frac{2726}{T}}\right)^3\right]\times 100\% \tag{5.6}$$

Figure 5.6 shows the comparison of biodiesel yield between experimental yield and model yield obtained from Eq. (5.6). The model yield compared well to the experimental results up to 80% biodiesel yield. More than 80% biodiesel yield, the significant deviations of the model yield from experimental results were observed, since the fitting data to derive this model yield conducted for 30 min reaction time that was the irreversible reaction region.

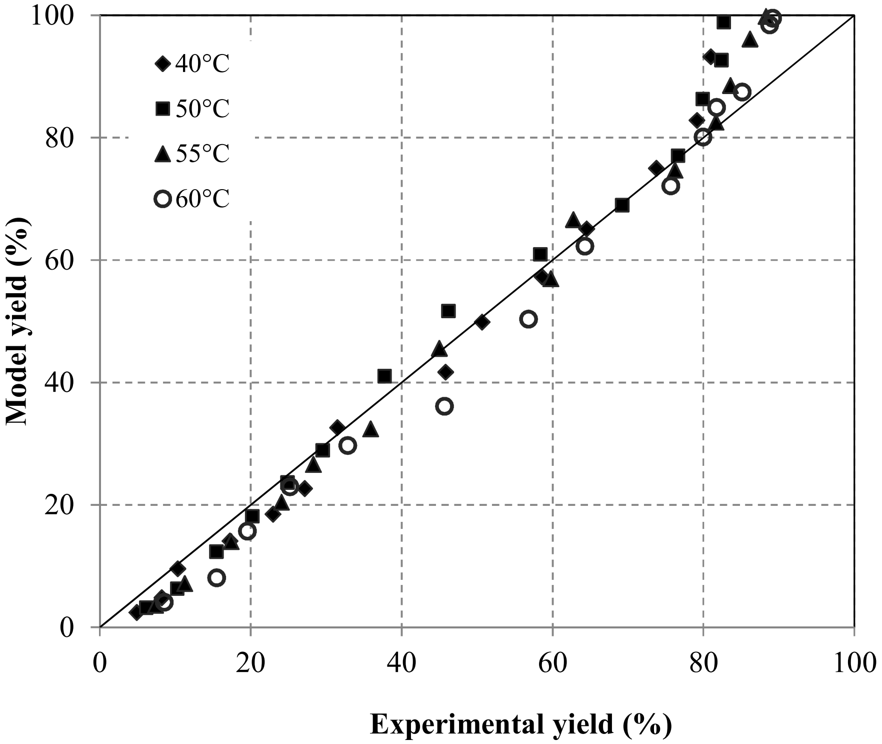


Figure 5.6: Comparison of experimental yield to model yield obtained from Eq. (5.6)

5.2.1.2 In-situ Transesterification with the mixture of methanol and isopropanol

The rate-controlling step was predicted from the graph of the progress reaction of *Jatropha curcas* particles with surrounding the mixture of methanol and isopropanol at different reaction temperature as shown in Figure 5.7. The graph demonstrates that the mechanism of internal diffusion and chemical reaction could be controlled the reaction rate. Meanwhile the mechanism of external mass transfer was not controlled the reaction rate due to the data of (1-X) was not fitted linearly against t/τ.

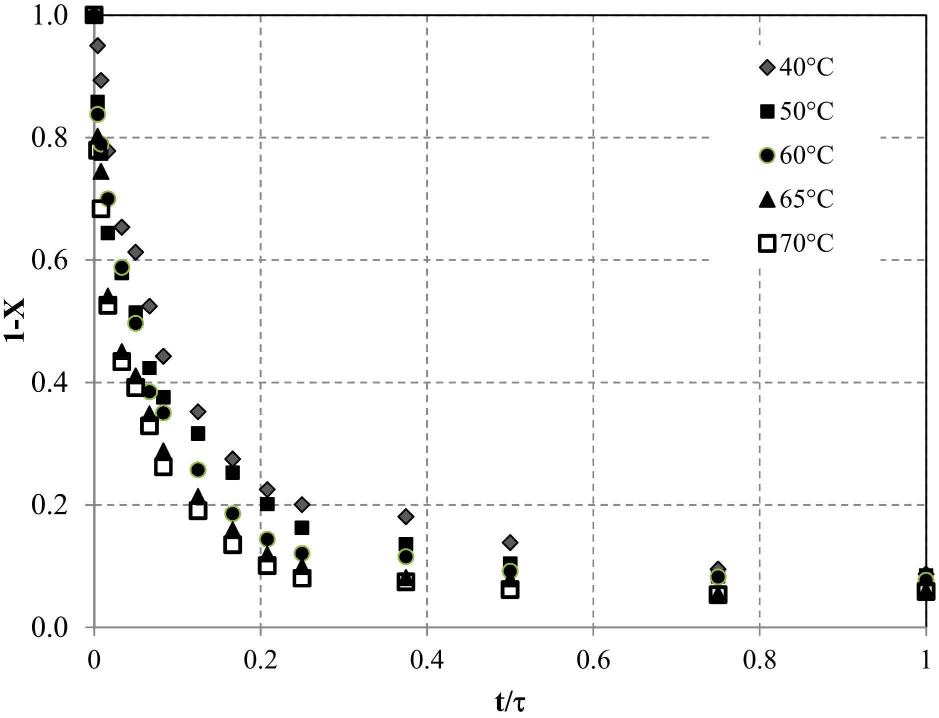


Figure 5.7: Progress reaction of *Jatropha curcas* particles with surrounding the mixture of methanol and isopropanol at different reaction temperature

Figures 5.8 and 5.9 describe linear regression through the origin of the data as a function of biodiesel fractional conversion according to the mechanism of internal diffusion (Eq. (5.1)) and chemical reaction (Eq. (5.2)).

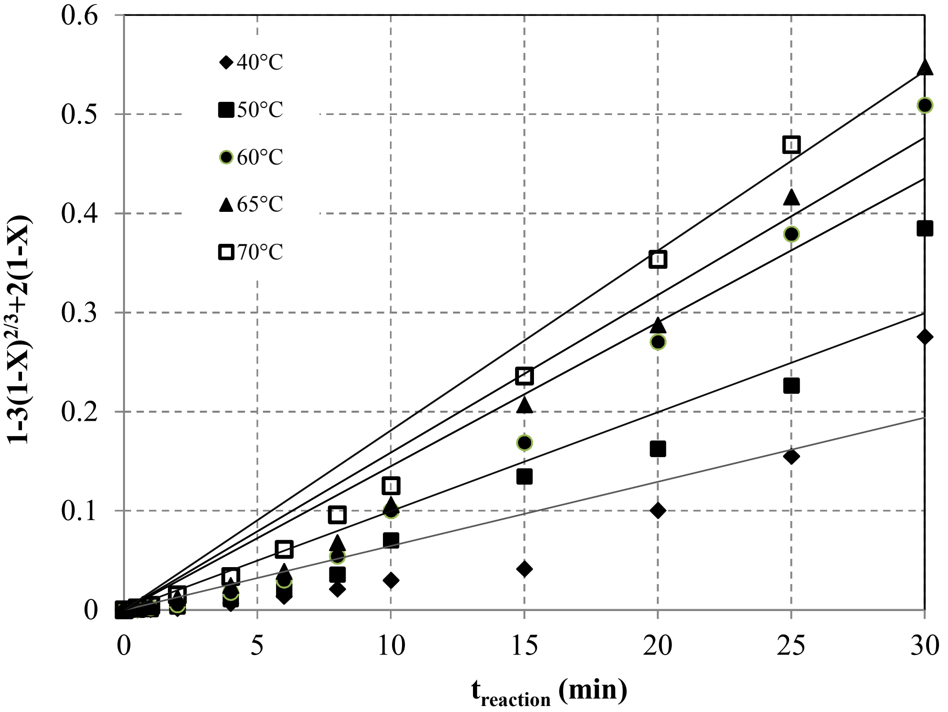


Figure 5.8: Plot of $[1\text{-}3(1\text{-}X)^{2/3}+2(1\text{-}X)]$ versus reaction time

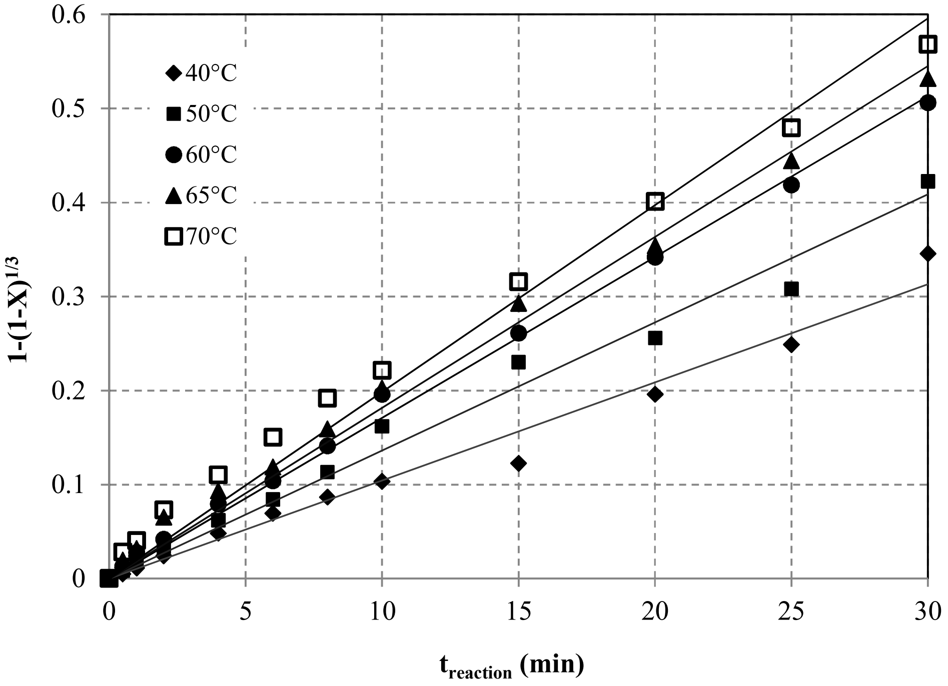


Figure 5.9: Plot of $[1\text{-}(1\text{-}X)^{1/3}]$ versus reaction time at different reaction temperature

Based on the values of correlation coefficient of the fitting curves as presented in Table 5.3, the mechanism of *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and isopropanol was controlled by chemical reaction.

Table 5.3: Slope (1/τ) and correlation coefficient of fitted linear curves

T	**Internal diffusion control**		**Chemical reaction control**	
(^{0}C)	**Slope (1/τ)**	**R^2**	**Slope (1/τ)**	**R^2**
40	0.0065	0.8219	0.0104	0.9795
50	0.01	0.905	0.0136	0.9852
60	0.0145	0.9378	0.0171	0.9966
65	0.0159	0.9492	0.0182	0.9911
70	0.0181	0.9675	0.0199	0.9825

The first-order rate constant for the surface reaction (k") was determined using the values of slope according to the Eq. (5.2). Table 5.4 indicates values of the first-order rate constant for the surface reaction (k") at various reaction temperatures. These first-order rate constants were higher than the first-order rate constant resulted from *in-situ* methanolysis of *Jatropha curcas* seeds. It revealed that *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and isopropanol could be proceeded faster than that with methanol.

Table 5.4: The first-order rate constant for the surface reaction

T (^{0}C)	**1/τ**	**k" ($m.s^{-1}$)**
40	0.0104	$7.7242.10^{-9}$
50	0.0136	$1.0101.10^{-8}$
60	0.0171	$1.2700.10^{-8}$
65	0.0182	$1.3517.10^{-8}$
70	0.0199	$1.4780.10^{-8}$

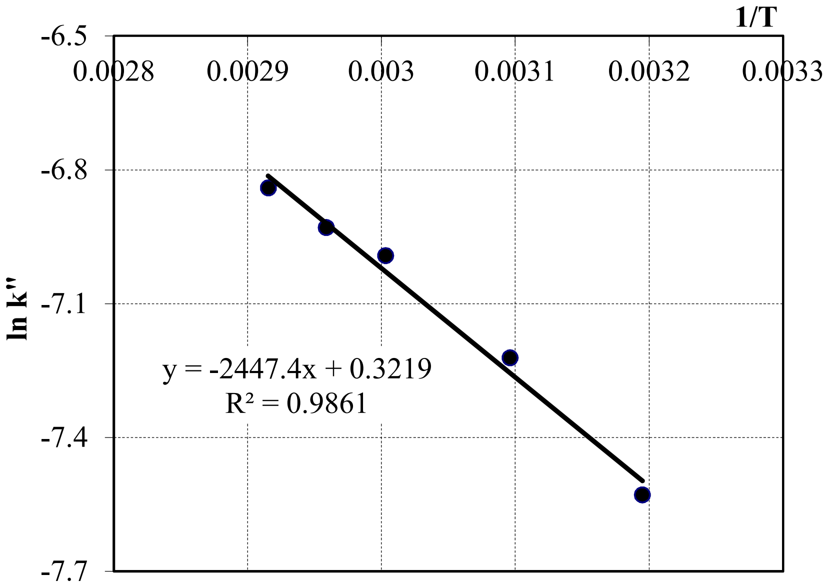


Figure 5.10: Reaction rate constant versus reaction temperature to determine the activation energy

The activation energy (E_a) for *in-situ* transesterification of *Jatropha curcas* seeds could be calculated using the Arrhenius equation. By plotting ln (k”) versus 1/T and fitting the data as the linear curve, the activation energy could be obtained from the slope of E_a/R. Figure 5.10 shows a high linearity between ln (k”) and 1/T. Using the slope of 2447.4, the activation energy (E_a) of 20.35 kJ/mole and pre-exponential factor of 1.380 could be determined, which was lower than the activation energy resulted from *in-situ* transesterification with methanol.

The empirical equation of biodiesel yield could be expressed as a function of reaction temperature and reaction time according to the shrinking core model with the reaction mechanism of chemical reaction control as shown in Eq. (5.8).

$$1-(1-X_B)^{\frac{1}{3}} = \frac{b.C_{Al}t}{\rho_B.R}k_0 e^{-\frac{Ea}{RT}} \tag{5.7}$$

$$Y = \left[1-\left(1-26.3168\cdot t\cdot e^{-\frac{2447}{T}}\right)^3\right]\times 100\% \tag{5.8}$$

Figure 5.11 demonstrates the comparison of biodiesel yields between experimental yield and model yield obtained from Eq. (5.8). The model yield compared well to the experimental results up to around 90% biodiesel yield. More than 90% biodiesel yield, the model yields were significantly difference from the experimental yields, since the fitting data to derive the model yield conducted for 30 min reaction time or in the region of irreversible reaction.

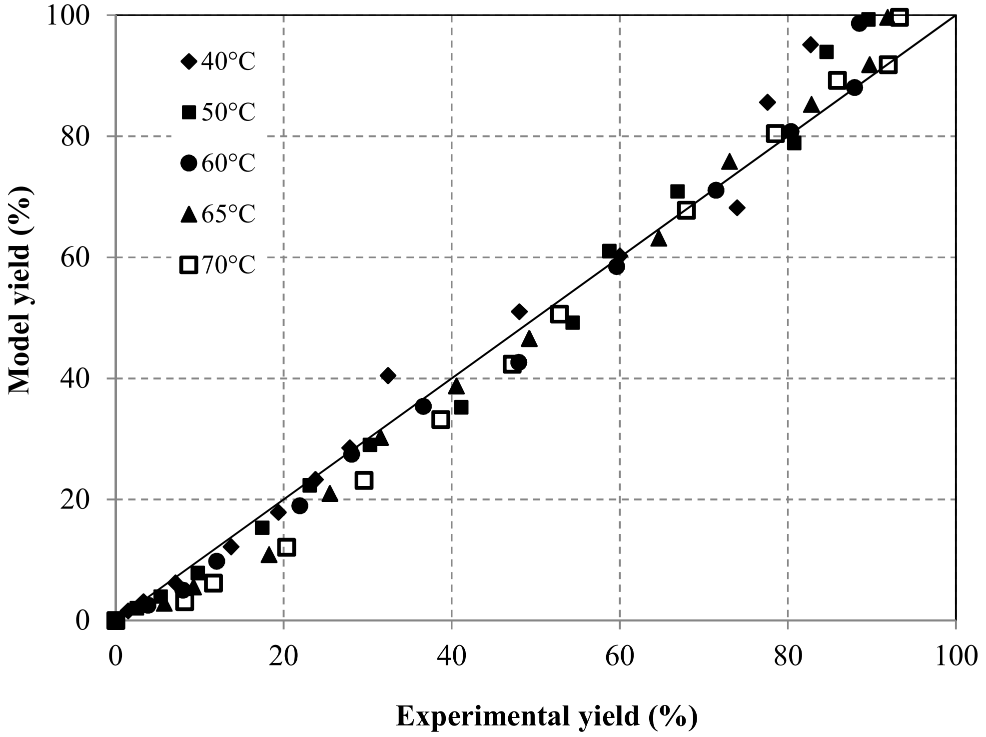


Figure 5.11: Comparison of experimental yield to model yield

5.2.2 Effect of particle size

The effect of particle size on biodiesel yield was used to determine the mechanism and kinetics of *in-situ* methanolysis of *Jatropha curcas* seeds using shrinking core model. The yields of biodiesel from three difference particle size of $600<D_P<1180$ μm, $425<D_P<600$ μm, and $D_P<425$ μm discussed in section 4.3.4 for methanol and in section 4.4.3 for the mixture of methanol and isopropanol were converted into fractional conversions of biodiesel and were used to predict the rate-controlling step.

5.2.2.1 In-situ transesterification with methanol

Figure 5.12 describes the progress reaction of spherical particle of *Jatropha curcas* seeds with surrounding methanol for different particle size. The mechanism of *in-situ* transesterification of *Jatropha curcas* seeds with methanol was not controlled by external mass transfer since the curves in Figure 5.12 were not linear. The reaction mechanism might be controlled by internal diffusion or chemical reaction.

Figure 5.13, Figure 5.14 and Table 5.4 demonstrate that the mechanism of *in-situ* transesterification of *Jatropha curcas* seeds with methanol for different particle size was chemical reaction control according to its higher correlation coefficients than that of internal diffusion control.

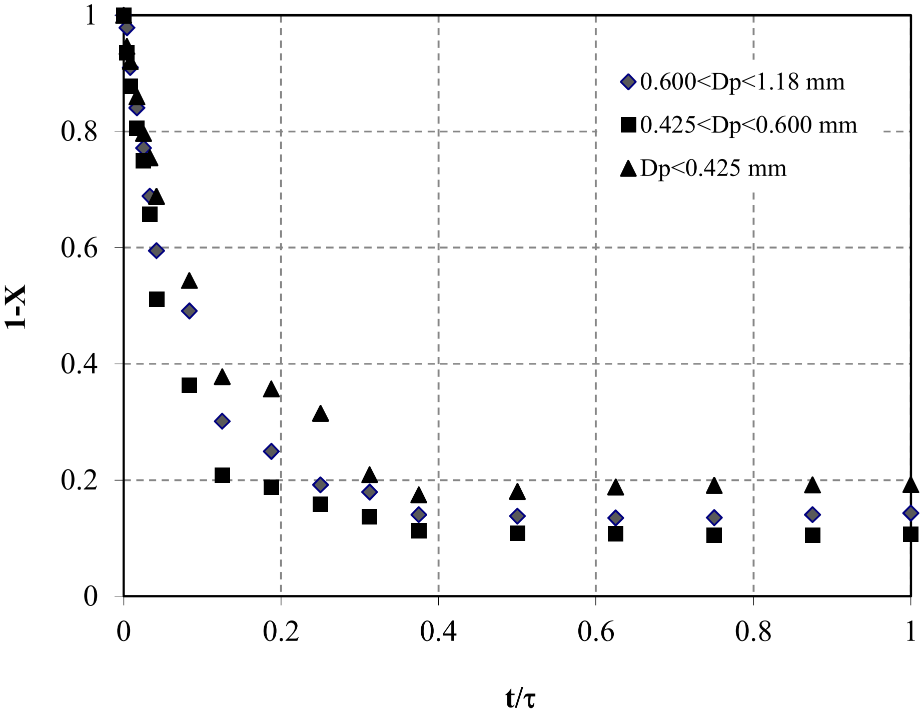


Figure 5.12: Progress reaction of spherical particle of *Jatropha curcas* seeds with surrounding methanol for different particle size for methanol

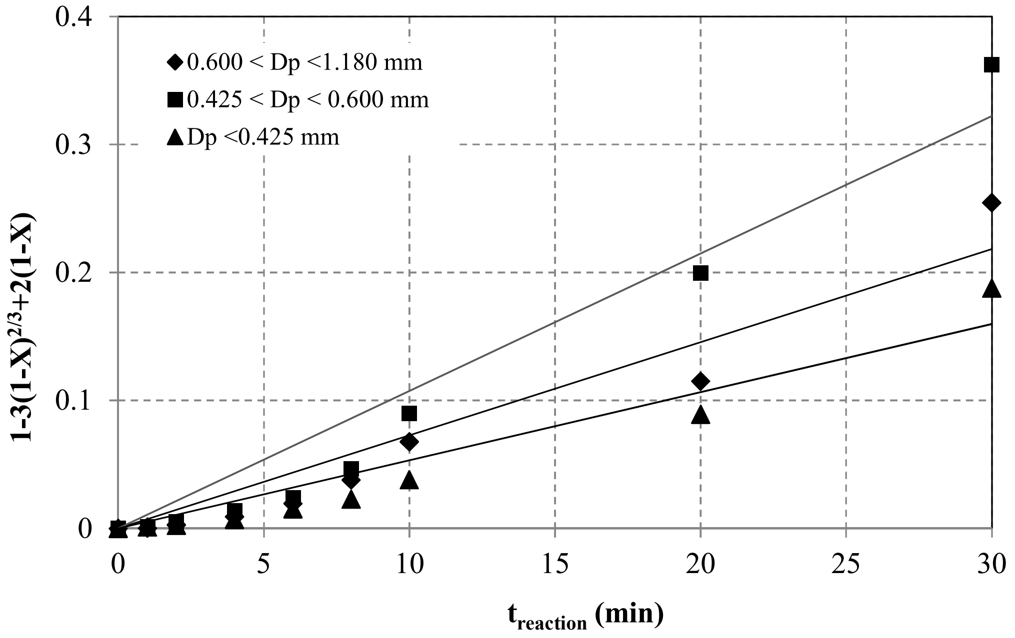


Figure 5.13: Plot of [1-3(1-X)$^{2/3}$+2(1-X)] versus reaction time for different particle size

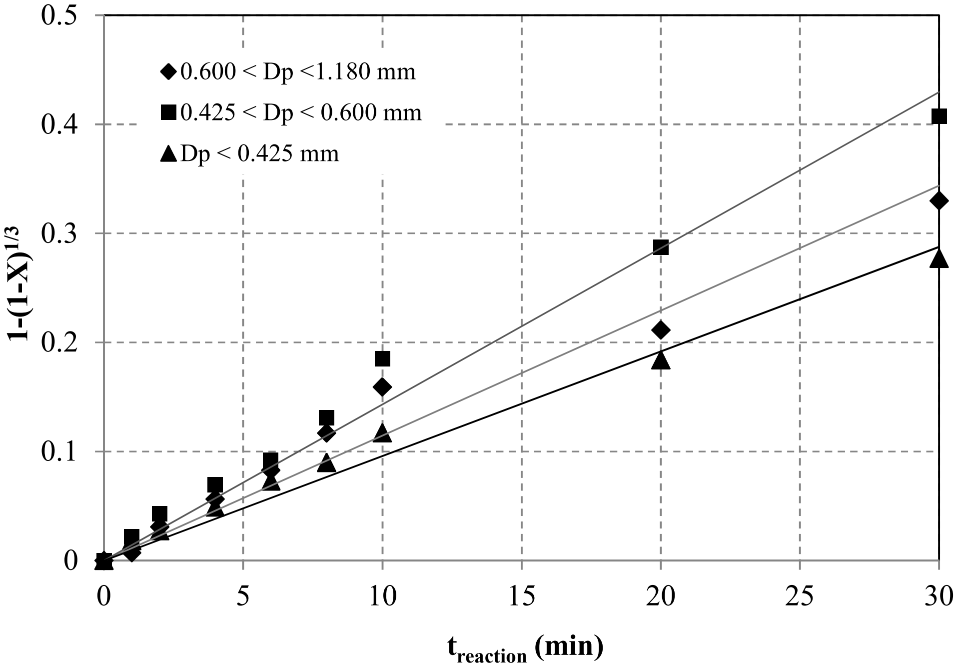


Figure 5.14: Plot of [1-(1-X)$^{1/3}$] versus reaction time for different particle size

Table 5.5: Slope ($1/\tau$) and correlation coefficients (R^2) of linear curve for different particle size

Dp	**Internal diffusion control**		**Chemical reaction control**	
(mm)	**Slope ($1/\tau$)**	**R^2**	**Slope ($1/\tau$)**	**R^2**
0.600<Dp<1.180	0.0073	0.9309	0.0115	0.9625
0.425<Dp<0.600	0.0107	0.9458	0.0143	0.9797
Dp<0.425	0.0053	0.9257	0.0096	0.9797

The first-order rate constant for the surface reaction (k") should be determined from the correlation between $1/\tau$ (slope) and $1/R_p$, with R_p as particle radius in Eq. (5.2). By plotting $1/\tau$ versus $1/R_p$ and fitting the data into linear curve through origin point of (0,0), the first-order rate constant could be obtained directly from the slope of the curve. However, Figure 5.15 reveals that the first-order rate constant could not be determined from *in-situ* methanolysis of *Jatropha curcas* seeds with different particle size, since the highest biodiesel yield was achieved from the middle particle size ($0.425 < D_P < 0.600$ mm), not from the smallest one ($D_P<0.425$ mm). Hence, the linear curve of $1/\tau$ and $1/R$ was not obtained and the first-order rate constant could not be determined.

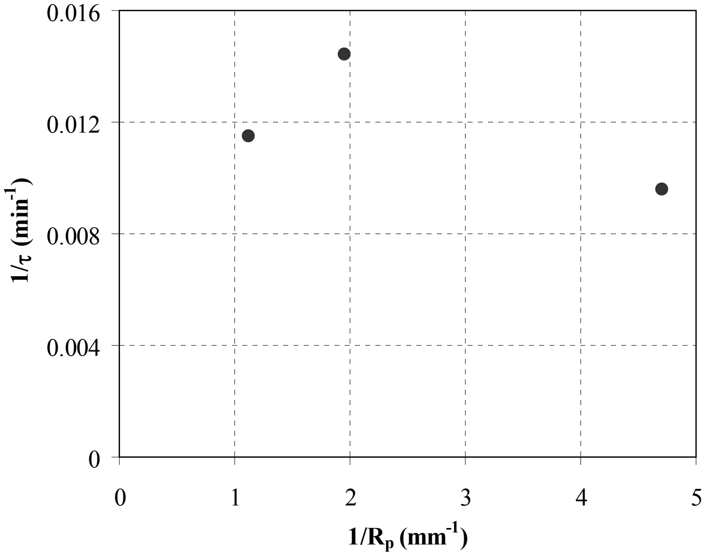


Figure 5.15: Effect of particle size on the time required for complete the reaction

5.2.2.2 In-situ transesterification with the mixture of methanol and isopropanol

The effect of particle size on the yield of biodiesel could be used to study the mechanism and kinetics of *in-situ* transesterification of *Jatropha curcas* seeds using the mixture of methanol and isopropanol. The steps of reaction mechanism determination were the same as previous discussions. The biodiesel yields which are displayed in Figure 5.16 could be expressed as biodiesel fractional conversion (X). The prediction of reaction mechanism was determined by plotting (1-X) versus reaction time, and then the curves were compared to Figure 5.1. The mechanism of *in-situ* transesterification was likely controlled by internal diffusion or chemical reaction.

Further study was required to ascertain the rate-controlling step by fitting the data as a function of fractional conversion of biodiesel according to Eq. (5.1) for internal diffusion control and Eq. (5.2) for chemical reaction control, which are presented in Figures 5.17 and 5.18, respectively.

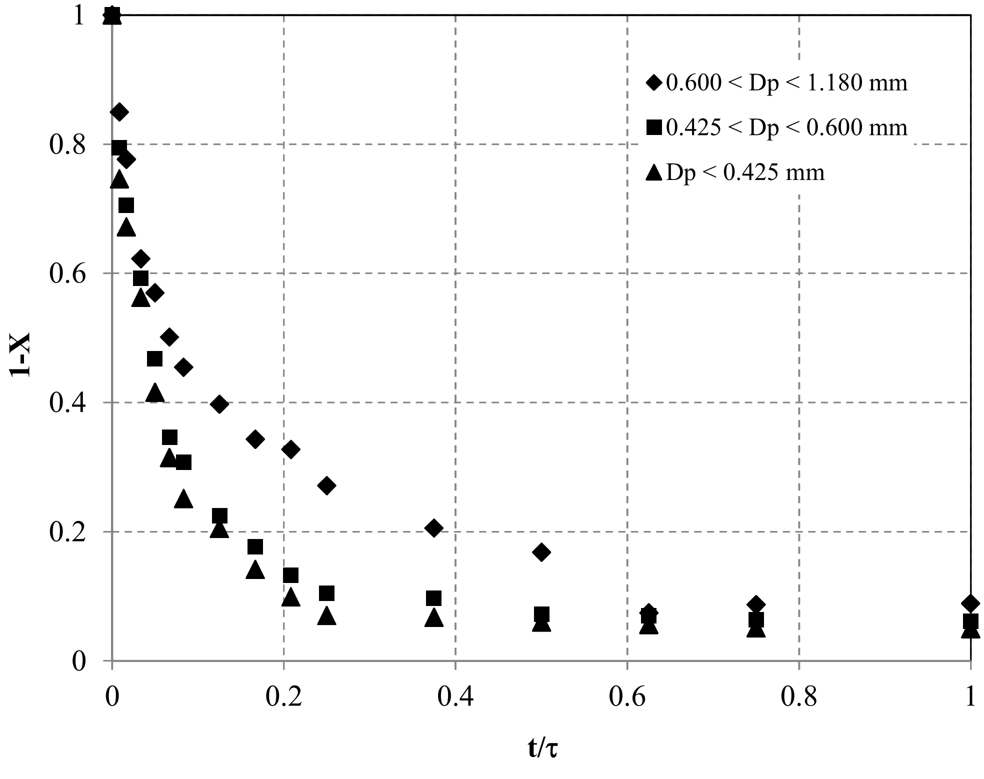


Figure 5.16: Progress reaction of *Jatropha curcas* seeds with surrounding methanol and isopropanol

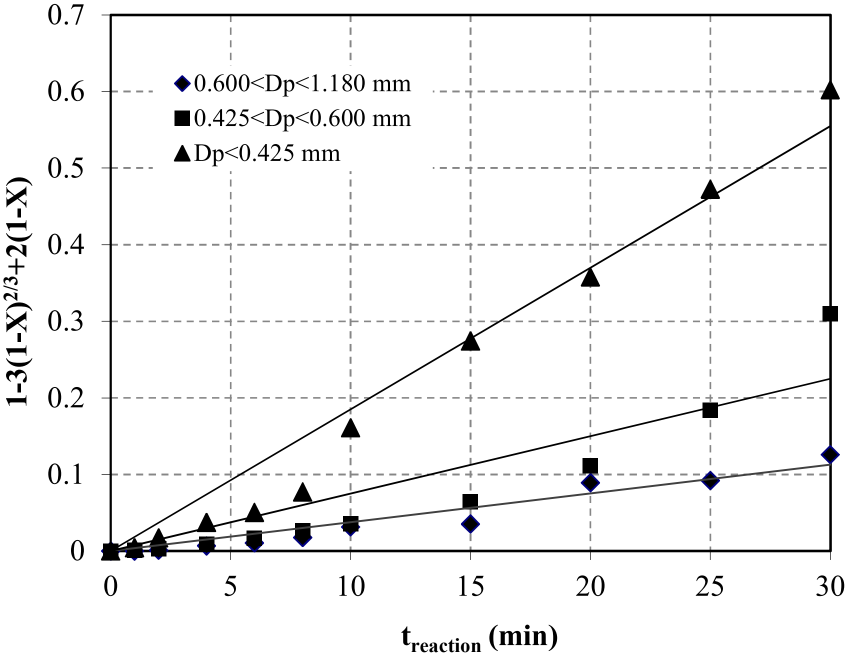


Figure 5.17: Plot of [1-3(1-X)$^{2/3}$+2(1-X)] versus reaction time for different particle size

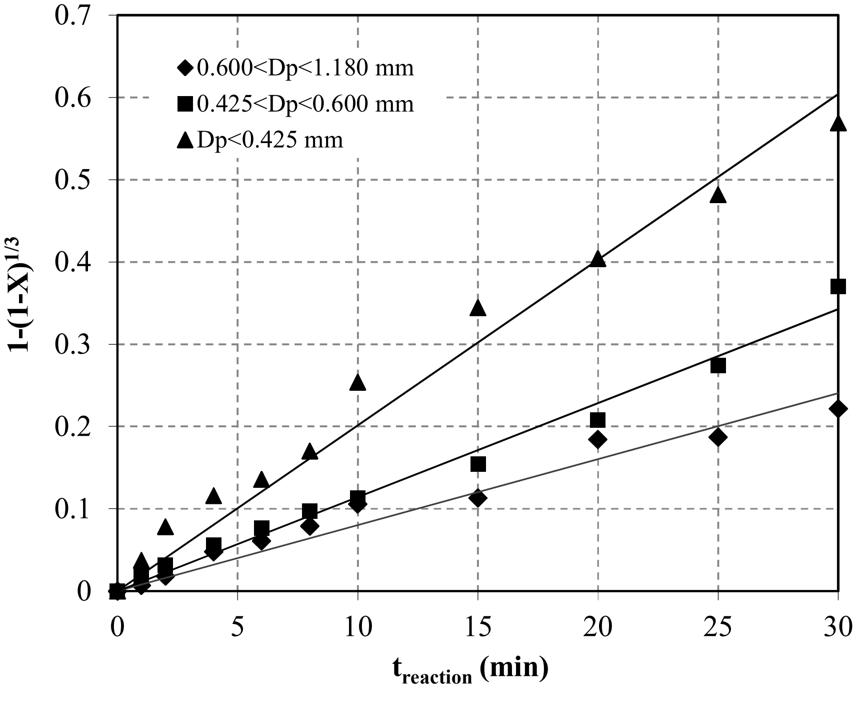


Figure 5.18: Plot of [1-(1-X)$^{1/3}$] versus reaction time for different particle size

According to the values of correlation coefficient (R^2) as shown in Table 5.6, the mechanism of *in-situ* transesterification was chemical reaction control.

Table 5.6: Slope ($1/\tau$) and correlation coefficients (R^2) of linear curve for different particle size

Dp (mm)	**Internal diffusion control**		**Chemical reaction control**	
	Slope ($1/\tau$)	**R^2**	**Slope ($1/\tau$)**	**R^2**
0.600<Dp<1.180	0.0038	0.9158	0.008	0.9586
0.425<Dp<0.600	0.0075	0.8423	0.0114	0.9854
Dp<0.425	0.0185	0.8689	0.0201	0.9733

The first-order rate constant could be determined at this condition by plotting $1/\tau$ versus $1/R_p$ and by fitting data into linear curve through origin point of (0,0). From Eq. (5.2), the first-order rate constant could be obtained directly from the slope of the curve.

$$\frac{1}{\tau} = \frac{bk"C_{Al}}{\rho_B R} \qquad (5.2)$$

Figure 5.19 shows the plot of $1/\tau$ versus $1/R_p$ to determine the first-order rate constant. The linear curve has a slope of 0.0026. Using Eq. (5.2) then the first-order rate constant (k") of $8.7378.10^{-9}$ m.s^{-1} is obtained.

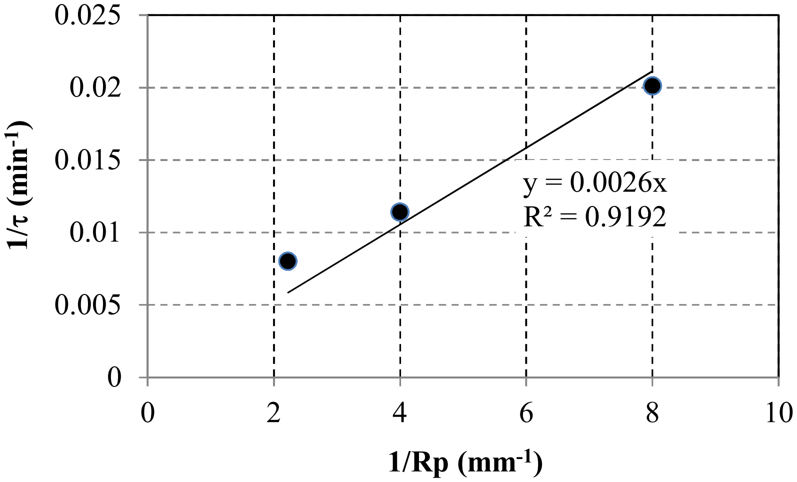


Figure 5.19: Effect of particle radius on determination of the first-order rate constant

The empirical model of biodiesel yield could be obtained by substituting the constant variables and the first-order rate constant into Eq. (5.2) and was expressed as a function of particle size and reaction time according to the shrinking core model with the reaction mechanism of chemical reaction control.

$$1-(1-X_B)^{\frac{1}{3}}=\frac{b\cdot C_{Al}\cdot k''\cdot t}{\rho_B.R_p} \tag{5.2}$$

$$Y=\left[1-\left(1-\frac{0.007948\cdot t}{R_p}\right)^3\right]\times 100\% \tag{5.9}$$

Figure 5.20 shows the comparison of biodiesel yield between experimental yield and model yield obtained from Eq. (5.9). The model yield fitted to the experimental results up to around 90% biodiesel yield. More than 90% biodiesel yield, the model yields were significantly difference from the experimental yields, since the fitting data to obtain the model yield conducted for 30 min reaction time that was in the region of irreversible reaction.

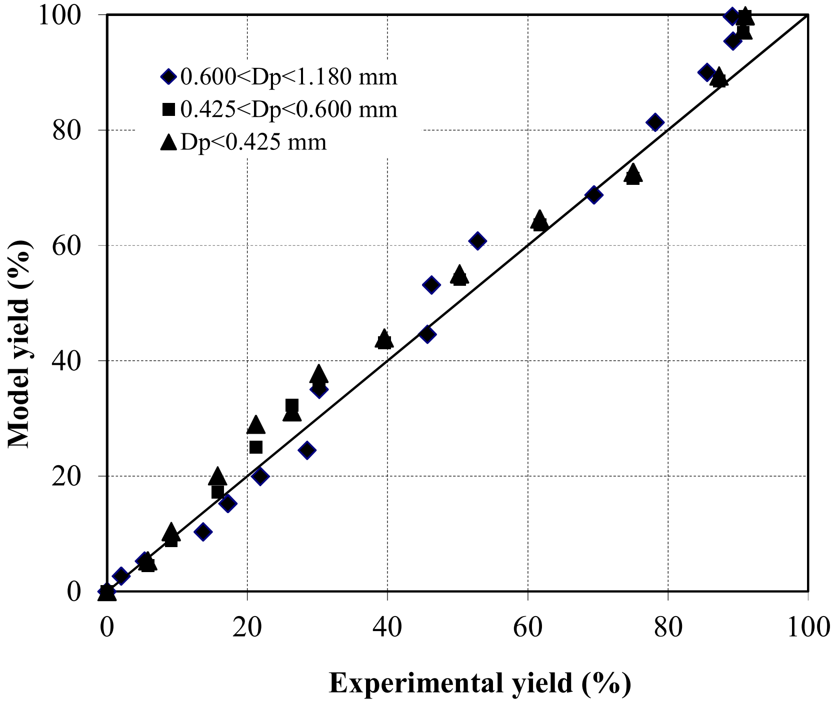


Figure 5.20: Comparison of experimental yield to model yield of Eq. (5.9)

5.3 Mechanism of two phase catalytic transesterification

Reaction between two liquids depends on their relative solubility. Solubility of methanol in oil phase is limited while isopropanol is more readily soluble. In spite of their solubility, oil and alcohol do not react to form biodiesel without an alkali or acid catalyst. Understanding the mechanism of how the catalyst acts to achieve the conversion is required to develop reaction kinetic model.

It is well recognized that alcohol in presence of an alkali forms NaOR alkoxide complex. Transfer of alkoxide complex in alcohol phase to oil phase could trigger the conversion of triglycerides in oil phase to biodiesel and formation of another complex NaDG which can in turn diffuse back to alcohol phase. The complex NaDG in alcohol phase could initiate reactions with alcohol to produce diglycerides, monoglycerides and glycerin along with biodiesel while releasing the NaOR complex. Thus, the transfer of reaction complexes NaOR and NaDG between the two phases leads to conversion to biodiesel. A schematic of the proposed mechanism of alkaline-catalyzed transesterification of *Jatropha curcas* oil in seeds with alcohols is presented in Figure 5.21, which was adapted from a reaction mechanism for transesterification of oil with alcohol using phase transfer catalysts proposed by Hailegiorgis, *et al.* [114].

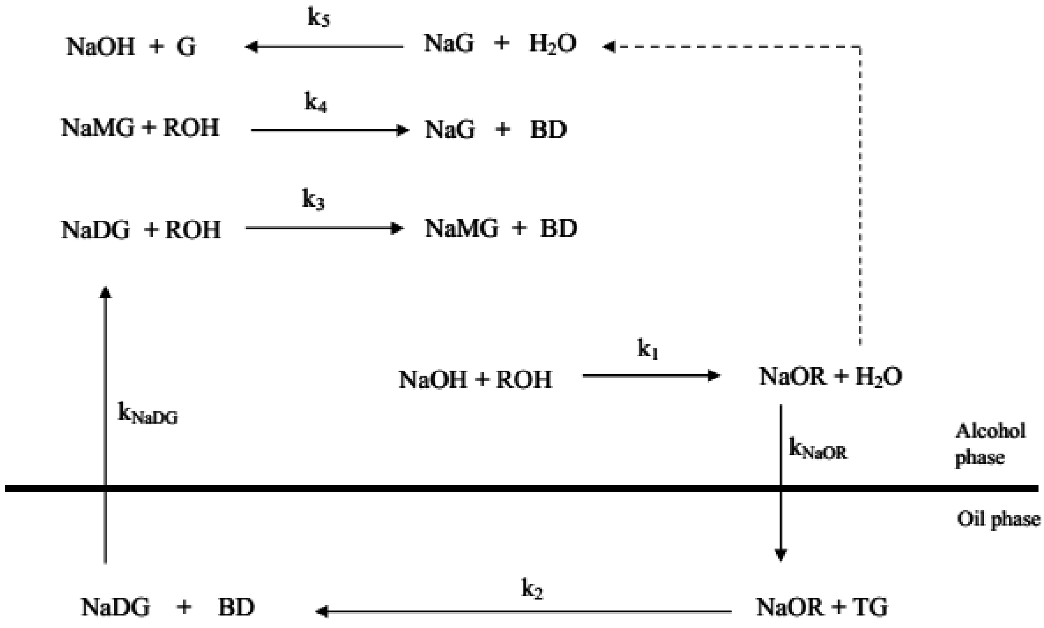


Figure 5.21: Schematic representation of the mechanism of alkaline-catalyzed *in-situ* transesterification of *Jatropha curcas* seeds

Possible reaction steps are described in the following subsections.

Step 1:Formation of NaOR in alcohol phase

Sodium hydroxide reacts with alcohol to form the reactant complex of sodium alkoxide (NaOR).

$$\text{ROH} + \text{NaOH} \xrightarrow{k_1} \text{NaOR} + \text{H}_2\text{O} \qquad (5.10)$$

The complex NaOR in alcohol phase diffuses into oil phase by mass transfer.

Step 2: Diffusion of NaOR into Oil phase

NaOR reacts with triglyceride (TG) to produce biodiesel (BD) and sodium-diglyceride complex (NaDG)

$$\text{NaOR} + \text{TG} \xrightarrow{k_2} \text{BD} + \text{NaDG} \qquad (5.11)$$

Step 3: Diffusion of NaDG to alcohol phase

The complex NaDG diffuses to alcohol phase and reacts with ROH to form biodiesel and another complex NaMG.

$$\mathrm{ROH + NaDG} \xrightarrow{k_3} \mathrm{NaMG + BD} \tag{5.12}$$

Step 4: Conversion of NaMG in alcohol phase

The complex NaMG in alcohol phase further reacts with alcohol to produce sodium-glycerol complex (NaG) and biodiesel (BD).

$$\mathrm{ROH + NaMG} \xrightarrow{k_4} \mathrm{NaG + BD} \tag{5.13}$$

Step 5: Conversion of NaG in alcohol Phase

Furthermore, NaG complex reacts with water which is produced from (5.8) to form sodium hydroxide (NaOH) and glycerol (G).

$$\mathrm{H_2O + NaG} \xrightarrow{k_5} \mathrm{NaOH + G} \tag{5.14}$$

The ratio of concentration of the complex in oil phase to that in alcohol phase is defined as partition coefficient.

$$M_{\mathrm{NaOR}} = \frac{[\mathrm{NaOR}]_o}{[\mathrm{NaOR}]_a} \tag{5.15}$$

$$M_{\mathrm{NaDG}} = \frac{[\mathrm{NaDG}]_o}{[\mathrm{NaDG}]_a} \tag{5.16}$$

The process of *in-situ* transesterification relies on the transfer rate of the complexes between two phases.

5.3.1 Model equation of reaction kinetics

Model equation of reaction kinetics is discussed in the following section.

a) The rate of triglyceride conversion in oil phase is

$$-\frac{d[TG]_o}{dt} = k_2[TG]_o[NaOR]_o \tag{5.17}$$

b) The overall mass balance of the active complexes, NaOR in the oil phase and the alcohol phase can be expressed as follows.

$$\begin{pmatrix}\text{Rate of Change}\\ \text{of } (NaOR)a\end{pmatrix}_a = \begin{pmatrix}\text{Rate of formation}\\ \text{of } (NaOR)a\\ \text{from}(NaOH)\end{pmatrix}_a - \begin{pmatrix}\text{Rate of mass transfer}\\ \text{of } (NaOR)a\end{pmatrix}_{ao}$$

$$\left(\frac{dV_a[NaOR]_a}{dt}\right) = k_1V_a[ROH]_a[NaOH]_a - k_{NaOR}A\left([NaOR]_a - \frac{[NaOR]_o}{M_{NaOR}}\right) \tag{5.18}$$

$$\begin{pmatrix}\text{Rate of change}\\ \text{of } (NaOR)_o\end{pmatrix}_o = \begin{pmatrix}\text{Rate of Mass Transfer}\\ \text{from alcohol phase}\end{pmatrix}_{ao} - \begin{pmatrix}\text{Rate of Consumption}\\ \text{in oil phase}\end{pmatrix}_o$$

$$\frac{dV_o[NaOR]_o}{dt} = k'_{NaOR} \cdot A\left([NaOR]_a - \frac{[NaOR]_o}{M_{NaOR}}\right) - k_2 \cdot V_o[TG]_o[NaOR]_o \tag{5.19}$$

c) The overall mass balance of the active complexes, NaDG in the oil phase and the alcohol phase can be expressed as follows.

$$\begin{pmatrix}\text{Rate of Change}\\ \text{of } (NaDG)_0\end{pmatrix}_o = \begin{pmatrix}\text{Rate of formation}\\ \text{of } (NaDG)o\end{pmatrix}_o - \begin{pmatrix}\text{Rate of mass transfer}\\ \text{of } (NaDG)o\end{pmatrix}_{ao}$$

$$\left(\frac{dV_o[NaDG]_o}{dt}\right) = k_2V_o[TG]_o[NaOR]_o - k_{NaDG}A([NaDG]_o - M_{NaDG}[NaDG]_a) \tag{5.20}$$

$$\begin{pmatrix}\text{Rate of Change}\\ \text{of } (\text{NaDG})\text{a}\end{pmatrix}_{\text{a}} = \begin{pmatrix}\text{Rate of mass transfer}\\ \text{of } (\text{NaDG})\text{a}\\ \text{from } (\text{NaDG})_{\text{o}}\end{pmatrix}_{\text{oa}} - \begin{pmatrix}\text{Rate of consumption}\\ \text{of } (\text{NaDG})\text{a}\end{pmatrix}_{\text{a}}$$

$$\left(\frac{dV_a[\text{NaDG}]_a}{dt}\right) = k_{\text{NaDG}}A\left([\text{NaDG}]_o - M_{\text{NaDG}}[\text{NaDG}]_a\right) - k_3V_a[\text{ROH}]_a[\text{NaDG}]_a \tag{5.21}$$

d) The overall mass balance of the active complexes, NaMG in the oil phase and the alcohol phase can be expressed as follows.

$$\begin{pmatrix}\text{Rate of Change}\\ \text{of } (\text{NaMG})\text{a}\end{pmatrix}_{\text{a}} = \begin{pmatrix}\text{Rate of formation}\\ \text{of } (\text{NaMG})\text{a}\end{pmatrix}_{\text{a}} - \begin{pmatrix}\text{Rate of consumption}\\ \text{of } (\text{NaMG})\text{a}\end{pmatrix}_{\text{a}}$$

$$\left(\frac{dV_a[\text{NaMG}]_a}{dt}\right) = k_3V_a[\text{NaDG}]_a[\text{ROH}]_a - k_4V_a[\text{ROH}]_a[\text{NaMG}]_a \tag{5.22}$$

e) The overall mass balance of the active complexes, NaG in the oil phase and the alcohol phase can be expressed as follows.

$$\begin{pmatrix}\text{Rate of Change}\\ \text{of } (\text{NaG})\text{a}\end{pmatrix}_{\text{a}} = \begin{pmatrix}\text{Rate of formation}\\ \text{of } (\text{NaG})\text{a}\end{pmatrix}_{\text{a}} - \begin{pmatrix}\text{Rate of consumption}\\ \text{of } (\text{NaG})\text{a}\end{pmatrix}_{\text{a}}$$

$$\left(\frac{dV_a[\text{NaG}]_a}{dt}\right) = k_4V_a[\text{NaMG}]_a[\text{ROH}]_a - k_5V_a[H_2O]_a[\text{NaG}]_a \tag{5.23}$$

The initial amount of sodium hydroxide added to the system is given by Eq. (5.24).

$$V_a[\text{NaOH}]_0 = V_o\left([\text{NaOR}]_o + [\text{NaDG}]_o\right) + V_a\left([\text{NaOR}]_a + [\text{NaDG}]_a + [\text{NaMG}]_a + [\text{NaG}]_a\right)$$

or

$$[\text{NaOH}]_0 = \frac{V_o}{V_a}\left([\text{NaOR}]_o + [\text{NaDG}]_o\right) + [\text{NaOR}]_a + [\text{NaDG}]_a + [\text{NaMG}]_a + [\text{NaG}]_a \tag{5.24}$$

The initial condition of the species, at t = 0, can be written as,

$[TG]_0 = [TG]_{o,0}$

$[NaOH]_a = [NaOH]_{a,0}$

$[NaOR]_{a,0} = [NaDG]_{a,0} = 0$

$[NaOR]_{o,0} = [NaDG]_{o,0} = 0$

$[NaMG]_{a,0} = 0$

$[NaG]_{a,0} = 0$

At excess amount of alcohol, the catalyst reactant complexes are dispersed steadily between the phases.

$$\frac{d[NaOR]_o}{dt} = \frac{d[NaOR]_a}{dt} = \frac{d[NaDG]_o}{dt} = \frac{d[NaDG]_a}{dt} = \frac{d[NaMG]_a}{dt} = \frac{d[NaG]_a}{dt} = 0 \tag{5.25}$$

Then Eq. (5.18) to Eq. (5.23) can be expressed as,

$$k_1 \cdot V_a [ROH]_a [NaOH]_a - k'_{NaOR} \cdot A\left([NaOR]_a - \frac{[NaOR]_o}{M_{NaOR}}\right) = 0 \tag{5.26}$$

$$k'_{NaOR} \cdot A\left([NaOR]_a - \frac{[NaOR]_o}{M_{NaOR}}\right) - k_2 \cdot V_o [TG]_o [NaOR]_o = 0 \tag{5.27}$$

$$k_2 \cdot V_o [NaOR]_o [TG]_o - k'_{NaDG} \cdot A\left([NaDG]_o - M_{NaDG}[NaDG]_a\right) = 0 \tag{5.28}$$

$$k'_{NaDG} \cdot A\left([NaDG]_o - M_{NaDG}[NaDG]_a\right) - k_3 \cdot V_a [ROH][NaDG]_a = 0 \tag{5.29}$$

$$k_3 \cdot V_a [ROH][NaDG]_a - k_4 \cdot V_a [ROH][NaMG]_a = 0 \tag{5.30}$$

$$k_4 \cdot V_a [ROH][NaMG]_a - k_5 \cdot V_a [H_2O][NaG]_a = 0 \tag{5.31}$$

Combining Eq. (5.28) and (5.29) and rearranging, the concentration of sodium diglyceride in the oil phase and the alcohol phase can be obtained as,

$$[\mathrm{NaDG}]_a = \frac{k_2 \cdot V_o[\mathrm{TG}]_o[\mathrm{NaOR}]_o}{k_3 \cdot V_a[\mathrm{ROH}]} \tag{5.32}$$

$$[\mathrm{NaDG}]_o = k_2 \cdot V_o[\mathrm{TG}]_o[\mathrm{NaOR}]_o\left(\frac{1}{k'_{\mathrm{NaDG}} \cdot A} - \frac{M_{\mathrm{NaDG}}}{k_3 \cdot V_a[\mathrm{ROH}]}\right) \tag{5.33}$$

Using Eq. (5.32) then Eq. (5.30) can be rewritten to be,

$$[\mathrm{NaMG}]_a = \frac{k_2 \cdot V_o[\mathrm{TG}]_o[\mathrm{NaOR}]_o}{k_4 \cdot V_a[\mathrm{ROH}]} \tag{5.34}$$

By substituting $[\mathrm{NaMG}]_a$ with Eq. (5.34), Eq. (5.31) can be expressed as,

$$[\mathrm{NaG}]_a = \frac{k_2 \cdot V_o[\mathrm{TG}]_o[\mathrm{NaOR}]_o}{k_5 \cdot V_a[\mathrm{H_2O}]} \tag{5.35}$$

Eq. (5.26) can be rearranged to be,

$$[\mathrm{NaOR}]_a = \frac{k_1 \cdot V_a[\mathrm{NaOH}]\ \ [\mathrm{ROH}]}{k'_{\mathrm{NaOR}} \cdot A} + \frac{[\mathrm{NaOR}]_o}{M_{\mathrm{NaOR}}} \tag{5.36}$$

According to reaction (5.10), consumption of [NaOH] to form $[\mathrm{NaOR}]_a$, and reaction (5.14), formation of [NaOH] from $[\mathrm{NaG}]_a$, the concentration of sodium hydroxide can be obtained.

$$[\mathrm{NaOH}] = -\frac{k_5[\mathrm{NaG}][\mathrm{H_2O}]}{k_1[\mathrm{ROH}]} \tag{5.37}$$

Using Eq. (5.35) to be substituted into Eq. (5.37), then Eq. (5.36) can be expressed as,

$$[\mathrm{NaOR}]_a = \frac{[\mathrm{NaOR}]_o}{M_{\mathrm{NaOR}}} - \frac{k_2 \cdot V_o[\mathrm{TG}]_o[\mathrm{NaOR}]_o}{k'_{\mathrm{NaOR}} \cdot A} \tag{5.38}$$

Eq. (5.32) to Eq. (5.38) is substituted into Eq. (5.24) to obtain the concentration of sodium hydroxide as a function of sodium alkoxide concentration in the oil phase.

$$
\begin{aligned}
[\mathrm{NaOH}]_0 = {} & \frac{V_o}{V_a}[\mathrm{NaOR}]_o + \frac{V_o^2 \cdot M_{\mathrm{NaDG}} \cdot k_2[\mathrm{TG}]_o}{V_a^2 k_3[\mathrm{ROH}]}[\mathrm{NaOR}]_o + \frac{V_o^2 \cdot k_2[\mathrm{TG}]_o}{V_a \cdot k'_{\mathrm{NaDG}} \cdot A}[\mathrm{NaOR}]_o \\
& - \frac{V_o \cdot k_2[\mathrm{TG}]_o}{k'_{\mathrm{NaOR}} \cdot A}[\mathrm{NaOR}]_o + \frac{1}{M_{\mathrm{NaOR}}}[\mathrm{NaOR}]_o + \frac{V_o \cdot k_2[\mathrm{TG}]_o}{V_a \cdot k_3[\mathrm{ROH}]}[\mathrm{NaOR}]_o \\
& + \frac{V_o \cdot k_2[\mathrm{TG}]_o}{V_a \cdot k_4[\mathrm{ROH}]}[\mathrm{NaOR}]_o + \frac{V_o \cdot k_2[\mathrm{TG}]_o}{V_a \cdot k_5[\mathrm{H_2O}]}[\mathrm{NaOR}]_o
\end{aligned}
\tag{5.39}
$$

Eq. (5.17) shows the equation of triglyceride consumption in the oil phase to produce biodiesel.

$$
-\frac{d[\mathrm{TG}]_o}{dt} = k_2[\mathrm{TG}]_o[\mathrm{NaOR}]_o \tag{5.17}
$$

$$
\begin{aligned}
k_{app} &= k_2[\mathrm{NaOR}]_o \\
[\mathrm{NaOR}]_o &= \frac{k_{app}}{k_2}
\end{aligned}
\tag{5.40}
$$

$$
-\frac{d[\mathrm{TG}]_o}{dt} = k_{app}[\mathrm{TG}]_o \tag{5.41}
$$

The conversion of triglyceride is defined as,

$$
X_{TG} = 1 - \frac{[\mathrm{TG}]_o}{[\mathrm{TG}]_{o,0}} \tag{5.42}
$$

The reaction rate can be expressed as a function of triglyceride conversion.

$$
-\ln(1 - X_{TG}) = k_{app} \cdot t \tag{5.43}
$$

The value of k_{app} can be obtained from the slope of linear regression curve of experimental results through the origin by plotting – ln (1-X_{TG}) versus reaction time at various reaction condition. Hence, Eq. (5.39) can be rearranged by substituting Eq. (5.40).

$$\begin{aligned}\frac{[\mathrm{NaOH}]_0}{k_{app}} &= \frac{V_o^2}{V_a^2}\left(\frac{M_{NaDG}\cdot[\mathrm{TG}]_o}{k_3[\mathrm{ROH}]}+\frac{V_a[\mathrm{TG}]_o}{k_{NaDG}\cdot A}\right)\\ &+\frac{V_o}{V_a}\left(\frac{1}{k_2}+\frac{[\mathrm{TG}]_o}{k_3[\mathrm{ROH}]}-\frac{V_a[\mathrm{TG}]_o}{k_{NaOR}\cdot A}+\frac{[\mathrm{TG}]_o}{k_4[\mathrm{ROH}]}+\frac{[\mathrm{TG}]_o}{k_5[\mathrm{H_2O}]}\right)\\ &+\frac{1}{k_2\cdot M_{NaOR}}\end{aligned} \tag{5.44}$$

Eq. (5.44) can be simplified to be Eq. (5.45).

$$\frac{[\mathrm{NaOH}]_0}{k_{app}} = a\varphi^2 + b\varphi + c$$

or

$$\overline{dy}\;\overline{dy}$$

$$dx\;dx\;\_$$

$$\frac{1}{k_{app}} = \left(\frac{a}{[\mathrm{NaOH}]_0}\right)\varphi^2 + \left(\frac{b}{[\mathrm{NaOH}]_0}\right)\varphi + \left(\frac{c}{[\mathrm{NaOH}]_0}\right) \tag{5.45}$$

where,

$$\varphi = \frac{V_o}{V_a}$$

$$a = \frac{M_{NaDG}[\mathrm{TG}]_o}{k_3[\mathrm{ROH}]} + \frac{V_a[\mathrm{TG}]_o}{k_{NaDG}\cdot A}$$

$$b = \frac{1}{k_2} - \frac{V_a[\mathrm{TG}]_o}{k_{NaOR}\cdot A} + \frac{[\mathrm{TG}]_o}{k_3[\mathrm{ROH}]} + \frac{[\mathrm{TG}]_o}{k_4[\mathrm{ROH}]} + \frac{[\mathrm{TG}]_o}{k_5[\mathrm{H_2O}]}$$

$$c = \frac{1}{k_2\cdot M_{NaOR}}$$

5.3.2 Effect of ratio of alcohol volume to seed weight (φ)

5.3.2.1 In-situ transesterification with methanol

In-situ transesterification with methanol was conducted at different ratio of alcohol volume to seed weight of 5 to 10 ($mL \cdot g^{-1}$). Figure 5.22 presents plots of – ln (1-X) versus reaction time and the slope of linear curve of – ln (1-X) versus reaction time through the origin demonstrated the value of k_{app}, which depicted in Table 5.7.

Table 5.7: Effect of ratio of methanol to *Jatropha curcas* seeds on the apparent rate constant (k_{app})

Ratio of methanol volume to seed weight (mL/g)	**Volume ratio of oil to methanol**	$\mathbf{k_{app}}$
5	0.110	0.0301
6	0.092	0.0338
7	0.079	0.0352
8	0.069	0.0361
9	0.061	0.0343
10	0.055	0.0319

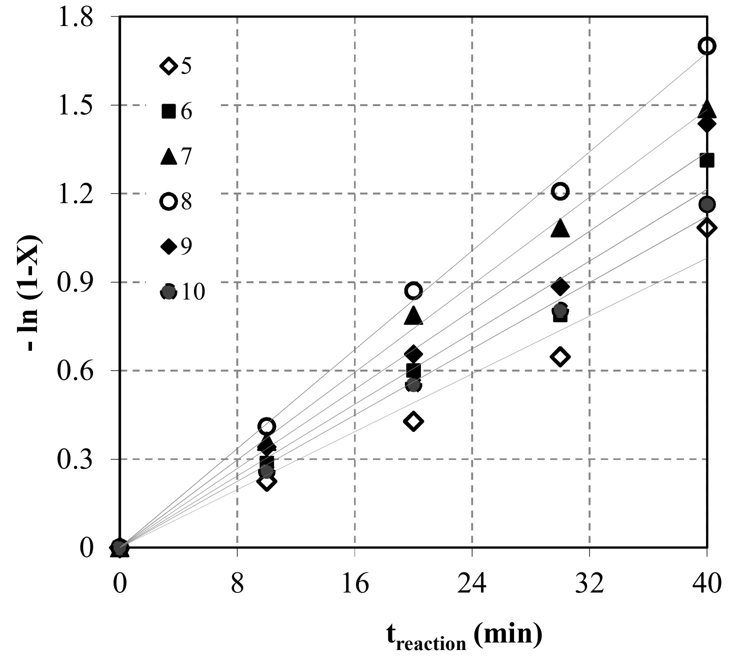


Figure 5.22: Plot of – ln (1-X) versus reaction time for methanol

Data in Table 5.7 is presented in Figure 5.23. The second-order polynomial equations are obtained by fitting the experimental data as presented in Figure 5.24.

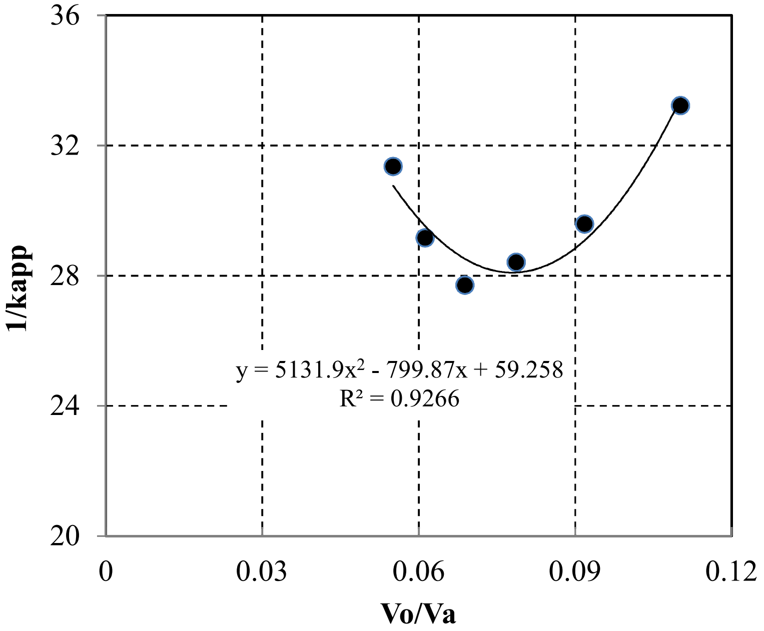


Figure 5.23: Effect of Vo/Va on k_{app} for methanol

The regression coefficients in the second-order polynomial equations are used in Eq. (5.45) to obtain the unknown model parameters.

$$\frac{1}{k_{app}} = 5131.9\varphi^2 - 799.87\varphi + 59.258 \tag{5.46}$$

Thus,

$$\frac{M_{NaDG}[TG]_o}{k_3[ROH][NaOH]} + \frac{V_a[TG]_o}{k_{NaDG} \cdot A[NaOH]} = 5131.9$$

$$\frac{1}{k_2[NaOH]_0} - \frac{V_a[TG]_o}{k_{NaOR} \cdot A[NaOH]_0} + \frac{[TG]_o}{k_3[ROH][NaOH]_0} + \frac{[TG]_o}{k_4[ROH][NaOH]_0} + \frac{[TG]_o}{k_5[H_2O][NaOH]_0} = -799.87$$

$$\frac{1}{k_2 \cdot M_{NaOR}[NaOH]_0} = 59.258$$

Using the experimental parameter values, the unknown parameters can be expressed as follows.

$$\frac{0.269698 \cdot M_{NaDG}}{k_3} + \frac{1}{k_{NaDG} \cdot A} = 1.75236 \cdot 10^6 \tag{5.47}$$

$$\frac{11.6}{k_2} - \frac{1}{k_{NaOR} \cdot A} + \frac{0.269}{k_3} + \frac{0.269}{k_4} + \frac{6.67 \cdot 10^3}{k_5[H_2O]} = -2.7313 \cdot 10^5 \tag{5.48}$$

$$\frac{1}{k_2 \cdot M_{NaOR}} = 1742 \tag{5.49}$$

The values of second-order reaction rate constant of k_2, k_3, k_4, and k_5 are large since the reactions are ionic in nature. Value of M_{NaOR} is also expected to be extremely low as the concentration of $[NaOR]_o$ in oil phase is very low due to its consumption by the very fast reaction with triglycerides in oil phase [TG]o. Hence, Eq. (5.47) to Eq. (5.49) can be written as

$$\frac{1}{k_{NaDG} \cdot A} = 1.752356 \cdot 10^6$$

$$\frac{1}{k_{NaOR} \cdot A} \cong 2.7313 \cdot 10^5 \tag{5.50}$$

$$\frac{1}{k_2 \cdot M_{NaOR}} = 1742$$

The mass transfer coefficients k_{NaOR} and k_{NaDG} are expected to be function of their diffusion coefficients which in turn are expected to be inversely proportional to their molecular weights. Molecular weight of NaOR and NaDG are 54 and 631.78 respectively. Then,

$$\frac{k_{NaDG} \cdot A}{k_{NaOR} \cdot A} \propto \frac{2.7313 \cdot 10^5}{1.752356 \cdot 10^6} \propto \left[\frac{M_W(NaOR)}{M_W(NaDG)}\right]^n \propto \left[\frac{54}{631.78}\right]^n \tag{5.51}$$

This observation corresponds to a value of 0.76 for n which is generally expected to be in the range of 0.5 to 1.

Model equation of reaction kinetics of two phases catalytic of transesterification can be written as,

$$\frac{1}{k_{app}} = \left[\frac{V_o^2}{V_a^2}\left(1.75236 \cdot 10^6 V_a [TG]_o\right) - \frac{V_o}{V_a}\left(2.7313 \cdot 10^5 V_a [TG]_o\right) + 1742\right] \frac{1}{[NaOH]_0} \tag{5.52}$$

Figure 5.24 shows the comparison between experimental $1/k_{app}$ and model $1/k_{app}$ obtained from Eq. (5.52). The model $1/k_{app}$ fitted to the experimental $1/k_{app}$ with the correlation coefficient of 0.9158.

According to Eq. (5.43), the conversion of triglycerides to biodiesel can be written as,

$$X = 1 - e^{-k_{app} \cdot t} \tag{5.53}$$

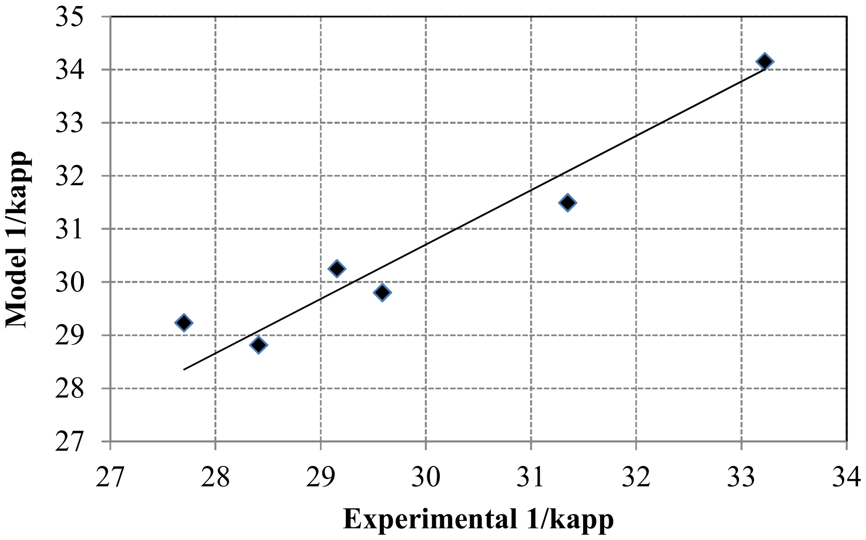


Figure 5.24: Comparison between model 1/k_{app} and experimental 1/k_{app} for methanol

Using Eq. (5.52) to substitute k_{app} in Eq. (5.53), the model predicted biodiesel yield could be evaluated.

$$Y_{BD} = \left(1 - e^{-\left(\frac{[\mathrm{NaOH}]_0}{\left[\frac{\mathrm{V}_o^2}{\mathrm{V}_a^2}\left(1.7524 \cdot 10^6\, \mathrm{V}_a [\mathrm{TG}]_o\right) - \frac{\mathrm{V}_o}{\mathrm{V}_a}\left(2.731 \cdot 10^5\, \mathrm{V}_a [\mathrm{TG}]_o\right) + 1742 \right]} \right) \cdot t} \right) \times 100\% \tag{5.54}$$

Figure 5.25 shows the comparison of the experimental observed biodiesel yield to the model predicted biodiesel yield obtained from Eq. (5.54).

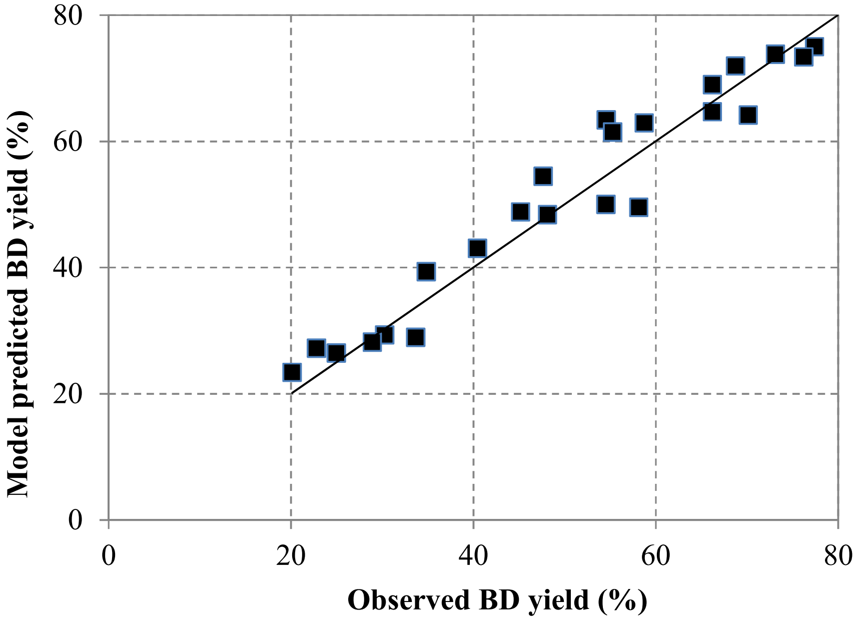


Figure 5.25: Model biodiesel yield versus observed biodiesel yield for methanol

The predicted biodiesel yields obtained from Eq. (5.54) were fitted well to the experimental observed biodiesel yield. Some data demonstrated that the model yields deviated from the experimental one. These deviations might be due to soap formation from saponification at high temperature or lack conversion of diglyceride and monoglyceride at shorter reaction time.

5.3.2.2 In-situ transesterification with the mixture of methanol and isopropanol

In-situ transesterification with the mixture of methanol and isopropanol was conducted at various ratio of alcohol volume to seed weight of 8 to 13 (mL·g^{-1}). Figure 5.26 shows plots of – ln (1-X) versus reaction time and the slope of linear curve of – ln (1-X) versus reaction time through the origin revealed the value of k_{app}, which depicted in Table 5.8.

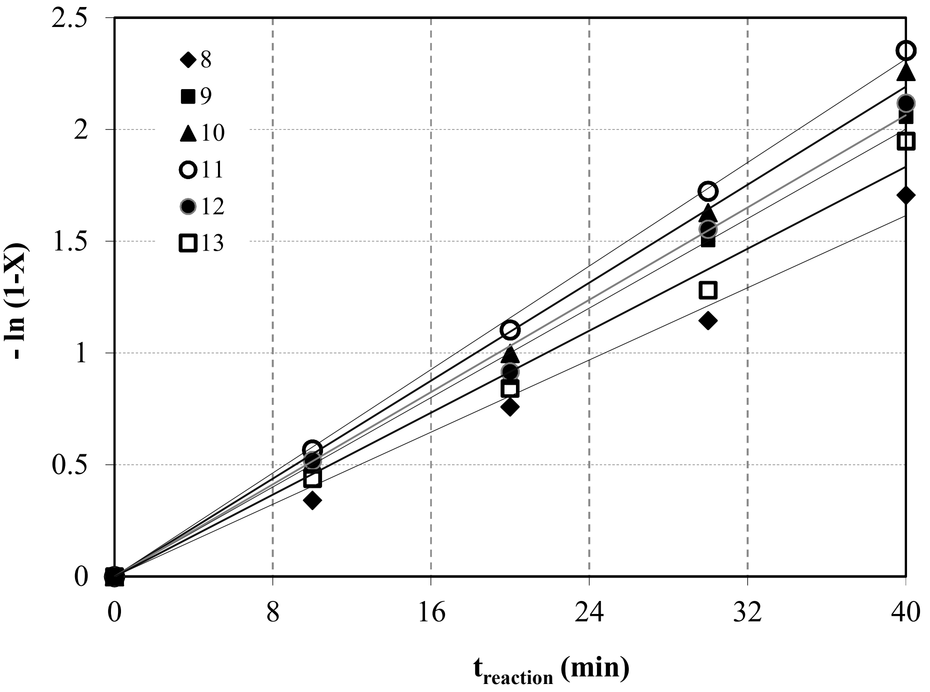


Figure 5.26: Plot of – ln (1-X) versus reaction time for the mixture of methanol and isopropanol

Table 5.8: Effect of ratio of the mixture of methanol and isopropanol to *Jatropha curcas* seeds on the apparent rate constant (k_{app})

Ratio of mixture volume to seed weight (mL/g)	**Volume ratio of oil to the mixture MeOH and IPA**	**k_{app}**
8	0.068	0.0441
9	0.061	0.0494
10	0.054	0.0522
11	0.050	0.0542
12	0.045	0.0502
13	0.042	0.0452

Data in Table 5.8 is presented in Figure 5.27. The second-order polynomial equations are obtained by fitting the experimental data as presented in Figure 5.27.

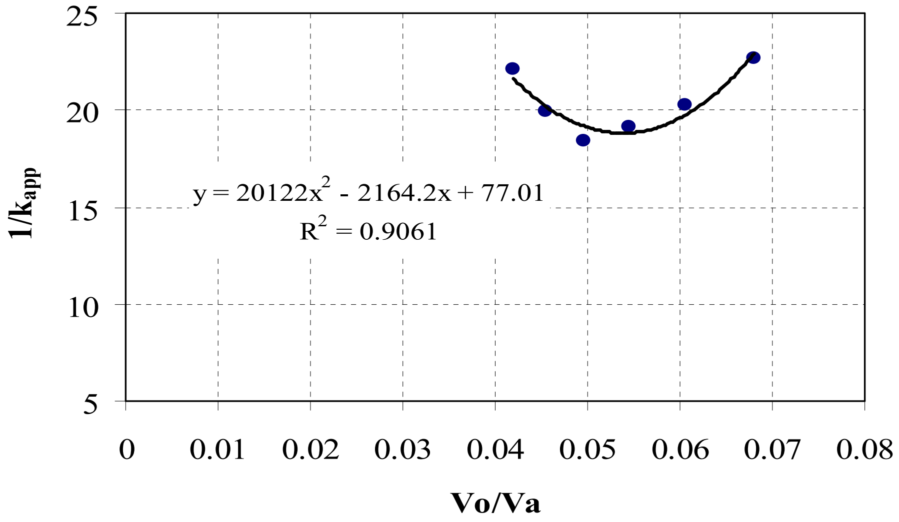


Figure 5.27: Effect of Vo/Va on k_{app} for the mixture of methanol and isopropanol

The regression coefficients in the second-order polynomial equations are used in Eq. (5.45) to obtain the unknown model parameters.

$$\frac{1}{k_{app}} = 20122\varphi^2 - 2164.2\varphi + 77.01 \tag{5.55}$$

Then,

$$\frac{M_{NaDG}[TG]_o}{k_3[ROH][NaOH]} + \frac{V_a[TG]_o}{k_{NaDG} \cdot A[NaOH]} = 20122$$

$$\frac{1}{k_2[NaOH]_0} - \frac{V_a[TG]_o}{k_{NaOR} \cdot A[NaOH]_0} + \frac{[TG]_o}{k_3[ROH][NaOH]_0} + \frac{[TG]_o}{k_4[ROH][NaOH]_0} + \frac{[TG]_o}{k_5[H_2O][NaOH]_0} = -2164$$

$$\frac{1}{k_2 \cdot M_{NaOR}[NaOH]_0} = 77.01$$

Using the experimental parameter values, the unknown parameters can be expressed as follows.

$$\frac{0.4128\cdot M_{NaDG}}{k_3}+\frac{1}{k_{NaDG}\cdot A}=5.2584\cdot 10^6 \tag{5.56}$$

$$\frac{11.5}{k_2}-\frac{1}{k_{NaOR}\cdot A}+\frac{0.4128}{k_3}+\frac{0.4128}{k_4}+\frac{6.67\cdot 10^3}{k_5[H_2O]}=-5.6551\cdot 10^5 \tag{5.57}$$

$$\frac{1}{k_2\cdot M_{NaOR}}=1733 \tag{5.58}$$

The values of second-order reaction rate constant of k_2, k_3, k_4, and k_5 are large since the reactions proceed fast. Value of M_{NaOR} is also expected to be extremely low as the concentration of [NaOR]o in oil phase is very low due to its consumption by the very fast reaction with triglycerides in oil phase [TG]o. It may be noted that $[1/k_2M_{NaOR}]$ is same for methanol alone as well as the present case. Hence, Eq. (5.55) to Eq. (5.58) can be written as

$$\frac{1}{k_{NaDG}\cdot A}=5.2584\cdot 10^6$$

$$\frac{1}{k_{NaOR}\cdot A}\cong 5.6551\cdot 10^5 \tag{5.59}$$

$$\frac{1}{k_2\cdot M_{NaOR}}=1733$$

It may be noted that the mass transfer coefficients for k_{NaOR} and k_{NaDG} for the mixture of methanol and isopropanol system are smaller compared to the values obtained for methanol alone.

Model equation of reaction kinetics of two phases catalytic of transesterification can be written as,

$$\frac{1}{k_{app}}=\left[\frac{V_o^2}{V_a^2}\left(5.2584\cdot 10^6 V_a[TG]_o\right)-\frac{V_o}{V_a}\left(5.6551\cdot 10^5 V_a[TG]_o\right)+1733\right]\frac{1}{[NaOH]_0} \tag{5.60}$$

Figure 5.28 shows the comparison between experimental 1/k_{app} and model 1/k_{app} obtained from Eq. (5.60). The model 1/k_{app} fitted well to the experimental 1/k_{app}.

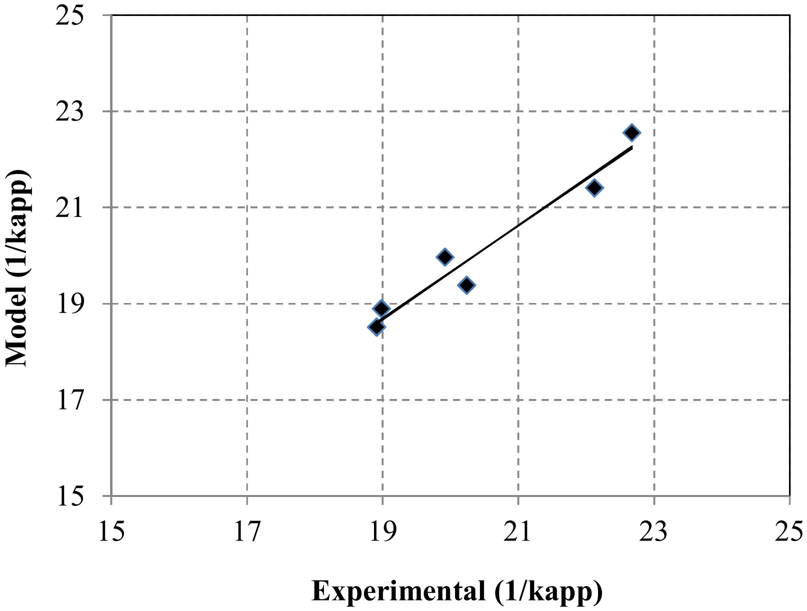


Figure 5.28: Comparison between Model 1/k_{app} and experimental 1/k_{app}

Using Eq. (5.43) and substituting Eq. (5.60) to k_{app}, the model predicted biodiesel yield for methanol and isopropanol can be determined.

$$Y_{BD} = \left(1 - e^{-\left(\frac{[\mathrm{NaOH}]_0 \cdot t}{\left[\frac{\mathrm{V_o^2}}{\mathrm{V_a^2}}\left(5.2584 \cdot 10^6\, \mathrm{V_a}\, [\mathrm{TG}]_o\right) - \frac{\mathrm{V_o}}{\mathrm{V_a}}\left(5.6551 \cdot 10^5\, \mathrm{V_a}\, [\mathrm{TG}]_o\right) + 1733\right]}\right)}\right) \times 100\% \tag{5.61}$$

Experimental observed biodiesel yields were compared to the predicted biodiesel yield obtained from the model equation (5.61) as presented in Figure 5.29. The predicted biodiesel yields by model equation (5.58) are fitted well to the experimental biodiesel yields. Less deviation of predicted biodiesel yields from the experimental one are observed than that for methanol.

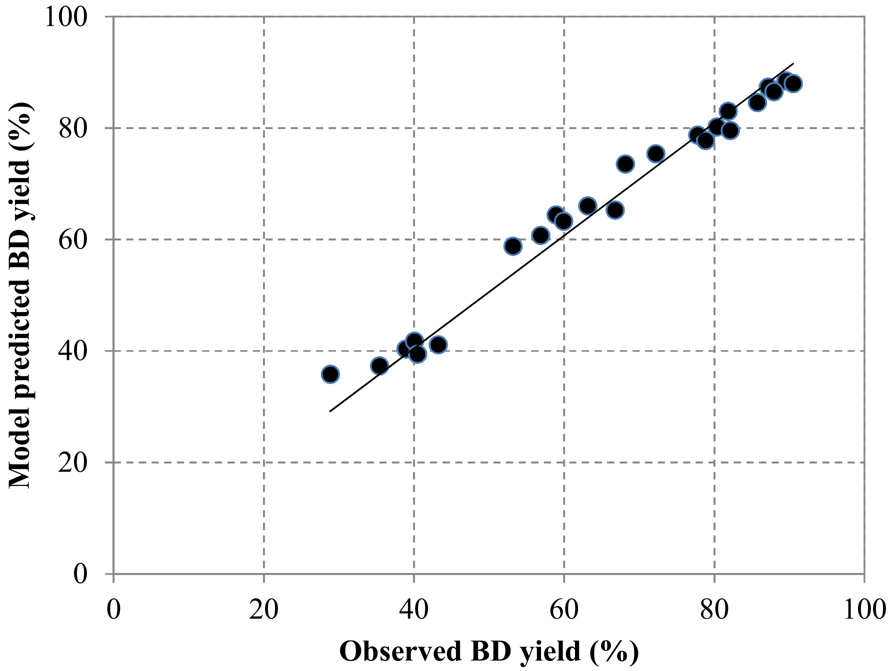


Figure 5.29: Model biodiesel yield of Eq. (5.59) versus experimental biodiesel yield

5.3.3 Effect of sodium hydroxide concentration

The effects of sodium hydroxide concentration on the apparent rate constant are displayed in Figure 5.30 for methanol and Figure 5.32 for the mixture of methanol and isopropanol. Increasing sodium hydroxide concentration enhanced the conversion of triglyceride to produce biodiesel up to concentration of 2 wt.% shown by the maximum k_{app} of 0.0383 min^{-1} at 2 wt.% sodium hydroxide concentration for methanol and 0.056 for the mixture of methanol and isopropanol. However rising sodium hydroxide concentration, more than 2 wt.%, the k_{app} decreased. It was observed at this condition the higher sodium hydroxide concentration led the saponification which consumed sodium hydroxide to produced soaps instead of biodiesel. Soaps disturbed the separation of biodiesel from the impurities. Thus, the parabolic curve was obtained in graph of $1/k_{app}$ versus 1/[NaOH].

5.3.3.1 In-situ transesterification with methanol

In order to study the effect of sodium hydroxide on the conversion of triglyceride to biodiesel, the reaction was conducted at different concentration of sodium hydroxide from 1.25 wt.% to 2.5 wt.% at the constant of Vo/Va of 0.068 for methanol. The plot of – ln (1-X) versus reaction time at different sodium hydroxide concentration for methanol is shown in Figure 5.30.

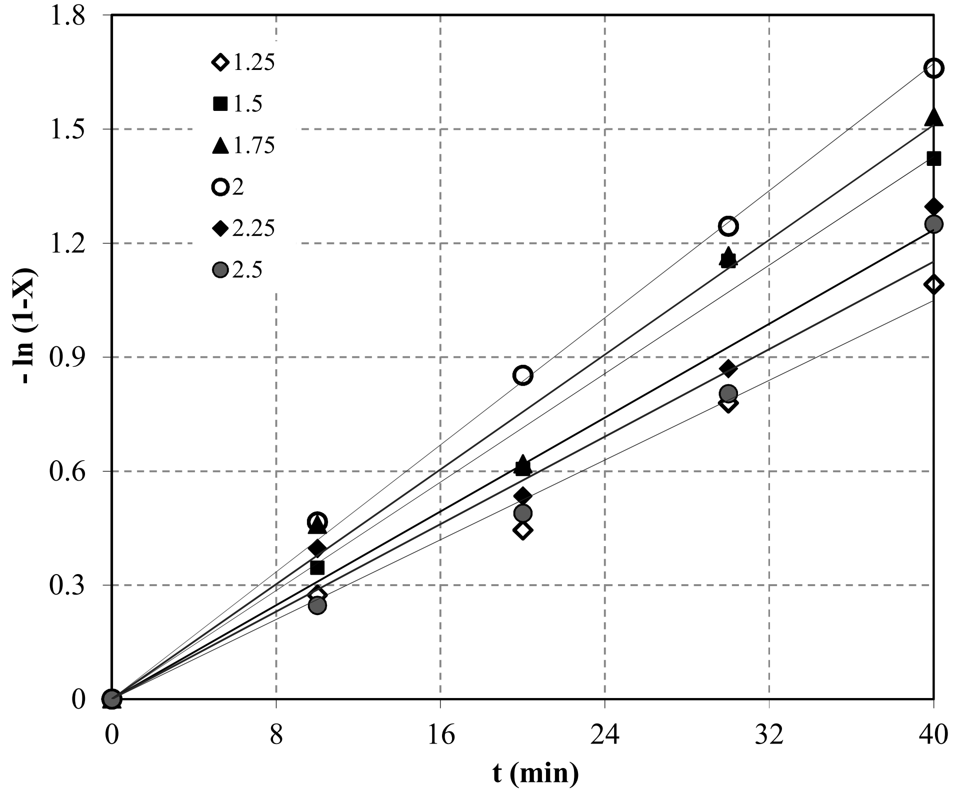


Figure 5.30: Plot of – ln (1-X) versus reaction time at different sodium hydroxide concentration for methanol

Table 5.9 describes the effect of various sodium hydroxide concentrations on the value of the first-order apparent rate constant (k_{app}) for methanol.

Table 5.9: Effect of sodium hydroxide concentration on the first-order apparent rate constant (k_{app})

Sodium hydroxide concentration (wt.%)	$\mathbf{k_{app}}$
1.25	0.0262
1.50	0.0357
1.75	0.0378
2.00	0.0383
2.25	0.0309
2.50	0.0258

Data in Table 5.9 is presented in Figure 5.31. The first-order polynomial equations are obtained by fitting the experimental data for sodium hydroxide concentration of 1.25 wt.% to 2 wt.% as presented in Figure 5.31. Data of sodium hydroxide concentration more than 2 wt.% was not used in curve fitting due to saponification process occurred and this condition must be avoided in biodiesel production.

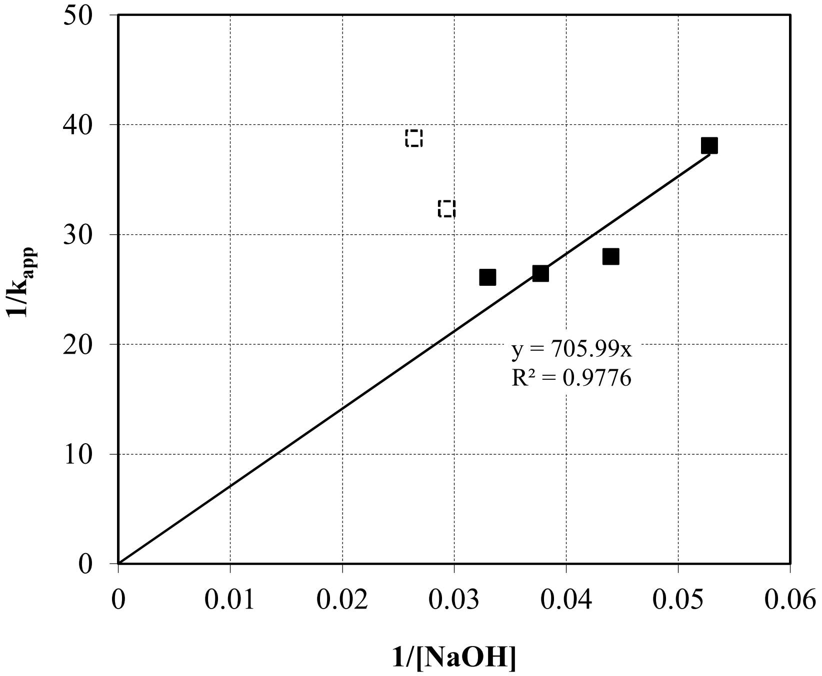


Figure 5.31: Effect of NaOH concentration on the apparent rate constant for methanol

By substituting parameters with the experimental data, then Eq. (5.52) could be expressed as a function of sodium hydroxide concentration as shown in Eq. (5.62).

$$\frac{1}{k_{app}} = 840.54 \frac{1}{[NaOH]_0} \tag{5.62}$$

It can be observed that there is a difference between the value of slope obtained from curve fitting and that from model equation.

5.3.3.2 In-situ transesterification with the mixture of methanol and isopropanol

In order to study the effect of sodium hydroxide on the conversion of triglyceride to biodiesel, the reaction was carried out at various concentration of sodium hydroxide from 1 wt.% to 2.5 wt.% at the constant of Vo/Va of 0.0504 for the mixture of methanol and isopropanol. The plots of – ln (1-X) versus reaction time at different sodium hydroxide concentration displays in Figure 5.32 for the mixture of methanol and isopropanol. Table 5.10 describes the effect of various sodium hydroxide concentrations on the value of the first-order apparent rate constant (k_{app}) for the mixture of methanol and isopropanol.

Table 5.10: Effect of sodium hydroxide concentration on the first-order apparent rate constant (k_{app})

Sodium hydroxide concentration (wt.%)	**k_{app}**
1.00	0.034
1.25	0.044
1.50	0.0504
1.75	0.0535
2.00	0.056
2.25	0.051
2.50	0.045

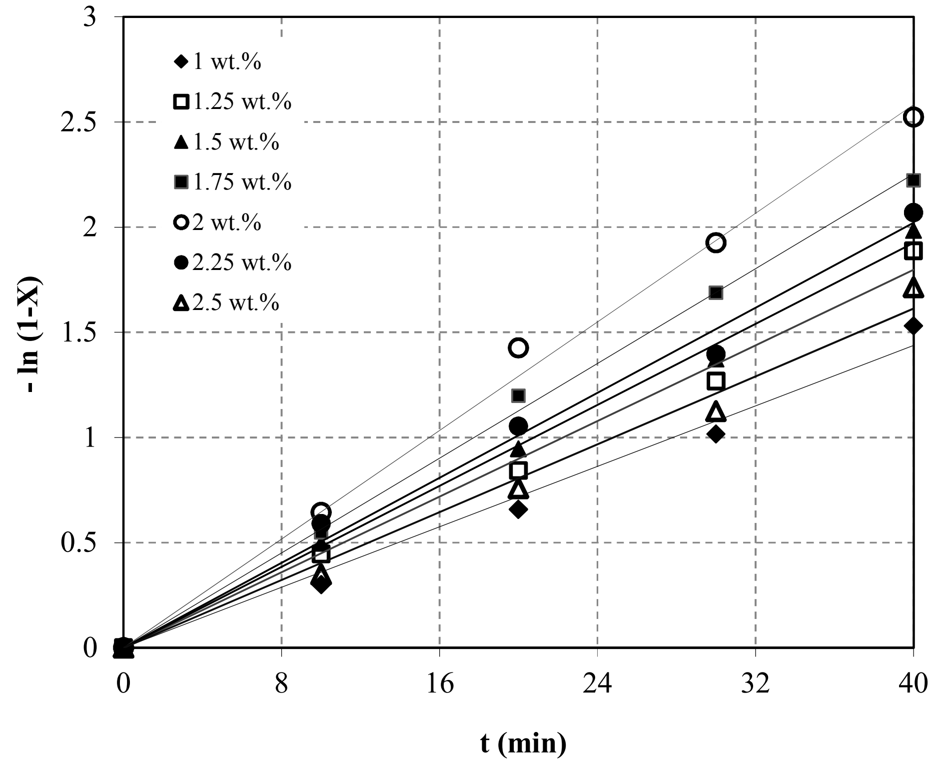


Figure 5.32: Plot of – ln (1-X) versus reaction time at different sodium hydroxide concentration for the mixture of methanol and isopropanol

Data in Table 5.10 was fitted linearly as described in Figure 5.33. The first-order polynomial equations are obtained by fitting the experimental data for sodium hydroxide concentration of 1 wt.% to 2 wt.% as presented in Figure 5.33. Data of sodium hydroxide concentration of 2.25 wt.% and 2.5 wt.% were not used in curve fitting since in this concentration, saponification process occurred and it must be avoided in biodiesel production.

By substituting parameters with the experimental data, then Eq. (5.60) could be expressed as a function of sodium hydroxide concentration as shown in Eq. (5.63).

$$\frac{1}{k_{app}} = 429\frac{1}{[\mathrm{NaOH}]_0} \tag{5.63}$$

It can be observed that there is a difference between the value of slope obtained from curve fitting and that from model equation.

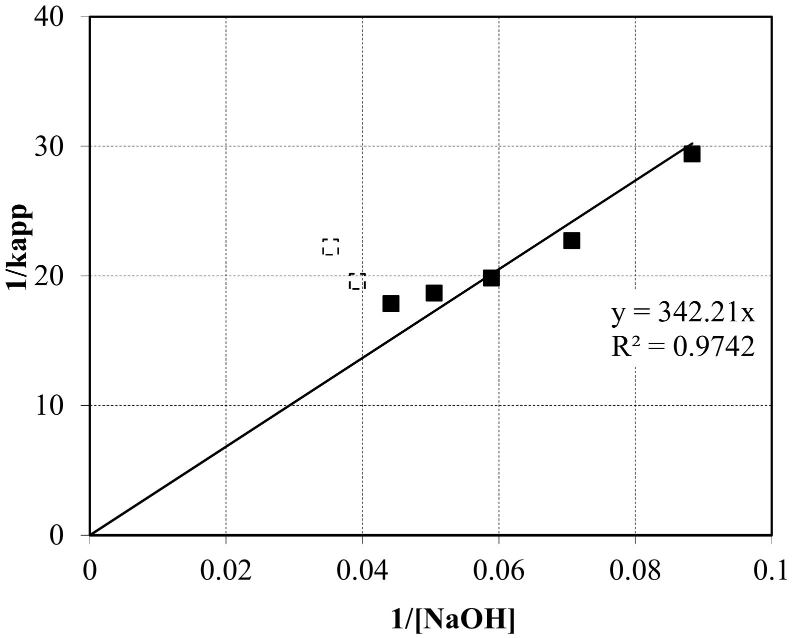


Figure 5.33: Effect of NaOH concentration on the apparent rate constant for the mixture of methanol and isopropanol

5.4 **Summary**

Reaction mechanism and kinetics of alkaline-catalyzed *in-situ* methanolysis and *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and isopropanol in a batch reactor at different reaction temperature and particle size had been investigated by applying the shrinking core model.

The mechanism of *in-situ* methanolysis and *in-situ* transesterification of the mixture of methanol and isopropanol were chemical reaction controls. Both of *in-situ* transesterification had the first-order reaction rate with the reaction rate constant from $5.15x10^{-9}$ to $8.76x10^{-9}$ $m \cdot s^{-1}$ and from $7.72x10^{-9}$ to $1.49x10^{-8}$ $m \cdot s^{-1}$, respectively. Arrhenius activation energies of the reactions were determined to be 2266 $J \cdot mol^{-1}$ for *in-situ* methanolysis and 2035 $J \cdot mol^{-1}$ for *in-situ* transesterification of the mixture of methanol and isopropanol.

Based on the reaction mechanism studied for two phase catalytic transesterification of *Jatropha curcas* oil, alkaline-catalyzed transesterification kinetics model equations were developed. The effects of alcohol volume to seed weight ratio and NaOH concentration on triglyceride conversion were evaluated using the model equations. The results were compared well with the experimental observation. The prediction of model equation on the apparent reaction rate constant and the yield of biodiesel were fitted well with the experimental results.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions can be drawn from the present research on optimization and reaction kinetics of *in-situ* transesterification of *Jatropha curcas* seeds,

1. *In-situ* transesterification of *Jatropha curcas* seeds to produce biodiesel catalyzed by sodium hydroxide was investigated with isopropanol, methanol, the mixture of methanol and isopropanol.

   a. The maximum yield of isopropyl ester of (85.32 ± 0.30)% was obtained. This yield was not high since isopropanol was less polarity than methanol which led saponification during biodiesel separation from the impurities of glycerol, isopropanol, NaOH, and water.

   b. Sodium hydroxide concentration, methanol volume to seed weight ratio, and reaction temperature are the important factors affecting methyl ester yield. Increasing values of these factors up to a certain level enhanced the yield of methyl ester. However, further increasing these factors decreased methyl ester yield. The maximum methyl ester yield was achieved at optimum reaction conditions including NaOH concentration of 1.94 wt.%, methanol volume to seed weight ratio of 8.08 ($mL\cdot g^{-1}$) at 57°C reaction temperature. The particle size had significant effect on the methyl ester yield. Grinding *Jatropha curcas* seeds into particle size of $425 < Dp < 600$ µm, the reaction time to obtain the equilibrium condition could be reduced from 90 min to 60 min.

   c. Mixing methanol with isopropanol could enhance the yield of biodiesel. The most significant variable affected methyl-isopropyl yield was NaOH concentration followed by the ratio of mixture volume to seed weight and the volume ratio of methanol to the mixture of methanol and isopropanol. Raising these parameters into certain values augmented the yield of methyl-isopropyl ester. Subsequent increasing these parameters reduced the yield of methyl-isopropyl ester. The maximum methyl-isopropyl ester yield of (94.78 ± 0.29)% that was insignificantly difference from the predicted yield of 95.31% was obtained at the optimum conditions included 1.99 wt.% NaOH concentration, 10.82 ($mL \cdot g^{-1}$) the mixture volume to seed weight ratio, 0.46 volume ratio of methanol to the mixture of methanol and isopropanol at 68°C reaction temperature. Reducing the particle size to less than 425 μm could shorten reaction time to reach the equilibrium condition from 60 min to 30 min. The properties of methyl-isopropyl ester were similar to the properties of methyl ester which were in agreement with the standards of EN 14214 and ASTM D6751.

2. *In-situ* transesterification is a catalytic liquid-liquid reaction in the presence of inert solid phase. Reaction mechanism and kinetics of *in-situ* methanolysis and *in-situ* transesterification of *Jatropha curcas* seeds with the mixture of methanol and isopropanol in a batch reactor at different reaction temperature and particle size had been investigated by applying the shrinking core model. The mechanism of *in-situ* methanolysis and *in-situ* transesterification of the mixture of methanol and isopropanol were chemical reaction controls.

    a. For *in-situ* methanolysis, the reaction was first order with the rate constant from 5.15×10^{-9} to 8.76×10^{-9} $m \cdot s^{-1}$, Arrhenius activation energy of 22.66 $kJ \cdot mol^{-1}$ and pre-exponential factor of 1.813 $m \cdot s^{-1}$.

    b. For *in-situ* transesterification with mixture of methanol and isopropanol, the reaction was also first order with the rate constant from 7.72×10^{-9} to 1.49×10^{-8} $m \cdot s^{-1}$, Arrhenius activation energy of 20.35 $kJ \cdot mol^{-1}$ and pre-exponential factor of 1.380 $m \cdot s^{-1}$.

Based on the reaction mechanism studied for two phase catalytic transesterification of *Jatropha curcas* oil, alkaline-catalyzed transesterification kinetics model equations were developed. The effects of alcohol volume to seed weight ratio and NaOH concentration on triglyceride conversion were evaluated using the model equations. The results were compared with the experimental observation. The prediction of model equation on the apparent reaction rate constant and the yield of biodiesel were comparable with the experimental results.

3. *In-situ* transesterification of *Jatropha curcas* seeds using methanol mixed with n-hexane to enhance the oil extraction had been investigated in Soxhlet extractor. The methyl ester yield of (83.61 ± 0.57)% which was insignificant to the predicted methyl ester yield of 84.67% was obtained at the optimum conditions of 8.4 ($mL \cdot g^{-1}$) mixture volume to seed weight ratio, 1.92 wt.% NaOH concentration, 0.52 ($mL \cdot mL^{-1}$) volume ratio of methanol to mixture at 60°C reaction temperature for 1 h reaction time. Prolong reaction time to 3 h; higher methyl ester yield of 90.74% was achieved.

6.2 Recommendations

The following recommendations are proposed:

1. In the present study, the experiments have been conducted in batch reactor. One experiment has been performed in Soxhlet extractor which almost similar to the packed bed reactor. Therefore, designing and building-up the efficient and economical continuous reactor to produce biodiesel by *in-situ* transesterification of non-edible oilseeds or algae is important to be investigated since the volume of actual commercial biodiesel production is very large.
2. The reaction mechanism and kinetics of batch process differs from the continuous process. Hence, developing the reaction mechanism and kinetics of continuous reactor should be conducted as well.

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APPENDIX

Table A 1: Alkaline-catalyzed transesterification

Feedstock **References**	**Alcohol**	**Alcohol:oil**	**Catalyst**	**Cat. amount (wt.%)**	**T (°C)**	**t (min)**	**BD yield (%)**
Beef fat	methanol	1 : 6	KOH	1.25	65	120	87.4
Chicken fat	methanol	1 : 6	KOH	1.25	65	120	89
Used cooking oil	methanol	1 : 6	KOH	1.25	65	120	88.3
Hoque, *et al*., 2011 [137]							
Tucum oil (*Astrocaryum vulgare*)	Methanol	5 : 1 w/w	NaOH	1.0	60	30	92
Lima, *et al*., 2008 [138]	ethanol	5 : 2 w/w	NaOH	1.0	60	30	90
Mixed soybean oil and rapeseed oil 50:50 weight ratio of oils; n-hexane was used as co-solvent Qiu, *et al*., 2011 [139]	Methanol	1 : 5	NaOH	0.8	55	120	94
Waste cooking oil Phan, *et al*., 2008 [140]	methanol	1 : 7 – 1 : 8	KOH	0.75	30-50	80-90	88-90

Feedstock **References**	**Alcohol**	**Alcohol:oil**	**Catalyst**	**Cat. amount (wt.%)**	**T (°C)**	**t (min)**	**BD yield (%)**
Iranian bitter almond oil Atapour and Kariminia, 2011 [141]	methanol	1 : 7	KOH	0.9	50	60	90.8
Palm kernel oil blender was used as reactor Alamu, *et al*., 2008 [142]	ethanol	5 : 1 w/w	KOH	1.0	60	120	96
Used cooking oil $NaOCH_3$, $KOCH_3$, NaOH, KOH were test as catalyst, Encinar, *et al*., 2007 [127]	ethanol	1 : 12	KOH	1.0	78	120	74.2
Sunflower oil	methanol	1 : 25	KOH	1.4	23	3	99.3
tetrahydrofuran (THF) was used	ethanol	1 : 25	KOH	1.4	23	6-7	98.4
as co-solvent	ethanol	1 : 25	KOH	1.4	60	2	98.8
Zhou, *et al*., 2003 [64]	1-butanol	1 : 25	KOH	1.4	23	30	98.0
Soybean oil tetrahydrofuran (THF) as co-solvent (0.87 v/v), Boocock, *et al*., 1998 [134]	methanol	1 : 27	NaOH	1.0	23	7	99.4

Feedstock **References**	**Alcohol**	**Alcohol:oil**	**Catalyst**	**Cat. amount (wt.%)**	**T (°C)**	**t (min)**	**BD yield (%)**
Rapeseed (*Brasica napus*) oil $NaOCH_3$, $KOCH_3$, NaOH were tested as catalyst as well, Rashid and Anwar, 2008 [58]	methanol	1 : 6	KOH	1.0	65	120	95-96
Neat canola oil, ester content = 98 wt.%	methanol	1 : 6	NaOH	1.0	40-45	60	93.5
Used canola frying oil, ester content = 94.6 wt.% Leung and Guo, 2006 [59]	methanol	1 : 7	NaOH	1.1	60	20	88.8
Milkweed (Asclepsia) seed oil	methanol	1 : 6	$NaOCH_3$	1.1	60	60	>99
Holser and Harry-O'Kuru, 2006[60]	ethanol	1 ; 6	$NaOC_2H_5$	1.1	60	60	97.7
Moringa Oliefera oil Kafuku and Mbarawa, 2010 [143]	methanol	30 wt.%	KOH	1.0	60	60	82
Malaysian RBD palm oil ester content = 88%	methanol	1 : 6	NaOH	1.0	60	60	81
Shahbazi, *et al*., 2012 [144]			KOH	1.0	60	60	90

Feedstock **References**	**Alcohol**	**Alcohol:oil**	**Catalyst**	**Cat. amount (wt.%)**	**T (°C)**	**t (min)**	**BD yield (%)**
Rice bran oil Sinha, *et al*., 2008 [145]	methanol	1 : 9	NaOH	0.75	55	60	90.18
Dairy waste scum Sivakumar, *et al*., 2011 [61]	methanol	1 : 6	KOH	1.2	75	30	96.7
mixtures of canola and used cooking oil, 40:60 weight ratio of used cooking oil to canola oil, Issariyakul, *et al*., 2008 [62]	ethanol	1 : 6	KOH	1.0	50	120	97.7
Muskmelon (Cucumis melo) seed oil Rashid, *et al*., 2011 [146]	methanol	1 : 5.8	KOH	0.79	55	72.5	89.5
Karanja (*Pongamia pinnata*) oil initial AV is 5.06 mg KOH/g Meher, *et al*., 2006 [63]	methanol	1 : 6	KOH	1.0	65	180	97-98
***Jatropha curcas* oil** Chitra, *et al*., 2005 [147]	methanol	1 : 5	NaOH	1.0	60	90	98

Feedstock **References**	**Alcohol**	**Alcohol:oil**	**Catalyst**	**Cat. amount (wt.%)**	**T (°C)**	**t (min)**	**BD yield (%)**
Cottonseed (*Gossypium hirsutum* L.) oil, NaOH, KOH, $KOCH_3$ were tested as catalyst as well, Rashid, *et al*., 2009 [66]	methanol	1 : 6	$NaOCH_3$	0.75	65	120	96.9
Mahua (*Madhuca Indica*) oil Engine performance test show that methyl ester as a fuel does not differ greatly from that of diesel, Puhan, *et al*., 2005 [148].	methanol	9 : 2 w/v	NaOH	0.75	60	120	92
Spent coffee ground oil oil content : 15.28% Al-Hamamre, *et al*., 2012 [149]	methanol	1 : 9	KOH	2.5	65	240	85
Okra (*Hibiscus esculentus*) seed oil; oil content : 12%; Anwar, *et al*., 2010 [150]	methanol	1 : 7	$NaOCH_3$	1.0	65	120	96.8
Canola oil	methanol	1 : 9	KOH	1.0	60	60	95
Corn oil	methanol	1 : 9	KOH	2.0	80	60	96
Patil and Deng, 2009 [78]							

Feedstock **References**	**Alcohol**	**Alcohol:oil**	**Catalyst**	**Cat. amount (wt.%)**	**T (°C)**	**t (min)**	**BD yield (%)**
***Pongamia pinnata* oil** oil content : 25% Karmee and Chadha, 2005 [151]	methanol	1 : 10	KOH	1.0	60	90	92
***Idesia polycarpa* var. *vestita* fruit oil**; oil content: 26.26%; Yang, *et al*., 2009 [152]	methanol	1 : 6	KOH	1.0	30	40	>99
Sunflower oil; oil content: 45-50%; Rashid, *et al*., 2008 [153]	methanol	1 : 6	NaOH	1.0	60	120	97.1
Pumpkin (*Cucurbita pepo* L.) seed oil; oil content: 45%; Schinas, *et al*., 2009 [154]	methanol	1 : 6	NaOH	1.0	65	60	97.5
Roselle (*Hibiscus sabdariffa* L.) oil; oil content: 18% Nakpong and Wootthikanokkhan, 2010 [155]	methanol	1 : 8	KOH	1.5	60	60	99.4

Feedstock **References**	**Alcohol**	**Alcohol:oil**	**Catalyst**	**Cat. amount (wt.%)**	**T (°C)**	**t (min)**	**BD yield (%)**
Palm oil; Reaction in continuous stirred-tank reactor (CSTR) Darnoko and Cheryan, 2000 [129]	methanol	1 : 6	KOH	1.0	60	60	97.3
Sunflower oil	methanol	1 : 6	NaOH	1.0	65	240	85.9
all catalysts have methyl ester concentration of near 100 wt.%	methanol	1 : 6	KOH	1.0	65	240	91.67
Vicente, *et al.*, 2004 [65]	methanol	1 : 6	$NaOCH_3$	1.0	65	240	> 98
	methanol	1 : 6	$KOCH_3$	1.0	65	240	> 98
Sunflower oil	methanol	1 : 6	$NaOCH_3$	0.5	60	60	98
Freedman, *et al.*, 1984 [15]	ethanol	1 : 6	$NaOCH_3$	0.5	75	60	97
	1-butanol	1 : 6	$NaOCH_3$	0.5	114	60	96
Coconut oil	methanol	0.25% v/v	KOH	0.5% w/v	58	20	98-99
palm oil	methanol	0.25% v/v	KOH	0.5% w/v	60	20	98-99
Satyanarayana and Muraleedharan, 2011 [156]							

APPENDIX III

Table A 2: Acid-catalyzed transesterification

Feedstock **References**	**Alcohol**	**Molar ratio Oil to alcohol**	**Catalyst**	**Cat. amount (wt.%)**	**Reaction condition**			**BD yield (%)**	**Acid value**	
					Temp	**time**	**Stirring speed**		**Initial**	**Final**
Sunflower oil Berrios, *et al*., 2007 [67]	methanol	1 : 60	H_2SO_4	5.0	60°C	2 h	-	-	5-7	1
***Zanthoxylum bungeanum* seed oil** Zhang and Jiang, 2008 [68]	methanol	1 : 24	H_2SO_4	2.0	60°C	80 min	600 rpm	-	45.51	1.16
Oleic acid applied ultrasonic irradiation Hanh, *et al*., 2009 [69]	ethanol	1 : 3	H_2SO_4	5.0	60°C	2 h	1800 rpm	> 95		
Waste frying oil Zheng, *et al*., 2006 [70]	methanol	1 : 245	H_2SO_4	3.8 mole%	70°C	4 h	400 rpm	99 ± 1	12	2

Feedstock References	Alcohol	Molar ratio Oil to alcohol	Catalyst	Cat. amount (wt.%)	Reaction condition			BD yield (%)	Acid value	
					Temp	time	Stirring speed		Initial	Final
Soybean Oil hydrochloric, formic, acetic, and nitric acids were evaluated Goff, *et al.*, 2004 [71]	methanol	1 : 9	H_2SO_4	0.5	100°C	8 h	-	> 99	-	-
Corn oil dimethyl ether (DME) as co-solvent; shaking speed of 2.6 Hz Guan, *et al.*, 2009 [72]	methanol	1 : 6	p-toluene sulfonic acid (PTSA)	4.0	80°C	2 h	-	97.1	7.5	-
Crude karanj oil water content: 10.2% Khayoon, *et al.*, 2012 [73]	methanol	1 : 6 1 : 6	H_2SO_4 H_3PO_4	2.0 2.0	65°C 65°C	5 h 5 h	600 rpm 600 rpm	89.8 20	15.62 15.62	0.09 0.09

Table A.3: Two steps homogeneous catalyzed transesterification

Feedstock References	**Alcohol**	**molar ratio oil : alcohol**	**Catalyst**	**amount (wt.%)**	**Reaction condition**			**AV (mg KOH/g)**	**Y (%)**
					Temp.	**time**	**stir speed**		
Polanga (*Calophyllum inophyllum* L.) oil	methanol	1 : 6	H_2SO_4	0.65 v.%	65°C	4 h	450 rpm	18	
Sahoo, *et al*., 2007	methanol	1 : 9	KOH	1.5	65°C	4 h	450 rpm	< 4	85
***Zanthoxylum bungeanum* seed oil**	methanol	1 : 24	H_2SO_4	2.0	60°C	80 min	600 rpm	45.51	
Zhang and Jiang, 2008 [68]	methanol	6.5:1	KOH	0.9	60°C	90 min	600 rpm	1.16	98
Rice bran oil	methanol	1 : 5	H_2SO_4	1.0 – 5.0	60°C	2 h	300 rpm	> 40	55 - 90
Zullaikah, *et al*., 2005 [75]	methanol	1 : 5	H_2SO_4	2.0	60°C	2 h	300 rpm	1.6	> 98
Rubber seed oil	methanol	1 : 6	H_2SO_4	0.5	45°C	30 min	-	34	-
Ramadhas, *et al*., 2005 [52]	methanol	1 : 9	NaOH	0.5	45°C	30 min	-	0.118	> 99
Tobacco (*Nicotiana tabacum* L.)	methanol	1 : 18	H_2SO_4	2.0	60°C	25 min	400 rpm	70	
seed oil Veljkovic, 2006 [76]	methanol	1 : 6	KOH	1.0	60°C	30 min	400 rpm	< 4	91

Feedstock	Alcohol	molar ratio	Catalyst	amount	Reaction condition			AV (mg	Y
References		oil : alcohol		(wt.%)	Temp.	time	stir speed	KOH/g)	(%)
Chicken tallow	methanol	1 : 30	H_2SO_4	1.25	50°C	24 h	130 rpm		99.01
Bhatti, *et al*., 2008 [157]	methanol	3.58:1 (g·mL^{-1})	KOH	1.5	30°C	1 h			88.14
Mutton tallow	methanol	1 : 30	H_2SO_4	2.5	60°C	24 h	130 rpm		93.21
Bhatti, *et al*., 2008 [157]	methanol	3.58:1 (g·mL^{-1})	KOH	1.5	30°C	1 h			78.33
Castor seed oil	methanol	1 : 40	H_2SO_4	1.0	60°C	1 h	300 rpm	3.3	
Hincapié, *et al*., 2011 [33]	methanol	1 : 20	KOH	1.0	60°C	1 h	300 rpm	< 1.0	98
Used cooking oils	methanol	7.14:1 (g·mL^{-1})	NaOH	0.42	25°C	30 min		6.5	73
Çaylı and Küsefoğlu, 2008 [77]	methanol	17:1 (g·mL^{-1})	NaOH	0.18	25°C	30 min		-	94
Karanja (*Pongamia glabra*) oil	methanol	1 : 6	H_2SO_4	1.0	60°C	45 min	-	28	
Patil and Deng, 2009 [78]	methanol	1 : 9	KOH	0.5	60°C	30 min		2	80
***Jatropha curcas* oil**	methanol	1 : 6	H_2SO_4	0.5	60°C	2 h	-	36	
Patil and Deng, 2009 [78]	methanol	1 : 9	KOH	2.0	60^{0}C	2 h		2	90–95
***Jatropha curcas* L. seed oil**	methanol	0.60 (w/w)	H_2SO_4	1.0	50^{0}C	1 h	400 rpm	30	

Feedstock	Alcohol	molar ratio	Catalyst	amount	Reaction condition			AV (mg	Y
References		oil : alcohol		(wt.%)	Temp.	time	stir speed	KOH/g)	(%)
Berchmans and Hirata, 2008 [48]	methanol	0.24 w/w	NaOH	1.4	65°C	2 h	400 rpm	2	90
Mahua (*Madhuca indica*) oil	methanol	0.35 v/v	H_2SO_4	1.0% v/v	60°C	1 h	-	38	
Ghadge and Raheman, 2005 [79]	methanol	0.25 v/v	KOH	0.7% w/v	60°C	1 h	-	4.84	98
Rubber (*Heava brasiliensis*) seed oil	methanol	0.75 v/v	H_2SO_4	1.0% v/v	63(±2)	1 h	-	48	
Satyanarayana and Muraleedharan, 2011 [156]	methanol	0.30 v/v	KOH	0.5 w/v	55(±2)	1 h	-	1.72	98-99
Kusum (*Schleichera triguga*) oil	methanol	1 : 10	H_2SO_4	1.0% v/v	50± 0.5	1 h	-	21.30	
Sharma and Bhaskar Singh, 2010 [81]	methanol	1 : 8	KOH	0.7% w/w	50± 0.5	1 h	-	0.94	95
***Ceiba pentandra* oil**	methanol	1 : 8	H_2SO_4	1.834	65°C	140 m	600 rpm	28.71	
Sivakumar, *et al*., 2013 [158]	methanol	1 : 9	KOH	1.0	65°C	45 min	600 rpm	1.68	99.5

Feedstock	**Alcohol**	**molar ratio**	**Catalyst**	**amount**	**Reaction condition**			**AV (mg**	**Y**
References		**oil : alcohol**		**(wt.%)**	**Temp.**	**time**	**stir speed**	**KOH/g)**	**(%)**
***Jatropha curcas* L. oil**	methanol	12% w/w	H_2SO_4	1.0	70°C	2 h		14	
Lu, *et al*., 2009 [82]	methanol	1 ; 6	KOH	1.3	64°C	20 min		1	98
Waste frying sunflower oil	methanol	1 : 6	KOH	1.0	30°C	30 min	400 rpm	-	
Predojevic, 2008 [159]	methanol	1 : 6	KOH	1.0	60°C	30 min	400 rpm	-	88.92-92.45
Karanja (*Pongamia pinnata*) oil	methanol	1 : 6	H_2SO_4	0.5	65°C	1 h	-	41.9	
Naik, *et al*. [160]	methanol	1 : 6	KOH	1.0	65°C	1 h	-	3.9	96.6–97
***Euphorbia lathyris* L**	methanol	1:10	H_2SO_4	0.8	60°C	45 min	600 rpm	25.4	
OC: 43.31 wt.%; convr: 97.9%	methanol	1:6	KOH	0.8	60°C	30 min	600 rpm	1.29	86.2
***Sapium sebiferum* L.**	methanol	1:8	H_2SO_4	0.4	60°C	30 min	600 rpm	4.83	
OC: 24.93 wt.%; convr: 97.9%	methanol	1:6	KOH	1.0	60°C	30 min	600 rpm	0.68	88.3
***Jatropha curcas* L.**	methanol	1:8	H_2SO_4	0.4	60°C	30 min	600 rpm	9.37	
OC: 38.09 wt.%; convr: 98.3%	methanol	1:6	KOH	1.0	60°C	30 min	600 rpm	0.72	86.2
Wang, *et al*., 2011 [161]									