In Situ Transesterification of Jatropha Curcas for Biodiesel Production

By

FARIZUL HAFIZ KASIM

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ABSTRACT

Biodiesel is primarily produced by transesterification of edible oils. Increasing concern about using food supplies for fuel has generated interest in alternative raw materials. Furthermore, there are numerous steps between harvesting of oilseeds and final production of biodiesel that can be integrated, thereby simplifying the process and making it more suitable for distributed production. Hence, in this study, the production of biodiesel via *in situ* transesterification of non-edible *Jatropha curcas* seed has been investigated. The main aim was to investigate the parameters of the process, with a view to reducing the substantial excess of methanol required. A significant secondary aim was to investigate the possibility of utilising other compounds that come out from the process. "Design of experiments" was employed to study the parameters at lab-scale, with the matrix boundary being determined beforehand using one-at-a-time experiments. The reduction of methanol excess was attempted by use of two co-solvents, hexane and diethylmethane (DEM), and by replacing methanol with methyl acetate. It was found that *in situ* transesterification run using particle sizes below 0.71 mm, a 400:1 molar ratio of methanol to oil, 60 minutes, and a minimum of 300 rpm mixing intensity yielded the highest biodiesel yield of 83 wt %. NaOH concentration and reaction temperature were not found to be significant variables, and were set at 1.0 N and 30^{o}C respectively. DEM was a more effective co-solvent than hexane. The addition of DEM to the process at 400:1 molar ratio experiment increased the yield from 83 to 92 wt %. When methyl acetate was used to replace methanol, the requirement of molar ratio of solvent:oil reduced significantly to 175:1 to achieved 86.8 wt% of biodiesel. The solid meal was shown to contain substantial amounts of protein, making it a valuable co-product stream. Previously *J. curcas* meal had had little value as animal feed due to its toxicity, but this may be reduced or removed by this

process.

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NOMENCLATURE

Re	- Reynolds number
W_{oc}	- Oil content, %
W_m	- Moisture and volatile content, %
W_{AV}	- Acid value content, mg/g KOH
W_{FFA}	- Acidity, %
C	- Ester content, %
M_{ME}	- Mass of methyl ester after Soxhlet extraction, g
M_R	- Mass of methyl ester after re-extract, g
M_T	- Mass of triglyceride in the sample, mg
M_g	- Mass of glycerol, mg
M_T	- Mass of extraction, g
M_O	- Mass of other compounds, g
M_C	- Mass of catalyst, g
M_{AA}	- Mass of acetic acid added for neutralisation, g
M_S	- Mass of soap, g
M_P	- Mass of phenol, g
D_{AB}	- Diffusion coefficient, m^2/s
T	- Temperature, K
μ	- Viscosity, Pa.s
j	- Molar flux relative to mass average velocity, kg mol/s.m^2
ΔC	- Concentration gradient, kg mol/ m^3
ΔX	- Length, m
k_c	- Mass transfer coefficient, kg mol/s.m^2
ν	- Kinematic viscosity, centistokes
U	- Liquid velocity, m/s
d_p	- Particle diameter, mm
Sh	- Sherwood number
Sc	- Schmidt number

ABBREVIATIONS

ACMA	- Advanced Chemical and Materials Analysis
ANOVA	- Analysis of variance
BS	- British Standard
CCD	- Central Composite Design
CFPP	- Cold filter plugging point
CHN	- Carbon-hidrogen-nitrogen analyser
CN	- Cetane number
DAMG	- Diacetinmonoglyceride
DDGS	- Distiller's dried grains with soluble
DEM	- Diethoxymethane
DoE	- Design of Experiments
FAME	- Fatty acid methyl ester
FFA	- Free fatty acid
FAO	- Food and Agriculture Organisation
FID	- Flame ionisation detector
GC	- Gas chromatography
GC-MS	- Gas chromatography-mass spectroscopy
HPLC	- High-performance liquid chromatography
IIP	- Indian Institute of Petroleum
IS 1	- Internal standard 1 for GC-MS
IS 2	- Internal standard 2 for GC-MS
IV	- Iodine value
MADG	- Monoacetinediglyceride
MBDOE	- Million Barrels per Day Oil Equivalent
MBM	- Meat/bone meal
MSTFA	- N-methyl-N-trimethylsilyfluoraacetamide
MW	- Molecular weight
PEG	- Polyethylene glycol
PIG	- Process Intensification Group
RSM	- Response surface methodology
SEM	- Scanning electron microscopy

UV-VIS - Ultra violet-visible spectrophotometer

1 INTRODUCTION

1.1 Research Background and Problem Statement

The increasing world population and rapid economic growth are driving up global energy demand. Today, the world is very much dependent on petroleum as a main transport fuel resource. In its report "Tomorrow's Energy" [1], ExxonMobil predicted that global energy demand will increase from 230 Million Barrels per Day Oil Equivalent (MBDOE) in 2005 to 334 MBDOE by 2030. The greatest proportions of the fuel supplied will be required for transportation and in industry [2]. As the reserves of crude oil are finite and the demand for petroleum is ever-increasing, the need to find alternative energy sources is becoming increasingly important.

Since 1900, when Dr. Rudolf Diesel demonstrated his diesel engine with peanut oil in a Paris exhibition [3], the possibility of using vegetable oil as a fuel has been investigated by many researchers. Nevertheless, because of its high price and the compatibility issues with the diesel engine, interest in vegetable oil-based diesel never fully developed. Geopolitical tensions in the Middle East and the price volatility of crude oil have, however, recently revived interest in vegetable oil-based diesel. Research on biodiesel prior to 1990 mostly centred on the use of raw vegetable oils in their pure form or with partial blend [4-7]. But, despite success in engine performance tests of less than 10 hours duration, problems occurred after longer periods of operation, such as clogging of engine parts [4].

The major problems with using raw vegetable oil, as listed by Pryde [6], were: i) coking on the injectors to such an extent that fuel atomization does not occur properly, or is completely prevented by the plugging of orifices; ii) carbon deposits; iii) oil ring sticking; and iv) the thickening and gelling of lubricating oil as a result of contamination with vegetable oil. These

problems occur due to the higher viscosity and lower volatility of vegetable oil, and the reactivity of unsaturated hydrocarbon chains. These problems were addressed by Peterson *et al*. [8], who used winter rape oil in a diesel engine. It was noted that the polyunsaturated fatty acid in the oil polymerized and form a layer of gum in the engine chamber, resulting in carbon deposits and piston sticking. Darcey *et al*. [5] also reported that the use of blended crude sunflower oil in diesel engines resulted in contamination by solids in the lubricating oil.

Other early researchers, such as Goering and Fry [9] and Ziejewski *et al*. [10] effectively reduced the viscosity of vegetable oil using a process called microemulsion. Furthermore, the hybrid oil obtained reduced engine wear [9]. However, greater deposits of carbon on the injector tips, intake valve and the tops of cylinder liners were observed [9, 10]. In addition, incomplete combustion and an increase in lubricating oil viscosity were reported [10], and consequently this process has never been commercialised

Thermal cracking [11, 12] and transesterification [13, 14] have also been reported. The composition of fuels produced by thermal cracking was similar to that of diesel [11, 12], but the equipment used was too expensive for modest throughputs, as the process was very energy-intensive [15].

Conversely, some researchers believed that they had found a suitable process in transesterification. In transesterification, oil is chemically converted to biodiesel and glycerol by reacting it with alcohol and catalyst. Not only was the quality of biodiesel produced using this method comparable to that of petroleum diesel, but the process could also be operated at low temperatures and pressures. In addition, the oil was observed to perform well in engine tests [14]. Researchers however started to use higher quality of raw materials, such as refined

vegetable oils when it was discovered later that ester yields were reduced by the presence of gums and extraneous material in crude vegetable oil [13].

The 1990s witnessed considerable research focussing on transesterification, with the effects of various parameters and new raw materials being reported throughout the decade. The research generally focussed on utilisation of vegetable oil as a raw material. In most cases, edible vegetable oil was used. However, a major problem arose when the market price of these vegetable oils increased. With higher prices of raw materials, the production costs of biodiesel also increased, causing it to become unprofitable. It has been suggested in the literature that transesterification would only be profitable if the price of vegetable oils was below 400 US$ per metric ton [16]. To overcome this problem, alternative, less expensive materials were investigated, including many types of inedible oil. Apart from using non-edible and low cost materials, the possibility of introducing new process route now become a main focus of current research [17, 18].

In 1985 Harrington and D'arcy-Evans [19] introduced an *in situ* transesterification process [19, 20]. Although like transesterification, in which vegetable oil is chemically converted to biodiesel and glycerol, *in situ* transesterification converts the oil within the seed directly to biodiesel rather than from the extracted oil. In this process, the seeds are reacted directly with the alcohol (containing the catalyst), producing biodiesel and glycerol. This removed various process stages, and could make biodiesel production more profitable. Work on *in situ* transesterification was reported, particularly by Harrington and D'Arcy-Evans [19, 20] on sunflower seed oil. Among their noteworthy conclusions was the claim that the process results in fatty acid esters qualitatively similar to, but quantitatively greater than, yields obtained from the treatment of pre-extracted oil [19, 20].

As shows in Figure 1.1, *in situ* transesterification did not receive a great deal of attention when it was first introduced in 1984. It took almost 20 years before researchers started to show interest towards the process. Haas *et al*. in particular published numerous papers on the subject [21-23]. Meanwhile, the Process Intensification Group (PIG) in Newcastle University began looking at the process circa 2005, and published its first peer-reviewed article 5 years later, in 2010.

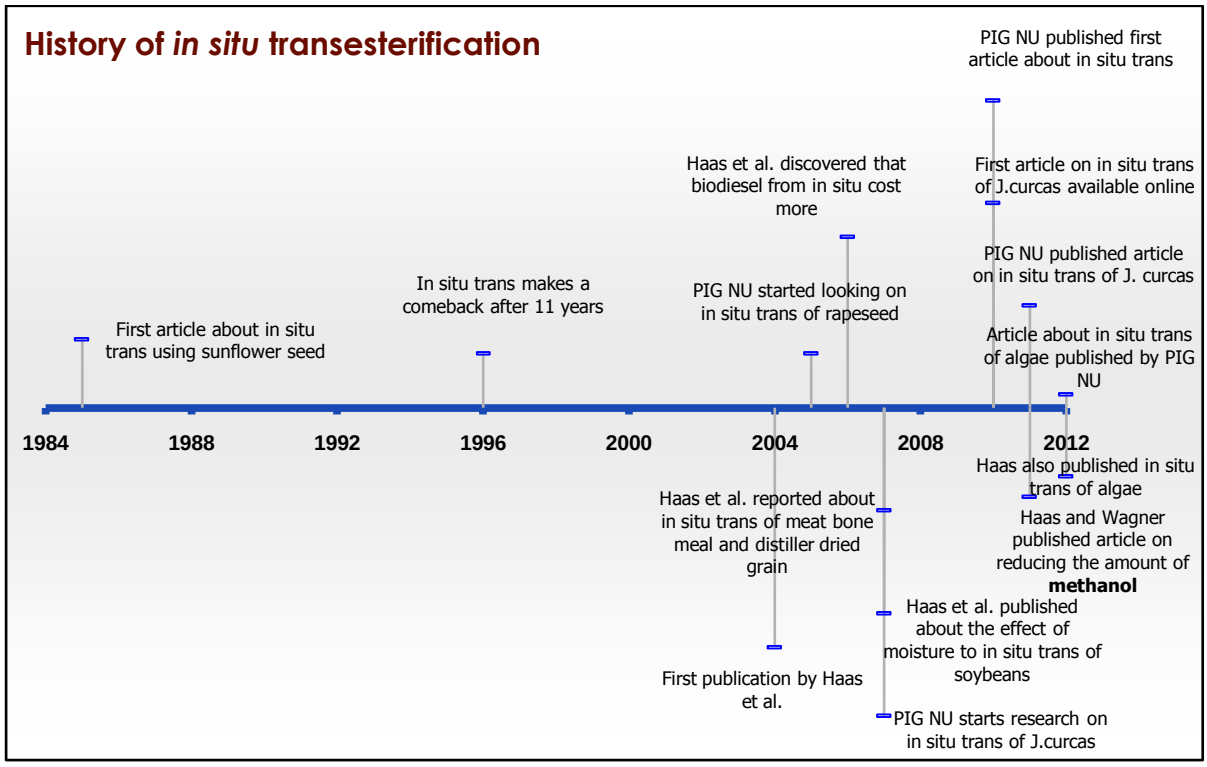


Figure 1.1 Brief history of *in situ* transesterification

In this study attempts were made to produce biodiesel based on *in situ* transesterification. This will avoid the use of solvents, such as hexane. Although hexane is considered to be an environmental friendly solvent [24] because of its high volatility and low solubility in water, prolong and intense exposure in workplace can cause several effect to the workers including toxicity to nerves and muscle weakness which leads to paralysis [25]. In order to avoid the problem of instability in the price of edible seeds, non-edible seeds are used as the raw material in this project.

1.2 Problem Statement

Although it has been proved that *in situ* transesterification is a feasible method to produce biodiesel, considerable effort is still required to fully understand the fundamental processes within the method. Furthermore, it is clear that the process parameters are raw material dependant, which implies that optimal process condition must be established independently for every new raw material.

Unlike conventional transesterification, where the kinetic modelling and reaction mechanism are well-researched, there are no publications concerning those topics in *in situ* transesterification,

The major issue with *in situ* transesterification is the huge amount of alcohol needed to get a desirable yield of product. To 6:1 ratio of alcohol-oil in conventional transesterification, *in situ* transesterification needs about 300/400:1. There is also a gap on the effort on reducing the amount of reagent used in *in situ* transesterification process. To date, only Haas and Wagner, in 2011, published on the issue [26]. They showed that a 20-fold reduction of methanol ratio usage, from previous ratio of 181, was achieved by combining flaking, extrusion and drying regime as a pre-treatment to the seed. Whilst the article discussed various mechanical treatments to seeds to address the problem, there are no reports concerning changing the process route of the process, which might be an alternatives to reduce the methanol requirement.

1.3 Aims and Objectives of the Research

The present study attempts to produce biodiesel based on *in situ* transesterification using non edible seeds as the raw material. To achieve this aim, every parameter involved in the process was investigated.

It is an objective of the study to develop a process that is feasible and practical for the biodiesel market. Thus, the research also aims to minimize the huge volumes of alcohol previously used in the process, which is a significant barrier hindering the commercial application of this technology. At the same time, the work also includes a study on waste streams, to examine the potential of these wastes to become valuable co-products. Economic evaluation on the process was performed at the end of the study.

Inedible *Jatropha curcas (J.curcas)* seeds, Figure 1.2, from India were used as the raw material in this research, since this species has been suggested in the literature to be a potential future source of biodiesel. No new catalysts were investigated in the study, as the catalysts which were discussed in previous work were found to be effective for the process. Therefore, the study uses existing commercially available transesterification catalysts.

Figure 1.2 *J. curcas* seeds, the raw material for the study

2 LITERATURE REVIEW

This review starts with a brief summary of the nature of biodiesel, followed by an explanation of the transesterification process and then *in situ* transesterification. Previous studies of *in situ* transesterification are discussed according to the variables identified as affecting the process. The drawbacks of *in situ* transesterification are then clarified and current work on overcoming the problems is described. In the final part, a critical assessment is presented of the selection on *J. curcas* as the raw material used in this study.

2.1 Biodiesel

Biodiesel is an alternative fuel made from vegetable oils and animal fats. Chemically, it consists of mono alkyl esters of the long chain fatty acids present in the triglycerides of vegetable oils or animal fats. Since the feedstock is plant- or animal-derived, biodiesel is a renewable fuel. It contains very small quantities of sulphur, polycyclic aromatic hydrocarbons or metals, whereas, petroleum diesel, for example, can contain up to 20% polycyclic aromatic hydrocarbons [27].

Biodiesel has similar properties to those of petroleum diesel. Its flashpoint is higher than diesel oil and so it is safer to handle. Biodiesel also has a higher cetane number and diesel index. Biodiesel's lower sulphur content and ash content make it more environmentally friendly than any fossil fuels [28].

Most biodiesel today is produced via a process based on the transesterification reaction: a basic scheme is shown in Figure 2.1. Refined, bleached vegetable oil is usually used as a raw

material. In the transesterification this oil reacts with methanol and base catalysts, such as potassium hydroxide or sodium hydroxide to form biodiesel and glycerol. The layers of crude biodiesel and crude glycerol are subsequently separated and refined to yield biodiesel and glycerol. The methanol is recovered and can be recycled into the process.

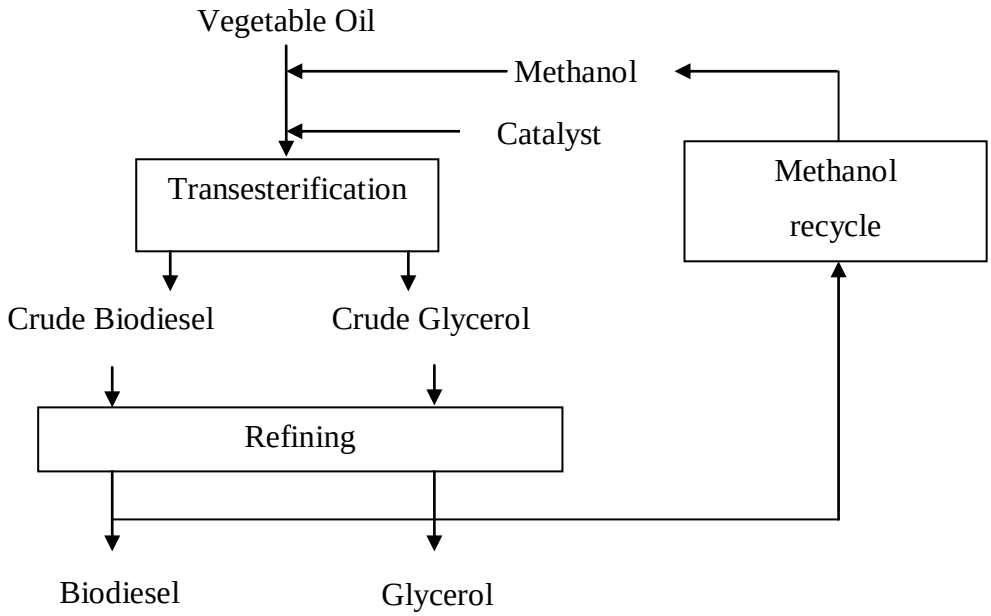


Figure 2.1 Basic scheme for biodiesel production via transesterification process with alkali catalyst

2.2 The Transesterification Reaction

Transesterification is a reaction between a triglyceride and an alkyl alcohol, producing alkyl esters (biodiesel) and glycerol. Figure 2.2 below depicts the transesterification reaction.

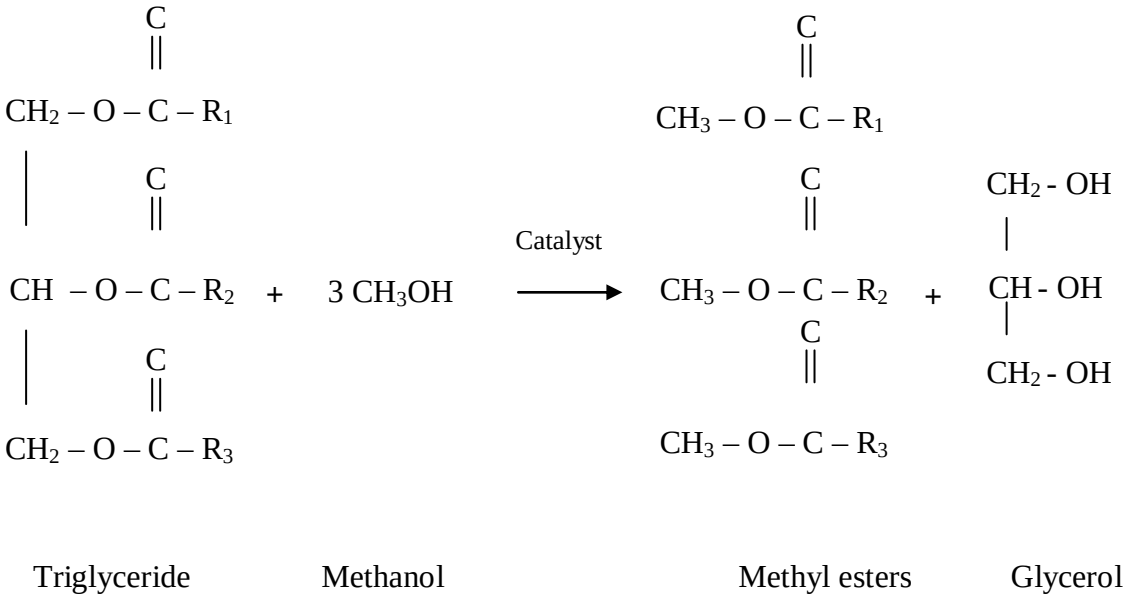


Figure 2.2 Transesterification reaction. One mole of triglyceride reacting with three moles of methanol produces three moles of methyl esters and one mole of glycerol.

R_1, R_2 and R_3 in the equation above are long fatty acid chains. Listed in Table 2.1 below are the typical fatty acid chains (R) found in oilseed and animal fats [29].

Table 2.1 Common fatty acid chains in soybean oil and animal fats [29]

Fatty acid chain	Name	Description
- $(CH_2)_{14} – CH_3$	Palmitic	16 carbons, (including one in the triglyceride backbone), 0 double bond (16:0)
- $(CH_2)_{16} – CH_3$	Stearic	18 carbons, 0 double bond (18:0)
- $(CH_2)_7 CH = CH(CH_2)_7CH_3$	Oleic	18 carbons, 1 double bond (18:1)
- $(CH_2)_7 CH = CH\text{-}CH_2\text{-}CH = CH(CH_2)_4CH_3$	Linoleic	18 carbons, 2 double bond (18:2)
- $(CH_2)_7 CH = CH\text{-}CH_2\text{-}CH = CH\text{-}CH_2\text{-}CH=CH\text{-}CH_2\text{-}CH_3$	Linolenic	18 carbons, 3 double bond (18:3)

Although an excess of methanol is typically used, the transesterification process can take place with only three moles methanol per mole of triglycerides. An excess is used to increase

the conversion, typically to over 95% completion. Either acid or alkali catalysts can be used to accelerate the process.

2.2.1 Competing Reactions

The presence of free fatty acids and water in the oil can trigger side reactions which affect the yield of the main product.

2.2.1.1 Free Fatty Acids

Free fatty acids are carboxylic acids. They are formed here when carbon chains become disconnected from the glycerol backbone. Figure 2.3 below shows the structure of oleic acid, which is a common free fatty acid in vegetable oils.

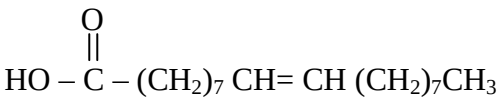


Figure 2.3 Example of free fatty acid (oleic acid) contains 18 carbon, 34 hydrogen and 2 oxygen atoms.

Free fatty acids react with alkali catalysts such as potassium or sodium hydroxide and produce soap (Figure 2.4, below) via the saponification reaction. This consumes the catalyst and prevents it from being used to catalyse the main reaction.

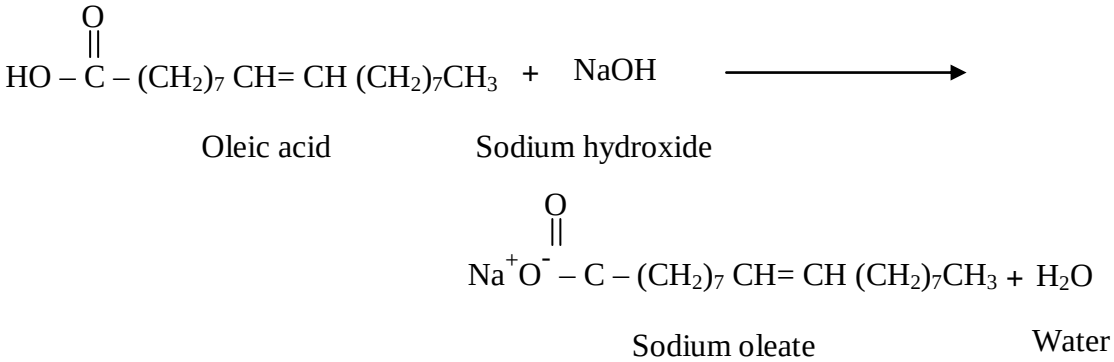


Figure 2.4 Side reaction in the transesterification process. Free fatty acid reacts with alkali catalyst producing soap and water

2.2.1.2 Water

Water in oil reacts with triglyceride and hydrolyses it to form free fatty acids. With the presence of free fatty acids, saponification occurs and soap is produced together with the biodiesel.

The saturated fatty acid soaps solidify at room temperatures and therefore the reaction mixture forms a semi-solid mass which is difficult to recover [29]. Figure 2.5 illustrates the reaction between triglyceride and water, producing diglyceride and fatty acid.

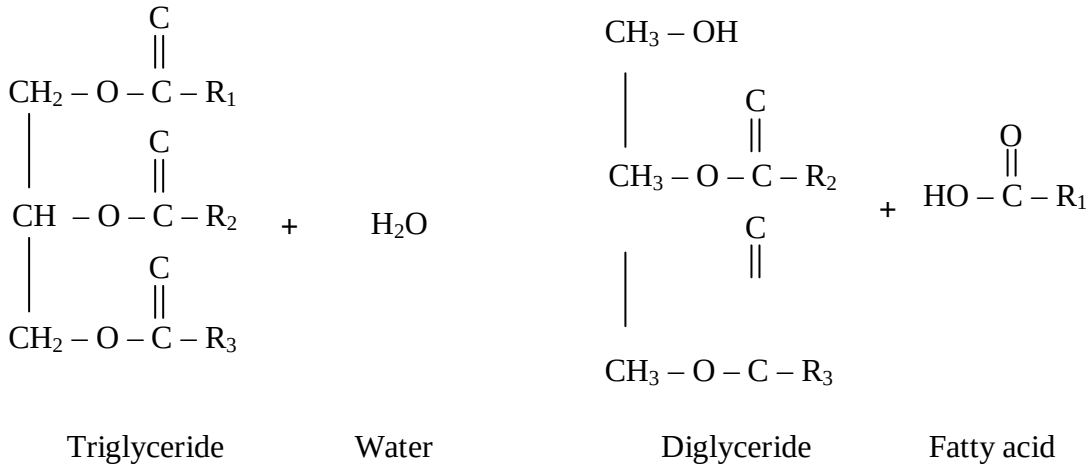


Figure 2.5 Side reaction of the transesterification process. Triglyceride reacts with water producing diglyceride and fatty acid [29].

2.3 *In situ* Transesterification

2.3.1 Definition

In situ transesterification is the direct transesterification of oil-bearing materials. The seed fragments are reacted with alcohol and a catalyst, producing alkyl fatty acid esters. This contrasts with conventional biodiesel transesterification, which uses raw materials of pre-extracted oil from oil-bearing seeds.

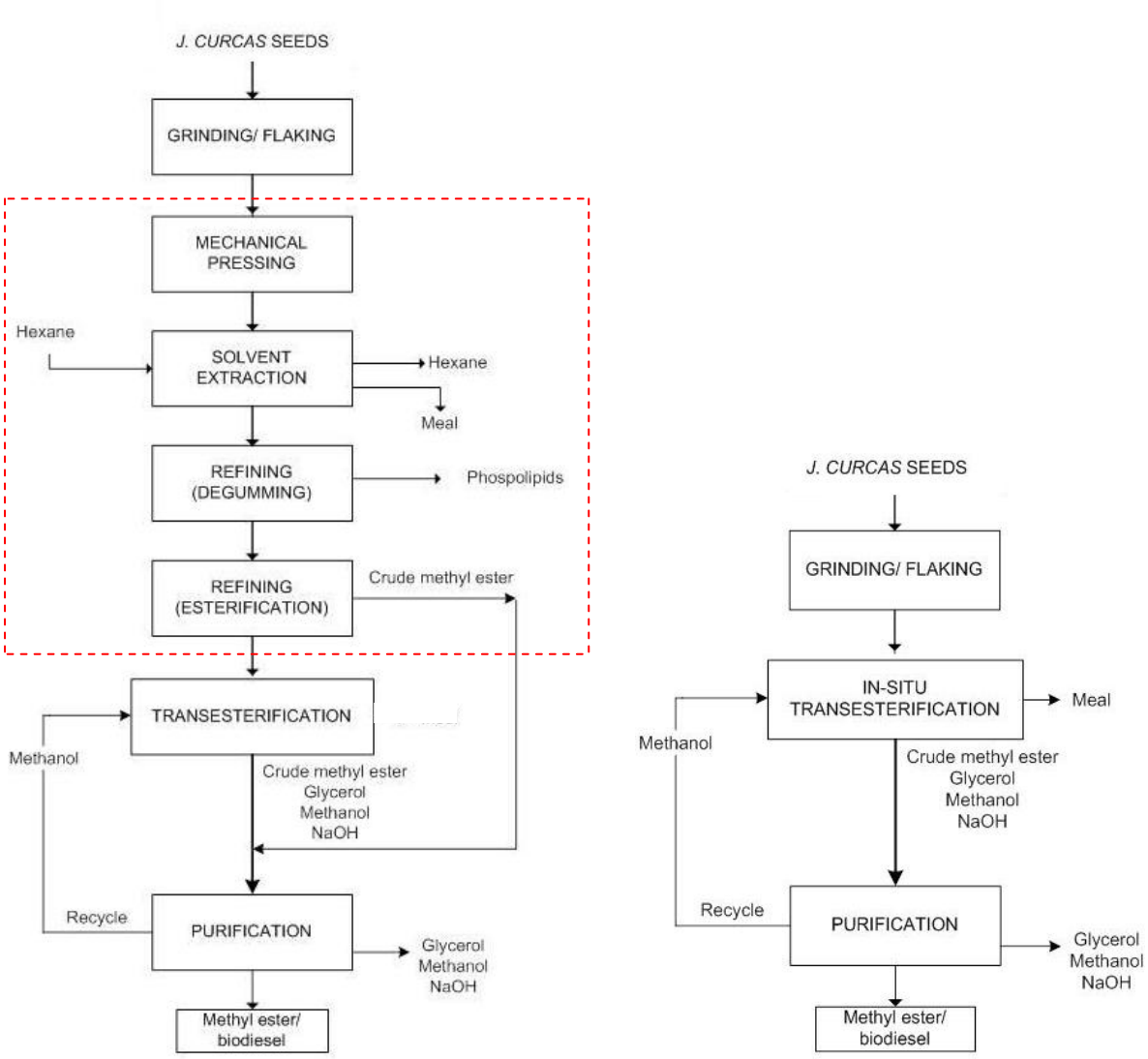


Figure 2.6 Conventional biodiesel process versus *in situ* transesterification process. In this case, high free fatty acid content material (*J.curcas*) was used as the raw material.

Figure 2.6 summarizes the two processes. Both involve a grinding stage, where the seeds are reduced in size. In the conventional process, after the grinding, the seed has to undergo several processes: mechanical pressing, solvent extraction, degumming and esterification. These four processes are not required in *in situ* transesterification. This is an advantage of *in situ* transesterification, as fewer unit operations are required, hence capital cost would be reduced. The next stage is the reaction. In conventional processes, the outputs from this process are crude methyl ester, glycerol, methanol and sodium hydroxide whereas for *in situ* transesterification, there is also the meal, the residual solid material from the seeds. The presence of the solid in *in situ* transesterification makes the purification step different from that in conventional processes as a filtering process to separate the meal from other products is required. The purification step of both processes is similar, involving methanol recycling process, water washing and separation of methyl ester from the other products by gravitational settlement.

2.3.2 Variables in *In situ* Transesterification

2.3.2.1 Raw Materials

Various traditional oil-bearing seeds such as rapeseed and sunflower seed, or even materials such as distiller's dried grains with solubles (DDGS) and meat/bone meal (MBM) have been studied by researchers [23] for use as feedstocks in *in situ* transesterification. The fatty acid profiles of the oils produced from these materials vary substantially and, consequently, the parameters of the *in situ* transesterification process differ. However, even though fatty acid profiles are known to influence biodiesel properties, such as cetane number and cold filter plugging point, no research has reported this with respect to *in situ* transesterification [30]. The *in situ* approach, as shown in Table 2.2, can be applied to almost any lipid-bearing material ranging from oil-bearing fruits such as sunflower seed and soybean, non-fruit

materials like meat and bone meal and palm oil pulp, to the unusual sources, such as waste water sludge. The table also shows that *in situ* transesterification can be performed on materials with oil contents as high as 51%, and as low as 2%. Interestingly, from the materials listed in the table, only two have the majority of their oil in saturated form: they are palm oil pulp and sewage sludge, with 44.3% and 42% of palmitic acid (16:0) respectively.

To investigate whether or not *in situ* transesterification is applicable to all lipid-bearing materials, Haas *et. al.*, performed experiments on distillers dried grains with soluble (DDGS) and meat and bone meal (MBM). Both types of raw material contain low percentages of oil, but the oil fractions of DDGS and MBM were successfully converted to methyl ester at rates of 91% and 93% respectively [23]. Using acid catalysis, Dufreche *et. al*., noted that the *in situ* transesterification of sewage sludge achieved 6.23 % (wt/wt) conversion, compared to 0.38 wt% when hexane extraction and acid transesterification was used. Even the 3.44 wt% conversion achieved when a mixture of hexane, methanol and acetone was used to extract the oil was less effective than *in situ* transesterification. Clearly, this significant difference might render use of low oil content feedstocks for biodiesel product economically viable.

Table 2.2 Raw materials and their composition used by researchers in *in situ* transesterification

Raw material	Oil (%)	Fatty Acid Methyl Ester (FAME) No of carbon atom: no of double bond (%)	References
Sunflower seed	38	16:0(6.8), 18:0(5.0), 18:1(19.6), 18:2(68.6)	[19]
Soybean	23	16:0(12.0), 18:0(5.0), 18:1(25.0), 18:2(52.0), 18:3(6.0)	[31]
Distiller dried grains with soluble (DDGS)	8.8	16:0(12.9), 18:0(1.6), 18:1(28.5), 18:2(55.5), 18:3(1.4)	[22]
Meat and bone meal (MBM)	9.1	16:0(25.2), 18:0(19.7), 18:1(35.6), 18:2(1.9), 18:3(0.3)	[23]
Palm oil pulp	80	12:0(0.3), 14:0(0.8), 16:0(44.3), 18:0(5), 16:1(0.2), 18:1(39.1), 18:2(10.1), uk(0.2)	[31]
Cottonseed	31.6	16:0(28.7), 18:0(0.9), 18:1(13.0), 18:2(57.4)	[32]
Rapeseed	42	16:0(4.0), 18:0(1.9), 18:1(62.1), 18:2(32.0)	[33]
Jatropha curcas	54	16:0(16.0), 18:0(7.0), 18:1(45.0), 18:2(32.0)	[34]
Wastewater sludge (primary sludge)	2	16:0(42.0), 18:0(14.0), 18:1(28.0), 18:2(10.0), 20:1(6.0)	[35], [36]
Microalga (*S. limacinum*)	51[b]	14:0(2.06), 16:0(35.5), 18:0(0.81), 22:5(8.58), 22:6(53.05)	[37]
Microbial biomass (*L.starkeyi*)	50[b]	14:0(0.4), 16:0(33.0), 17:0(0.4), 18:0(4.7), 16:1(4.8), 18:1(55.1), 18:2(1.6)	[38]
Microbial biomass (*R. toruloides*)	58[b]	14:0(0.7), 16:0(24.3), 17:0(0.6), 18:0(7.7), 16:1(1.1), 18:1(54.6), 18:2(2.1), uk(8.9)	[38]
Microbial biomass (*M.isabellina*)	53[b]	14:0(1.2), 16:0(28.2), 18:0(1.0), 16:1(5.8), 18:1(55.5), 18:2(5.8), 18:3(2.4), uk(0.1)	[38]

[a]uk:unknown

[b]total lipid extraction

2.3.2.2 Catalyst

It is well-documented that *in situ* transesterification is unable to proceed without a catalyst [31, 39]. Acid or alkali catalysts help to break down the cell wall of the oilseeds, thereby allowing methanol to access the oil in the cotyledon cells. Ren *et. al.*, investigated the *in situ* transesterification of canola using scanning electron microscopy (SEM) and light microscope [40]. Lipid staining showed that this reactive extraction followed a shrinking core model, where the area of cells containing lipid could clearly be seen to shrink as the extraction progressed.

Harrington and D'Arcy-Evans found that the total mass yield of extraction by *in situ* transesterification (40.9%) was greater than that obtained by conventional transesterification (30.3%) [19]. It was claimed that this was due to the capability of *in situ* transesterification to extract materials that were not extracted from the seed by hexane [19], such as phospholipid. As a non-polar solvent, hexane can only extract non-polar substances such as triglycerides. When acidified/alkaline methanol was used instead of hexane, both polar and non-polar substances, like phospholipid and triglyceride, respectively, were extracted from the seed. Dufreche *et. al.*, also claimed that a higher percentage of material was extracted from sewage sludge when using methanol (19.39%) rather than hexane (1.94%) [35]. The sharp increase attributed to methanol extraction in this case was probably due to the presence of large amounts of phospholipids in the form of microorganism cell membranes in the sewage.

Acids, and in particular sulphuric acid, were the preferred catalysts in the early research into *in situ* transesterification for biodiesel production, pioneered by Harrington and D'Arcy-Evans [19, 20]. Acid catalysis has often been investigated for the treatment of raw materials

with high levels of free fatty acids (FFA). Alkaline catalysts will react with the FFA to produce soap and glycerol, decreasing the amount of catalyst available, or even consuming it altogether. Furthermore, soap acts to emulsify the product, rendering the separation of alkyl esters from glycerol more difficult. Acid catalysis, in contrast, does not promote saponification. Mondala *et. al.*, for instance, used sulphuric acid as the catalyst for the conversion of their raw material of municipal sewage sludge, which contained 65% by weight of FFA [36]. Ozgul-Yucel *et. al.,* investigated extraction from rice bran and used acid catalysts because the acidity of rice bran oil was unpredictable and usually high [41-43].

Most researchers reported high level of conversion to methyl esters when using acid catalysts. Harrington and D'Arcy-Evans achieved the 98% conversion of sunflower seed oil to FAME using a methanol/sulphuric acid mixture [19, 20]. Siler-Marinkovic and Tomasevic also worked with a sunflower seed/methanol/sulphuric acid system, and observed conversion rate of over 90% with a wide range of experimental conditions [44]. Shuit *et. al.,* reported that 90% of oil was extracted from *J. curcas* seed when using acid-catalysed *in situ* transesterification, and all of it was converted to fatty acid methyl ester (FAME) [34]. Obibuzor *et. al.,* similarly reported a high conversion rate (97%) of oil to FAME from the reactive extraction of oil palm waste pulp using a methanol/sulphuric acid mixture [45]. Acid catalysis also works efficiently in the reactive extraction of oleaginous microbial biomass. Lipid contents from three different types of oleaginous biomass, *L. starkeyi*, *M. isabellina* and *R. toruloides*, were successfully converted to FAME at 97 wt% , 91 wt % and 98 wt % respectively [38]. Liu *et. al.,* investigated the *in situ* transesterification of cellular biomass from yeast and fungi using an acid catalyst and methanol. They found that both sulphuric and hydrochloric acids could produce moderate ester yields of 60% and 53% respectively.

Significantly lower yields (10%) were achieved when phosphoric acid was used, but no explanation for this was offered [38].

Researchers have also observed that reaction times are longer when using acid rather than alkaline catalysts. Shuit *et. al.,* for instance, found that the 90% conversion of *J curcas* using sulphuric acid required 24 hours [34]. Obibuzor *et. al.,* on the other hand obtained the same level of conversion in 12 hours when using reactively extracted palm oil pulp waste with sulphuric acid [45]. These were long compared to reactions using alkaline catalysts, which usually take less than 30 minutes to reach the same level [21, 46].

The first *in situ* transesterification using an alkaline catalyst was reported by Haas *et. al.,* in 2004 [21]. Their experiment was conducted using soybean flakes as raw material, and the highest percentage of methyl ester was produced using 12.5 mL of methanol and 0.18 N of sodium hydroxide. This is equivalent to a molar ratio of 226:1:1.6 methanol/oil/NaOH. Compared with the ratio of 6:1:0.22 in conventional transesterification experiments by Freedman *et. al.,* [47], it is clear that *in situ* transesterification requires substantially more methanol and more catalyst. Haas *et. al.,* observed three main things when comparing the effectiveness of acid and alkali catalysts. Firstly, the flaked, as opposed to pulverized seeds produced a high yield when used in *in situ* transesterification with alkali. All the previous study with acid catalysts used pulverized materials [19, 20, 31]. Secondly, less reagent was required, along with moderate process conditions. Thirdly, higher yields of methyl ester are obtained [21]. The former two advantages are repeatedly found in the literature. For instance, a molar ratio of 553:1 methanol to oil in experiments by Harrington and D'Arcy-Evans using sunflower seeds and sulphuric acid achieved 97% conversion [19], Georgogianni *et. al.,*'s 163:1 molar ratio using sunflower seeds and sodium hydroxide achieved 95-97% conversion

[48]. However, it seems that both types of catalyst produce comparable yields of methyl ester, but not at the same rates. Harrington and D'Arcy Evans' reaction was 4 hours, while Georgogianni *et. al.*, only needed 2 hours to produce the same yield. Furthermore, in the latter study, 94% of the oil had already been converted to methyl ester after 40 min.

The levels of conversion of oil to methyl esters reported in the literature are typically very high when using methanol and sodium hydroxide, for example 97% with both sunflower seeds and cottonseeds [32, 48], 88% with soybean [21], and over 95% with cottonseed [39]. *In situ* transesterification using alkaline catalyst has also been conducted with a number of non-oilseed feedstocks.

Table 2.3 lists the different raw materials, catalyst and solvents used by researchers to produce biodiesel through *in situ* transesterification. All researchers listed used methanol as a solvent. The molar ratio of solvent to oil ranged from 100:1 for sodium hydroxide catalyst to 1400:1 in a process involving sulphuric acid catalyst. Noticeably, sulphuric acid and sodium hydroxide was a preferred acid and alkali catalyst respectively. In general, reaction time for experiments with acid catalysts was longer than the one with alkali catalyst.

Table 2.3 Combinations of raw material, catalyst and alcohol used by reserachers to produce biodiesel through in situ transesterification

Raw material	Solvent	Catalyst (mol/L)	Molar ratio solvent/oil	Reaction time (h)	Temp. (^oC)	Conversion (oil basis) (%)	Ref	Notes
Sunflower	Methanol	H_2SO_4 (0.75)	532:1	5	65	93	[20]	
Sunflower	Methanol	H_2SO_4 (0.7)	300:1	4	64.5	98.2	[44]	
Soybean	Methanol	H_2SO_4 (0.75)	281:1	10	65	23.3	[31]	
Soybean	Methanol	H_2SO_4 (0.75)	150.1	3	121	83	[49]	CO_2 cosolvent, Pressure=7.38 bar
J. curcas	Methanol	H_2SO_4 (0.2)	300:1	24	60	99.8	[34]	Hexane cosolvent
Microbial biomass	Methanol	H_2SO_4 (0.2)	830:1	20	70	96.8 (*L.starkeyi*) 91.0 (*M.Isabellina*) 98.1 (*R.toruloides*)	[38]	
Primary sewage sludge	Methanol	H_2SO_4 (0.9)	1400:1	24	75	66	[36]	
Soybean	Methanol	NaOH (0.09)	543:1	8	23	88	[21]	

Raw material	Solvent	Catalyst (mol/L)	Molar ratio solvent/oil	Reaction time (h)	Temp. (^{o}C)	Conversion (oil basis) (%)	Ref	Notes
DDGS	Methanol	NaOH (0.4)	655:1	1.2	35	91.1	[23]	
MBM	Methanol	NaOH (2.0)	550:1	0.2	35	93.3	[23]	
Cottonseed	Methanol	NaOH (0.4)	673:1	0.3	60	95	[32]	Ultrasound
Cottonseed	Ethanol	NaOH (0.4)	613:1	0.7	80	98	[32]	Ultrasound
Sunflower	Methanol	NaOH (0.4)	476:1	0.7	60	97	[48]	Ultrasonic
Sunflower	Ethanol	NaOH (0.4)	434:1	0.7	80	98	[48]	Ultrasonic
Sunflower	Methanol	NaOH (0.2)	101:1	13	20	98	[50]	DEM cosolvent
J. curcas	Methanol/ ethanol mix	NaOH (0.02)	512:1	1	60	87	[51]	
J. curcas	Methanol	NaOH (0.04)	100:1	1	60	70	[52]	

Various steps were employed to improve *in situ* transesterification processes: For example, co-solvents and ultrasound, have been used, particularly to enhance oil extraction. This can hopefully reduce the molar ratio of solvents used in the process. The table shows that the use of CO_2 and diethoxymethane (DEM) as co-solvents successfully reduced the molar ratio of solvent to oil from 280 to around 150 and from 512 to 100, respectively. The use of CO_2 also reduced the reaction time of the reaction, from usually 24 hours to only 3 hours. However, the CO_2 combination was executed at 121^{o}C, the highest temperature among the combination listed in the table, whereas the process is usually performed slightly over or below methanol boiling point, 65^{o}C.

2.3.2.3 Moisture Content

In conventional transesterification, the presence of water in the process causes soap formation and frothing. This results in increased viscosity, gel formation and difficulty in separating the glycerol and alkyl ester-rich phases [15]. In addition, the saponification process consumes triglyceride, thereby reducing the potential yield of methyl ester.

After reducing the moisture content prior to *in situ* transesterification, Haas *et. al.,* found that the amount of alcohol required for the process significantly lowered. They reported a 60% reduction of methanol and a 56% reduction of sodium hydroxide when soybean flakes were dried in a convection oven until the water content reached zero. Experiments with samples containing 2.6% water reduced the methanol and sodium hydroxide requirements by 40 % and 33 % respectively [53].

In situ transesterification has been shown to require higher alcohol to oil ratios than conventional transesterification. Even though the application of *in situ* transesterification eliminates the need for pre-extracted oil, Haas asserts that the resulting biodiesel is still more expensive than that produced by conventional transesterification [20]. The reduction of water content however, was able to reduce the estimated cost of biodiesel production from $3.14 to $1.02 per gallon. [54]. A similar trend has been reported by Qian *et. al.,* where methyl ester conversion was found to increase significantly from 80% to 98% when the moisture content was reduced from 8.7% to 1.9%. Further reductions in moisture content, however, had very little effect on level of conversion [39].

By contrast, research at Newcastle University [55] on the *in situ* transesterification of rapeseed using methanol and sodium hydroxide has shown that drying the seeds from 6.7% to 0% water content neither reduces the solvent requirements, nor increases the yield of ester significantly. It was found that ester yields were only reduced when there was more than 2% water in the solvent [33]. This indicates that, for rapeseed at least, the drying stage may be unnecessary, which should reduce the cost of biodiesel production by this method.

2.3.2.4 Mixing Intensity

Two studies by Georgogianni *et. al.,* compared the use of a mechanical stirrer at 600 rpm and low frequency ultrasound (24 kHz) as a means of agitation in *in situ* transesterification reactions [32, 48]. When the experiments were conducted using methanol, no significant difference was noticed, and both agitation methods led to high conversion rates of methyl ester after 20 minutes of the reaction. However, when ethanol was used, the application of ultrasound produced high conversion rates more rapidly than mechanical stirring. At 40 minutes, 98% conversion was achieved with ultrasound, whereas mechanical stirring resulted

in lower yields of 88% with both sunflower and cottonseed. It was concluded that ultrasound produced less soap because no stirring was required, although unfortunately no further experiments were conducted to confirm this hypothesis. However, saponifications occurs as a result of the reaction between sodium hydroxide and free fatty acid (FFA) and, as with any reaction, its occurrence will depend to some extent upon the degree of mixing, but is unlikely to be dependent on the form of mixing, so this point is debatable. It may be that, since ethanol is a better solvent for triglycerides than methanol, more of the reaction takes place in the liquid phase, rather than in the seed leading to a sonochemical enhancement for ethanol but not for methanol.

Zeng *et. al.,* studied the *in situ* transesterification of sunflower seeds with diethoxymethane as co-solvent. They found that when using only agitation, the change of speed had no influence on biodiesel yield or FAME purity in the ranges tested (300-600 rpm). This may be thanks to the co-solvent used which extract the oil out from the seeds [50] or that the ranges tested for agitation did not produce a change in the flow region. Because the co-solvent has to be removed from biodiesel, the benefit of using it in the process must be balanced with this disadvantage.

2.3.2.5 Molar Ratio of Alcohol to Oil

All researchers agree that the required molar ratio of alcohol to oil in *in situ* transesterification is extremely high compared to that in the conventional transesterification of vegetable oil. Siler-Marinkovic and Tomasevic [44], for example, used a 300:1 ratio in their experiments with sulphuric acid as catalyst, while Haas *et. al.,* [21] applied a 543:1 ratio for sodium hydroxide. The typical ratio used for conventional transesterification is 6:1 [47]. Calculations performed by Haas' group indicate that the amount of methanol involved in this

process was the main reason for the high production costs of biodiesel [54], mainly because the purification of the biodiesel becomes more complicated.

Researchers are now trying to find ways of reducing the amount of alcohol required. The use of co-solvents in conventional transesterification is known to improve the solubility of alcohol, thereby increasing the rate of reaction [56]. Qian *et. al.,* [39] discussed the feasibility of using petroleum ether as a co-solvent in the process. The amount of oil extracted from seed and dissolved in methanol increased from 95% in one hour without co-solvent, to 98% with a mixture of petroleum ether and methanol. However, petroleum ether/methanol was only effective when it was below a volume ratio of 1:3. Above that, the concentration of oil became too low.

The application of co-solvents in *in situ* transesterification has also been investigated in detail by Zeng *et. al.*,[50]. They demonstrated that using diethoxymethane (DEM) as a co-solvent reduces the amount of methanol required. At a 58:1 molar ratio of DEM/oil, a molar ratio of methanol to oil of only 101:1 was required to produce a 96% yield of crude biodiesel. For comparison, the highest yield achieved by researchers working with sunflower seeds was 97%, but the methanol to oil molar ratio here was 476:1 [48].

The most recently reported attempt to lower the alcohol to oil ratio used CO_2 as a co-solvent [49] at temperatures and pressures at which methanol acts as a less polar solvent. This was expected to increase the rate of triglyceride extraction, and therefore the overall reaction rate. However, the addition of CO_2 only gave positive results when it was used with an acid catalyst (in this case sulphuric acid) rather than an alkali. When sodium hydroxide was used, sodium carbonate was detected in the system, suggesting that the methoxide was converted to

carbonate in the presence of CO_2, thereby reducing the amount of catalyst and, correspondingly the rate of the reaction. The authors claimed that, using sulphuric acid, the total volume of methanol can be lowered by one third without adversely affecting methyl ester yield. Not only was the volume of methanol needed lowered, but the rate of reaction increased by as much as 2.5 fold.

The reason for a large molar excess of alcohol being needed in *in situ* transesterification may be that the speed of diffusion of alcohol into the particles determines the rate of reaction. A high molar ratio would be required to overcome substantial mass transfer resistance in order for the reaction to proceed at an appreciable rate. Further evidence for this is the increase in reaction rates observed with decreasing particle size [31]. The thermodynamic driving force, known as free entropy also may have an effect to the diffusion of solvent into the seed particle.

2.3.2.6 Temperature

Haas *et. al.,* compared the reaction rates of *in situ* transesterification of soybeans using methanol and sodium hydroxide at room temperature, (23^oC) and 60^oC. Both conditions yielded high percentages of methyl ester [21], but, at room temperature more methanol was required. The optimal molar ratio of alcohol to oil was 2.4-times higher than at the higher temperature, whereas in a study at Newcastle University using rapeseed, increasing the temperature from 30 to 60^oC increased the initial rate of ester formation while the time needed to reach equilibrium (60 minutes) was comparable [33].

Noureddini and Zhu have observed that, in conventional transesterification, temperature influences mass transfer as well as conversion [57]. The mass transfer region, which was the

period when mass transfer is the reaction limiting factor, was reduced from 55 to 20 min when temperature was increased from 30 to 60^{o}C. This effect was very obvious when the reaction was conducted at a low mixing intensity of Re =3100 but became insignificant at a high mixing intensity of Re = 6200. This indicates that, at higher mixing intensities, the external mass transfer resistance is removed so that reaction rate is no longer dependent on temperature. Temperature should also not have a strong effect on *in situ* transesterification, since the reaction is believed to be largely mass transfer controlled. The results reported by Haas *et. al.*, [53] support this, where the conversions achieved with DDGS, methanol and sodium hydroxide at three different temperatures of 35, 45 and 55^{o}C were almost the same and the reactions were completed at the same time of 180 min.

Liu and Zhou [38], on the other hand, reported a considerable increase in reaction rate with increasing temperature when sulphuric acid catalyst was used with biomass and methanol. For a 20 hour reaction using 0.2 M sulphuric acid, the yield of ester increased from 44.8% to 96.8 % when the temperature was progressively increased from 40 to 70^{o}C. Since transesterification is generally much slower with an acid catalyst than alkaline, it is therefore conceivable that increases in temperature will produce a more significant effect with acid catalysts.

It should be noted that optimal temperature is likely to depend on the feedstock used. Different feedstocks will have varied internal structures and therefore different effective diffusivities. This may explain some of the apparent contradictions in findings reported in the literature.

2.3.2.7 Catalyst Concentration

Catalyst concentration has been identified as the most important factor in determining reaction rates of conventional transesterification [58, 59]. Zeng *et. al.,* measured the yield and purity of the biodiesel at different concentrations of sodium hydroxide for in situ transesterification. They found that while the catalyst concentration did not affect methyl ester yield, it did influence its purity, which is the methyl ester concentration, in the final product [50]. For instance, *in situ* transesterification using sunflower seeds, methanol and sodium hydroxide at low catalyst concentrations achieved 93% conversion with only 30% methyl ester purity, whereas at high concentrations, the conversion was 95% with 98% of methyl ester purity.

In contrast, Qian *et. al.,* reported that the conversion of oil to methyl ester for a cottonseed/methanol system increased from 33% to 97% when the concentration of sodium hydroxide was doubled to 0.1 mol/L [39]. Nonetheless, 0.05 mol/L in this case is equivalent to a 0.2:1 molar ratio of catalyst:oil, which is low compared to the levels in Zeng *et. al.,*'s experiments. The larger amount of catalyst used by Zeng *et. al.,* may explain the apparently contradictory results. Additionally, if the rate determining step is the diffusion of alcohol into the particles, the different feedstocks used may have contributed to the difference in findings, since different oilseeds have different internal structures.

2.3.2.8 Particle Size

Since the particle size of the seeds plays a very important role in conventional solvent extraction [60, 61], it should be similarly important in *in situ* reactive extraction. Kildiran *et. al.,* investigated two sizes of soybean seeds (<1 mm and <0.5 mm) at three different reaction times [31]. At 1 hr reaction time, the larger particle size gave the highest percentage of oil

dissolved in ethanol. However, when the reaction time was longer, i.e. at 3 hr and 5 hr, smaller particle sizes produced better yields.

Ren *et. al.,* investigated the effect of particle size in rapeseed *in situ* transesterification [40]. The light microscopy and SEM analysis showed that with seed samples at the smallest particle size all the lipids were removed after 1h. However, some lipids remained in the centre of particle of larger sizes, particles at this time, and it was evident from experiments with light microscopy and lipid staining that there was a shrinking core of oil-bearing material. As the particle size of the rapeseed fragments increased from 300 – 500 μm to 500 – 850 μm, and then to 1000-1400 μm, rates of conversion after 1 hour decreased from 86% to 65% to 43%. The results clearly suggest that, for rapeseed at least, the transport of the methanol into the seed particles determined the reaction rate.

2.3.2.9 Alcohol Type

At least five types of monohydroxy alcohols have been evaluated as reagents in *in situ* transesterification. Ozgul and Turkay used methanol, ethanol, propanol and butanol as reagents with rice bran oil [43]. The solubility of the oil increased with alcohol chain length. However, it was noted that, even though the amount of oil dissolved increased, the alkyl ester content decreased. The reduction in polarity of the alcohol molecule as chain length increases enables it to stabilise the emulsions formed during the course of the reaction. The emulsion formed can persist and adversely affect conversion.

2.3.3 Biodiesel Quality

One of the most important factors to be considered in the development of *in situ* transesterification is whether the process can provide the market with biodiesel of sufficient quality to meet the requirement of governing bodies. The two main standards are ASTM D6751 and EN 14214.

Haas and Scott examined methyl ester produced from soybean flakes via *in situ* transesterification and compared it to the ASTM D6751 standard [53]. The methyl ester passed all the tests except for the acid number test, for which it required additional washing before it passed the test. Table 2.4 shows the comparison reported by Haas and Scott against another standard, EN 14214.

Table 2.4 Comparison of soybean flake methyl ester obtained via *in situ* transesterification against ASTM D6751 and EN 14214

Property	Soybean methyl ester	ASTM D6751	EN 14214
Flash point (^{o}C)	160	>130	>101
Water and sediment (vol%)	0	0.05	0.05
Carbon residue (wt%)	<0.010	0.05	0.3
Sulfated ash (mass%)	0.000	0.020	0.02
Kinematic viscosity (cSt, at 40^{o}C)	4.017	1.9-6.0	3.5-5.0
Sulfur (wt%)	0.00035	0.05	0.001
Cloud point (^{o}C)	0.0	Report	Not specify
Cetane number		>47	>51
Copper corrosion	1a	Class 3	Class 1
Acid number (mg KOH/g)	0.04	0.80	0.50
Free glycerine (wt%)	0.000	0.02	0.02
Total glycerine (wt%)	0.071	0.240	0.25
Phosphorus (wt%)	0.000	0.001	0.001
Reduced pressure	350	360	n.a.

distillation (temperature at 90% recovery, ^{o}C)

2.4 Reducing the Volume of Reactant

The enormous amount of reactant used in *in situ* transesterification is one of the obstacles to commercialisation. The reactant needs to be removed from the product, and the separation process becomes more energy intensive and expensive when there is more reactant. This has an effect on the price of the biodiesel, making it uncompetitive compared to conventional biodiesel [54]. This problem has been investigated using two approaches: modifying the raw material or the process.

2.4.1 Modifying the Raw Material

The main concept in this approach is to disrupt the cell walls inside the raw material, thus helping the oil to travel within the cell with less resistance and making the oil body in the cell more easily accessible to the reactant. The combination of these effects is then expected to reduce the amount of reactant required.

Haas and Wagner pre-treated soybeans with four different physical treatments and investigated their performance as raw material in *in situ* transesterification. The four treatments were: 1) dehulling and flaking; 2) dehulling, flaking and passage through a twin screw extruder; 3) passage through an expender type extruder and 4) conversion to a flour-like consistency via disruption in a Pulsewave disintegrator [26]. The second treatment successfully reduced the methanol needed by 20-fold from a molar ratio of methanol to substrate fatty acids to of 181:1 to 9:1. The other pretreatment regimes were found to be ineffective in reducing the amount of methanol required.

Although the objective of reducing the reactant was achieved in this study, no further analysis was conducted of the energy requiremenst for the pretreatment stages. Whether such a combination of dehulling, flaking and extruder could work for other raw material also remains to be seen.

2.4.2 Modifying the Process

The process is usually modified by introducing other compounds to work as co-solvents. The idea here is that the co-solvent, which is usually non-polar, will help extract the oil from the raw material. Once the oil is extracted, it can be transesterified with methanol. Since methanol is not used as an extraction agent, the amount used can be reduced. Various non-polar solvents, such as hexane and diethoxymethane have been employed as co-solvents in *in situ* transesterification process [34, 39, 50].

Another novel approach is to change the reactant. The conventional reactant used in *in situ* transesterification process is a short-chain alcohol, such as methanol or ethanol. This results in the production of methyl ester (biodiesel) and glycerol which is a low value by-product. By replacing the short-chain alcohol with short-chain alkyl acetates such as methyl acetate and ethyl acetate, triacetin is produced instead of glycerol. Triacetin is a better "co-product" as it is used widely as a plasticiser or gelatinising agent in polymers [62], and as fuel additive [63]. The utilisation of methyl acetate as a reactant has been studied in the conventional transesterification of sunflower oil [64], as well as in transesterification with enzyme catalysts [65]. There is as yet no published report however, of the *in situ* transesterification of *J. curcas* using methyl acetate and either alkali or acid catalysts.

In “interesterification”, the alkyl group in triglyceride is replaced by alkyl group in methyl acetate, in contrast to transesterification, where the alkyl group in the triglyceride is replaced by an alcohol group from methanol instead, as shown in Figure 2.7. The absence of polar compounds in the reactant results in a change in the polarity of the mixture, from polar to non-polar. This affects the solubility of sodium hydroxide, where it becomes partially soluble in the mixture [66]. The addition of a phase transfer agent, such as polyethylene glycol (PEG) to the mixture can provide a solution to this problem.

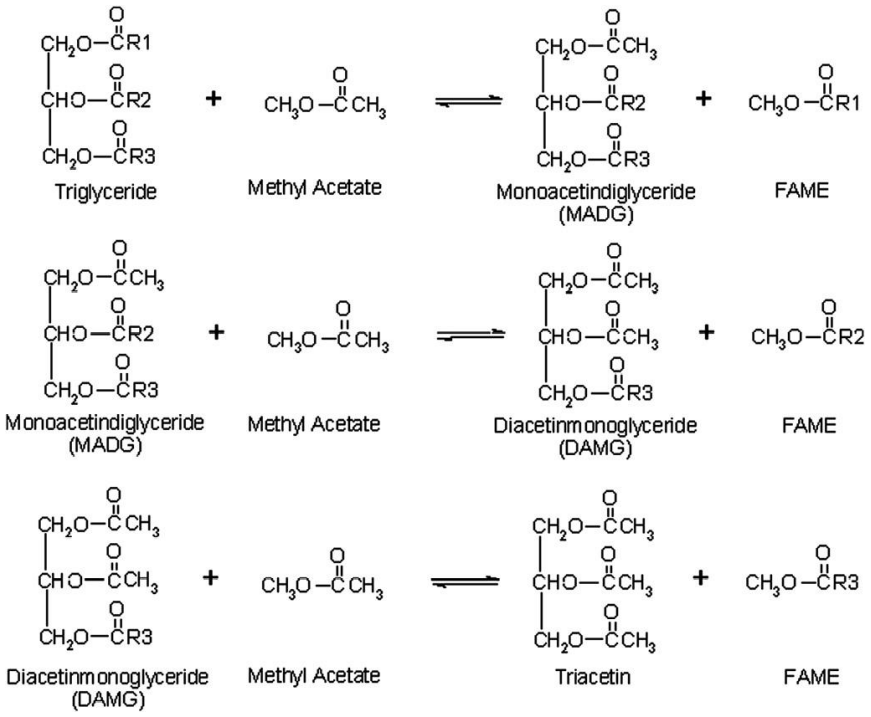


Figure 2.7 Process of producing FAME with methyl acetate as alkyl acceptor. The process is known as interesterification [66].

2.5 Raw Material: *Jatropha curcas (J. curcas)*

J. curcas was selected as a raw material because it is inedible and its oil properties are similar to that of rapeseed. It also grows on almost every type of soil. Research on biodiesel is

currently focused on utilizing inedible oils, and *J. curcas* is one of the most promising inedible oils that can be used for this purpose.

It was been reported that in 2008, 900 000 hectares *J. curcas* was planted globally with majority of it located in Asia, and the rest in Africa and Latin America. The report also made a projection of 12.8 million hectares of *J. curcas* plantation by 2015 [67].

2.5.1 General Background

Jatropha derives from two Greek words: *jatros* (doctor) and *trophe* (food). The literal meaning of the words implies the function of the tree as a medicinal plant.

Jatropha species, which belong to the *Euphorbiaceae* family, are small trees or large shrubs. The plants can grow up to seven metres tall and are able to survive in harsh conditions. Becker and Makkar [68] reported trials of growing *J. curcas* on degraded land. They showed that *J. curcas* successfully fruited after 9 months, even on land in very poor condition, such as coastal sand dunes, where the level of three important soil ingredients (organic carbon, total nitrogen and total phosphate) were very low compared to that of fertile land.

Most *Jatropha* species are toxic. The seed contains phorbol esters, curcin, and lectins, which are all toxic substances. However, a species found in Mexico was reported to be non-toxic, and is consumed by local people [69]. In general, the fact that the plant is toxic protects it from pests and diseases, and also from being a source of food for ruminant animals.

The fruit of the plant is comprised of seeds which can be divided into two components, the kernel and shell. Achten *et. al.,* [70] collected data from various sources on the composition of *Jatropha* kernels and shells as presented in Figure 2.8.

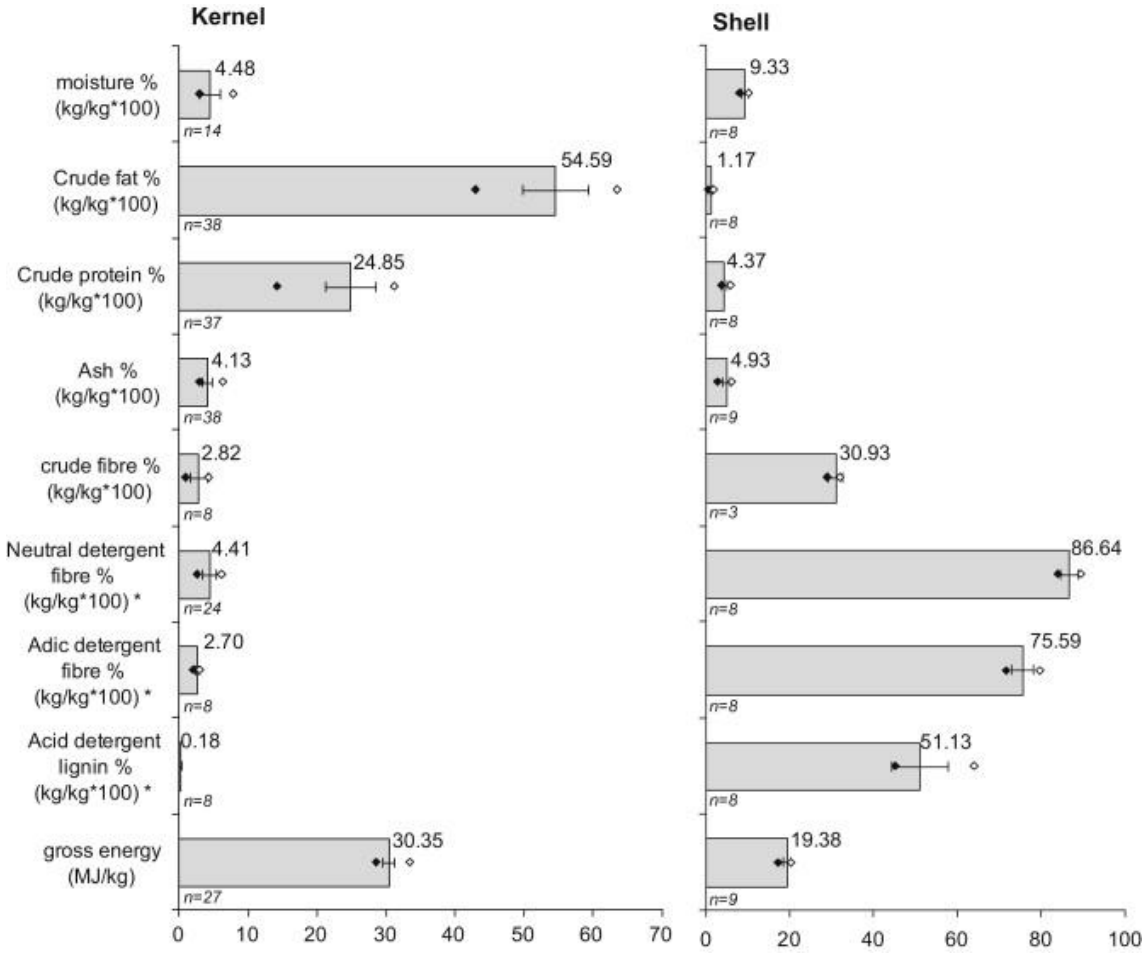


Figure 2.8 Major components in kernel and shell of Jatropha fruit [70]

From the figure, it can be observed that two major components in the kernel are crude fat and crude protein, while various types of fibre constitute the main components in the shell. The shell contains more moisture but less energy than the kernel.

There are 170 known *Jatropha* species, but the most commonly cited in the literature is *J. curcas*. It is believed that the genus *Jatropha* originally came from Central America but has

since spread all over the world, such as to the countries like India, Nigeria, Mozambique, Malaysia, and Thailand.

2.5.2 *Jatropha* as a Fuel Source

Jatropha has been investigated as a fuel source by many researchers. Banerji *et. al.,* [71] compared the fatty acid profile of four different species of *Jatropha*, namely *J. curcas, J. glandulifera, J. gossypifolia and J. multifida*, all of which were found to be suitable for methyl ester production. *J. curcas* was found to have the highest oil content at 48.5% and *J. multifida* had the highest energy value.

J. curcas is the most widespread species among the *Jatropha* species, particularly because of its high oil content. It has been used directly in engines [72] and transesterified to methyl ester [73-76] as well as blended with alcohol [72].

2.5.3 Composition and Characteristics of *J. curcas*

The composition and characteristics of *J. curcas* play significant roles in determining the suitability of the oil as a fuel source. Table 2.5 shows the fatty acid composition of *J. curcas* [68], which may vary from one plant to another, but generally most of the oil is oleic acid (C18:1) and linoleic acid (C18:2). Other major fatty acids include palmitic acid (C16:0) and stearic acid (C18:0). Small percentages of myristic acid (C14:0), palmitoleic acid (C16:1) linolenic acid (C18:3), arachidic acid (C20:0) and, in some cases, behenic acid (C22:0) are also present.

Table 2.5 Fatty acid composition of J.curcas oil [61]

Systematic name	C:D	Scientific name	Percentage (%)
Myristic	14:0	Tetradecanoic	0.1
Palmitic	16:0	Hexadecanoic	15.3
Heptadecanoic	17:0	Heptadecanoic	0.1
Palmitoleic	16:1	9-hexadecanoic	0.9
Stearic	18:0	Octadecanoic	6.6
Oleic	18:1	*cis*-9-octadecanoic	41
Linoleic	18:2	*cis*-9-12-octadecadienoic	35.3
Linolenic	18:3	9,12,15-octadecatrienoic	0.3
Arachidic	20:0	Eicosanoic	0.2
Behenic	22:0	Docosanoic	tr
Lignoceleric	24:0	Tetracosanoic	0.1

C:D = carbon chain: no. of double bond
tr = trace

Table 2.6 lists the typical physical and chemical properties of *J. curcas* seed oil [68, 70]. The calorific value (37.8 MJ/kg) of *J. curcas* oil, for example, is very similar to that of rapeseed oil (39.08), which is a main source of biodiesel in Europe [68, 77].

Table 2.6 Fatty acid composition in *J. curcas* oil [68, 70].

	Range
Specific gravity (g/cm^3)	0.860-0.933
Calorific value (MJ/ kg)	37.83 – 42.05
Pour point (^{o}C)	-3
Cloud point (^{o}C)	2
Flash point (^{o}C)	210 – 240

Cetane value	38.0 – 51.0
Iodine value	102*
Saponification number (mg/g)	102.9 – 209.0
Viscosity at 30^{o}C (cSt)	37.00 – 54.80
FFA % (kg/kg *100)	0.18 – 3.40
Unsaponifiable % (kg/kg *100)	0.79 – 3.80
Iodine Number (mg iodine/g)	92 – 112
Acid number (mg KOH/g)	0.96 – 6.16
Monoglycerides % (kg/kg *100)	nd – 1.7
Diglycerides % (kg/kg *100)	2.50 – 2.70
Triglycerides % (kg/kg *100)	88.20 – 97.30
Carbon residue % (kg/kg *100)	0.07 – 0.64
Sulfur content % (kg/kg *100)	0 – 0.13

*from [68]; nd = not detected

2.5.4 *J. curcas* versus Other Inedible Oils

Fatty acid composition plays a very important role in determining biodiesel properties. Plant oils have a wide variety of compositions. Table 2.7 lists fatty acid compositions of four different inedible oils [78]. These four types of plants are among the most studied inedible plant in biodiesel research.

Amongst the important factors in biodiesel quality are cetane number (CN) and iodine value (IV). CN is a measure of the ignition delay when the fuel is injected into the cylinder. Fuels with short ignition delay have higher CN, thus perform better as fuel. IV is used to measure the total level of unsaturation in the oil. High IV levels in oil generate problems such as the polymerisation of the oil, leading to deposits being formed on engine parts [79].

Table 2.7 Fatty acid composition in various non edible oils [78]

Fatty acid	C:D	*J. curcas*	*P. pinnata*	*S. oleidis*	*A. indica*
				(%)	
Capric	10:0			0.8	
Lauric	12:0			35.6	
Myristic	14:0	1.4		50.7	
Palmitic	16:0	15.6	10.6	4.5	14.9
Stearic	18:0	9.7	6.8		14.4
Oleic	18:1	40.8	49.4	8.3	61.9
Linoleic	18:2	32.1	19	0.1	7.5
Arachidic	20:0	0.4	4.1		1.3
Eicosenoic	20:1		2.4		
Behinic	22:0		5.3		
Lignoceric	24:0		2.4		

C:D = carbon chain: no. of double bonds

According to the European Standard EN 14214, the CN of biodiesel must be more than 51 while the IV must be less than 120g I_2/100g. Table 2.8 compares the four prominent inedible oils with respect to EN 14214 specifications.

Table 2.8 CN and IV of inedible oils compared to EN 14214 [78]

Property	Units	Limits		*Jatropha curcas*	*Pongamia pinnata*	*Salvadora oleidis*	*Azadarichta indica*
		Min.	Max.				
Cetane No.	-	51		52.31	55.84	7.6	57.83
Iodine Value	g I_2/100 g		120	93	80.9	66.13	69.3

In terms of CN and IV, neat oil from all of the plants listed falls within the limits set by EN 14214. CN levels are expected to increase once the neat oil is blended with diesel oil, as observed in a number of studies [68, 74]. Iodine values however, remain the same.

Other than CN and IV, the oxidation stability and cold filter plugging point (CFPP) must also be within EN 14214 specifications. CFPP is a criterion used to predict the performance of

biodiesel at cold temperatures. It has been suggested that CFPP depends on the length of the carbon chains in biodiesel [30], where the longer the chains, the worse the low-temperature properties will be [80]. However, this claim was made in a study of peanut biodiesel and no publications report the CFPP properties of inedible oils.

None of the inedible oils listed in Table 2.7, has carbon chains longer than arachidic acid (20:0), except for *P. pinnata*, 10% of whose carbon chains are longer than that. Although no study has reported the cold flow properties of *P. pinnata*, these may be expected to be worse than those of the other inedible oils.

Muniyappa *et. al.*, [81] investigated the correlation between the density, viscosity and cloud point of biodiesels from soybean and tallow oil. It was found that the high cloud point obtained for methyl ester from beef tallow oil was due to its high concentration of saturated fatty esters. Three of the inedible oils considered (*J. curcas, P. pinnata, A. indica*) also contain high percentages of unsaturated fatty acids, and so they are unlikely to suffer from this problem. *J. curcas* contains the highest percentage of unsaturated fatty acid at 72.9 %, while *P. pinnata* and *A. indica* each contain 68.4 %. The high percentage of fatty acid in *S. oleidis*, however, comes in saturated for fatty acid, especially as myristic and oleic acids. The combination of these fatty acids contributes about 96.3% of the overall composition of the oil. Therefore biodiesel from *S. oleidis* is likely to have poor cloud point properties, but no studies could be found to corroborate this.

A. indica is more renowned for its medicinal properties than its capability as a new raw material in biodiesel production. Exploited largely in India for medicinal purposes, *A. Indica* can also be used as a biopesticide [82]. The significant impact *A.indica* has made in medicine,

especially in India, is far more attractive than its prospects as a raw material for biodiesel production.

Out of the four inedible oils listed, *J. curcas* remains the best option as a raw material for biodiesel production. The CN of the oil may be the lowest of the four, but it is still within the EN 14214 minimum limit. The oil fatty acid composition in *J. curcas* is dominated by oleic (18:1) and linoleic (18:2) acids, both of which are unsaturated fatty acids and thus the high cloud points of oils with high percentages of saturated fatty acids will be avoided. The longest fatty acid chain in *J. curcas* is arachidic acid (20:0), which contributes to 0.4% of its overall composition. The lack of long fatty acids in *J. Curcas* will help to avoid the CFPP problem. This is very important in order to ensure that the biodiesel would be accepted at higher latitudes.

J. curcas is a toxic plant, so is not consumed by animals. Although one study has reported a non-toxic *J. curcas*, this variety was exclusive to Mexico [69]. *J. curcas* is capable of growing in very challenging environments, and Becker and Makkar [68] have listed the characteristic of soils in India where the plant fruited after 9 months as shown in Table 2.9

Table 2.9 Characteristic of soils (at 15 cm depth) in India where J.curcas fruited after 9 months [68]

Type of soil	Organic carbon (%)	Total nitrogen (kg/ha)	Available phosphate (kg/ha)
Rocky and hard soil	0.2	155	13
Heavy Black soil	0.5	465	2
Laterite soil	0.4	310	2
Red loam	0.2	181	2
Coastal sand dune	0.1	86	2
Fertile land	2	9000	100

The study proved that *J. curcas* can grow and fruit on poor and stony land. The plant can also tolerate long dry seasons and is resistant to disease. Because of the capability of *J. curcas* to grow in arid and semi-arid land, its cultivation would not reduce the amount of available fertile land used for food crops. It would also not affect current tropical forest, savannah or grassland environment, all of which are very important for the carbon cycle. Instead, by utilizing degraded, arid, semi-arid and barren farm land little or no carbon debt would be involved which would give advantages in terms of managing greenhouse gas emissions [83, 84], as well as favouring countries with vast areas of wasteland such as India. Issues in the reclamation of such wastelands, as well as prospects for increasing the socio-economic profiles of degraded areas from the planting of *J. curcas* have been discussed in detail by Francis *et. al.*, [85].

Even though the advantages of *J. curcas* are widely acknowledged, the acceptance of *J. curcas* as a raw material in biodiesel production still appears unpromising. Among the barriers that contribute to the reluctance to use it are the following:

- Lack of availability of detailed information about large-scale cultivation and harvesting, compared to its competitors, such as rapeseed, oil palm and soy.
- Limited agronomic studies so far, leading to various uncertainties. For example, it has been claimed that *J. curcas* has low nutrient requirements for growth, whereas recent studies have shown that an insufficient supply of nutrients will lead to reduced growth and crop production [86, 87].
- Lack of species development through special breeding programmes.
- Availability of competitor species.
- Lack of investigation into the utilisation of by-products. Since the by-products contain toxic components, they cannot be sold as animal feed.

- Lack of information about the dangers of processing, due to the toxicity and carcinogenicity of *J. curcas*.

2.5.5 Biorefining Opportunity

J. curcas seeds contain many valuable chemical compounds. Among those identified are proteins [88, 89], phenol [90] and phorbol ester [91, 92]. In biodiesel production, the proteins usually concentrate in the solid waste of seeds extraction. Rapeseed meal has been reported as an excellent source of protein-rich meal for animals [93]. However, meal from *J. curcas* has been found to be toxic and needs further treatment before it can be utilised as animal feed [94]. Makkar and Becker identified the protein types in *J. curcas* meal and found that it is very similar in this respect to soybean meal, with all essential amino acids present [95]. Phenolic compounds, meanwhile, offer nutritional benefits in the form of antioxidants [79]. Phorbol esters can be used as high value biopesticides and insecticides [96]. They may also possess medicinal value, as one study has shown that a phorbol ester isolated from *J. gossypifolia*, a near relative of *J. curcas*, successfully inhibits cancer cell activity [97].

2.6 Summary of Literature Review

Biodiesel is conventionally produced via the transesterification process, where the refined, bleached vegetable oil reacts with alcohol in the presence of acid or alkali catalysts. Because refined, bleached vegetable oil is used as a raw material in this process, its cost alone can account for 75% of overall expenditure [98], which subsequently affects the price of biodiesel. On top of that, edible oils have usually been the preferred feedstocks, but their price is volatile, so biodiesel prices will fluctuate in response. The debate on food versus food

has become intense, as the demand for agricultural crops increases year-on-year with the accelerating use of edible oil seeds for biodiesel production [99].

To address this problem, the possibility of using another reaction route, called *in situ* transesterification, on non-edible species seeds of *J. curcas* has been evaluated.

In situ transesterification is an approach to the production of biodiesel from oil-bearing materials instead of directly from the oil. To date, the reaction has been tried with various kinds of oil-bearing materials, and has successfully produced biodiesel. The process is influenced by many variables such as raw material particle size, the molar ratio of alcohol to oil, catalyst concentration, reaction temperature, reaction time and mixing speed intensity. The diversity of potential raw materials means that optimal operational conditions for the process vary widely.

The literature demonstrates that the *in situ* transesterification process can occur in the presence of either acid or alkali catalysts. However, it does not proceed without a catalyst. The major difference when these two catalysts are used is that acid catalysts take a longer time to complete the reaction compared to alkali catalysts. Both, nonetheless, produce high yields of biodiesel.

The use of excessive alcohol has hindered the commercial development of this process, and the possibility of reducing the amount of reagent in *in situ* transesterification has therefore been investigated. Compared with the 6:1 - 9:1 molar ratio of alcohol to oil in the conventional process, *in situ* transesterification require ratio of about 300:1 to 500:1 to produce significant biodiesel yields. The recovery of this huge amount of alcohol from the

product by distillation would be very energy intensive and strongly affects the final production cost.

It has also been shown that the reaction is able to progress at moderate temperatures. The maximum temperature used is usually just under the boiling point of the alcohol used. The performance of the reaction in high pressure conditions has never been studied, since it already progresses sufficiently well at atmospheric pressure. Reports of the effect of mixing intensity on the reaction are scarce. Generally, researchers have used a minimum of 200 rpm in mixing.

Short-chained alcohols such as methanol and ethanol are commonly used for this reaction. Longer chained alcohols have been tried, but the reduced solubility of the catalysts in alcohol decreases the biodiesel yield. The drying step has proven to be important for certain feedstocks such as soybean, but has no impact on other feedstocks, such as rapeseed.

The possible utilisation of non-edible raw materials in biodiesel production has reignited interest in this process. Four different non-edible oils, namely *J. curcas, P. pinnata, A. indica and S. oleidis,* have been compared in terms of their suitability as raw materials. *J. curcas*-derived biodiesel has CN and IV within EN 14214 limit, and contains short carbon chains (not more than 20 carbons), and has a high percentage of unsaturated fatty acid which will help in avoiding high CFPPs and will reduce the cloud point. Therefore it was used as a raw material for biodiesel production in the present study. The possibility of producing high value co-products from *J. curcas,* such as protein, phenol and methyl ester has also been discussed.

3 MATERIALS AND METHODS

Since *J. curcas* seeds are used as raw material in the process investigates in this research, the need to understand the seeds themselves prior to converting them into biodiesel is paramount. Among the characteristics studied are the oil content, moisture content, free fatty acid content, acid value and fatty acid profile.

Following the study of seed characteristics, the first experiments investigated the relationship between seed particle size and biodiesel yield. From this experiment, a suitable particle size was selected and used throughout the study. The effect of moisture on the reaction was also investigated. The reaction was then tested with a range of different catalysts, in order to determine the most suitable catalyst to be utilised in the process.

The parameters involved in the process were then investigated using a design of experiments-based matrix. However, because no data were available to set the highest and lowest values of the parameters used, one-at-a-time experiments were carried out first. Here, one parameter was varied while the others were held constant. The parameters studied were: molar ratio of alcohol to oil, catalyst concentration, reaction temperature, reaction time and mixing speed. In the design of experiment investigation, a full factorial design (2^5) was employed. Then, a response surface methodology was utilised to inspect the findings at higher mathematical orders, the results of which were subsequently used to suggest the optimal conditions for running the experiment. Time profile experiments were then performed to observe the behaviour of the process as the reaction progressed. In these experiments, information about impurities in the biodiesel including diglyceride and triglyceride, was also collected.

This was followed by series of modified experiments, the aim of which was to address the problem of the excess volumes of methanol used in the *in situ* transesterification process. Firstly, the performance of two different co-solvents (hexane and dimethylethoxymethane) in the reaction was evaluated. Then, methyl acetate was used as a substitute for methanol.

The economic feasibility of the process also depends on the added value of the by-products generated, and in this study the fate of phenols and protein was investigated.

The analytical methods used throughout this study were gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). Light microscopy and Scanning Electron Microscopy (SEM) were used to get more information from the experiments result.

3.1 Characterisation of the Oilseeds

Characterisation of the oilseeds was performed to understand and confirm their properties. This was essential as the difference between *J. curcas* batches can be significant. In this section, the kernel oil, moisture and volatile matter content were checked. The acid value and the acidity of the oil, as well as the fatty acid profile were investigated and reported.

3.1.1 Determination of Kernel Oil Content

J. curcas seeds were provided by the Indian Institute of Petroleum (IIP), Dehradun, Uttarakhand, India. Seeds were received in batches and stored in opaque air-tight containers.

The oil content of *J. curcas* was determined according to the procedure described by the British Standards Institution (BS EN ISO 659:2009).The seed coats were separated manually

from the kernels prior to the experiments. The kernels were ground and sieved until their size was not greater than 2 mm. The ground seeds were then dried in an oven at 80^{o}C until the difference in mass between before and after drying was less than 10%. 10g samples of the ground seed kernels were weighed and put inside a cellulose thimble and plugged using cotton wool.

The thimble was placed in the Soxhlet apparatus, while hexane (Fisher Scientific, UK) was poured into the flask connected to the bottom of the apparatus. 1 mg of anti-bumping granules (Fisher Scientific, UK) was added to the solvent. Heating was performed so that the rate of reflux was at least 3 drops per second. The extraction was left to run for 4 hours. After that it was allowed to cool and the thimble was removed and left to dry.

The extracted seed kernels were then put into the grinder again, and ground for 7 minutes, before being extracted again for another 2 hours. This procedure was then repeated one more time for another 2 hours. Hexane was then removed from the solvent using a heated rotary evaporator (Buchi, Switzerland) under vacuum conditions. The flask was then placed in the oven at 103^{o}C to eliminate any remaining traces of hexane. After cooling in a desiccator, the flask was weighed and the mass was recorded as m_1. The flask was then reheated for 30 minutes and the mass subsequently recorded again as m_2. $m_2 - m_1$ must not equal more than 5 mg, otherwise the sample has to be reheated and weighed again. The oil content, *w*, was then calculated from the equation below

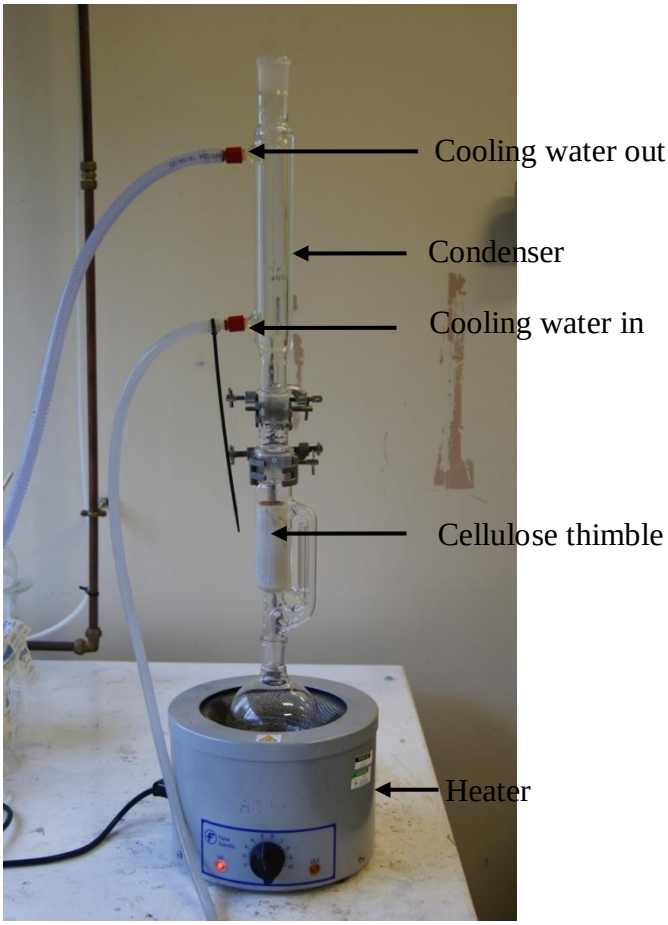


Figure 3.1. Picture of Soxhlet extraction apparatus.

$$w_{oc} = \frac{m_2}{m_1} \times 100$$ **Equation 3.1**

where m_1 = mass (g) of the test portion

m_2 = mass (g) of the dried extract

3.1.2 Moisture and Volatile Matter Content

Moisture and volatile matter content were determined using a procedure described by the British Standards Institution (BS EN ISO 665:2000).

A flat-bottomed vessel (with lid) was dried for 1 hour at 103°C and then weighed after being placed in a desiccator to cool, giving m_0. 5 g of *J. curcas* seeds were put into the vessel and weighed again, m_1. The seeds were ground to a size of less than 2 mm and were used without particle distribution analysis. The test sample in the vessel (lid removed) was then placed in the oven (Memmert, Germany), with the temperature set at 103°C. After 3 hours, the vessel's lid was closed and it was cooled in a desiccator. The vessel (with lid) was weighed once it reached room temperature, giving m_2.

The determination was considered as finished if $m_2 - m_1$ was equal to or less than 0.005g. The above procedure was repeated if the difference between weighing was greater than 0.005g. However, instead of 3 hours of drying, 1 hour as used for the second, third and subsequent dryings, until the determination was complete. Equation 3-2 below was employed to calculate the mass percentage of moisture and volatile matter in the sample mass.

$$w_m = \frac{m_1 - m_2}{m_1 - m_0} \times 100\% \qquad \textbf{Equation 3.2}$$

where m_0 = mass (g) of the vessel

m_1 = mass (g) of the vessel and sample before drying

m_2 = mass (g) of the vessel and sample after drying

3.1.3 Acid Value and Acidity

The determination of acid value and acidity (percentage of free fatty acids) was carried out based on a titration method. The procedure used in the experiments is described in detail by the British Standards Institution (BS EN ISO 660: 2009).

50 mL of ethanol (96%, Fisher Scientific, UK) containing 0.5 mL of phenolphthalein indicator (Fisher Scientific, UK) was boiled in the flask. The solution was then neutralised using 0.1 mol/l potassium hydroxide (Fisher Scientific, UK) while its temperature was still above 70^{o}C. The titration endpoint occurred when a single drop of potassium hydroxide produced a slight but definite colour change lasting for about 15 seconds.

The neutralised ethanol was then mixed with 10 g of *J. curcas* oil. The mixture was then titrated with potassium hydroxide solution while being vigorously agitated. The titration was considered to be complete when the first permanent pink colour appeared for at least 15 seconds.

The acid value, w_{AV} (mg/g KOH), was calculated from Equation 3.3, while acidity w_{FFA} (%) was calculated using Equation 3.4:

$$w_{AV} = \frac{56.1 \times cV}{m} \qquad \textbf{Equation 3.3}$$

$$w_{FFA} = \frac{V\ c\ M \times 100}{1000 \times m} \qquad \textbf{Equation 3.4}$$

where c = concentration (mol/l) of the potassium hydroxide used

V = volume (mL) of potassium hydroxide used

m = mass (g) of *Jatropha* oil used

M = molar mass (g/mol) of the acid. In this case, it was oleic acid (282 g/mol).

3.1.4 Fatty Acid Profiles

The fatty acid profile of *J. curcas* oil was determined by comparing the retention time of the peaks eluted from the sample chromatogram with standard peak values, allocating the peaks accordingly and quantifying the amounts versus the internal standard. Before undergoing gas chromatography analysis the oil had to be converted into the ester form, to reduce its boiling point. The transesterification method, as described in details by the British Standards Institution (BS EN ISO 5509:2001), was used to convert the oil into its ester.

The procedure for converting the oil started by dissolving 60 mg of oil in 4 mL of isooctane (Sigma Aldrich, UK). 200 µl methanolic potassium hydroxide solution (2 mol/l), which was prepared beforehand, was then added to the solution. The mixture was shaken vigorously for about 30 seconds and 1 g of sodium hydrogen sulfate monohydrate (Sigma Aldrich, UK) was added to neutralise the potassium hydroxide. The upper layer was decanted and was then injected into the gas chromatography column.

The eluted peaks then were compared to those individual fatty acid standards. Standards were available for methyl laurate, methyl palmitate, methyl stearate, methyl oleate and methyl linoleate, purchased from Sigma Aldrich, UK.

3.2 *In situ* Transesterification Experiment

A 250 mL Schott bottle was used as the reaction vessel for the *in situ* transesterification experiment. A pre-determined amount of methanol was added to the bottle together with the relevant amount of catalyst. The mixture was then placed in the programmable incubator shaker (IKA, Germany) and shaken at 400 rpm until the catalyst had completely dissolved. At the same time, the mixture was pre-heated to the desired reaction temperature. When the methanolic solution had reached the desired temperature, 10 g of *J. curcas* seeds were introduced to the solution. All of the physical parameters (temperature, agitation speed and time) were controlled from the incubator. Figure 3.2 shows the IKA programmable incubator shaker employed throughout the study.

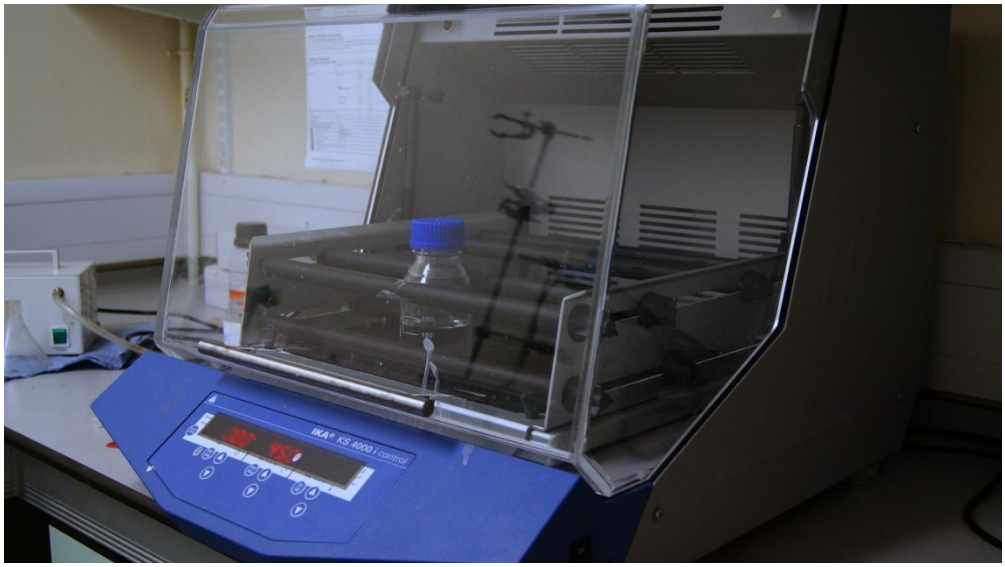


Figure 3.2 Programmable incubator shaker used to control temperature, agitation speed and time, with Schott bottle used as reaction vessel.

Figure 3.3 below shows the next stages of the experiment which were conducted after the bottle had been removed from the incubator. A vacuum pump (KNF, Germany) together with a filter were used to separate the solid and liquid under vacuum conditions. Glacial acetic

acid (Fisher Scientific, UK) was added to the liquid part to neutralise the base catalyst, thus ensuring that the transesterification reaction had completely stopped.

Most of the methanol was removed from the mixture using a rotary evaporator (Buchi, Switzerland) with the temperature set at 55^{o}C - 60^{o}C under vacuum conditions. 10 mL of hexane (Fisher Scientific, UK) was then added to the remaining mixture, in which the methyl ester in the mixture dissolved. Because of the difference in polarity between methanol and hexane, the glycerol, catalyst and un-reacted triglycerides were dissolved in methanol whilst the methyl ester dissolved in hexane. The mixture was then transferred to a separating funnel for gravitational separation.

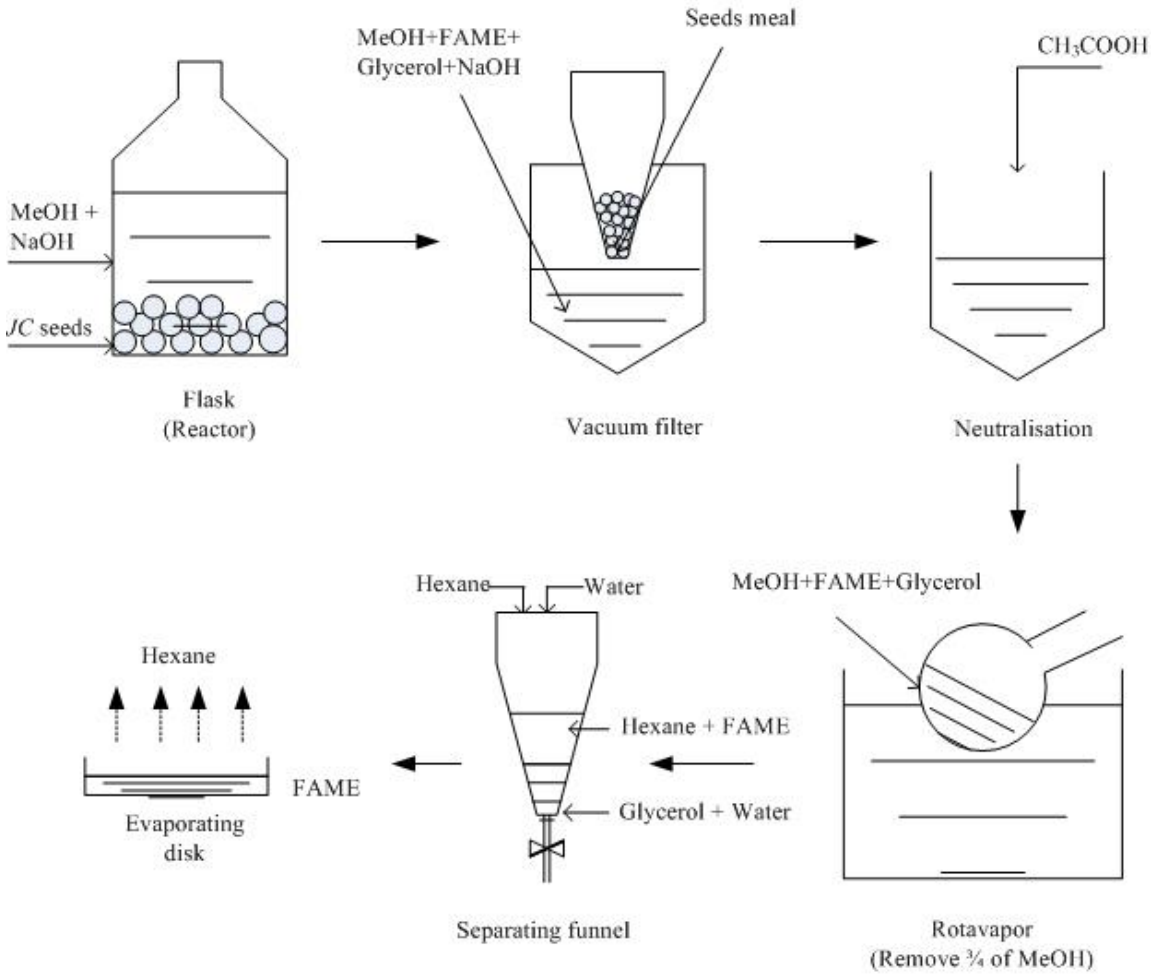


Figure 3.3 Schematic representation of the process flow of *in situ* transesterification process

The biodiesel-rich layer appeared in the top half with the glycerol-rich layer in the bottom half. The mixture was then washed with warm water. After the glycerol layer had drained out, the upper hexane/biodiesel layer was put on an evaporating disc and heated at 60°C using a hot plate in a fume cupboard. The mass of the biodiesel was recorded, and it was then analysed by GC to determine the percentage of methyl ester. Equation 3.5 was used to calculate the methyl ester yield:

$$Yield_{methyl\ ester}(wt\%) = \frac{mass\ of\ ester\ phase\ \times 0.995}{mass\ of\ triglycerides\ in\ the\ seed} \times 100$$

Equation 3.5

3.2.1 Study of Process Parameters

In order to study all variables, experiments were conducted by changing each parameter of interest whilst holding other parameters constant. Table 3.1 below summarises the settings of all parameters used in the experiments. The settings were based on previous work by Haas *et. al.*, and Harvey and Zakaria [21, 100]

Table 3.1 Parameter settings for the process parameters study

Experiment	Parameters					
	Seed size (mm)	Mixing speed (rpm)*	Reaction temperature (°C)	Reaction time (min)	Catalyst concentration (N)	Alcohol:oil molar ratio
Effect of particle size	<0.5-4	400	60	60	0.1	400
Effect of mixing speed	<0.71	100-400	60	60	0.1	400
Effect of reaction temperature	<0.71	400	30-60	60	0.1	400
Effect of reaction time	<0.71	400	60	10-60	0.1	400
Effect of	<0.71	400	60	60	0.1-0.2	400

catalyst concentration						
Effect of methanol to oil molar ratio	<0.71	400	60	60	0.1	200:1-600:1

*To convert RPM to G Force [g = (1.118 x 10^{-5}) x RPM x 2 cm]

3.2.2 Study of Main Parameters and Their Interaction using Design of Experiments (DoE)

The Design of Experiment technique was used to determine the main factors in the process, as well as to study the interaction between factors in the experiments. Design Expert® Version 7 software (StatEase, USA) was utilised for this purpose. The data set was first tested using two-level factorial design and then with the response surface methodology (RSM).

3.2.2.1 Two-level Factorial Design

Factorial design was used to identify the most important factors among many experimental factors and also to investigate the interaction between factors. The method can also suggest a first order equation that fits the data, and furthermore can give recommendations as to whether or not higher order testing is needed by analysing the presence of curvature within the data.

Five factors were considered in the design stage, which were molar ratio of methanol to oil, catalyst concentration, reaction time, reaction temperature and mixing speed. High (+1) and low (-1) levels of each factor were determined earlier in the process parameters study. Table 3.2 lists all the factors considered with their respective high and low levels.

Table 3.2 Factors involved in full factorial design with their respective levels

Factor	Unit	Code	Level	
			-1	+1
Molar ratio of methanol to oil	-	A	100	400
NaOH concentration	N	B	0.1	0.2
Reaction time	Min	C	10	60
Reaction temperature	^{o}C	D	30	60
Mixing speed	rpm	E	100	400

A full factorial design was used to evaluate the effect of the factors involved. A number of centre points were also tested to provide information about curvature as well as the stability of the process. The dependent variable (response) selected was the yield (wt %) of methyl ester obtained from the experiments.

3.2.2.2 Response Surface Methodology (RSM)

The Central Composite Design (CCD) in response surface methodology was utilised to fit the data in a second-order model as well as to optimise the process. Additional experiments were added to the design to provide the data at the 'star point'; in order to create a central composite design. Table 3.3 below lists the star points, encoded as –α and +α.

Table 3.3 Star points of the design for RSM experiments

Factor	Unit	Code	Level	
			-α	+α
Molar ratio of methanol to oil	-	A	26.0	474.0
NaOH concentration	N	B	0.08	0.22
Reaction time	Min	C	0	72.0
Reaction temperature	^{o}C	D	23.0	67.0
Mixing speed	Rpm	E	26.0	474.0

3.2.3 Time Profile of the Reaction

A different set-up was used to study the development of the reaction as time progressed. This was necessary since it was difficult to monitor the reaction as it progressed using the procedure described in Section 3.2. For this purpose, a 1 L three neck flask was used as the reaction vessel. The mechanical stirrer (VWR International, UK) was connected in the middle neck while the condenser was attached to the other neck. The flask was half submerged in the heated water bath (Fisher Scientific, UK) which was set to the reaction temperature. Figure 3.4 shows the experimental setup for the experiment.

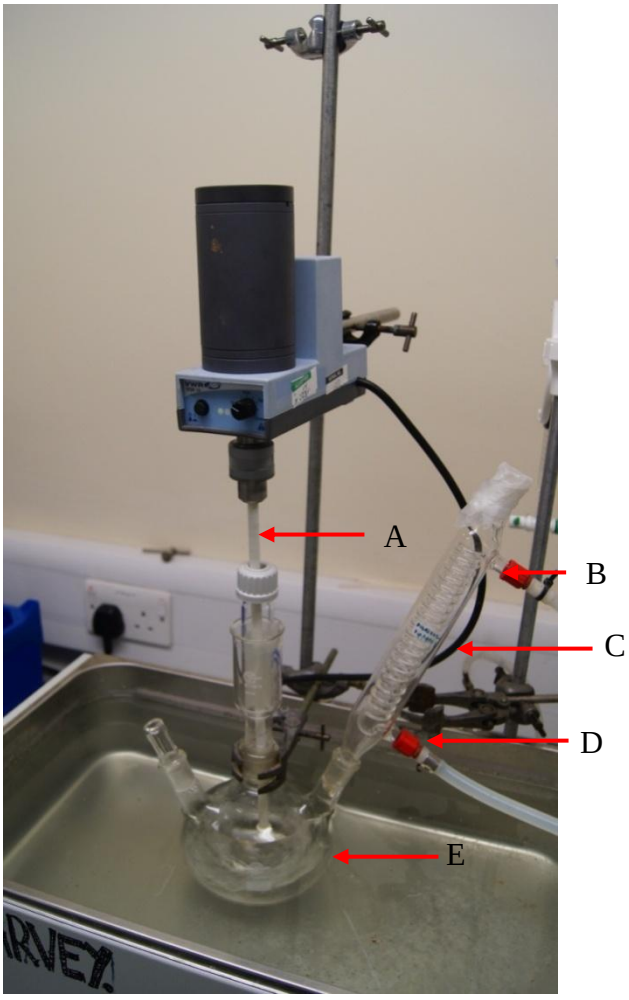


Figure 3.4. Experimental setup for time profile study. A-stirrer; B-cooling water out; C-condenser; D-cooling water in, E-three neck flask

Methanol was put into the flask first, followed by NaOH. The mixture was stirred until all of the NaOH had dissolved in methanol. The temperature of the methanolic solution was checked to ensure that it had reached the desired reaction temperature. 40 g of the pre-ground *J. curcas* seed was then put into the flask.

1 mL of reaction mixture was taken from the flask at different times. 5 µl of acetic acid was added to neutralise the NaOH. To separate the solid that was accidentally taken out during sample withdrawal, the mixture was passed through a 13 mm, 0.45 µm membrane syringe filter (VWR International, UK). The small portion of the sample then underwent gas chromatography analysis (Section 3.5.2). The remaining sample was heated until its mass was constant, to remove the methanol from the sample. The product was separated into two layers after it had gravitationally settled. The top ester layer was taken out and analysed to determine the concentrations of monoglyceride, diglyceride and triglyceride (Section 3.5.3.2 and 3.5.3.3).

3.2.4 Effect of Moisture

To examine the effect of moisture on the methyl ester yield, the *in situ* transesterification reaction was applied to the *J. curcas* seed samples with different levels of moisture content. This was achieved by adding water to the dry samples. Samples were spiked with 0.5, 1, 3, 5, 7, 9 wt% of water. The other parameters were set at 60^{o}C, 400 rpm and 0.1 N sodium hydroxide catalyst. The time of the reaction was 1 hour and the methanol-oil molar ratio was 400:1.

3.2.5 Catalyst Type

A comparison was made between an alkali-based catalyst (sodium hydroxide), an acid-based catalyst (sulphuric acid) and a methoxide catalyst (sodium methoxide). All of the catalysts were utilised in the *in situ* transesterification process with 10 g of *J. curcas* seeds. The other parameters were set at a molar ratio of methanol-oil of 400:1, an agitation speed of 400 rpm, a reaction temperature of 60^{o}C, and a reaction time of 1 hour. Sodium hydroxide was tested in three different concentrations of 0.05 N, 0.1 N and 0.2 N. Sodium methoxide was examined in four different concentrations of 0.025 N, 0.05 N, 0.01 N and 0.2 N, while sulphuric acid was only tested at one concentration, 0.08 N.

3.3 Modifying the *In situ* Transesterification Process

3.3.1 Co-solvent Experiments

As shown in Table 3.4, the effect of adding co-solvents to the *in situ* transesterification process was examined by using two solvents, hexane and dimethoxymethane (DEM) (Sigma Aldrich, UK). Both solvents were used at three different solvent/oil molar ratios; 10, 30 and 50:1. The minimum methanol/oil molar ratio investigated was 25, whilst the maximum was 100. Sodium hydroxide concentration was fixed at 0.1 N. Experiments with hexane were conducted at 60^{o}C whilst those with DEM were at 40^{o}C. The reactions were monitored continuously for 60 minutes.

Table 3.4. Experiment matrix for investigation of hexane and DEM as co-solvents for the *in situ* transesterification process

Co-solvent	Methanol/ Oil molar ratio	Co-solvent/oil molar ratio
Hexane Reaction temperature: 35^{O}C NaOH concentration: 0.1 N Reaction time: 60 min	50	10
		30
		50
	100	10
		30
		50
	200	10
		30
		50
DEM Reaction temperature -35^{O}C NaOH concentration– 0.1 N Reaction time - 60 min	50	10
		30
		50
	100	10
		30
		50
	200	10
		30
		50

3.3.2 Methyl Acetate as a Reactant

Sodium methoxide (CH_3NaO) was used as a catalyst in this experiment and because it is insoluble in methyl acetate, a suitable co-solvent to dissolve it was introduced, polyethylene glycol (PEG) was used for this purpose following the work by Casas and co-researchers [64]. The *in situ* transesterification reaction was conducted as mentioned in section 3.2. The bottle was charged with a pre-determined amount of polyethylene glycol (PEG) and the corresponding amount of CH_3NaO catalyst was then added to the liquid. To dissolve CH_3NaO in the PEG, the bottle was then placed in the incubator at 50°C and 400 rpm for 5 minutes. After this dissolution period, the pre-measured volume of methyl acetate and 10g *J. curcas* were added to the catalyst mixture. The reaction was then treated at 50°C and 400 rpm and left for a duration of 90 minutes.

Table 3.5 Factors involved in experiment with their respective levels

Factor	Description	Level			Units
		-1	0	+1	
A	Molar ratio of PEG/catalyst	3:1	51.5:1	100:1	-
B	Molar ratio of methyl acetate/oil	50:1	175:1	300:1	-
C	Catalyst concentration	0.05	0.13	0.20	mol/L

A Design of Experiments (DOE) matrix was employed to determine the effect of the selected process parameters on the yield of FAME. Three factors were considered to be independent variables: the molar ratios of polyethylene glycol to catalyst and methyl acetate to oil, and catalyst concentration, as shown in Table 3.5.

3.4 Phenol, Protein and Soap Analysis

3.4.1 Phenol

The method described by Singleton *et. al.,* [101] was used to determine the amount of phenol present throughout the reaction. The analysis required a ultra violet-visible spectrophotometer (UV-VIS) at 760 nm. The UV-VIS sample was prepared by adding 50 mg of reaction sample to 35 mL of distilled water and 2.5 mL Follin-Ciocalteu reagent in a 50 mL volumetric cylinder. The mixture was left for three minutes, where upon 7.5 mL of 20% sodium carbonate solution was added. Distilled water was then added up to the 50 mL mark. The mixture was left again for two hours before analysis using the UV-VIS spectrophotometer.

3.4.2 Protein

The protein analysis was carried out by determining the nitrogen content via elemental analysis in a carbon-hydrogen-nitrogen analyser (CHN) (Perkin Elmer, UK). The analysis

was conducted with fresh seeds before the reaction and seeds after reaction. The amount of nitrogen was multiplied by a factor 5.53 to derive the amount of protein in the sample [102].

3.4.3 Soap

The amount of soap was quantified for use in the mass balance calculation. The soap analysis procedure was adapted from the Official Methods and Recommended Practices of the AOCS [103]. Acetone (2 % vol. water) (Sigma Aldrich, UK) was prepared and 0.5 mL of an indicator, bromophenol blue (Sigma Aldrich, UK), was added to it. The solution was titrated with 0.1 N hydrochloric acid (HCl) solutions until the acetone became yellow.

Both ester and glycerol phases after the reaction were analysed. A 5g sample was used from the ester phase whilst 0.5g was taken from the glycerol phase. Each sample was put into a test tube and 1 mL of water was added. 50 mL of neutralised acetone was then added. The mixture was titrated with 0.1 N HCl slowly until the colour changed from blue to yellow. The total volume of HCl was recorded as mL_a.

The amount of soap was calculated using Equation 3.6

$$Percentage\ of\ soap\ (wt\%) = \frac{Volume\ of\ HCl, ml_a\ X\ 0.1N\ X\ MW\ Sodium\ Oleate\ (304.4)}{1000\ X\ sample\ mass\ (g)}$$

Equation 3.6

3.5 Analytical Methods

Throughout the study, a number of analytical instruments were used to analyse the results. Gas chromatography (GC) was used to provide data on FAME yield and mass percentage,

whilst gas chromatography equipped - mass spectrometry (GC-MS) was used to analyse levels of mono, di and triglycerides and glycerol. Seed morphology was investigated using a light microscope and Scanning Electron Microscope (SEM).

3.5.1 Gas Chromatography – Total Ester Mass Fraction Calculation

A flame ionisation detector (FID) HP5890 Series II (Hewlett Packard, USA) gas chromatograph fitted with a BPX70 column, 30 m long x 0.32 mm ID x 0.25 μm film thickness (SGE, Australia) was used to analyse the samples. Helium was used as the carrier gas at a pressure of 7 psi and oven temperature was maintained at 230°C for 30 minutes.

The data was acquired and processed using Clarity Chromatography Station for Windows (DataApex, Czech Republic). This software allowed the integration of peaks on the chromatogram to be performed.

An internal standard, methyl heptadecanoate solution was prepared prior to sample preparation. To prepare the solution, methyl heptadecanoate was weighed to approximately 500 mg in a 50 mL volumetric flask. Heptane was then added up to the 50 mL mark.

A 250 mg of the sample was then weighed and placed in the vial, before 5 mL of methyl heptadecanoate solution was added. The mixture was mixed thoroughly using a MS1 Minishaker (IKA, Germany). 1 μL of the sample was then injected into the GC using a 5 μL microsyringe (SGE, Australia).

The total methyl ester mass fraction was calculated according to the guidelines given by British Standards Institution (BS EN 14103:2003). The chromatogram obtained was treated

before it could be used in the equation. Integration of the peaks was performed in order to eliminate the solvent peak from the calculation. The ester content in the sample, C, expressed as a mass fraction percentage, was then calculated using Equation 3.7 below:

$$C = \frac{\sum A - A_{EI}}{A_{EI}} \times \frac{C_{EI} \times \mathrm{V_{EI}}}{\mathrm{m}} \times 100\%$$

Equation 3.7

where

$\sum A$ = total peak area from the methyl ester $C_{14} - C_{24:1}$

A_{EI} = peak area corresponding to methyl heptadecanoate

C_{EI} = concentration in mg/mL of the methyl heptadecanoate solution

V_{EI} = volume in mL of the methyl heptadecanoate solution used

m = mass of the sample (mg)

3.5.2 Gas Chromatography Technique – Calculation of Methyl Ester Mass

From Equation 3.7, it can be observed that the calculation of FAME yield is dependent upon the mass of the ester phase, which was obtained after numerous downstream processing steps and because these steps were conducted manually, it was very difficult to maintain accuracy for each run. This method did however, allow the mass of methyl ester to be calculated directly from step 3 (as shown in Figure 3.3) and therefore any inaccuracy from the downstream processing was minimised. This was crucial since a majority of the experiments involved small amounts of raw material.

The difference between this technique and the one described in Section 3.5.1 above is the chemical used as the internal standard. In this technique, methanol was used instead of heptane to dissolve the methyl heptadecanoate. This was because the sample now consisted of a mixture of methanol and methyl ester. This mixture could dissolve in an internal standard with methanol but not with heptane because of the difference in polarity between the two solvents.

In preparing the sample for injection, 1 mL of internal standard stock solution was used instead of 5 mL. Equation 3.7 still applies in calculating the mass fraction of the methyl ester in the sample, C. The mass of methyl ester was then calculated by multiplying C by the mass of the total filtered mixture (step 3, Figure 3.3) as in Equation 3.8:

$$Methyl\ ester\ mass\ (g) = C\ (\%)\ \times Mass\ of\ total\ filtered\ mixture\ (g)$$

Equation 3.8

This value was then used in Equation 3.7 to calculate the weight percentage of the FAME yield.

3.5.2.1 Validity of the Technique

A series of tests were conducted to validate the technique. A known methyl ester mass was dissolved in methanol and then injected into the GC. The C value (from Equation 3.7) was calculated and multiplied by the mass of methanol and methyl ester (Equation 3.8). The result was then compared with the actual methyl ester mass.

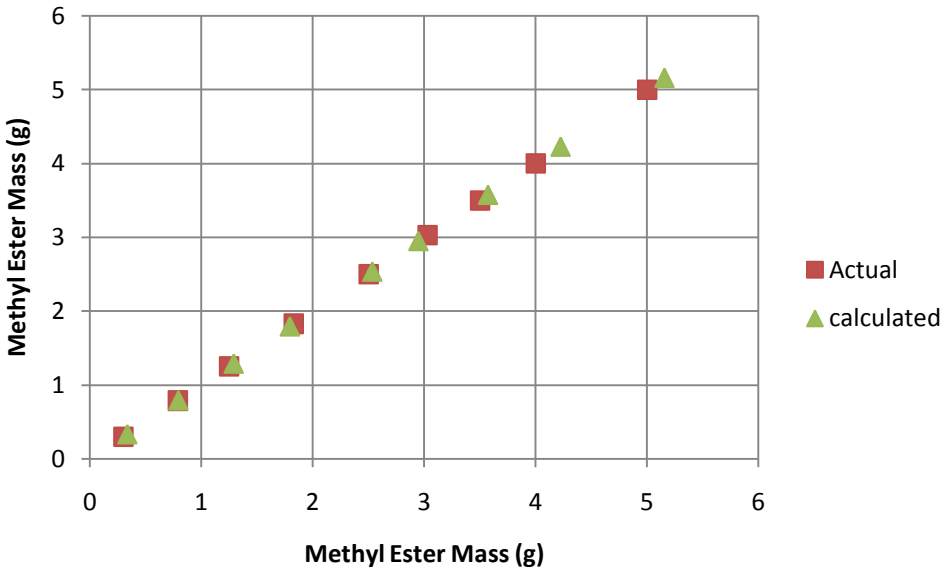


Figure 3.5 Comparison of actual methyl ester mass with calculated methyl ester mass

The comparison was made using with methyl ester ranging from 0.3g to 5g. Figure 3.5 shows that the agreement between actual and calculated values was very good (R^2=0.99). Therefore, it was concluded that this technique was reliable to use in calculating the mass of methyl ester dissolved in methanol.

3.5.3 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was used to determine the total glycerol and mono-, di-, and triglyceride content in the samples. In this procedure, N-methyl-N-trimethylsilyfluoraacetamide (MSTFA) (Sigma Aldrich, UK) was used to transform all the components into silylated derivatives, which are more volatile. The procedure is explained in detail by the British Standards Institution (BS EN 14105:2003).

Two internal standard solutions were prepared prior to the analysis. The first internal standard (IS 1), 1,2,4-butanetriol (Sigma Aldrich, UK), was added for the determination of the free

glycerol whilst the second internal standard (IS 2), 1,2,3-tricaproylglycerol (Sigma Aldrich, UK), also known as tricaprin, was used in the determination of the glycerides. Both were prepared using pyridine (Sigma Aldrich, UK).

Calibration curves for all the compounds (glycerol, monoglycerides, diglycerides and triglycerides) must be created before calculating the concentrations of these components. The calibration curves were created by calculating the ratio of the components' area to the internal standard area. Table 3.6 shows the mixing proportion of each component in preparing the calibration curves.

Table 3.6 Calibration solutions mixing proportion

Solution	1	2	3	4
Glycerol (µl)	10	40	70	100
Monoolein (µl)	50	120	190	250
Diolein (µl)	10	40	70	100
Triolein (µl)	10	50	100	200
IS 1 (µl)	80	80	80	80
IS 2 (µl)	100	100	100	100

3.5.3.1 Calibration of Glycerol and Glycerides

Equation 3.9 below was used to create a calibration curve for glycerol. For the quantification of glycerol, the concentration of glycerol stock solution used was 0.5 mg/mL (Sigma Aldrich, UK). It was prepared by mixing 50 mg of glycerol with pyridine in a 10 mL volumetric flask. 1 mL of this solution was then put into another 10 mL flask. Pyridine was added to make it

up the 10 mL mark. The concentration of stock solution for IS 1 was 1 mg/mL whilst for IS 2 it was 8 mg/mL. Both were prepared by dilution in pyridine.

$$\boldsymbol{M_g/M_{ei1} = a_g(A_g/A_{ei1}) + b_g}$$

Equation 3.9

Where:

M_g = mass of glycerol (mg)

M_{ei1} = mass of internal standard No 1 (mg)

A_g = peak area of glycerol

A_{ei1} = peak area of internal standard No 1

a_g and b_g = constants from regression method of glycerol

The calibration curves for the glycerides were calculated from Equation 3.10 for monoglyceride, Equation 3.11 for diglyceride and Equation 3.12 for triglyceride.

For monoglyceride, the calibration curve was prepared by injecting a 4 monoolein stock solution with the amount indicated in Table 3.6. The concentration of the monoolein stock solution was 5 mg/mL (Sigma Aldrich, UK), whilst the concentration of IS 2 was 12.6 mg/mL.

For diglyceride and triglyceride, the concentration of standard di- and triolein stock solutions prepared were 5 mg/mL (Sigma Aldrich, UK). The IS 2 concentration was 0.5 mg/mL.

$$\boldsymbol{M_m/M_{ei2} = a_m(A_m/A_{ei2}) + b_m}$$

Equation 3.10

$$\boldsymbol{M_d/M_{ei2} = a_d(A_d/A_{ei2}) + b_d}$$

Equation 3.11

$$\boldsymbol{M_t/M_{ei2} = a_t(A_t/A_{ei2}) + b_t}$$

Equation 3.12

Where:

$M_{m,} M_{d,} M_t$ = mass of the monoolein, diolein and triolein (mg)

M_{ei2} = mass of internal standard No 2 (mg)

$A_{m,} A_{d,} A_t$ = peak area of monoolein, diolein and trioleion

A_{ei2} = peak area of internal standard No 2

A_m and b_m = constants from regression method of monoglycerol

A_d and b_d = constants from regression method of diglycerol

A_t and b_t = constants from regression method of triglycerol

All the calibration curves were calculated using linear regression and were only regarded as acceptable when the correlation coefficient was found to be equal to or higher than 0.95.

3.5.3.2 Sample Preparation for Monoglyceride

For monoglyceride quantification, a 20 mg of the sample was mixed with 20 µl of IS 2 (12.6 mg/mL). 20 µl of N-methyl-N-trimethylsilyfluoraacetamide (MSTFA) was then added and the mixture was left for 15 minutes at room temperature to allow it to silylate. 2 mL of heptane (Sigma Aldrich, UK) was added after 15 minutes and 0.5 µl was injected into the GC-MS.

3.5.3.3 Sample Preparation for Diglyceride and Triglyceride

Because the detection limits for diglyceride and triglyceride are lower than that for monoglyceride, each sample was injected twice in order to quantify all three components. For diglyceride and triglyceride, 20 µl of sample was added to 100 µl of IS 2 (0.5 mg/mL). 20 µl of MSTFA was then added and left to silylate for 15 minutes. 0.5 mL of heptane was added to the mixture after 15 minutes and 4 µl was injected into the GC-MS.

3.5.3.4 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS used was a Perkin Elmer Clarus 500 and 560D (Perkin Elmer, UK) which was fitted with a Perkin Elmer Col-elit column (PE-5HT) (Perkin Elmer, UK). The column dimensions were 15 m in length, 0.25 µm internal diameter and 0.1 µm film thickness. Helium was used as the carrier gas at a flowrate of 62 cm^3/s. The GC oven was set at 50^oC for 1 minute, then heated to 180^oC at a rate of 15^oC/min, then to 230^oC at a rate of 7^oC/min and finally to 370^oC at a rate of 10^oC/min. At 370^oC, the temperature was held for 10 minutes, giving a total run period of 31.5 min. The injector was set at 350^oC and the detector at 370^oC. The temperatures of the MS source and MS inlet line were 250^oC and 270^oC respectively.

3.5.3.5 Identification of the Peaks

The identification of the peaks was determined by comparing *J. curcas* oil peaks to the relative retention times of the standards. Figure 3.6 is the chromatogram of *J. curcas* oil under conditions described in Section 3.5.3. The peak at 5.02 minutes was the first internal standard peak (IS 1). From 11.13 to 12.63 minutes, two different trimethylsily acids were identified. The peak at 11.13 minutes was hexadecanoic acid, also known as palmitic acid. The other fatty acid, whose peak was between 12.60 and 12.85 minutes was stearic acid.

The next peak at 17.85 minutes was monooleoglycerol. The peak for IS 2 eluted at 22.92 minutes. This was followed by peaks from 25.86 to 26.99 minutes, which were diglyceride.

Starting from 29.49 minutes onwards, the peaks represent different components of triglycerides. Peaks at 29.49, 31.31 and 32.85 minutes represent octadecenoic acid, and those at 35.35 and 38.36 minutes both represent trilinolein.

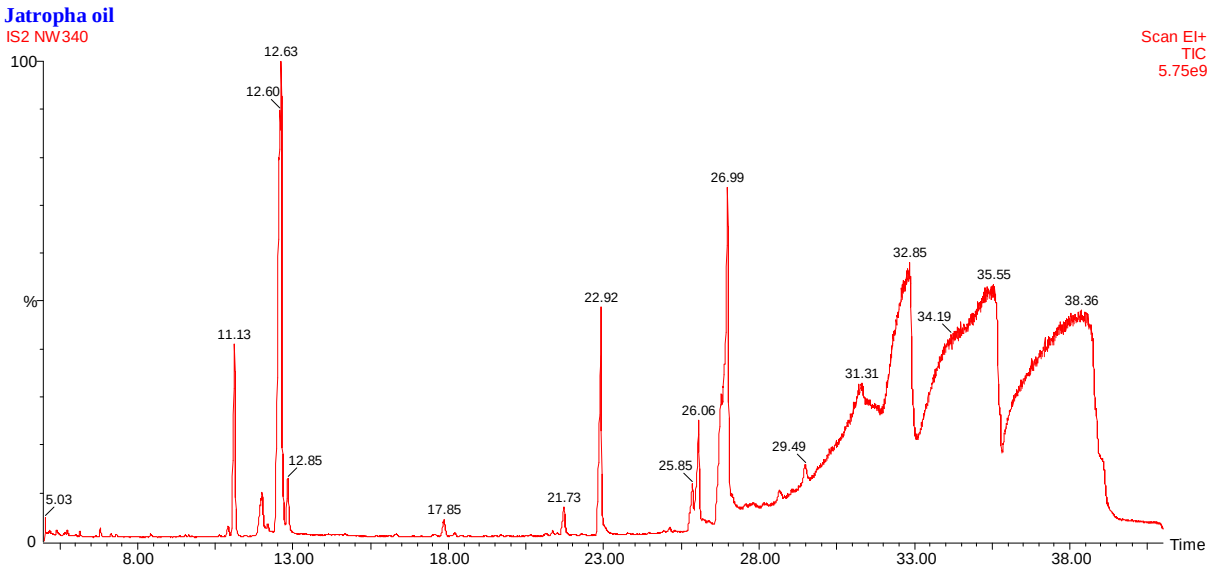


Figure 3.6 Chromatogram of *J. curcas* oil methyl ester

3.5.4 Seed Morphology

The effect of the reaction on the seed particles' morphology was evaluated using light microscopy and Scanning Electron Microscopy (SEM).

3.5.4.1 Light Microscopy

Sections of the seed were cut and mounted on glass slides. The protocol was carried out by Newcastle University Electron Microscopy Research Services. To observe the lipids, the glass slides were immersed in Sudan Black B solution. This stained the lipids and made them visible under the microscope. The Sudan Black B was prepared by mixing Sudan Black B powder in 70% ethanol until it became saturated. The solution was then filtered and diluted with five parts of 70% ethanol.

The excess Sudan Black B solution on the slides was removed by washing with 90% ethanol, followed by air drying. The slides were then examined by Olympus BX41 light microscope

equipped with a Sony camera. The captured images were processed using the Altra20 Soft Imaging System.

3.5.4.2 Scanning Electron Microscope (SEM)

The procedure was carried out by Advanced Chemical and Materials Analysis (ACMA), Newcastle University. The seeds were dried in a desiccator for 48 hours prior to analysis, and were then coated in gold and scanned using high vacuum in a FEI XL30 ESEM-FEG microscope (FEI, The Netherlands) at 500X magnification.

3.6 Mass Balance Calculation

The total amount of triglyceride available for the reaction was calculated from the mass of oil in *J. curcas*. This mass (M_T) was obtained from the Soxhlet extraction as described in Section 3.1 above.

The product of the reaction was two separate layers of liquid. The upper layer contained methyl ester and methanol and the bottom layer consisted of a mixture of glycerol, NaOH, acetic acid and other methanol-extracted products. The product was placed in a separating funnel and the bottom layer drained out. The upper layer was then washed with hexane (10 mL) three times. Another layer appeared, separating the hexane and methyl ester (non-polar) and methanol (polar). The non-polar layer was then taken out and the hexane removed using a vacuum evaporator. The remainder, which was the methyl ester, was weighed using an A&D HR-200 (A&D, Japan) scale and compared with the mass obtained from the method described in Section 3.5.2. This was marked as M_{ME}

The seed meal after Soxhlet extraction was dried in the oven to remove the methanol and then re-extracted to quantify the mass of oil left in the seed. This was marked as M_R. The sum of M_R and M_{ME}, should theoretically be the same as the total oil, M_T, as shown in Equation 3.13.

$$M_T = M_{me} + M_R$$

Equation 3.13

The bottom layer of the product of *in situ* transesterification process contained a mixture of various compounds, therefore it was difficult to quantify the amount of glycerol experimentally. To overcome this, the amount of glycerol was calculated using the stochiometric ratio of ester to glycerol, which is 3 to 1.

$$\boldsymbol{Glycerol\ mass(M_G) = \frac{Mole\ of\ Methyl\ Ester\ X\ MW\ of\ Glycerol\ (92.09)}{3}}$$

Equation 3.14

The difference between the mass of the extraction (M_T) and the combination of methyl ester, glycerol mass was then defined as the mass of other components (M_O) as in Equation 3.15.

$$M_O = M_T - (M_{ME} + M_G)$$

Equation 3.15

The mass of other components includes the combined masses of catalyst (M_C), acetic acid added for neutralisation (M_{AA}), soap (M_S) and phenol (M_P). Equation 3.16 shows the compositions of the mass of other components.

$$M_O = M_C + M_{AA} + M_S + M_P$$

Equation 3.16

4 RESULTS AND DISCUSSION

The results of all of the experiments conducted in this research are presented and discussed in this chapter. The chapter starts with the results for characterisation of *J. curcas*. To select the catalyst most suitable for the raw material and the process involved, three different catalysts were subjected to the screening process. After the selection of the catalyst, each parameter involved in *in situ* transesterification was investigated using a "one-at-a-time" method. The information yielded by that experiment was then used as the basis for the next set of experiments, based upon a "design of experiments" approach. Attempts to reduce the amount of alcohol required using a co-solvent are discussed in section 4.6.1. Findings on the use of methyl acetate instead of methanol are described in section 4.6.2. The chapter ends with a discussion of biorefining aspects of this process.

4.1 *J. curcas* Characterisation

There are two parts to a *J. curcas* seed, the kernel and shell. Fat (54%) and protein (25%) are the two main types of compound in kernel, whilst fibre (87%) is the main component of the shell [70]. The characteristics of the *J. curcas* used in this research are shown in Table 4.1 below.

The oil content of the *J. curcas*, at 36.0 ± 0.2%, tested was 4% less than that reported by Azam *et. al.*, [78] by 4%. The oil content of seeds depends on many factors such as soil characteristics, fertilisers, irrigation and annual rainfall [70, 104, 105]. The FFA content was 9.2 ± 0.2%, which is considered unsuitable for conventional biodiesel production with alkali catalyst. The fatty acid content analysis revealed that 80.4% of the fatty acids in the oil were

unsaturated, which is desirable for biodiesel, since oils with high saturated fatty acids contents, such as palm oil, produce biodiesels with CFPPs that are too high.

Table 4.1. Main characteristics of the *J. curcas* used as the raw material.

Test	Assay method	Unit	
Oil content	BS 659: 2008	%	36.0 ± 0.2
Moisture content	BS 665:2000	%	7.4 ± 0.2
FFA content	BS 660:2009	%	9.2 ± 0.2
Acid value	BS 660:2009	mg/g KOH	18 ± 0.5
Fatty acids content	BS 684:2001 + BS 15304	%	C16:0 (13.6), C18:0 (6.0), C18:1 (42.3), C18:2 (38.1)

4.2 The Water Tolerance of *In situ* Transesterification of *J. curcas*

It has been reported that the presence of moisture in seeds significantly reduces the methyl ester yield when soybeans are used as the raw material [53]. In alkaline-catalysed transesterification, the presence of water in the system promotes saponification rather than the transesterification reaction during the process, promoting soap rather than methyl ester formation, thereby reducing yield, and rendering downstream separations more difficult. This phenomenon was, however, proven to be seed-specific to some extent, as other researchers have found that for rapeseed, the presence of water in the system did not significantly affect the methyl ester yield [55].

To determine the effect of moisture on the process when *J. curcas* seed was used as raw material, two sets of sample, dry and wet were *in situ* transesterified at three different molar ratios. "Dry" refers to samples which underwent a drying process in which the samples were placed in the oven until the samples' mass were constant, prior to the experiments, whereas wet samples were not. The effect of dry and wet samples to the FAME yield at different molar ratios was shown in Figure 4.1, below. At all ratios, the FAME yields of experiments with dry samples were higher than in the wet samples, indicating that the presence of moisture did affect the yield. However, the magnitude of the effect was minimal: the biggest difference was 4%, occurring at a molar ratio of 300. The difference between 400 and 500 molar ratio was 1.2%. In order to determine the level of significance of such differences, a T-test analysis was performed on the results.

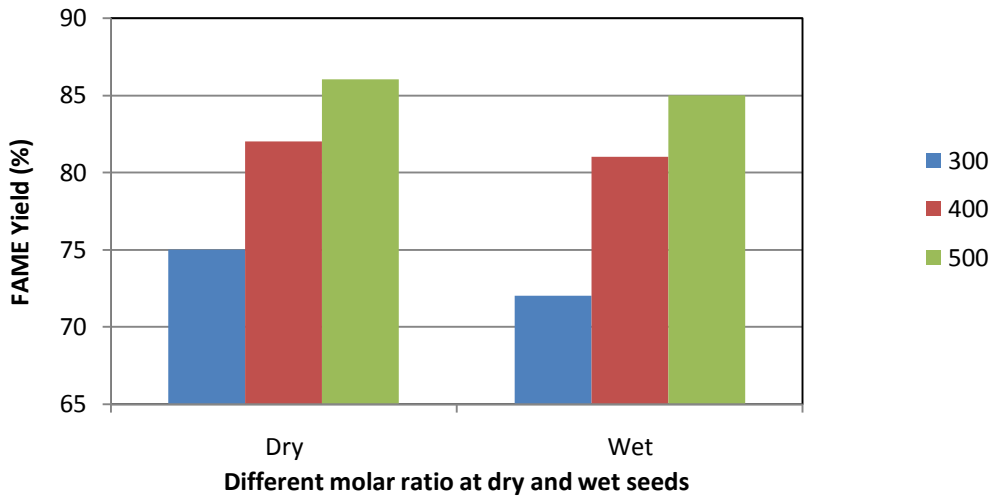


Figure 4.1 Drying effect on FAME yield at different molar ratios. NaOH concentration = 0.1 N, reaction temperature = 60^{O}C, reaction time = 1 hr, agitation speed = 400 rpm, seed size <0.71 mm.

It was found that, in this process, the drying of the *J. curcas* seed was not significant with respect to FAME yield, as the p-value derived was 0.318 which is greater than the significance level of 0.05. This finding is in agreement with that of Zakaria [55], but contrasts

with work by Haas and Scott [53], which reported that after drying the soybeans prior to the experiments, the amount of methanol needed to achieve maximum FAME yield decreased by 60%. Therefore, it is clear that the necessity for drying depends on the seeds used. This result has a significant effect on the overall process, especially in terms of energy use reduction associate with the drying stage.

The finding is significant since it can affect the overall *in situ* transesterification process. It would reduce the number of process steps required, because the process is tolerance towards the water content in *J. curcas*.

4.3 Catalyst Screening

I*n situ* transesterification process uses homogenous catalysts. In this work, three different catalysts, namely sodium hydroxide (NaOH), sodium methoxide (CH_3NaO) and sulphuric acid (H_2SO_4), were compared and contrasted. Sodium methoxide was investigated as a substitute for sodium hydroxide mainly because it provides more direct way of producing the methoxide ions. Furthermore, the preparation of methoxide ions from sodium hydroxide and methanol produces water as a by-product, as shown in Equation 4.1.

$$OH^- + CH^3OH \rightarrow CH_3O^- + H_2O$$

Equation 4.1

The formation of water is critical in the conventional transesterification process, since it will initiate triglyceride and methyl ester hydrolysis, which will convert those components into free fatty acids and subsequently to soap. This has been shown to affect the FAME yield significantly[106]. NaOH and CH_3NaO are compared in Figure 4.2 below.

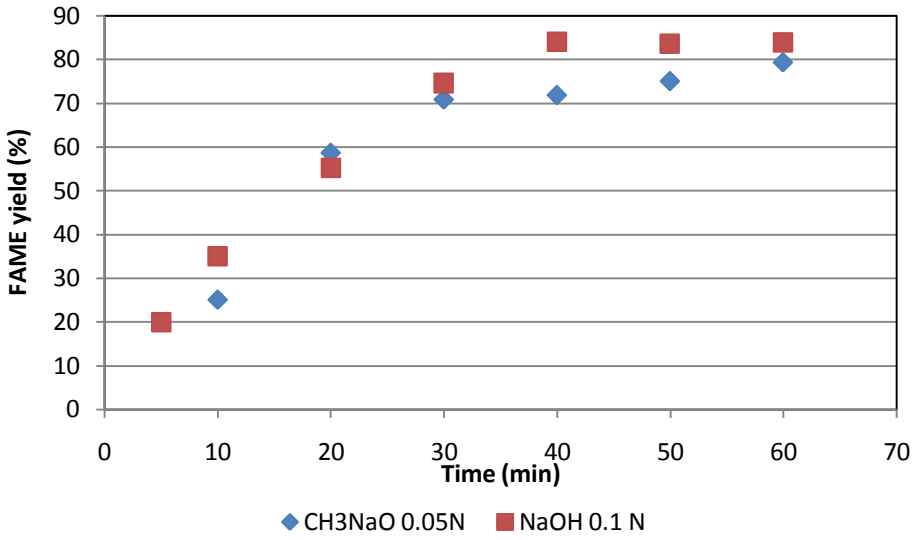


Figure 4.2 Comparison of sodium methoxide and sodium hydroxide as catalysts in the process. Reaction temperature = 60^{O}C, agitation speed = 400 rpm, seed size <0.71 mm.

In this case, however, both catalysts exhibited very similar trends during the reaction. As discussed in the section 4.2, the presence of water has an insignificant effect on the *in situ* transesterification of *J. curcas* seeds up to a few percent, because of the excess of methanol in the system.

When sulphuric acid was employed as a catalyst in the reaction, the reaction rate was significantly lower, as shown in Figure 4.3. This finding agrees with Shuit *et. al.*, [34], who found that the *in situ* transesterification of *J. curcas* with an acidic catalyst took 24 hours to reach equilibrium. However, they also reported that the maximum FAME yield achieved was higher at between 95-99% compared to 83% with sodium hydroxide.

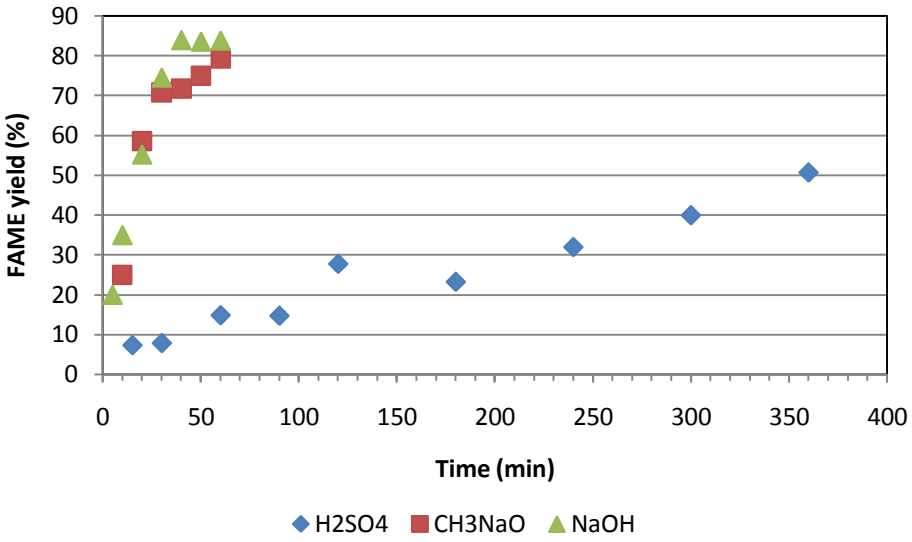


Figure 4.3 Comparison of sulphuric acid, sodium methoxide and sodium hydroxide as catalysts in the process. Reaction temperature = 60^{O}C, agitation speed = 400 rpm, seed size <0.71 mm.

In situ transesterification has the same reaction mechanism as conventional transesterification: the triglyceride is converted to diglyceride, monoglyceride and finally glycerol with an ester liberated at each stage. Therefore, due to reaction via electrophilic attack in the acid-catalysed reaction against nucleophilic attack in the base-catalysed, the activity of acid-catalysed transesterification was much slower than that of base-catalysed transesterification [107-109]. Figure 4.4 and Figure 4.5 illustrate the chemical pathway for both acid and base-catalysed transesterification respectively. The important step in acid-catalysed transesterification is the protonation of the carbonyl oxygen, as in step 1, Figure 4.4. The protonation exposes the adjoining carbon atom to nucleophilic attack, as the electrophilicity of that carbon atom increases (step 2). The tetrahedral intermediate formed after the necleophilic attack then breakdown because of proton migration [108].

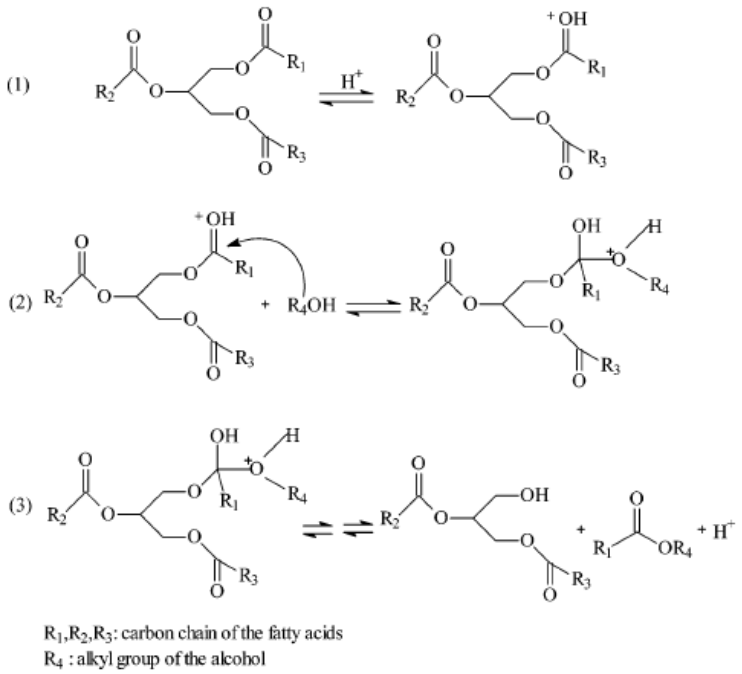


Figure 4.4 Chemical pathway for acid-catalysed transesterification [108]

In the base-catalysed reaction, a strongly nucelophilic alkoxide ion is formed directly, as illustrated in step 2 in Figure 4.5, below. The ion then attacks the carbonyl group on triglyceride (step 3) to form tetrahedral intermediate, which then breaks down to form diglyceride and ester [108].

This explains the findings in the above experiments, where for acid-catalysed transesterification, 360 minutes was required to get 50% FAME yield, whilst base-catalyst only needed less than 20 minutes to reach the same point.

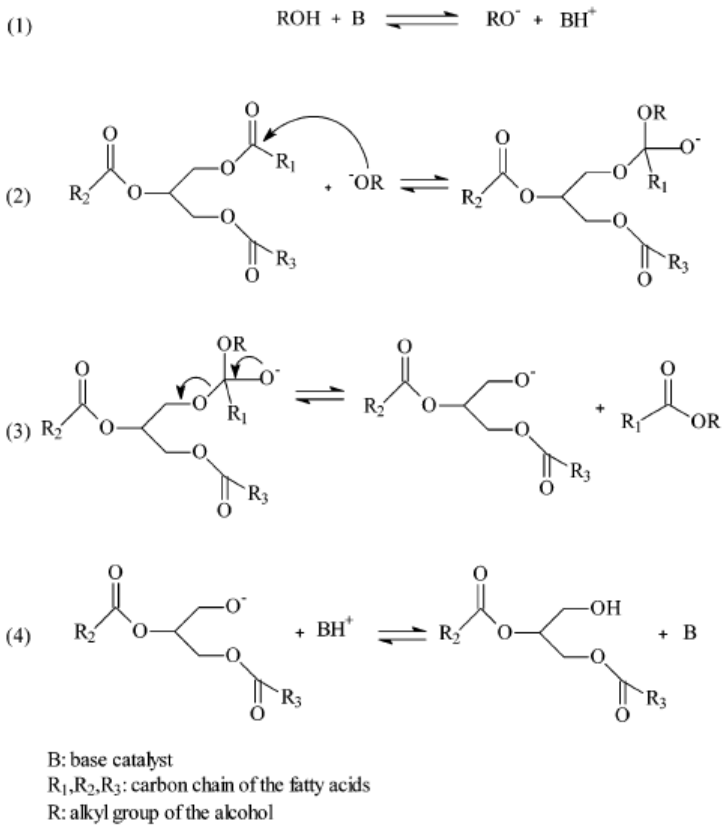


Figure 4.5 Chemical pathway for base-catalysed transesterification [99]

4.4 Study of Individual Parameters

It was suspected that a number of parameters would have some effect on the *in situ* transesterification reaction. This section discusses the results of individually investigating particle size, mixing speed, reaction temperature, reaction time, catalyst concentration and molar ratio of methanol-oil.

4.4.1 Particle Size

Figure 4.6 shows that the yield of methyl ester decreases with increasing particle size when it is larger than a threshold value of around 0.71 mm. No significant difference was found between the yields for the smallest two particle size groups, which were <0.5 mm and 0.5 –

0.71 mm, with the former yielding 86.1% and the latter 83.7%. The largest particle size range of 2–4 mm produced the lowest yield, at 35.5%.

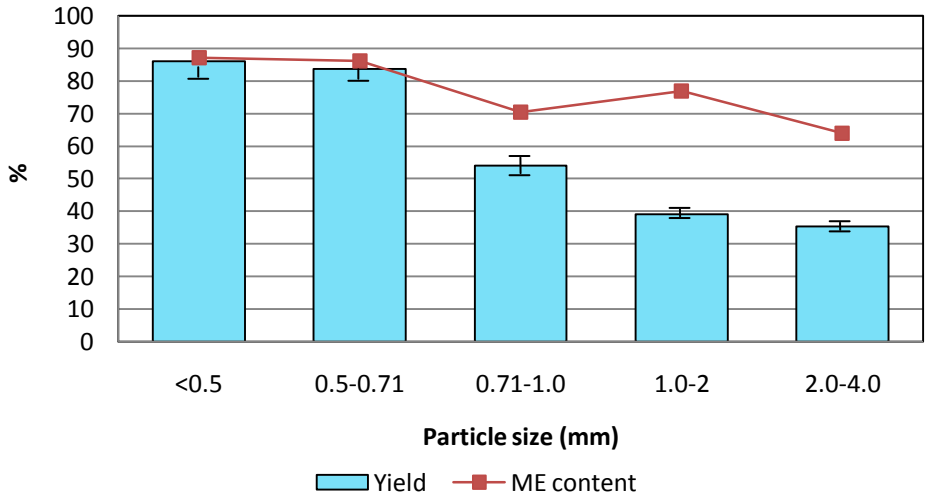


Figure 4.6 Percentage of methyl ester yield and methyl ester content for various particle sizes. Alcohol to oil ratio = 400:1; NaOH concentration = 0.1N; reaction temperature = 60^{o}C; mixing speed = 400 rpm; reaction time = 1 hr

The diffusion of fluid to solid particle is controlled by various factors, among others are the boundary layer, the reaction itself [110] as well as thermodynamics of the system, in this case the free entropy. The boundary layer decreases with decreasing particle size and increasing fluid velocity [111]. In this research, the seed particles were surrounded with a thin boundary layer, because the velocity of the fluid was high at 400 rpm, and therefore has small external resistance to the diffusion. As the boundary layer is small, the internal diffusion becomes the rate-limiting step.

In the results, it was clear that the yield increased as particle size reduced, from 2.0-4.0 mm particle size group to 1.0-2.0 mm and 0.71-1.0 mm. This is because the time needed for the methanol-sodium hydroxide mixture to diffuse inside the seed particle is longer than the time for the reaction to occur on the interior surface. At the smaller particle size groups of <0.5

mm and 0.5-0.71 mm, the mixture takes less time to diffuse into the particle, and thus, internal diffusion control is reduced.

This finding is consistent with those reported in the literature, where the authors observed the increased of oil dissolved in methanol with the decreased of soybean particle size [39]. They also claimed that further decreased of the soybean size from it optimum point, 0.3 mm, has negligible effect to the extraction of oil from the particle [39].

In terms of methyl ester content, only the two smallest particle sizes produced more than 80% of methyl ester in the sample. The other particle sizes produced lower methyl ester contents: size 0.71-1 mm gave 70.5% methyl ester, size 1-2 mm 77.0% and 2-4 mm 64.0%. No further analysis was conducted to determine other compounds in the samples, but the probable suggestion would be the presence of non-reacting glycerides in the form of mono-, di- and triglycerides [112, 113]. Because of settling and hexane washing, the chances are small of other compounds, such as glycerol and polar lipids being present.

4.4.2 Mixing Speed

From Figure 4.7, it is noticeable that the mixing intensity is not a rate-limiting factor once it reaches 300 rpm. This finding suggests that there is no point in increasing agitation beyond this level, as it will not lead to any significant improvement in yield. However, decreasing the speed to 200 rpm, and further to 100 rpm, decreased the yield from 94.8% to 85.7% and then further to 37.2%. At low agitation speeds, it was apparent that the distribution of seeds was not uniform. The seeds settled on the bottom of the reaction vessel and this reduced the biodiesel yield.

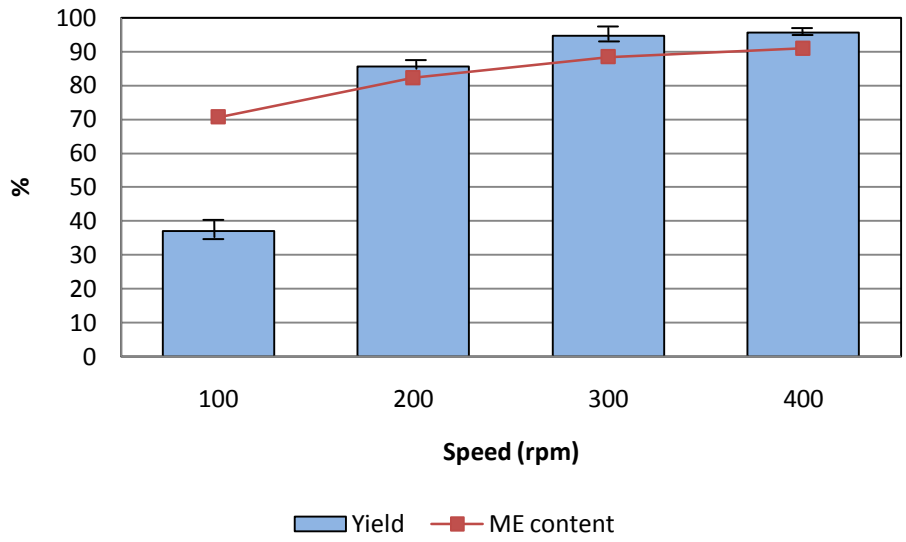


Figure 4.7 Plotted data for the effect of mixing intensity to *in situ* transesterification of *J. curcas.* Alcohol to oil ratio = 400:1; NaOH concentration = 0.1N; reaction temperature = 60°C; seeds size = <0.71 mm; reaction time = 1 hr

Transport of methanol-sodium hydroxide mixture from bulk into seed particles involves diffusion through an external boundary layer, which is a function of fluid velocity. The thickness of this boundary layer is in inverse proportion to the fluid velocity. At 100 rpm, where the velocity of the fluid was at its low, the FAME yield obtained was at the lowest, at 37.2%. The yield then increased to 85.7% when the fluid velocity increased with the increases of mixing speed from 100 rpm to 200 rpm. This indicates that at 200 rpm, the boundary layer was thinner than at 100 rpm. The resistance of this boundary layer, however, became negligible in comparison to other resistances in the system when the mixing speed was set at 300 rpm and 400 rpm.

4.4.3 Reaction Temperature

Four different temperatures (30, 40, 50 and 60°C) were used in *in situ* transesterification of *J. curcas* seeds. The data obtained are plotted in Figure 4.8 below.

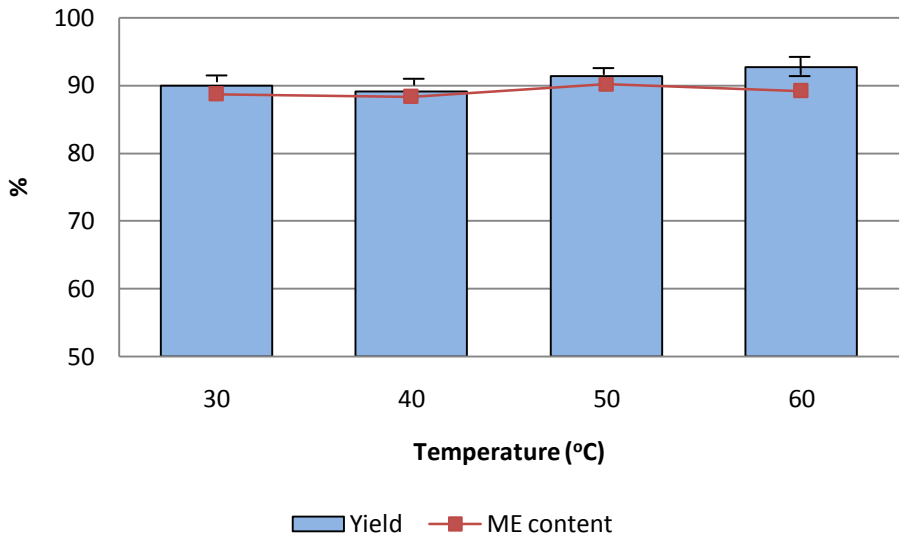


Figure 4.8 Methyl ester yield and methyl ester content of *J.curcas* seed at four different temperatures. Alcohol to oil ratio = 400:1; NaOH concentration = 0.1N; seeds size = <0.71 mm; mixing speed= 400 rpm; reaction time = 1 hr

After 1 hour, the temperature did not seem to have any significant effect on biodiesel yield. Although this result is in agreement with the observations of Haas and his co-workers that triglyceride can be converted to biodiesel at both low and high temperatures [21], it should be noted that probably at 1 hour reaction time, the *in situ* transesterification reaction already completed and therefore, the change in yields were unnoticeable.

However, to further understand the dependency of *in situ* transesterification on temperature, time profile experiments were conducted at 30, 40 and 60^{o}C. Figure 4.9 shows the time profile of methyl ester yield at those temperatures.

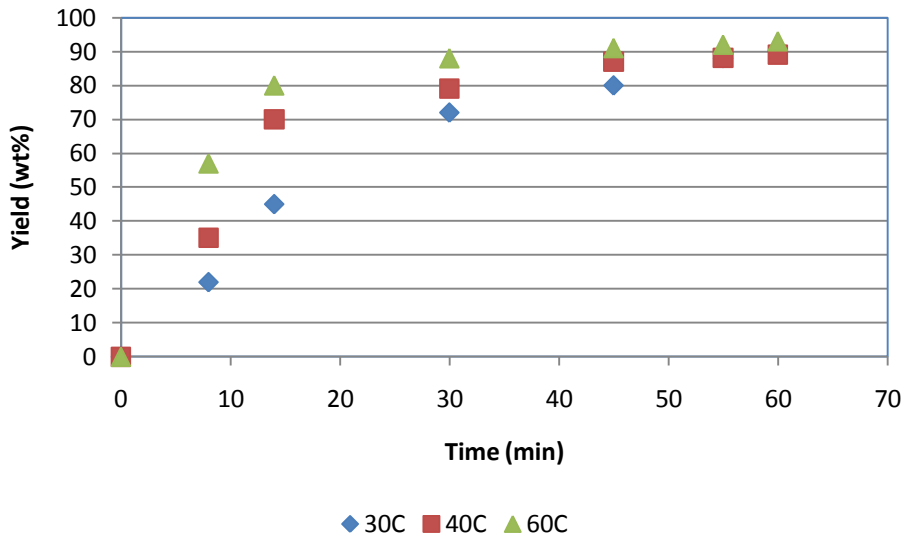


Figure 4.9 Time profile of methyl ester yield of *J. curcas* seed at three different temperatures. Alcohol to oil ratio = 400:1; NaOH concentration = 0.1N; seeds size = <0.71 mm; mixing speed= 400 rpm; reaction time = 1 hr

Examination of Figure 4.9 indicates that the rate of reaction increases with temperature, although the final equilibrium methyl ester yield for each temperature ended at almost similar point. The relationship between diffusivity and temperature in liquid-liquid phase, like in the triglyceride-methanol system can be described by Equation 4.2 [111].

$$D_{AB}(T_2) = D_{AB}(T_1)\frac{\mu_1}{\mu_2}\left[\frac{T_2}{T_1}\right]$$

Equation 4.2

where;

D_{AB} = diffusion coefficient of species from A to B (m2/s)

T_1 = temperature at initial condition (K)

T_2 = temperature at final condition (K)

μ_1 = viscosity of species at initial condition (cP)

μ_2 = viscosity of species at final condition (cP)

Using diglyceride diffusion coefficient at 60^{o}C as an example [55], the diffusion coefficients for 30, 40, 50 and 70^{o}C were calculated (Appendix 1) and plotted, to show the dependency of diffusion of diglyceride in methanol on temperature. The change in diffusion coefficient was predicted to increase exponentially with temperature [111].

4.4.4 Reaction Time

It has been shown that the *in situ* transesterification reaction is a "fast" reaction. Haas *et. al.*, [21], proved that the reaction produced 80% of FAME within 15 minutes, and reaction time up to 6 hours did not increase the yield significantly. However, based on the literature, this is only true with alkali-based catalyst since the *in situ* transesterification of *J.curcas* seeds with acid catalyst needs considerably more time to complete [34].

Figure 4.10 shows that the methyl ester yield exhibited minimal change from 30 minutes onward. It is therefore probable that the reaction was complete between 20 and 30 minutes. It can also be observed from Figure 4.10 that reactions of less than 20 minutes did not achieve the highest yields. This is in agreement with other findings which have reported rapid increases in yield within the first 30 minutes of the reaction [21, 39].

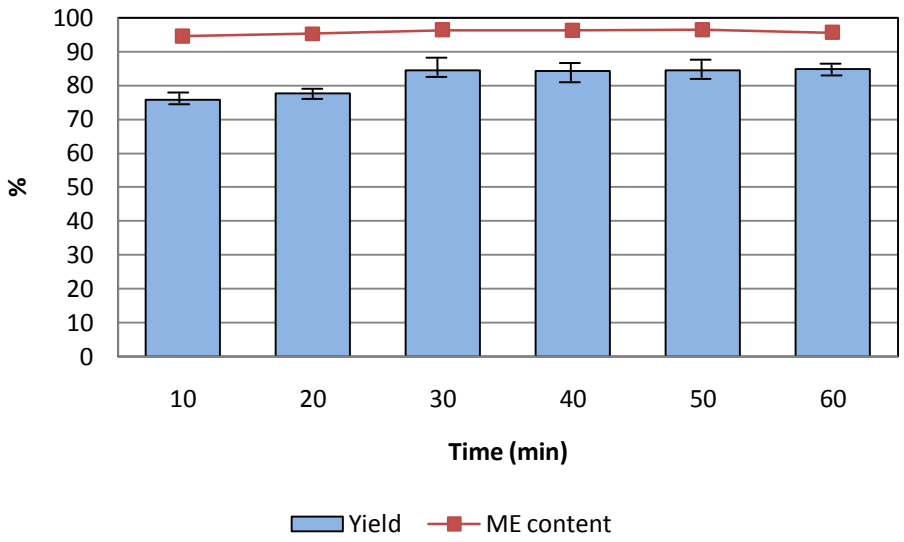


Figure 4.10 Methyl ester yield and methyl ester content of *J. curcas* seed at various reaction times. Alcohol to oil ratio = 400:1; NaOH concentration = 0.1N; reaction temperature = 60°C; seeds size = <0.71 mm; mixing speed= 400 rpm

4.4.5 Catalyst Concentration

The transesterification reaction does not proceed without a catalyst. A comparison of *in situ* transesterification with and without catalyst is presented in Table 4.2.

Table 4.2 Comparison of *in situ* transesterification with hexane extraction (8-hours) and methanol extraction. The condition of *in situ* transesterification: alcohol to oil = 400:1, mixing speed = 400 rpm, reaction temperature = 60°C, seeds size = <0.71 mm, reaction time = 2 hours.

Extraction method	Mass of oil extracted (g)	Extraction efficiency (%)	Methyl ester yield (%)
Hexane-soxhlet	5.53	100	0.0
Methanol-NaOH	4.66	84.3	81.9
Methanol only	0.8	14.5	0.0

Solvent extraction with methanol yielded some extract, but no methyl ester was detected in the samples. The extract thus probably consists of polar components such as phospholipids, which can be extracted by polar solvents [114]. The methanol-NaOH *in situ* transesterification produced 4.66g of a possible 5.53g of oil, and 3.8g (89.1%) of it was converted to biodiesel. In conventional transesterification, Om Tapanes *et. al.*, achieved a

96.3% yield using a 9:1 methanol alcohol ratio [75], indicating that if they started with the same amount of oil (5.53%), they would get 5.3g of biodiesel. However, they used refined, bleached, deodorized *J. curcas* oil, rather than the *J. curcas* seed, and each of the preliminary stages would be associated with a loss of yield, as well as implications for capital and running costs. These effects must be weighed against one another to determine the economic viability of this process.

The table above also shows that adding sodium hydroxide to methanol significantly increases extraction efficiency. Ren *et. al.,* [115] studied lipid content in rapeseeds during *in situ* transesterification with methanol, both with and without sodium hydroxide. In the latter case, lipid staining and microscopy clearly demonstrated that lipids were still present and the morphology of the seed was unchanged. With sodium hydroxide present, almost all of the lipids in the seeds were removed: the presence of catalyst is essential for *in situ* transesterification to take place. Furthermore, the oil-containing part of the seed clearly shrank as the reaction progressed, indicating that the reaction takes place largely within the seed.

Figure 4.11 presents the data from the present study of NaOH concentration. Three different catalyst concentrations were subjected to experimentation. The experiments with 0.25, 0.3 and 1.0 N NaOH concentration was also executed, but failed to produced any methyl ester. Instead, the products from these experiments were soap.

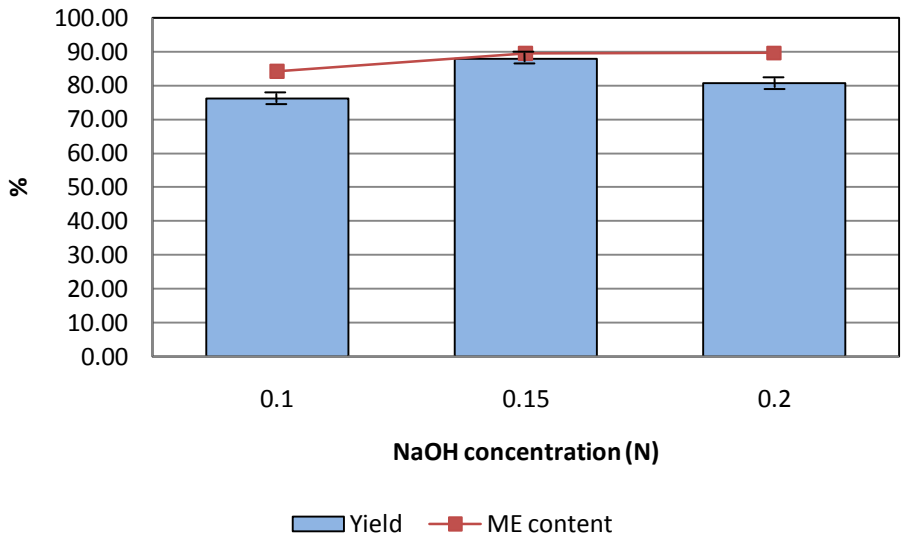


Figure 4.11 Methyl ester yield and methyl ester content of *J.curcas* seed at different NaOH concentrations. Alcohol to oil ratio = 400:1; reaction temperature = 60°C; seeds size = <0.71 mm; mixing speed= 400 rpm; reaction time = 1 hr.

From the methyl ester yield data, it is clear that the addition of NaOH, albeit in small amounts (0.1 N) has a significant effect on conversion to methyl ester. Increments in NaOH concentration to 0.15 N increased methyl ester yield from 76.2% to 87.8%. However, a further increase in concentration, to 0.2 N, decreased the yield to 80.8%. It is interesting to note that, with further increase in NaOH concentration, an emulsion started to form consequently reducing yield. The most likely cause of the emulsion is the formation of soap which is a competing reaction in the alkali-catalysed transesterification process.

The formation of soap occurs through two different mechanisms: hydrolysis of a triglyceride and saponification [112]. The mechanism of both processes was explained in detail in Section 2.2.1. In conventional transesterification with alkali catalysts, the formation of soap emulsions occur when there are high levels of free fatty acids [116]. To overcome this problem, the feedstock is usually pre-treated with acid catalyst to esterify the free fatty acids prior to the transesterification process with alkali catalyst [117]. Generally, *J. curcas* oil has a high acid value number, which is why the majority of researchers have adopted this route to

produce biodiesel [118-120], although there have also been reports of various other routes of reaction, such as acid catalyst *in situ* transesterification [34], supercritical reactive extraction [121], direct acid catalyst transesterification [122] and by using heterogeneous catalyst [123]. De Oliveira and co-researchers [122], for example, in particular, reported that when *J. curcas* oil was transesterified using sodium hydroxide as catalyst, a stable emulsion formation was observed in the sample, which limited the final yield to 68%. Interestingly, it is apparent from Figure 4.11 that the high free fatty acid content of *J. curcas* oil had no negative effect on the yield until the NaOH concentration reached 0.2 N.

Figure 4.11 also shows that increasing catalyst concentration from 0.1 N to 0.15 N had a positive impact on FAME conversion. However, a further increase from 0.15 N to 0.2 N did not affect methyl ester content, presumably due to the increased formation of soap.

4.4.6 Methanol-Oil Molar Ratio

The methanol volume required in *in situ* transesterification is very high compared to that of conventional processes [21]. In this study, the molar ratio of methanol to oil was ranged from 100 to 600. The results are shown in Figure 4.12.

No methyl ester was produced at a molar ratio of 100, even though 18.1% of the mass was extracted from the initial 10g of seeds. A 52% yield of methyl ester was obtained at the 200 ratio which then increased steadily with the ratio. The yield at a molar ratio of 300 was 74.7% and at 400, 500 and 600 yields were 81.9%, 85.7% and 86.9% respectively.

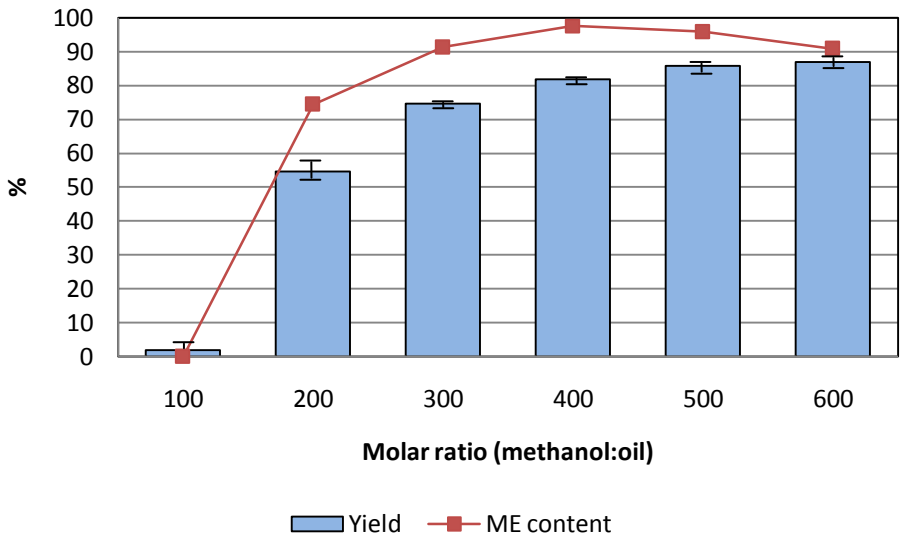


Figure 4.12 Methyl ester yield and methyl ester content of *J. curcas* seed at various molar ratios of methanol to oil. Other parameters: NaOH concentration = 1 N; reaction temperature = 60°C; seeds size = <0.71 mm; mixing speed= 400 rpm; reaction time = 1 hr.

The results suggest that the amount of methanol must be very high in order to achieve an appreciable yield. This is presumably necessary to drive the penetration of alkaline methanol into the seed, as described in Fick's law of diffusion and observed by Ren *et. al.*, [115]. Any further excess of methanol (e.g.600) does not greatly increase the yield, so is undesirable since it will increase the load on the downstream separation processes, especially on the process such as separation between FAME and glycerol as well as methanol recycling system.

4.5 Design of Experiments

4.5.1 Screening

The screening experiments were executed on 2^5 full factorial designs. To allow experimental error to be assessed, five central points were added to the design giving a total of 37 experiments overall. Table 4.3 shows a combination of the experimental matrix and responses

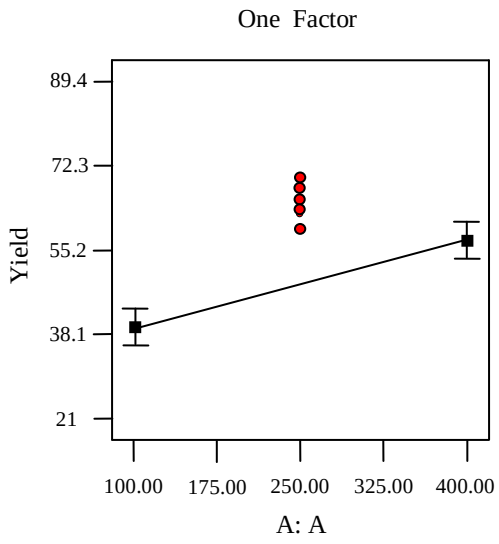
in standard order. To avoid bias, the experiments were conducted in random order. Run numbers 33 to 37 are the central point experiments.

Table 4.3 25 Full factorial experiments matrix with centre points in standard order

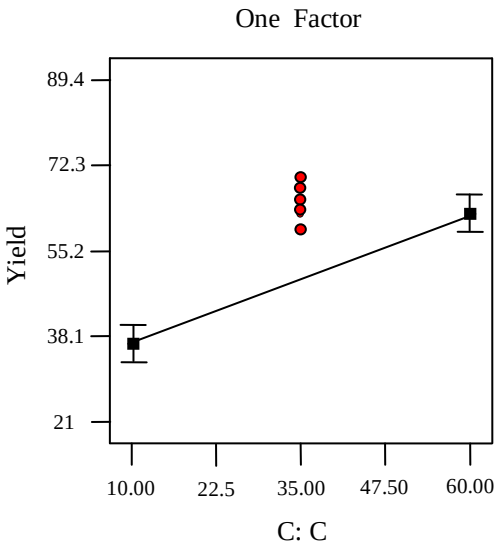
Standard run	A	B	C	D	E	Y (%)
1	100	0.1	10	30	100	30.9
2	400	0.1	10	30	100	37.4
3	100	0.2	10	30	100	30.0
4	400	0.2	10	30	100	54.0
5	100	0.1	60	30	100	35.2
6	400	0.1	60	30	100	66.8
7	100	0.2	60	30	100	48.3
8	400	0.2	60	30	100	68.7
9	100	0.1	10	60	100	33.5
10	400	0.1	10	60	100	40.2
11	100	0.2	10	60	100	46.1
12	400	0.2	10	60	100	21.0
13	100	0.1	60	60	100	38.5
14	400	0.1	60	60	100	58.9
15	100	0.2	60	60	100	34.2
16	400	0.2	60	60	100	68.6
17	100	0.1	10	30	400	28.7
18	400	0.1	10	30	400	35.5
19	100	0.2	10	30	400	48.4
20	400	0.2	10	30	400	34.3
21	100	0.1	60	30	400	44.3
22	400	0.1	60	30	400	76.2
23	100	0.2	60	30	400	53.2
24	400	0.2	60	30	400	77.8
25	100	0.1	10	60	400	32.2
26	400	0.1	10	60	400	46.5
27	100	0.2	10	60	400	34.2
28	400	0.2	10	60	400	40.8

29	100	0.1	60	60	400	60.3
30	400	0.1	60	60	400	89.4
31	100	0.2	60	60	400	58.7
32	400	0.2	60	60	400	87.2
33	250	0.15	35	45	250	62.4
34	250	0.15	35	45	250	64.9
35	250	0.15	35	45	250	68.0
36	250	0.15	35	45	250	63.0
37	250	0.15	35	45	250	59.1

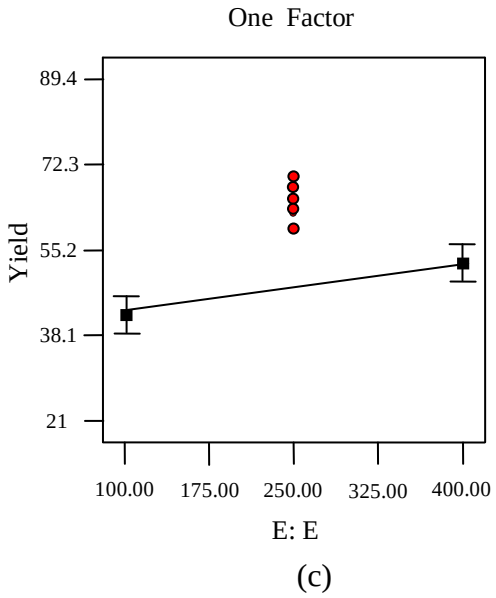
Figure 4.13 shows that molar ratio, reaction time and mixing speeds had positive effects on biodiesel yield. The p-values for these parameters were less than 0.05, indicating that these effects were significant.



(a)



(b)



(c)

Figure 4.13 The effect of various factors on biodiesel yield (a) molar ratio of methanol to oil; (b) reaction time and (c) mixing speed.

The yield increased with increase in molar ratio, reaction time and mixing speed. However, the centre points in all the figures clearly suggest the presence of curvature, indicating that correlations between parameters and yields are not linear and therefore, require further examination.

Two interactions, of molar ratio-reaction time and reaction time-agitation speed, also gave low p-values, indicating that these interactions have especially significant influences on biodiesel yield.

In Figure 4.14(a), both positive (C+) and negative (C-) reaction times at lower molar ratios gave low yields of 46% and 35% respectively. However, the effect of reaction time becomes more obvious at high molar ratios. At lower reaction times, a high molar ratio produced a low yield at 38%, which is almost the same percentage as at low molar ratio. However a high reaction time produced a high yield of 74%.

The same pattern can be seen in Figure 4.14(b), below, which shows the interaction between mixing speed and reaction time. At low reaction times, the yields obtained at low and high mixing speed were almost identical at 37% and 36% respectively. At higher reaction times, however, high mixing speed produced a higher yield of biodiesel (68%) than at low mixing speed (52%). As in Figure 4.13, the relationships between these factors and yield are clearly non-linear.

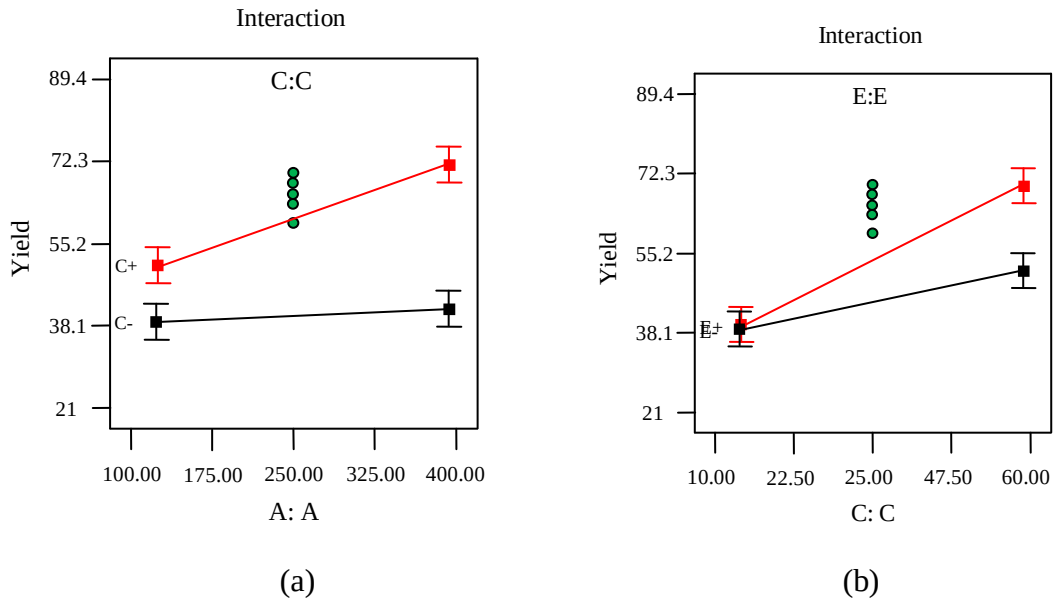
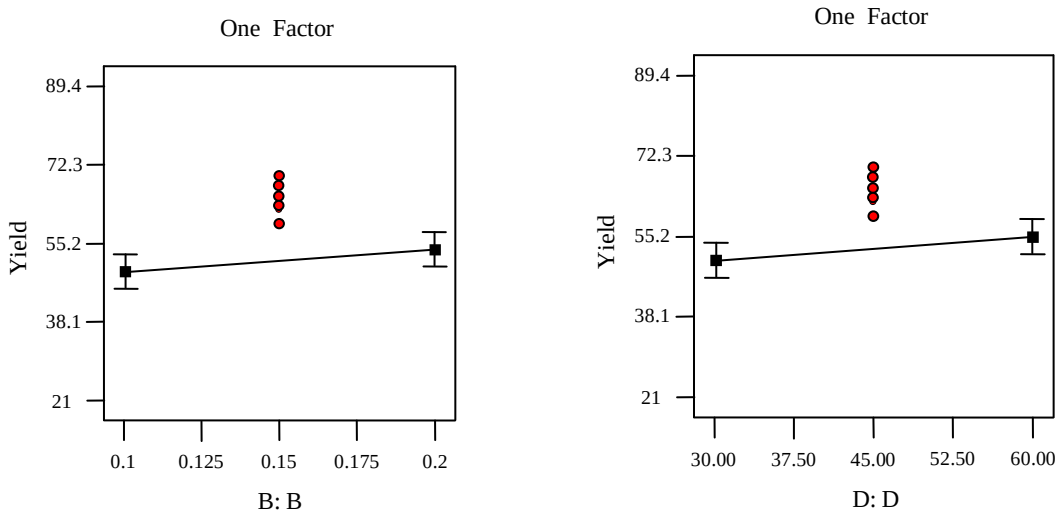


Figure 4.14 The interactions of (a) molar ratio and reaction time and (b) reaction time and mixing speed

The other two factors, NaOH concentration and reaction temperature were found to have a negligible influence on biodiesel yield, as shown in Figure 4.15.



(a) (b)

Figure 4.15 The negligible effect of sodium hydroxide concentration (a) and reaction temperature (b) on *in situ* transesterification of *J. curcas* oil.

A linear equation representing the results in coded factor terms is given in Equation 4.3

$$\mathbf{Y = 48.76 + 7.69A + 11.66C + 4.24E + 6.09AC + 3.78CE}$$

Equation 4.3

The p-value of the curvature for the process was 0.0005, which suggests that the presence of curvature is highly statistically significant. Therefore, a different higher order model must be considered to represent the data more accurately.

4.5.2 Non-linear Model

A response surface methodology was employed to fit the data to a non-linear model. This was required to fully describe the dependences due to curvature. Ten additional experiments were conducted to test the responses at +α and -α for each factor. Table 4.4 below lists the settings for the additional experiments against the response. A quadratic model was developed which including all five factors considered.

Table 4.4 Parameters and responses in additional experiments conducted for response surface methodology study

Standard Run	A	B	C	D	E	Y(%)
1	25.70	0.15	35	45	250	13.2
2	474.30	0.15	35	45	250	82.1
3	250	0.08	35	45	250	51.2
4	250	0.22	35	45	250	55.4
5	250	0.15	0	45	250	0
6	250	0.15	72.38	45	250	57.3
7	250	0.15	35	22.57	250	47.8
8	250	0.15	35	67.43	250	58.7
9	250	0.15	35	45	25.70	13.5
10	250	0.15	35	45	474.30	56.9

In coded terms, the yield is represented by the equation below;

$$Y = 43.44 + 23.04A + 1.40B + 19.16C + 3.64D + 14.51E - 0.67AB + 1.53AC - 2.31AD - 5.53AE + 11.34BC + 10.08BD + 7.38BE + 8.32CD + 6.63CE + 9.87DE + 2.24A^2 + 4.77B^2 - 6.26C^2 + 4.75D^2 - 3.33E^2$$

Equation 4.4

Equation 4.4 can be represented by a 3D surface plot to predict yield in the range of parameters studied. Figure 4.16 below shows the response predicted when molar ratio was plotted against other factors (B, C, D and E).

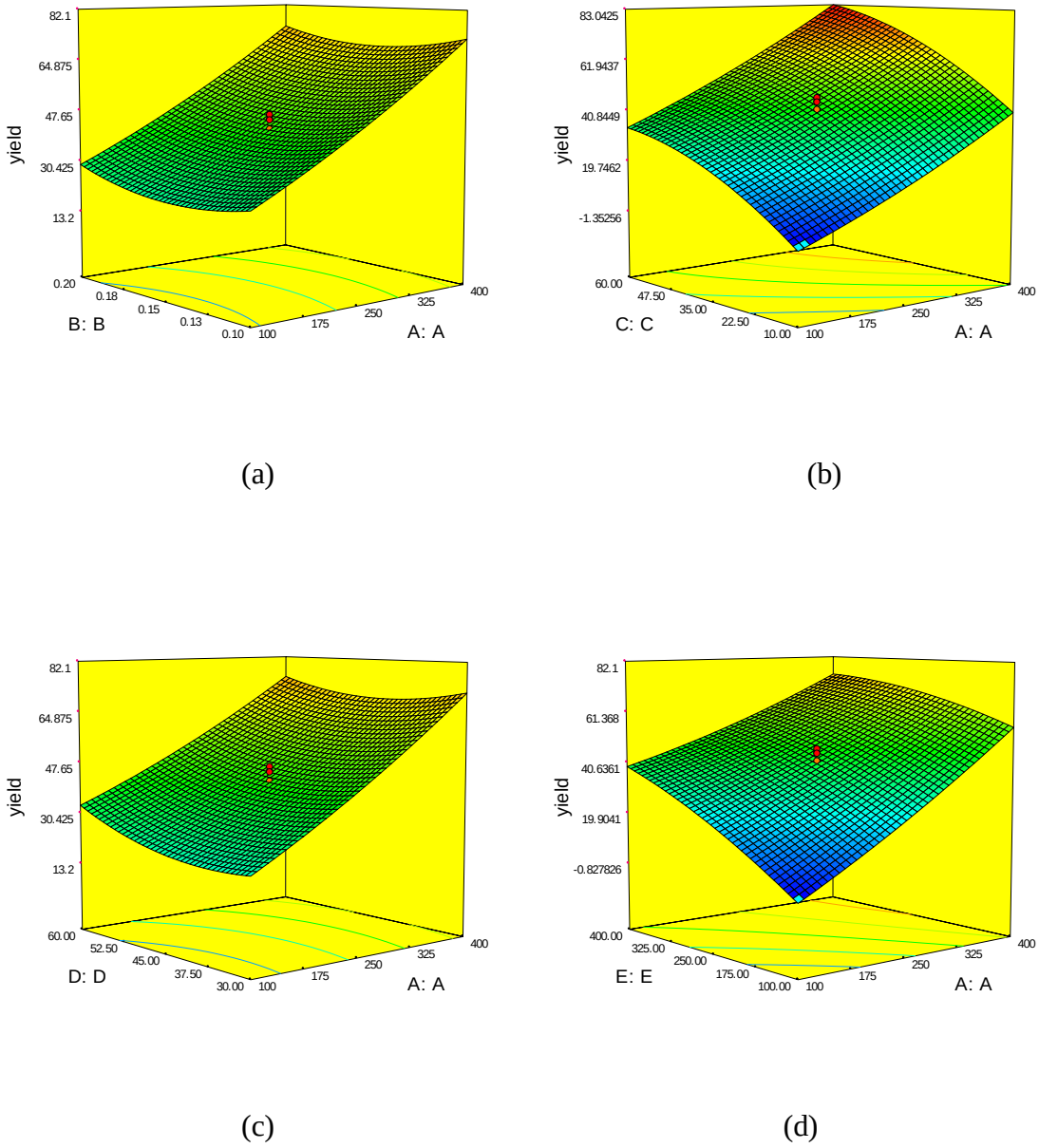


Figure 4.16 The 3D surface plot of interactions of (a) A with B, (b) A with C, (c) A with D and (d) A with E. A= molar ratio, B=NaOH concentration, C=reaction time, D=temperature and E=mixing speed.

Figure 4.16(a) shows the plot for the interaction of molar ratio and NaOH concentration against methyl ester yield. It can be seen that increasing NaOH concentration from 0.1 N to 0.2 N did not greatly influence methyl ester yield. However, the methyl ester yield clearly increased as the molar ratio of methanol to oil increased from 100 to 400. When molar ratio was plotted against reaction time as in Figure 4.16b, both factors were observed to influence

yield. The maximum yield of 83% was predicted at a molar ratio of 400 and 60 minutes reaction time. Figure 4.16(b) also suggests that the methyl ester yield approaches a plateau as reaction time increases. The positive interaction between molar ratio and biodiesel yield was linear, and the effect of reaction temperature was found to be insignificant (Figure 4.16c). Figure 4.16(d) shows that the methyl ester yield increased significantly with the increases of mixing speed from 100 to 300 rpm but beyond 300 rpm, the mixing speed produced small change of yield.

4.5.3 Discussion on the Design of Experiment Results

The relationship of the molar ratio of methanol to oil was similar to those reported by other researchers [21, 34, 39, 42], where the FAME increases with the increased of the ratio. However, as shown in Figure 4.17, disregarding the ratio of methanol to oil, the maximum yield was achieved after about the same time of 30 minutes. The same phenomenon was also observed by Mondala *et. al.*, [36] in their work with municipal sludge.

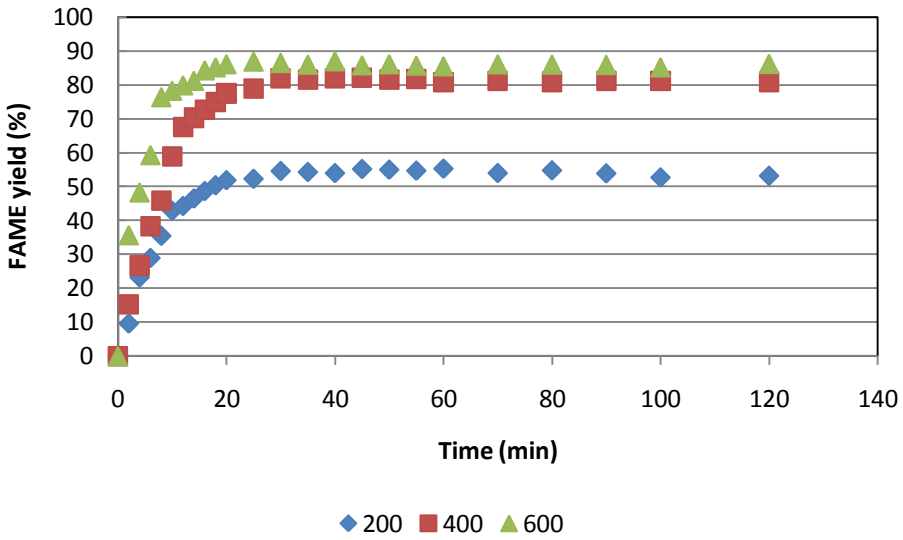


Figure 4.17 Effect of methanol-oil molar ratio on reaction time. NaOH concentration = 0.1 N, mixing speed = 400 rpm, seed size < 0.71mm

The amount of methanol needed in *in situ* transesterification must be enough to at least submerge all the seeds. In this study, at least 17 mL methanol (equivalent to a 100:1 methanol to oil molar ratio) were needed to fulfil that requirement. In conventional transesterification, if the suggestion of 6:1 ratio by Freedman *et al*. [13], is applied, only 1 mL of methanol is required to transesterify the same amount of seeds (10 g of seeds = 3.6 g of oil, molecular mass of the *J. curcas* oil = 877 g/mol).

According to this calculation, the amount of methanol supplied to the *in situ* transesterification should be adequate to give a significant yield, but the experiments proved otherwise. At the minimum methanol-oil ratio of 100:1, the yield was only 1.9%. One likely explanation for this is the large amount of methanol required for the extraction.

According to the Fick's laws of diffusion cited below, the rate of diffusion, *j*, is proportional to the concentration gradient, ΔC, so that the steeper the latter, the faster the rate of diffusion accross the length, ΔX.

$$j = D_{AB}\frac{\Delta C}{\Delta X}$$

Equation 4.5

In this case, in the first 10 minutes, the concentration of oil in the seed was high compared to that in the bulk liquid. Therefore, the yield increased rapidly. As time lengthened, more products were extracted to the bulk liquid, decreasing the concentration gradient. Once the concentration in the bulk liquid was in equilibrium with that inside the seed, extraction then stopped.

When a vast amount of methanol is used, this will dilute the bulk liquid further and make the concentration gradient steeper. Consequently, this will produce higher yields than reactions with low molar ratios of methanol. That increasing the amount of solvent achieves higher yield has been reported by several researchers. Sayyar *et. al.*, for example, reported that oil extracted from *J. curcas* was found to increase with the amount of hexane [124], while Franco *et. al.*, [125] discussed increases in equilibrium yield when the amount of ethanol was increased during the extraction of oil and antioxidants from *Rosa rubiginosa L.*

In the case of mixing speed, this factor was found to be significant, since at the low mixing level (100 rpm) the reaction occurred in a zone of dependence on external mass transfer. The reaction was found to be free from external mass transfer dependency once the mixing rate was set above 300 rpm, as shown in Figure 4.18.

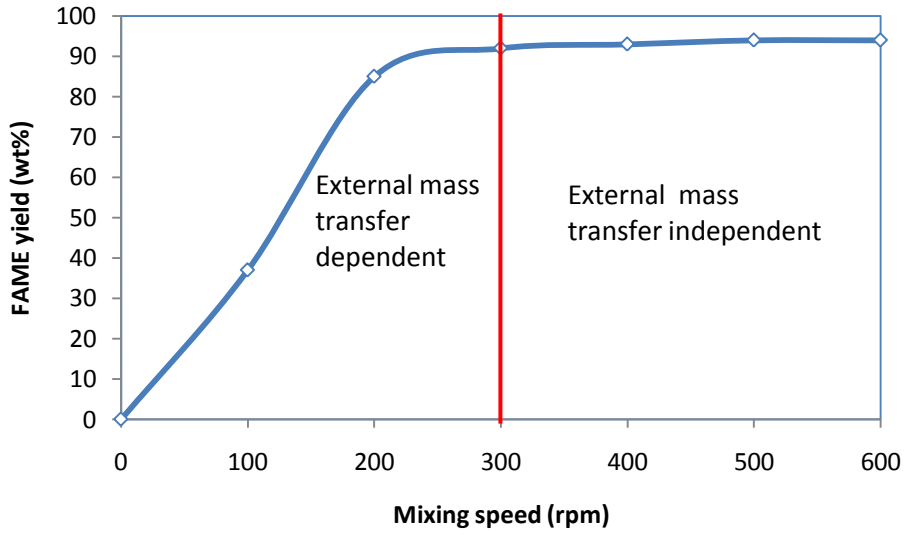


Figure 4.18 The external mass transfer regions in the *in situ* transesterification of *J. curcas*. Conditions: seed size = <0.71 mm, NaOH concentration = 0.1 N, methanol:oil = 400:1, reaction time = 1 hr, reaction temperature = 60°C

This phenomenon can be described using the Frossling correlation [111], where the mass transfer coefficient, k_c, kinematic viscosity, ν, diffusion coefficient, D_{AB}, liquid velocity, U, and particle diameter, d_p are correlated as in Equation 4.6.

$$\mathrm{Sh} = 2 + 0.6\mathrm{Re}^{1/2}\mathrm{Sc}^{1/3}$$

Equation 4.6

$$\mathrm{Sh} = \frac{k_c d_p}{D_{AB}}; \mathrm{Re} = \frac{\rho d_p U}{\mu} = \frac{d_p U}{\nu}; \mathrm{Sc} = \frac{\nu}{D_{AB}}$$

Equation 4.7

Replacing Sh, Re and Sc in Equation 4.6 with Equation 4.7 :

$$\frac{k_c d_p}{D_{AB}} = 2 + 0.6\left(\frac{d_p U}{\nu}\right)^{1/2}\left(\frac{\nu}{D_{AB}}\right)^{1/3}$$

Equation 4.8

The Sherwood and Reynolds numbers are in the thousands, so the number 2 in Equation 4.8 is negligible. Making k_c the subject gives:

$$k_c = 0.6\left(\frac{D_{AB}}{d_p}\right)\left(\frac{U d_p}{\nu}\right)^{1/2}\left(\frac{\nu}{D_{AB}}\right)^{1/3}$$

$$k_c = 0.6 \times \frac{{D_{AB}}^{2/3}}{\nu^{1/6}} \times \frac{U^{1/2}}{{d_p}^{1/2}}$$

Equation 4.9

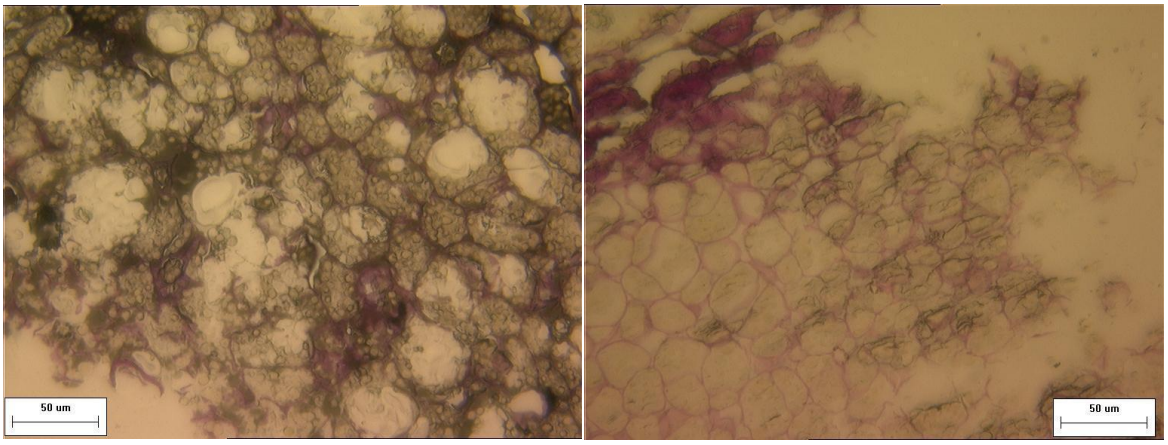
The diffusivity, D_{AB}, increases with temperature, and kinematic viscosity, ν, for a liquid, decreases with temperature. The second term, however, is a function of flow condition and particle size.

In this present case, all the terms were fixed during the experiments, except for U. k_c is proportional to U to the power 1/2, so when the mixing speed is increased from 100 to 300 rpm, k_c should increase by $3^{1/2}$.

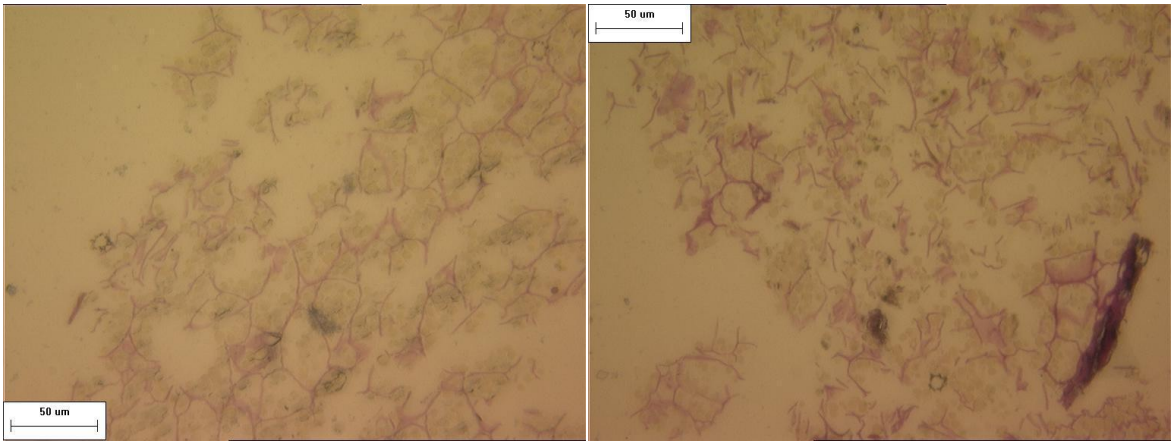
At low velocity, the reaction is limited by diffusion, as the mass transfer boundary thickness is large. As velocity is increased, thickness of the boundary layer decreases, and rate of reaction is no longer limited by the mass transfer across the boundary layer.

In this study, the reaction temperature was found to have an insignificant effect on FAME yield. The effect of temperature was unnoticed because in the experiment matrix, the yields data were acquired at 30 minutes and 60 minutes point. At that point, the reaction was most probably completed as shown in Figure 4.8. However, when the yield data were recorded as time progressed, as discussed in Section 4.4.3, the effect of temperature on the experiment were obvious.

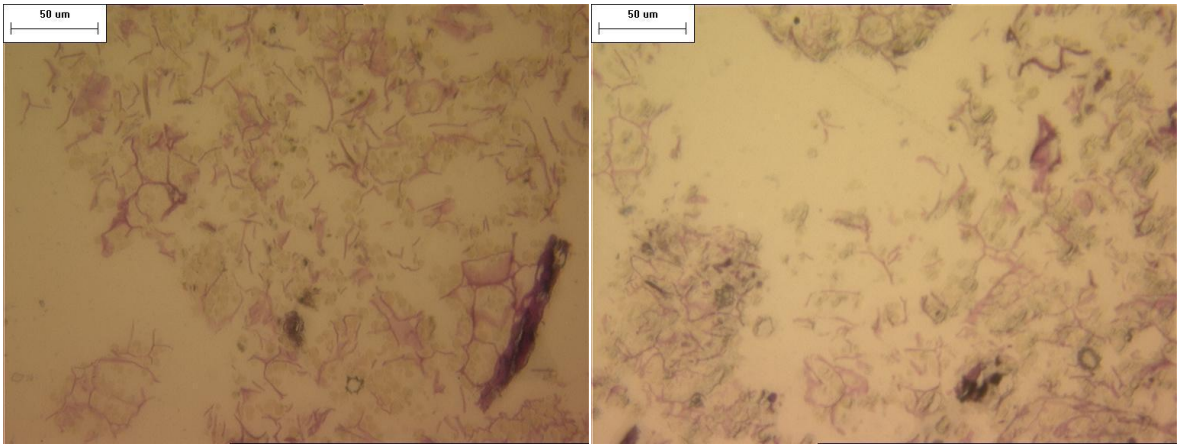
To provide further insight into the influence of molar ratio and reaction time on *in situ* transesterification, the seeds before and after reaction were examined under a light microscope. Figure 4.19(a) shows fresh seeds consisting of cells 10–40 µm in diameter. Each cell is surrounded by wall membranes 0.5-1.5 µm thick. Figure 4.19(b) - (f) show the condition of the seeds at 10, 20, 30, 40 and 50 minutes of reaction time respectively.



(a) (b)



(c) (d)



(e) (f)

Figure 4.19 Section of *J. curcas* seed cotyledon tissue before the *in situ* transesterification reaction (a). Areas stained red indicate cell wall polysaccharides. Other sections of the seed after 10 minutes reaction, (b) 20 minutes, (c) 30 minutes, (d) 40 minutes, (e) 50 minutes and (f) 60 minutes

The number of intact cells per area decreased from 1.23 x 10^5/cm^2 before the reaction to 1.10 x 10^5/cm^2 after 10 minutes of reaction, then to 0.3 x 10^5/cm^2 at 20 minutes and 0.1 x 10^5/cm^2 at 30 minutes. No intact cells were observed in the seeds after 40 or 50 minutes of reaction.

The intact cell calculation suggests that the membrane cell walls break down as the reaction progresses. This results in higher yields of methyl ester as the reaction progresses since the lipids are released from the cells and react with the methanolic solution. However, this

finding was totally different from that of rapeseed. In the rapeseed case, the cell walls were found to be intact after the reaction [55]. This was probably due to the different in cell wall composition and structure.

The correlation between number of intact cell that was calculated from the micrographs and the FAME yields is shown in 4.22. In general, the FAME yield increases as the number of intact cells decreases, up to 40 minutes onwards when all were broken.

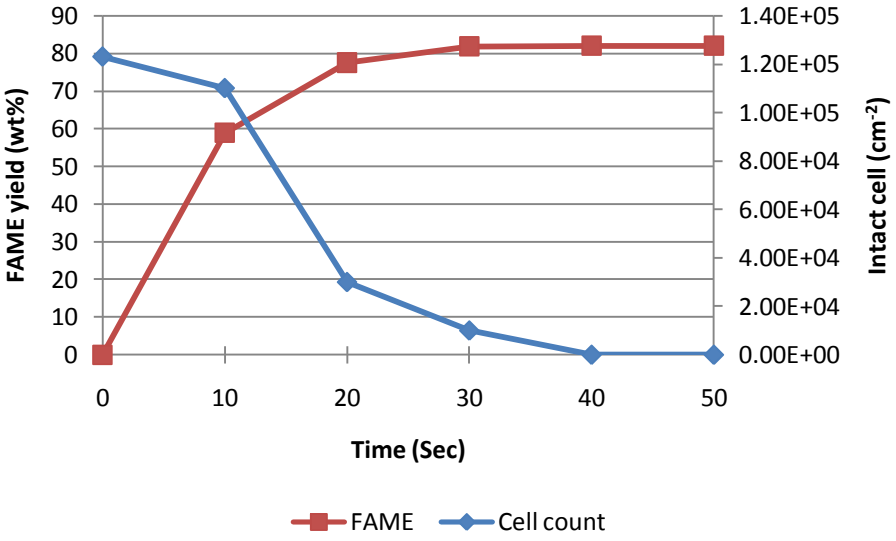
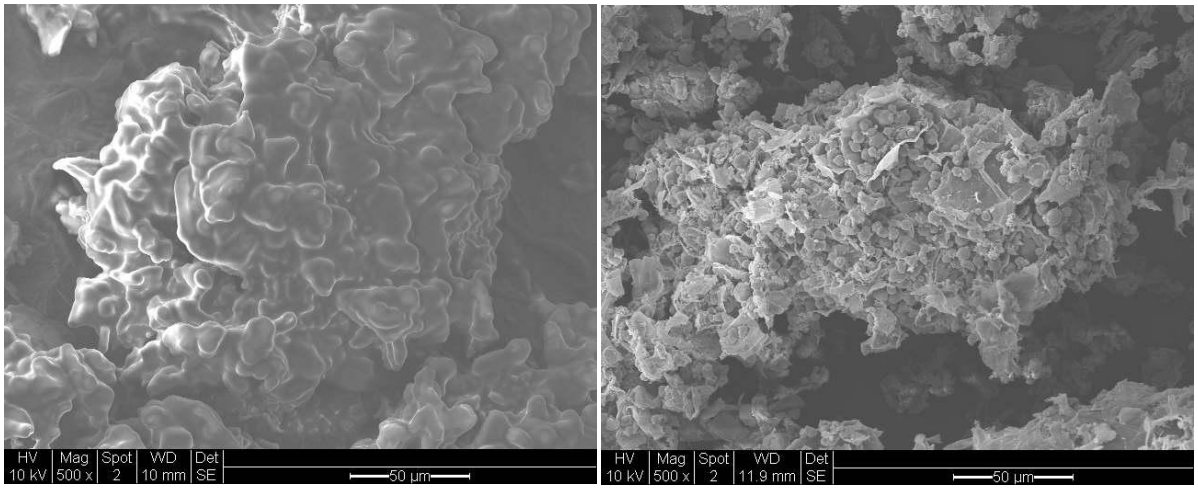


Figure 4.20 The relationship of FAME yield and intact cell count at same reaction time.

However, at 10 minutes, the reduction of the number of intact cell from 1.23 x 10^5/cm^2 to 1.10 x 10^5/cm^2 was relatively small compare to the big change in FAME yield from 0 to 59%. This phenomenon can be explained by

Figure 4.21 Figure 4.21 below.

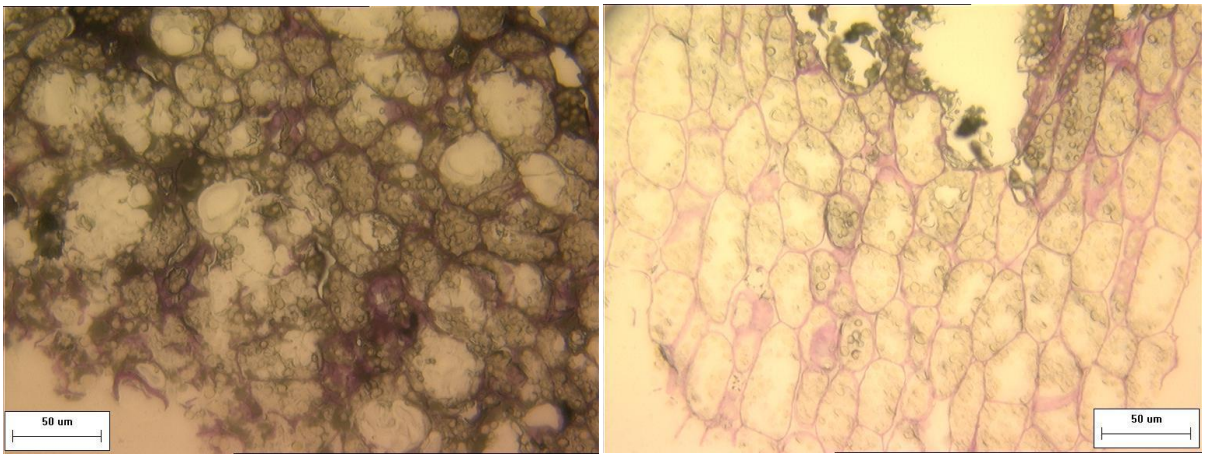


(a) (b)

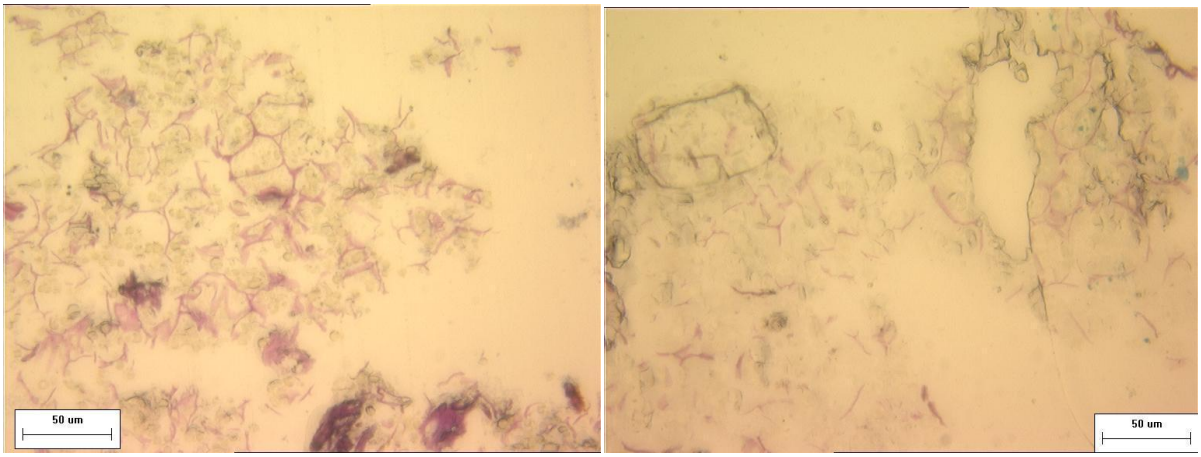
Figure 4.21 Micrographs of sections of *J. curcas* seed cotyledon tissue before the *in situ* transesterification reaction (a) and after *in situ* transesterification (b) taken by SEM. Scale bars for micrographs a – b = 50 µm.

Figure 4.21 (a) shows the seed condition prior to *in situ* experiment. The globules on the seed's surface is oil, released from the fractured cell during grinding. This outer surface oil reacted when it was mixed with methanolic methanol and therefore produced a high yield in the first 10 minutes of reaction. Figure 4.21 (b) meanwhile shows the seed condition after the reaction, and it is apparent that at 60 minutes, all the outer surface's oil had been removed. The shrinkage of the cell itself can also be observed.

The micrographs for different molar ratios shown in Figure 4.22 a-d also reveal that the same phenomena occured at different molar ratios.



(a) (b)



(b) (d)

Figure 4.22 Section of *J. curcas* seed cotyledon tissue before the *in situ* transesterification reaction (a). Areas stained red indicate cell wall polysaccharides. Other section of the seed after reaction with 100:1 molar ratio of methanol to oil, (b); 300:1 molar ratio of methanol to oil, (c) and 400:1 molar ratio of methanol to oil, (d). Scale bars for micrographs a – d = 50 µm.

The intact cell count for the seeds at a molar ratio of 100 was 1.06 x 10^5/cm^2, only 6 % less than in fresh seeds. This contributed to the lower methyl ester yield at this molar ratio. As the molar ratio increased to 300 and 400, the intact cell count decreased accordingly to 0.23 x 10^5/cm^2 and 0.03 x 10^5/cm^2 respectively.

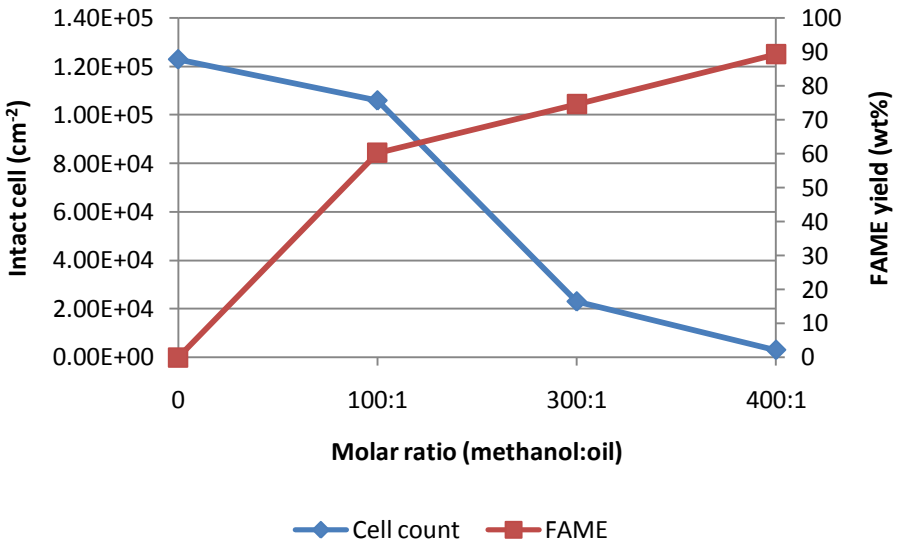


Figure 4.23 The relation of intact cell count and FAME yield at same molar ratio.

Similar to the relationship of FAME and intact cell in reaction time, the decreased of intact cell as molar ratio increases also affect the FAME yield positively, as shown in Figure 4.23. Again it was observed that as more intact cell break, higher percentage of FAME yield was obtained. Whilst a sufficient amount of methanol and an adequate reaction time are needed to release lipids from the cells, an appropriate mixing speed is also required to provide enough intensity in the *J. curcas* oil-methanol-sodium hydroxide system [46]. High mixing rates help to eliminate the boundary layer between the seed and the bulk solution thus enhance the transport of the methanol into the seeds, and at the same time help the *J.curcas* oil in the bulk phase to exceed the required mixing threshold, as suggested by Ma *et. al.*, (1999) [126].

4.6 Reducing the Molar Ratio

The large amount of alcohol required for *in situ* transesterification makes commercialisation difficult to envisage. A simulation on recovery of methanol by Dhar and Kirtania reported that the reboiler heat duty energy requirement to recover 80% of methanol for 10 stages distillation column was increased from 500 kW to 3000 kW for 6:1 and 50:1 molar ratio of methanol to oil respectively. Furthermore, the report also revealed that the energy requirement increases exponentially more than 80% recovery is desired [127]. This was considered by Core (2005) [54], who concluded that the price of biodiesel obtained by this method is higher than that of conventional methods.

Two different approaches have been tried to address this issue. The first was to employ co-solvents and the second to use methyl acetate as a replacement for alcohol. In co-solvent experiments, the idea was to facilitate oil extraction with non-polar solvents to enhance the

yield. The idea behind using methyl acetate was to change the reaction such that triacetin rather than glycerol is produced as by-product. Although there were reports on the use of methyl acetate as a replacement of methanol, it was limited to transesterification [66], enzymatic [128-130] and supercritical biodiesel production process [131, 132].

4.6.1 Co-solvents

The addition of co-solvents to the methanol should assist the oil extraction, thereby lessening the methanol requirement. Hexane and DEM are both non-polar solvents, and are both effective in extracting oil seeds. This will help to reduce the amount of methanol needed in the process. The utilisation of co-solvents in extraction has been extensively reported, especially by Young *et. al*., [133, 134] and other researchers [135, 136].

Figure 4.24 shows the FAME yields gained when DEM and hexane were added in three different molar percentages of 10, 30 and 50 to the *in situ* transesterification reaction at low methanol to oil molar ratios of 100 and 200. At a lower molar percentage of co-solvent of 30, the addition of hexane and DEM to the reaction at 100:1 methanol to oil molar ratio did not affect the FAME yield. However, when DEM was added at the 200:1 molar ratio, the reaction produced higher FAME yields than with hexane, at the same methanol:oil molar ratio. This pattern was notably observed when 30% molar ratios of DEM and hexane were introduced to the reaction. The reaction with DEM at a 100 molar ratio produced a higher yield than with hexane at a 200 molar ratio. This was observed again in experiments with 50% molar percentages of the co-solvents.

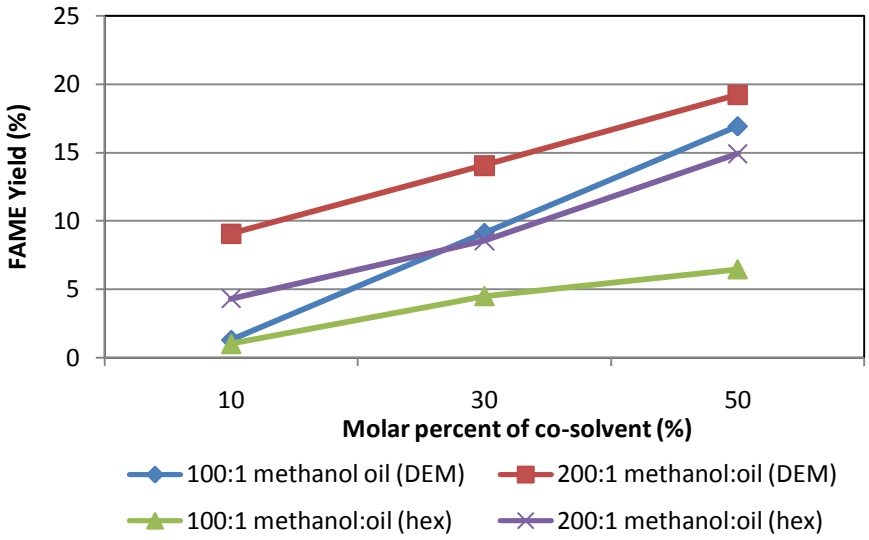


Figure 4.24 FAME yields for different molar ratios of DEM and hexane at three different molar percentages. Seeds = 5 g; temperature = 60°C for hexane, 40°C for DEM; NaOH concentration = 0.1 N, Mixing speed = 400 rpm, reaction time = 1 hr.

DEM was clearly a better co-solvent in these experiments. The addition of higher amounts of DEM at higher molar ratios of methanol to oil produced better yields than hexane. This agrees with work by Zeng *et. al.*, [50, 137], where the extraction rate with DEM was found to be higher than with hexane. At 50% molar percentage DEM and a ratio of methanol to oil of 200, the FAME yield was 19%, this was 64% less than the yield of 83% achieved at a molar ratio of 400 of methanol to oil. To investigate the effect of DEM at the higher molar ratio, a 50% molar percentage of DEM was added to the reactions with 400 and 500 molar ratios of methanol to oil.

The results shown in Figure 4.25 verify that DEM performed better in the reactions with high molar ratio. The experiments at 400:1 and 500:1 with DEM both produced better yields than the experiments without DEM.

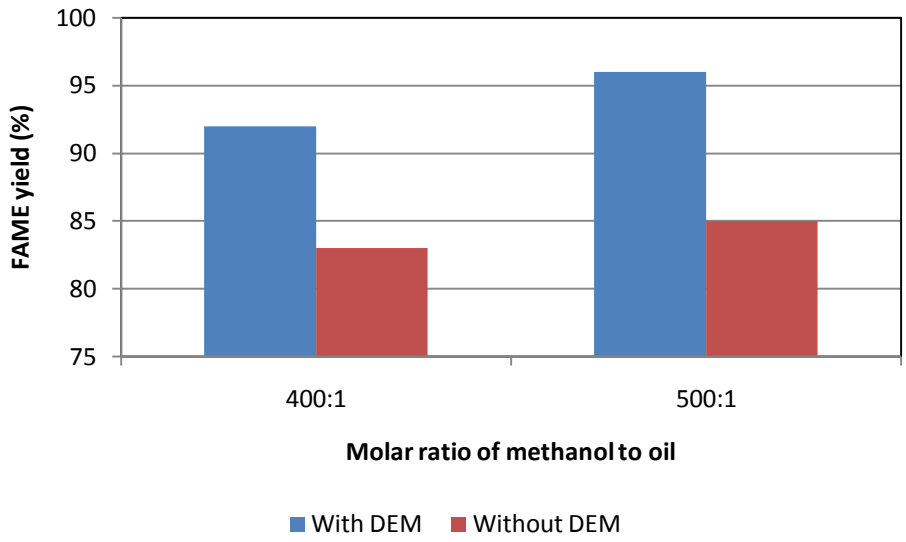


Figure 4.25 The difference between FAME yields for the *in situ* transesterification with and without DEM at 400:1 and 500:1 methanol to oil ratios. Seeds = 5 g; temperature = 60°C for hexane, 40°C for DEM; NaOH concentration = 0.1 N; mixing speed = 400 rpm, reaction time = 1 hr.

4.6.2 Methyl Acetate

Soxhlet extraction of *J. curcas* seed by three different solvents, methyl acetate, n-hexane and methanol yielded 38.2, 36.1 and 8.0 wt% of extraction product, respectively. This confirms methyl acetate capability to extract the oil from the seed. The difference in polarity of the compounds is vital in the extraction of the oil from the seed. As a non-polar compound, n-hexane was expected to yield the highest extraction product, followed by methyl acetate, which is a weak polar solvent, and then methanol, a polar solvent. However, from the information given below, concerning amounts of methyl acetate extraction, its yield of 38.2% was slightly higher than that of n-hexane at 36.1% and methanol at 8%. Methyl acetate extracted more, because it extracted polar and non-polar compounds, as it is not entirely polar. The same pattern of finding was reported by Su *et. al.*, [65], although the authors did not discuss possible reasons for the phenomenon.

The results indicate that of the three factors studied (molar ratio of polyethylene glycol (PEG) to catalyst, molar ratio of methyl acetate to oil and catalyst concentration) only the ratio of methyl acetate to oil had a significant effect on methyl ester yield. Figure 4.26 below illustrates these findings.

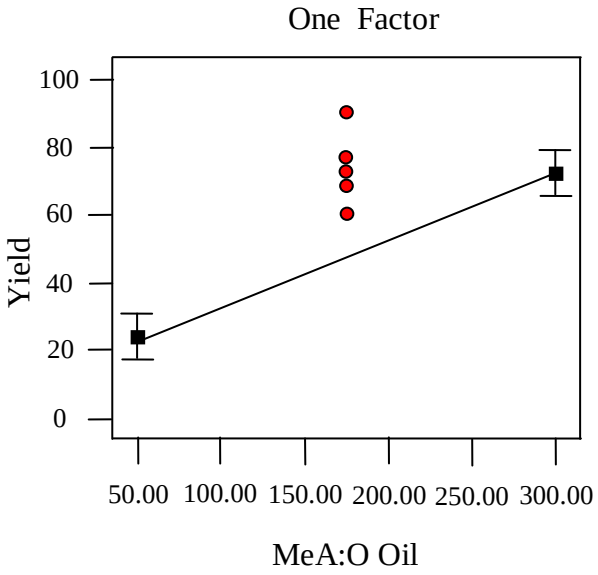


Figure 4.26 The effect of methyl acetate-oil ratio to methyl ester yield

The yield was 21.6% at the lower molar ratio (50), but at a higher molar ratio (300) a significant increase in yield to 75%, was recorded. At the centre point, where the ratio was 175:1, the average yield for 5 runs was 69.7%. The centre points also suggest that the methyl acetate ratio and methyl ester yield were not linearly correlated. This is also indicated in the analysis of variance (ANOVA) shown in Table 4.5.

The p-value of the source must be the same as or below p=0.005 in order to be considered significant, and the chart shows that whilst sources corresponding to methyl acetate-oil molar ratio, B, and curvature had p-values in the range of significance, the remainder exceeded this level.

Table 4.5 Analysis of Variance (ANOVA) Chart for the Experiments

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	p-value Prob > F
Model	6864.3	4	1716.08	7.43	0.0116
A-PEG:Cat	97.64	1	97.64	0.42	0.5363
B-MeA:Oil	4805.98	1	4805.98	20.81	0.0026
C-Cat. Conc	1851.86	1	1851.86	8.02	0.0253
Curvature	3656.1	1	3656.1	15.83	0.0053
Residual	1616.49	7	230.93		
Lack of Fit	1115.13	3	371.71	2.97	0.1605
Pure Error	501.35	4	125.34		

As illustrated in the 3D representation below, methyl ester yield increases to a maximum point of 90.9%. The operational conditions at this point are, a NaOH concentration of 0.20 mol/L, PEG-NaOH molar ratio of 3:1 and methyl acetate-oil ratio of 300:1. There also appears to be a local maximum value of 86.8% at the centre point of the operational conditions tested. At this point, the operating conditions were: a NaOH concentration of 0.13 mol/L, PEG-NaOH molar ratio of 3:1 and methyl acetate-oil ratio of 175:1. A comparison of these two sets of operating conditions indicates that the yield increased by just 6.8 wt% when the methyl acetate-oil ratio was doubled.

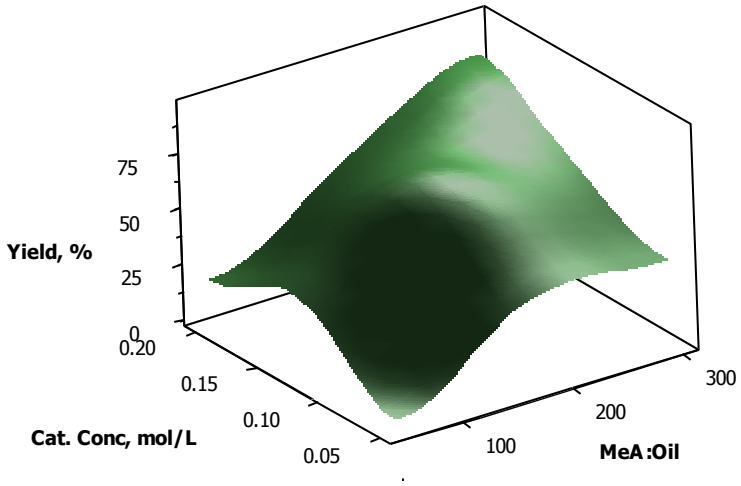


Figure 4.27 3D Representation of Yield against Various Parameters

The positive effect of methyl acetate on the yield is to be expected, since it had been proved in previous experiments that methyl acetate can operate as a solvent for oil. Its ability to extract oil leads to more of the reaction occurring in the bulk environment, rather than inside the seeds. Therefore, the mass transfer of the reagent to the seeds, which is a limitation on the reaction in *in situ* transesterification, was less important in this process.

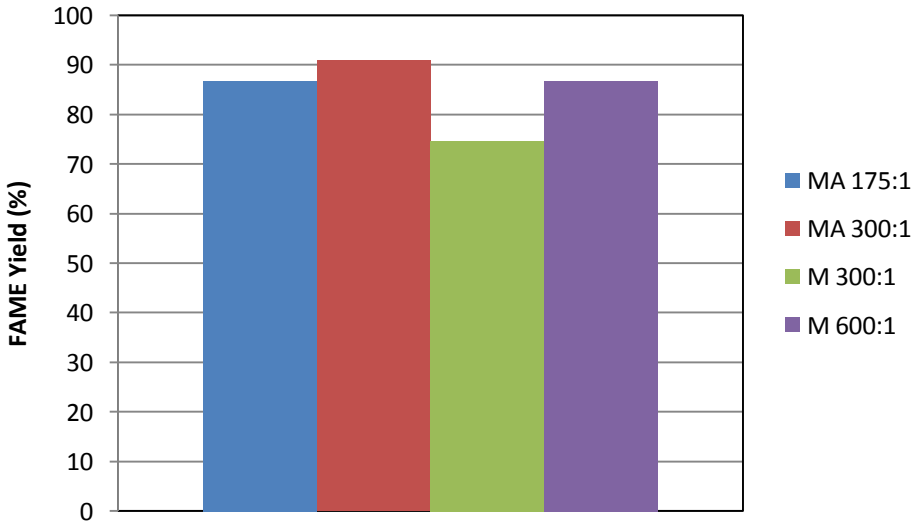


Figure 4.28 Comparison of FAME yield at various operation conditions. MA for the experiments with methyl acetate and M, with methanol.

By comparing the results with those from the process using methanol, it can be seen that the use of methyl acetate produced higher methyl ester yields. At the same molar ratio of 300:1, the FAME yield with methyl acetate surpassed that with methanol by about 16.2%. Even when the amount of methyl acetate was reduced, as in methyl acetate-oil ratio of 175:1, the yield was still better than methanol-oil ratio of 300:1. Doubling the amount of methanol to 600:1 increases the FAME yield by 12.1%, to 86.8%, but still gave a slightly lower yield than that achieved by methyl acetate at 300:1.

These findings have several repercussions for the design of an overall process. In term of the amount of reactant used in the process, at 175:1 molar ratio, 57 mL of methyl acetate required for 10 g of *J. curcas* seed. Meanwhile, at 300:1, 115 mL of methanol needed for the same amount of seed. Evidently the use of methyl acetate reduced the amount of reactant by half. The effect will cascade to downstream processing unit, in particular the reactant recycling unit. Although there was no information on reboiler heat duty during methyl acetate recycling process, generally the duty increases with increasing load and the percentage of recovery.

However, it should be noted that with the use of methyl acetate, PEG will be present in the outlet stream. Because PEG is soluble in water, it can be removed from the process during water washing. PEG is a non-toxic compound [138], so it can be released with the waste water to the environment. The waste water stream also contained phenolic compounds, which are hazardous due to their toxicity and persistent in the environment [139]. Interestingly, PEG was used as additive in the oxidation of phenolic compounds by peroxidise enzyme reaction to remove the phenolic compounds in waste water [140, 141].

One of the main advantages of using methyl acetate is that glycerol triacetate, known as triacetin, is produced instead of the glycerol resulting from the reaction of triglycerides with methanol. As stated before, triacetin at current price of £0.90/ kg is more valuable than glycerol (£0.20/ kg) and, therefore, can potentially improve the process economics of the whole operation.

4.7 Biorefining

Plant oil has been utilised for its high-value products in sectors like nutritional food, lubricants and ink manufacturing [142]. The richness of compounds in *J. curcas* offers a possibility for biorefineing, which could increase the economic viability of *in situ* transesterification. There are two main waste streams in *in situ* transesterification. The first is the meal, which is the solid remainder of the seed after *in situ* reaction. The second is the bottom phase (glycerol and other polar compounds) from the separator. These streams were evaluated to identify any valuable compounds within them.

4.7.1 Evaluation of Waste Stream

Figure 4.29 below, shows the mass ratios of glycerol-rich phase (bottom phase) and ester-rich phase (upper phase) in the final product of the *in situ* transesterification process. At 6.7 g glycerol-rich layer and 11.5 g ester-rich layer, the mass ratio of 5:10 was higher than that of the conventional process, which is usually at 1:10.

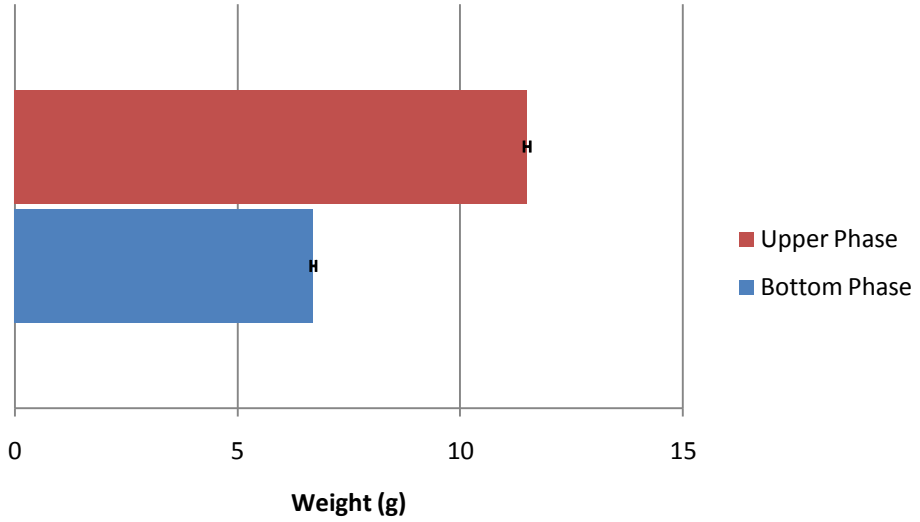


Figure 4.29 Comparison between glycerol-rich and ester-rich phase after the *in situ* transesterification of *J. curcas*. Seeds = 40 g, methanol:oil molar ratio = 400:1

Analysis of the compounds was tabulated in Table 4.6. The table suggested that higher mass ratio of upper phase to bottom phase was due to several reasons. The capability of methanol to extract polar compounds, such as phenolic compounds and soap contributes to this. However, the amount of phenolic compounds in the samples was found to be very small, only 0.55 g, which was equivalent to 0.03 g/g total extract. This is in accordance with Tongpoothorn *et. al.*,'s work, who found 0.04 g phenolic compounds per gram of total extract in the methanolic extract of *J. curcas* [143]. Soap, which also ended up in the bottom (glycerol-rich) phase accounted for 3.1 g. The soap was present in the bottom phase, as *J. curcas* contains high proportions of free fatty acids in its oil. These saponify and turn into soap in the glycerol phase. The rest of the mass was made up by the catalyst, NaOH, the acetic acid.

Table 4.6 Mass balance of triglyceride in the *in situ* transesterification product (40 g seeds, 400:1 methanol oil molar ratio, 60^{o}C reaction temperature, 0.1 N NaOH concentration, 1 hr reaction time, 450 rpm mixing speed)

Oil in seed (hexane Soxhlet extraction)	14.40 ± 0.005g
Total extract	18.00 ± 0.005 g
Upper phase	11.50 ± 0.07g
Ester	10.70 ± 0.08 g
Bottom phase	6.70 ± 0.06 g
Glycerol	1.10 ± 0.04 g
Re-extracted seed	
Oil	4.50 ± 0.08 g
Total oil recovery	= 9.70+4.50 = 14.20 ± 0.09 g
Uncounted oil	=14.40 – 14.20 = 0.2 ± 0.09 g
Other components in Bottom phase	
NaOH	1.10 ± 0.07 g
Acetic acid	1.70 ± 0.06 g
Methanol soluble compounds	= 6.70– 1.1 – 1.7 – 0.2 = 3.7 ± 0.07g
Other components in methanol soluble compounds	
Soap	3.10 ± 0.04g
Phenol	0.55 ± 0.07g
Phorbol Ester	0.05 ± 0.01g

Since the oil content in *J. curcas* oil was 36%, with 40 g of seeds, the maximum amount of ester it was possible to produce was 14.4 g. The total extract, which is the amount of liquid product after filtration of *J. curcas* meal, was 18.0 g. After separation, 11.5 g were in the upper phase, whilst 6.7 g was in the bottom phase.

The seeds were then re-extracted with hexane to extract oil that had remained inside. The amount of oil recovered at this stage was 4.5 g. This oil was then analysed by gas chromatography, and it was revealed that 54.3% of it was in ester form. This confirms the claim that *in situ* transesterification also occurs inside the seed. In equilibrium, when the concentration of methyl ester in the bulk methanol is equal to that in the seed, the latter would

not be extracted. Therefore, lower concentrations of methyl ester in the bulk will lead to more of it being removed from the seeds. This requires more methanol, hence a higher molar ratio of methanol to oil.

After the deduction of sodium hydroxide and acetic acid (used for neutralisation), the amount of methanol soluble compounds was 3.7 g. Of this amount, 3.1 g was soap and 0.55 g phenol compounds. The nature of the remaining 0.2 g is assumed as phorbol ester, which dissolves in methanol and is present in *J. curcas* oil. Various researchers have also reported the presence of phorbol ester in *J. curcas* oil [144, 145], and it solubility in methanol [146, 147]. In this study, the distribution of kernel and shell in *J. curcas* was 62.7 g to 37.3 g as shown in Figure 4.30. For 40 g of seed, the kernel contribution was 24 g and the amount of phorbol ester per gram kernel was 2.1 mg/g kernel. This amount was within the range stated by Devappa *et. al*., [144] which was 0.8 to 3.3 mg/g of kernel.

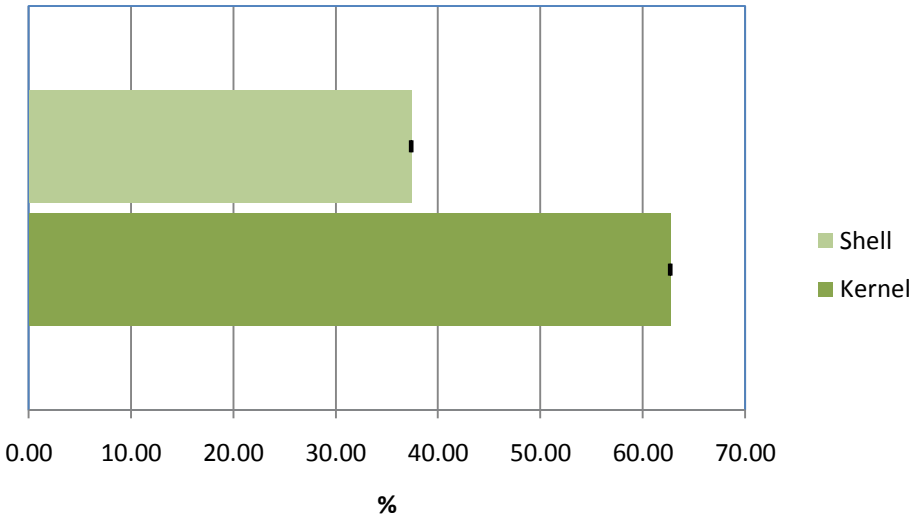


Figure 4.30 Distribution of shell and kernel in *J. curcas* used in this study

4.7.1.1 Protein

Defatted *J. curcas* contains high amounts of protein. Oskoueian *et. al*., reported that the amount of protein in defatted *J. curcas* seeds in their experiments was as high as 61.8% [148], which is very high compared to those found by other researchers, for example 26.6% by Oseni and Akindahunsi [149] and 22-28% by Devappa *et. al*., [150]. Achten *et al*., investigated 37 samples of *J. curcas* seeds originating from all over the world, and stated that the average protein amount in the kernel was 24.85% [70].

J. curcas protein was therefore determined using elemental analysis, where the nitrogen element was quantified. The nitrogen concentration was then multiplied by a factor of 5.53 [102], as described in Section 3.4.2. The same method has been used before to quantify protein in *J. curcas* meal [151]. Table 4.7 shows elements of nitrogen, carbon and hydrogen in the seed before and after the experiment.

Table 4.7 Elemental (CHN) analysis results for the seed before and after experiment

Sample	Found % N	Found % C	Found % H	Protein %
Before	6.64 ± 0.35	56.88 ± 1.10	9.19 ± 0.53	36.72
After	3.90 ± 0.35	40.54 ± 0.64	4.56 ± 0.25	21.57

The percentage of nitrogen after *in situ* transesterification reaction was 2.74% lower than the amount before reaction. Non-protein nitrogen in *J. curcas* seed is reported to be small: below 9%, as reported by Makkar and Becker [95]. Therefore, assuming that all of the nitrogen was protein-nitrogen, the protein percentage before experiment was 36.72% and then loss by 15% to 21.57%. This is comparable to the level obtained after mechanical pressing, reported to be in the range of 22-24% [89] and slightly higher than rapeseed meal, which usually contains 17 -20 % protein [152, 153]. Work by Makkar and Becker, also showed that the protein from

J. curcas kernels has good acid amino composition, which means it has all the essential protein comparable to Food and Agriculture Organisation (FAO) reference protein for growing child [89].

The only problem to utilise the meal as animal feed, is the existence of anti-nutritionals and phorbol ester in it. The main anti-nutritional presence in *J. curcas* are trypsin inhibitors, lectin and phytate [150]. Phorbol ester exists in small quantity, but even though minor, exhibit toxicity on different kind of animals when used as animal feed [154, 155]. However, since methanol and alkali are used in *in situ* transesterification, there is a high probability that the phorbol ester decomposed during the reaction, as it was reported that phorbol ester can be reduced by alkali treatment and methanol extraction [156]. Unfortunately this could not be definitively proved during the course of this research as the analytical method to determine phorbol ester required a high-performance liquid chromatography (HPLC) equipped with special column (reverse-phase C18 LiChrospher 100 [91]), which was not available. However, this is perhaps the highest priority further work, as it could substantially improve the economics of the process. Rapeseed meal in the UK is an important part of the economics of rapeseed farming.

4.8 Economic Evaluation

To estimate the price of biodiesel per unit kilogram, the economic evaluation analysis on two *in situ* transesterification scenarios was performed. The first scenario was on methanol-seed system (Case I) and the second case was methyl acetate-seed system (Case II). A number of limitations were set, in order to help with the analysis. The limitations were;

i. Data for the fixed capital cost, which is the equipment cost, was taken from literatures.

ii. The operating cost, which is the cost associate with raw material, chemicals, product and by-product was calculated using current prices.

iii. The amount of triacetin in Case II was acquired from mass balance calculation.

iv. For both cases, meal was considered saleable and can be used as animal food.

v. For both case, solvents were recycled at 80% from the start up run.

4.8.1 Stream Profiles

Table 4.8 below shows the stream profiles for both cases;

Table 4.8 Profile of the inlet and outlet stream for Case I and II

	Case I	**Case II**
General		
Solvent:oil molar ratio	400:1	175:1
NaOH concentration (mol/L solvent)	0.1	0.13
PEG (200): NaOH molar ratio	-	3:1
Oil amount (g)	14.4	14.4
Feed stream		
J. curcas seed (g)	40.0	40.0
Methanol (g)	210.0	-
NaOH (g)	0.9	1.2

Methyl acetate (g)	-	212.7
PEG (200) (g)	-	78.0
Outlet stream		
Biodiesel (g)	10.7	11.5
Glycerol (g)	1.1	-
Triacetin (g)	-	1.5
Soap (g)	3.1	3.0
Meal (g)	25.6	24.0

In both Case I and II, sodium hydroxide was used to catalyse the *in situ* transesterification process. Although the molar ratio of solvent to oil was low in Case II, at 175 compared to 400:1 in Case I, in terms of solvent mass, both were almost similar. The reason was the difference in molecular weight, where the methyl acetate molecular weight, at 74.1 g/mol, was more than double the methanol molecular weight, 32.0 g/mol. Both cases used 40 g of *J. curcas* seed, and with 36 % of oil content, the amount of oil was 14.4 g.

4.8.2 Capital Cost

The capital cost was calculated based on the process models developed by Haas et. al., [98] and Marchetti *et. al.*, [16]. Figure 4.31 shows the process flow diagram for homogenous transesterification process with preesterification, which is the process route for producing biodiesel from *J.curcas* oil.

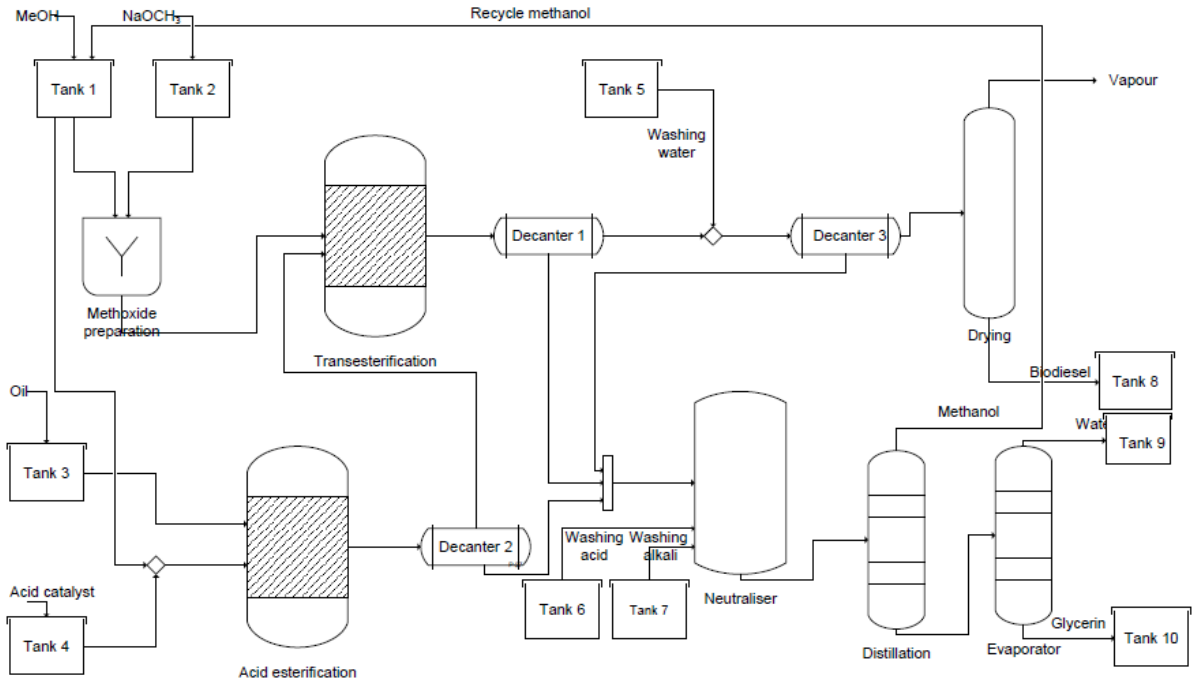


Figure 4.31 Process flow diagram of conventional transesterification process

The oil from Tank 3 was acid esterified with alkali catalyst and methanol from Tank 4 and Tank 1 respectively, in order to reduce the FFA content. The product then went through Decanter 2, where the oil phase was separated from the water phase.

In the upper half of the process, the oil phase from Decanter 2 became a feeder for transesterification reactor. The transesterification product then was passed through Decanter 1 to separate the biodiesel phase and glycerol phase. The biodiesel phase then was washed and passed through another decanter to separate the water and biodiesel. Finally the biodiesel was dried in the column and stored in Tank 8,

At the bottom half, the outlet stream from all decanters was combined and went to neutraliser reactor, where alkali, or acid was added to neutralise the compound. Methanol then was

separated using distillation column and recycled to Tank 1. The bottom product was put in the evaporator to separate water from crude glycerine.

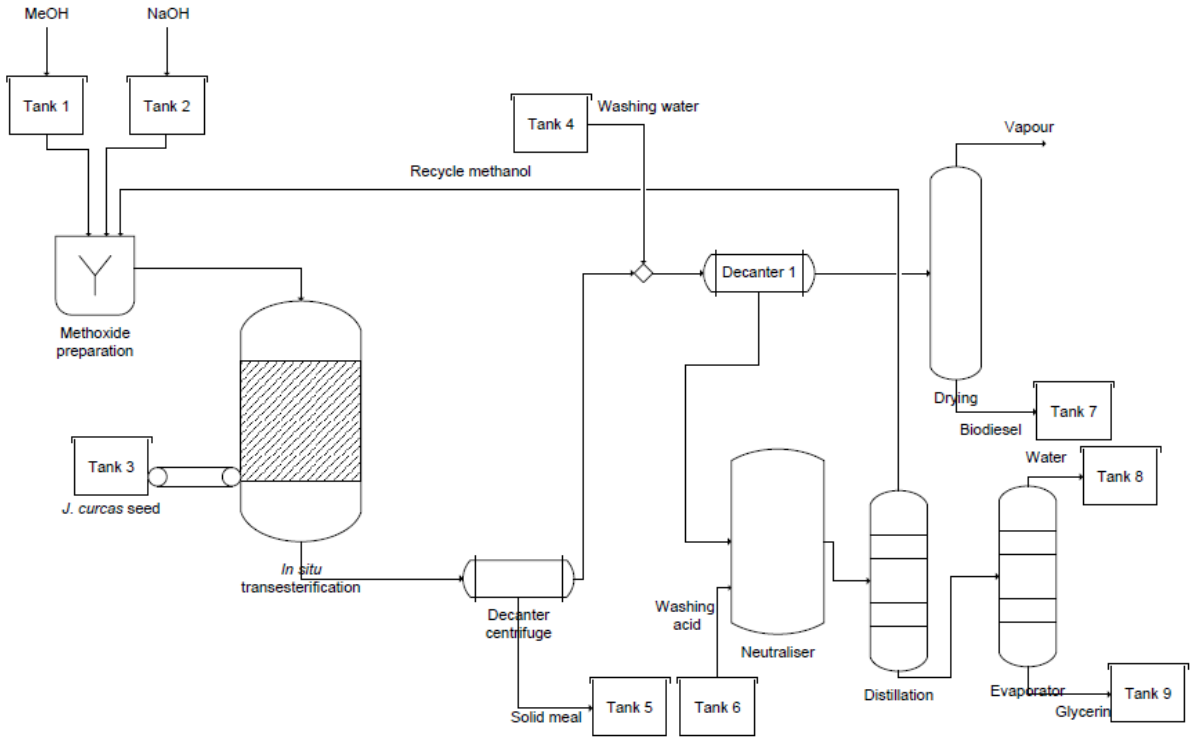


Figure 4.32 Process flow diagram of *in situ* transesterification process

The process flow diagram of *in situ* transesterification in Figure 4.32 shows less unit operation required for the process. The process started with ground *J. curcas* seed transferred to the *in situ* transesterification reactor, where it mixed with methoxide solution. The product passed through decanter centrifuge to separate the solid phase and liquid phase. The liquid phase then washed and passed through another decanter to separate the water phase and biodiesel phase. Top product from decanter, the biodiesel was dried in the drying column and stored in Tank 7. The bottom product from decanter meanwhile was neutralised in a batch reactor and then went to distillation where the methanol was separated from the glycerol phase. The methanol was recycled to Tank 1 whilst the glycerol phase was put into evaporator to get crude glycerol.

The cost of unit operations was taken from work by Haas *et. al*., and Marchetti *et. al*., which produced 34,000 and 36, 000 MT/ year of biodiesel respectively [16, 98]. For *in situ* transesterification process, the seed used in raw material was ground before stored in Tank 3. Table 4.9 below listed the price for each unit operation for both cases.

Table 4.9 Price of unit operation used in Case I and Case II. All prices are in US$ 1000

Equipment	Transesterification	*In situ* transesterification
Pre-mixer	50	50
Transesterification reactor	350	350
Acid esterification reactor	349	-
Sum of all decanters	69.6	46.4
Nutriliser reactor	13.5	13.5
Distillation column for biodiesel purification	60	60
Distillation column for methanol separation	40	40
Distillation column for glycerin separation	77.5	77.5
Tanks		
Methanol	24	24
NaOH	25	25
Oil	506	-
Biodiesel	447	447
Crude Glycerol	22	22
Washing water	35	35
Acid	25	25
Washing Acid	25	-
Washing Alkali	25	-
Wastewater	35	35
Solid meal	-	15
J. curcas seed	-	100
Total Equipment	2178.66	1365.4
Installation, @200% of	4357.32	2730.8

equipment cost		
Miscellaneous Improvements	500	500
Total Other Cost	4857.32	3230.8
Total Cost	7036	4596
Saving, %	0	34

The calculation revealed that total equipment cost for *in situ* transesterification was less than that of conventional by 37%. The main unit operations that affect the equipment cost were the acid esterification reactor and oil tank. The saving on the total cost, which included the installation and miscellaneous cost, was 34%.

4.8.3 Operating Cost

A breakdown of operating cost was presented in Table 4.10 for Case I and Table 4.11 for Case II.

Table 4.10 Operating cost for *in situ* transesterification using methanol as a solvent

Item	Price £/ kg	kg required/ kg biodiesel	Price £/ kg biodiesel
Methanol [157]	-0.26	3.9	-1.014
J. curcas seed [158]	-0.09	3.7	-0.333
NaOH [159]	-0.14	0.1	-0.014
Soap [160]	-0.02	0.3	-0.006
Wash water [161]	-0.06	0.1	-0.006
Biodiesel [162]	0.72	1	0.72
Glycerol [163]	0.20	0.1	0.02
Process water [164]	-0.01	0.1	-0.001
Meal [165]	0.28	2.6	0.728
			0.1

In each case, price (£) per kg of all the components in feed stream and outlet stream was listed. After that, the amount of each component, with respect to 1 kg of biodiesel was

calculated, using data from Table 4.8. The components in the feed stream were negative in value whilst the components in outlet stream were positive, except for the soap. This is because of the charge imposed by UK local authority to collect and treat it. Process water means the water used in the process, which was bought from local authority. Wash water meanwhile means the waste water, which collected by local authority with certain charge.

Table 4.11 Operating cost for *in situ* transesterification using methyl acetate as a solvent

Item	Price £/ kg	kg required/ kg biodiesel	Price £/ kg biodiesel
J. curcas seed	-0.09	0.45	-0.315
NaOH	-0.14	0.3	-0.063
Methyl Acetate [166]	-0.74	3.7	-2.738
PEG [167]	-0.90	6.8	-6.12
Soap	-0.02	0.3	-0.006
Wash water	-0.06	0.1	-0.006
Biodiesel	0.72	1	0.72
Triacetin [168]	0.90	0.1	0.09
Process water	-0.01	0.1	-0.001
Meal	0.28	2.1	0.588
			-7.85

Comparison of the unit price of biodiesel from both cases shows that *in situ* transesterification by methanol reduced the unit price from £0.72/ kg to £0.62/ kg, which is £0.1/ kg less than the current price of biodiesel. Meanwhile, in methyl acetate case, the price was increased by £7.85/ kg, to £8.57/ kg, almost 11 times higher than the current price. For comparison, the more complex techno-economic study by Haas on *in situ* transesterification of soybean, which considered all the cost associated with the operating cost, found that the price per gallon of biodiesel was 8 time higher than the current price in America [54]. The different probably due to the price of raw material, in their case was soybean which the current price is £0.40/ kg [169], compared to £0.09/ kg price of *J. curcas.*

For Case II, although the molar ratio of methyl acetate needed for Case II was a half less than that of methanol-oil in Case I, the high price of methyl acetate, £0.74 compare to £0.26 of methanol, affect the unit price of biodiesel greatly. The need to add PEG as a transfer phase agent worsens the economic balance as the PEG price was high at £0.90 per kilogram. Even though the by-product triacetin price £0.90 was higher that Case I's glycerol, £0.20, this was cancelled of by the use of PEG.

It is also interesting to note that for 1 kg of biodiesel, the value of meal was similar to that of biodiesel, £0.72. The reason for this is because the huge quantity of meal, 2.6 kg, obtained for every kilogram of biodiesel. The price of *J. curcas* meal, $430/ ton, and equivalent to £0.28/ kg is also high, compared to soybean meal, $300/ ton or £0.19/ kg. The explanation of this high price is because the meal contains more protein than for example rapeseed meal, as stated in Section 4.7.1.1.

Although the biodiesel price in Case I was lower than the current price, it should be noted that, the methanol recycling process was more energy consuming in *in situ* compared to conventional transesterification. According to Dhar and Kirtania [126], 500 kW of electricity required to recycle 80% of the methanol at 6:1 molar ratio, and this amount increased to 3000 kW for 50:1 molar ratio. By extrapolating the points for 400:1, which is the molar ratio in Case I, 22, 900 kW of electricity required to recycle 80% of methanol back to the process. At current electricity price of £0.68 kWh [170], the cost to operate the distillation column for 1 hour in *in situ* transesterification plant is £15,000 compared to £340 in conventional transesterification plant.

Further analysis shows that the electricity price can be lowered by decreasing the recovery level of excess methanol. Figure 4.33 shows the electricity price decreased from £15,000 to £12,000, £10,000 and £6,000 as methanol recovery percentage was lowered from 80% to 75, 70 and 65% respectively.

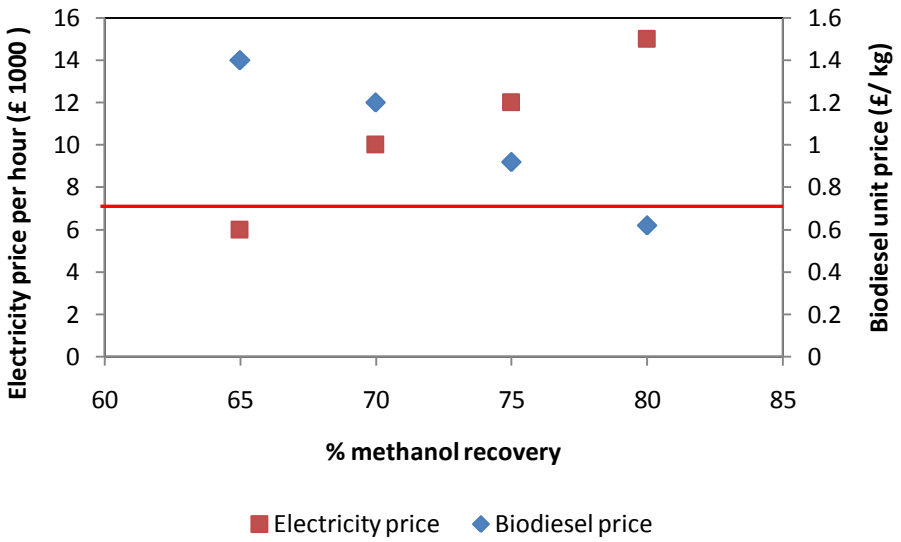


Figure 4.33 Relationship of percentage of methanol recovery with electricity and biodiesel price

However, as the methanol recovery percentage and electricity price decrease, the biodiesel price per unit kg increased above the current biodiesel price line, £0.72. Therefore, to ensure the biodiesel price from *in situ* transesterification process remains competitive, a balance combination between methanol recovery and electricity price must be considered.

5 CONCLUSIONS AND FURTHER WORK

5.1 Conclusions

The main aim of this research was to extensively study biodiesel production by *in situ* transesterification of *J.curcas* seed. It was found that generally, it is possible to produce fatty acid methyl ester (biodiesel) via *in situ* transesterification of *J. curcas* with alkali catalyst. This was possible despite its high free fatty acid content, which would conventionally mean that the oil has to undergo an acid esterification process before converted to biodiesel via the transesterification process. The *in situ* transesterification of *J. curcas* was also found to have high tolerance towards water content (5%), as opposed to *in situ* transesterification of soybean (0%). In terms of process, this will have significant effect since the drying stage can be eliminated from the process. The *J. curcas* seed was characterised and it was shown that it had 36 % triglyceride and 37 % protein. The triglyceride was dominated by unsaturated fatty acids, specifically, oleic and linoleic acids

Sodium hydroxide, sodium methoxide and sulphuric acid were investigated to find the most suitable catalyst for the process. Of the three catalysts used in the screening process, sodium hydroxide gave the highest yield at the shortest reaction time. Yield per unit time increased with the decreasing particle size until the size reach 0.71 mm.

6 parameters were investigated in initial one-at-a-time screening experiments: particle size, mixing speed, reaction temperature, reaction time, catalyst concentration and methanol-oil molar ratio were tested one by one. For the mixing speed, the parameter become insignificant once it reaches 300 rpm. The reaction temperature was found to be irrelevant to the yields end point, but when the time profile for different temperatures were plotted, it was obvious

that the increases in temperature increased the reaction rate. The FAME yield was observed exhibited minimal change beyond 30 minutes of reaction time, suggesting that the reaction completed within 20 to 30 minutes after the reaction start. The *in situ* transesterification were unable to proceed without the presence of catalyst, in this case the sodium hydroxide catalyst. Even the small amount of sodium hydroxide, 1.0 N, catalysed the reaction greatly. However, the addition of more than 0.2 N of sodium hydroxide had promoted soap formation instead. The most crucial parameter in *in situ* transesterification process is the molar ratio of the solvent to oil. The results suggest that the amount of methanol must be very high in order to achieve an appreciable yield, in this case as high as 400:1.

The data from these experiments were used to set the limits of the "Design of Experiments" matrix. The limits were: methanol to oil ratio, 100 – 400, NaOH concentration of 0.1-0.2 N, 10-60 minutes reaction time, 30-60^{o}C reaction temperatures and 100-400 rpm mixing speed. The design of experiments result shown that within the experiment matrix, out of 5 parameters (mixing speed, reaction temperature, reaction time, catalyst concentration and methanol-oil molar ratio), only three, which were mixing speed, reaction time and methanol-oil molar ratio gave significant effect to the FAME yield. It was found in screening process that the correlation between the parameters and FAME yield was not linear, and thus non-linear polynomial model has been suggested instead.

In the effort to reduce the solvent used in the *in situ* transesterification, the used of diethoxymethane (DEM) was found producing better yield than that of hexane. While the effect of DEM was not significant in lower molar ratio experiment, the addition of it in high molar ratio experiment increased the FAME yield by 11%. This however did not reduce the amount of methanol. One of the more significant findings to emerge from this study is that

the replacement of methanol with methyl acetate successfully reduced the amount of solvent required for the process. The used of 175:1 molar ratio of methyl acetate to oil produced almost similar amount of yield with 400:1 molar ratio of methanol to oil.

It also emerged from this study that there were various compounds that end up in the waste streams of the *in situ* transesterification process. The liquid waste stream mainly consists of glycerol, soap, phenol and phorbol ester, whilst the solid waste stream consists of meal which was rich in protein. As opposed to the conventional process, where the meal still contained toxic compounds and thus make it unsuitable for animal feed, *in situ* transesterification may have reduce or remove these compounds and increased it value as animal feed.

Taken together, the results of this study indicate that the *in situ* transesterification can offer alternative route in producing biodiesel from *J. curcas*. The advantages of this process lie on several factors. The first one is there is no dependency on edible oil, which the price is volatile, due to its main use as a food material. Secondly, the process eliminates few energy intensive, huge capital cost stages, such as oil extraction and acid esterification process. This will help reducing the overall capital cost although its impact on the overall techno-economic still has to be investigated. Thirdly, the other compounds that came out from the process' waste streams have their own value in the market. This possesses the possibility of biorefinery concept to be implemented and at the same time will help on the economic aspect of the overall project.

The findings from this study make several contributions to the current literature. First, this project was the first attempt of *in situ* transesterification of *J. curcas* seed with alkali catalyst.

Therefore, it consists of details and in-depth information on the effect of each parameter, specific to *J. curcas* seed. Secondly, the relationship of the effect of parameters with fundamental concept such as Fick's laws of diffusion for molar ratio effect, diffusivity-temperature relation for the effect of temperature and Frossling equation for the effect of mixing speed, has not been discussed in the *in situ* transesterification literature to date. Thirdly, the use of light microscope to look into the effect of some parameters to the seed's cell, were started from Process Intensification Group (PIG), and pioneered in *in situ* transesterification study. Fourthly, the modified technique to determine yield in bulk solvent phase, also invented within the group, and offered a simpler, consistent and reliable method to measure the yield. Although the used of hexane and DEM is not the pioneer in *in situ* transesterification, the use of methyl acetate as a replacement to the methanol is the first of its kind. There is high possibility that *in situ* transesterification capable to remove the toxic compound, phorbol ester from the meal. This is based on the literature that indicates that the phorbol ester was decomposed when mixed with methanol. The reason is because both methanol and phorbol ester are polar compounds. The economics evaluation on the operating cost, although briefly, provide a foundation for more extensive techno-economic study and never been published in current literature.

5.2 Further Work

It is recommended that further research be undertaken in the following areas:

i. The reactor design. The used of counter current extractor in many bio-based solid extraction has been proved to be more efficient than batch system [171, 172]. This will ensure that the seed received fresh solvent during the reaction and thus increase the rate of extraction. This will also minimise the solvent used, as the solvent will recycle throughout the process.

ii. The phorbol ester material balance. In this study, the amount of phorbol ester was determined by calculating the mass balance of the overall process. It will be more accurate if the phorbol ester is detected and quantified at all process stages. The detection and determination of phorbol ester is important in order to ensure the meal can be utilised as animal feed. In mechanical pressing extraction system, this compound remained in the meal. The process to quantify the amount of phorbol ester was developed by Makkar and describe in details in his publication [69]. The sample was prepared by mixing the ground seed with dichloromethane to extract all other non-polar compounds from the seeds. The liquid then was filtrated and the dried residue was mixed with tetrahydrofuran. This mixture then was passed through a filter and then injected into the HPLC, equipped with reverse phase C18 column. The condition of the HPLC also described in the publication.

iii. Kinetic Modelling. The modelling of *in situ* transesterification will help researcher to ascertain on the reaction pathway. Data on the kinetics involved in the reaction is important, for example to determine the rate limiting step. The data also used to develop a conceptual design of the plant with simulator. The hypothesis of the modelling can be based on shrinking

core model where the solid particle shrinks in liquid. Two processes occurred in series during the dissolution process, which were the escape of solute from the solid particle and the diffusion of the solute to the bulk phase. The rate of dissolution may be controlled by one of these two steps [173].

iv. Techno-economic study. The issue of biodiesel price is an intriguing one which could be usefully explored in further research. In the current study, the fixed capital cost, which is the cost associated with the equipment's price was adopted from literatures. To improve the economic analysis study, a conceptual design for each reaction scenario needs to be developed with the help of process simulator.

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7 APPENDICES

A: Calculation of methanol-oil molar ratio.

B: Calculation of diffusion coefficient, D_{AB}, for diglyceride-methanol system.

C: Publications

C1. Kasim, F.H., A.P. Harvey, and R. Zakaria, *Biodiesel production by in situ transesterification.* Biofuels, 2010. **1**(2): p. 355-365. [174]

C2. Kasim, F.H. and A.P. Harvey, *Influence of various parameters on reactive extraction of Jatropha curcas L. for biodiesel production.* Chemical Engineering Journal, 2011. **171**(3): p. 1373-1378.[46]

7.1 Appendix A: Calculation of methanol-oil molar ratio.

Calculation of methanol				
1	Percentage of oil in seed:	=	0.36	%
2	Mass of sample:	=	10	g
3	Mass of oil in the sample	=	0.36 x 10	
		=	3.6	g
4	MW of Jatropha oil:	=	877	
5	No of mol of sample:	=	3.6/ 877	
		=	0.004104903	mol
6	Ratio of alcohol:oil	=	100	
7	Mol of methanol required:	=	4.1E-03 x 100	
		=	0.410490308	mol
8	MW of methanol	=	32.04	
9	Mass of methanol needed	=	4.1E-01 x 32.04	
		=	13.15210946	g
10	Density of methanol	=	0.7918	g/cm3
11	Volume of methanol needed	=	13.15 x 0.7918	
		=	10.41217	cm3
Calculation of sodium hydroxide				
1	Catalyst concentration	=	0.1	mol/L NaOH
2	Mass of	=	791.8	g

	methanol for 1 L			
3	No of mol of 1 L MeOH	=	24.71285893	mol
4	MW of NaOH	=	40	
5	Mass of NaOH needed	=	4	g
6	Mass of NaOH needed for X g of methanol	=	0.066441573	g

7.2 Appendix B: Calculation of diffusion coefficient, D_{AB}, for diglyceride-methanol system

$D_{AB}(60^oC) = 1.62 \times 10^{-9}\ m^2/s$ [55]

Viscosity, μ, of the methanol

Temperature	Viscosity (cP)
30	0.521
40	0.469
50	0.399
60	0.366
70	0.314

$$D_{AB}(T_2) = D_{AB}(T_1)\frac{\mu_1}{\mu_2}\left[\frac{T_2}{T_1}\right]$$

For D_{AB} (70^oC):

$$D_{AB}(70^oC) = D_{AB}(60^oC)\frac{\mu_{60}}{\mu_{70}}\left[\frac{343.15}{333.15}\right]$$

$$= 1.62 \times 10^{-9}\frac{0.366}{0.314}\left[\frac{343.15}{333.15}\right]$$

$$= 1.94 \times 10^{-9}\ m^2/s$$

D_{AB} value for temperature ranging from 10-70°C

Temperature (K)	D_{AB} (m^2/s)
283.15	7.19×10^{-10}
303.15	1.04×10^{-09}
313.15	1.19×10^{-09}
323.15	1.44×10^{-09}
333.15	1.62×10^{-09}
343.15	1.94×10^{-09}

7.3 Appendix C: Publications

REVIEW

Biodiesel production by *in situ* transesterification

Biofuels (2010) 1(2), 355–365

Farizul Hafiz Kasim†, Adam Philip Harvey & Rabitah Zakaria

Biodiesel is conventionally produced by transesterification of vegetable oils using an alcohol (usually methanol) and a catalyst (usually hydroxides or methoxides of sodium or potassium). The process usually uses pre-extracted oil as the raw material, which is usually produced by pressing the oil-bearing seeds, often followed by solvent extraction to extract any remaining oil. Alternatively, biodiesel can be produced via *'in situ* transesterification' or 'reactive extraction'. In this process, oil-bearing seeds are ground, then reacted directly with the alcohol and catalyst, thereby eliminating the need for pre-extracted oil, and its associated capital and intensive running cost production methods. Various parameters play important roles in determining the conversion, reaction rate and quality of the biodiesel in *in situ* transesterification. These include: catalyst type, seed moisture content, agitation intensity, molar ratio of alcohol to oil, reaction temperature, catalyst concentration, seed fragment particle size and alcohol type. This article gives an overview of *in situ* transesterification, the parameters that have a significant effect on this process and the advantages and disadvantages of this process.

Since Dr Rudolf Diesel first demonstrated his compression ignition combustion engine using peanut oil at the 1900 Paris exhibition [1], the use of vegetable oil as a transport fuel has been known to be feasible. However, petroleum fractions that were compatible with the diesel engine became less expensive than vegetable oils, so vegetable oil-based fuels were not commercially viable. Over the last 20 years, factors such as geopolitical tension in the Middle East, leading to price volatility in crude oil and fears for security of supply, and the realization of global warming, have combined to stimulate interest in vegetable oil-based diesel fuels. Research into **biodiesel** prior to 1990 centered on using raw or modified vegetable oils [2–5]. Despite these fuels successfully passing shorter engine performance tests (less than 10 h duration), problems began to emerge after longer periods of use. The major problems (according to Pryde [4]) were:

- Coking and trumpet formation on the injectors, to such an extent that the fuel atomization does not occur properly or is even prevented altogether by total blockage of the injector;
- Carbon deposits;
- Oil ring sticking;
- Thickening and gelling of the lubricating oil as a result of contamination with vegetable oil.

These problems occur due to the higher viscosity of the vegetable oil, reduced volatility and the reactivity of unsaturated hydrocarbon chains. These problems were addressed by Peterson *et al.* when they assessed winter rape oil in diesel engine [6]. They suggested that polyunsaturated fatty acids in the oil tend to polymerize and subsequently form gums in the engine chamber. The gums cause the carbon deposit and sticking problems. Darcey *et al.* also reported that the use of blended crude sunflower oils in diesel engines resulted in solid contamination in the lubricating oil [3].

To reduce these problems, Goering and Fry [7] and Ziejewski *et al.* [8] tried to decrease the viscosity of the vegetable oils by creating 'microemulsions'. The process effectively reduced the viscosity of the vegetable oil. Furthermore, use of the hybrid oil obtained resulted in reduced engine wear [7]. However, deposits of carbon on

†Author for correspondence
School of Chemical Engineering and Advanced Material, Merz Court, Newcastle University, NE1 7RU, UK
Tel.: +44 191 222 7268; Fax: +44 191 222 3496; E-mail: f.h.kasim@ncl.ac.uk

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Key terms

Biodiesel: a renewable alkyl ester fuel made by the transesterification of oils or fats (triglycerides)

Transesterification: a reaction in which the alkoxy group of an ester is changed for another. In transesterification of triglycerides, the linkage to the 'glycerol backbone' is exchanged for a methyl (or sometimes ethyl) group from methanol (or ethanol), thereby splitting the resultant ester from the backbone, reducing the size of the molecule, and therefore its viscosity, allowing it to flow and combust in a similar manner to conventional 'petrodiesel' in a diesel engine

In situ transesterification: transesterification performed directly on lipid-bearing materials, usually oilseeds

Process intensification: a design philosophy intended to reduce the size of process plants and individual items of equipment by orders of magnitude

Oilseeds: oil-bearing seeds, many of which are used for biodiesel production, notably rapeseed, soya beans, sunflower seeds and palm kernels

the injector tips, intake valve and tops of cylinder liners were increased [7,8]. Incomplete combustion and abnormal increases in the viscosity of lubricating oil were also reported [8].

Thermal cracking [9,10] and **transesterification** [11,12] were also reported in this period (pre-1990). Thermally cracked fuels, after fractionation, were shown to have similar properties to diesel fuel [10]. The drawback of this technique was that the processes were too expensive for modest throughputs, because they were very energy intensive [13]. In transesterification however, researchers believed they had found the right process. Not only was the quality of biodiesel produced comparable to that of petroleum diesel, but the process could also be operated at low temperature (typically 60°C) and low pressure, resulting in relatively low energy consumption. In addition, the fuel performed well in engine tests [12]. However, it was also discovered that ester yields were reduced due to the existence of gums and extraneous material in the crude vegetable oil [11]. Research into ***in situ* transesterification** was also reported at this time, particularly by Harrington and D'Arcy-Evans [14,15]. Both reported experiments on *in situ* transesterification of sunflower seed oil. Among the noteworthy conclusions was the claim that this process yielded fatty acid esters that were qualitatively similar to those produced by the liquid-phase transesterification, and at a greater yield [14,15].

In the 1990s, most research was concerned with the transesterification of vegetable oil. The effects of the various process parameters and a range of raw materials were reported throughout the decade. Problems arose when the market price of these edible vegetable oils increased. This caused the profitability of the process to decrease. It was suggested that transesterification was only profitable at vegetable oil prices below US$400 per metric ton [16]. To overcome this problem, alternatives to the usual raw materials were investigated and, as a consequence, a myriad of inedible oils were investigated. The focus of current research is to introduce new, low-cost and often inedible oils. Apart from new raw materials, researchers also began to investigate new processes to reduce processing costs. Algae began to receive attention as a new raw material [17,18] as it has the potential to provide the possibility of 20-fold increases in oil yield per hectare.

This review critically discusses *in situ* transesterification, which is a process of producing alkyl ester directly from oil-bearing material, usually ground **oilseeds**. Since its introduction by Harrington and D'arcy Evans [14], numerous researchers have investigated the performance and feasibility of this process. However, whether it can replace the current transesterification technology remains to be seen. The possibility of producing biodiesel via *in situ* transesterification can only be materialized once the process as a whole has been fully characterized [19]. In conventional transesterification, the raw material usually comes from edible oil, where oil was extracted from the seeds using the conventional extraction processes of crushing, perhaps followed by solvent extraction. Haas *et al.* estimated that the cost of refined soybean oil in biodiesel transesterification accounted for 88% of the total production cost [20]. It was also asserted by Haas that biodiesel produced by *in situ* transesterification is more expensive than biodiesel produced via conventional transesterification, due to the large amounts of methanol required for the process. The production cost of biodiesel by *in situ* transesterification of soya was estimated at US$3.14 compared with 0.38 by transesterification [19].

Ongoing research into *in situ* transesterification may render it more economically attractive. Inedible oils, such as *Jatropha curcas* and *Pongamia piñata,* may be grown on 'marginal' land, and, for this reason and because they have greater yields per hectare than soya and canola, may prove to be inexpensive feed. *J. curcas* for example, contains phorbol esters, which are cocarcinogen and toxic. The *Jatropha* oil obtained by cold pressing and solvent extraction still contains such substances. However, biodiesel produced from *Jatropha* oil by *in situ* transesterification has been shown to not contain such chemicals [21]. The application of *in situ* transesterification therefore not only **intensifies** the process, but also removes the need for a specific step to extract these toxic substances in the downstream processing, reducing the exposure of workers to such substances.

Definition

In situ transesterification is the direct transesterification of ground oil-bearing seeds. The seed fragments are reacted with alcohol and catalyst, producing alkyl fatty acid esters. This should be contrasted with conventional biodiesel transesterification, in which the raw materials are pre-extracted from oil-bearing seeds.

Figure 1 summarizes the two processes: *in situ* transesterification has fewer steps than conventional processing. The crushing and solvent extraction steps that are required in the conventional process, but not in *in situ* transesterification, are usually the most capital and running cost-intensive.

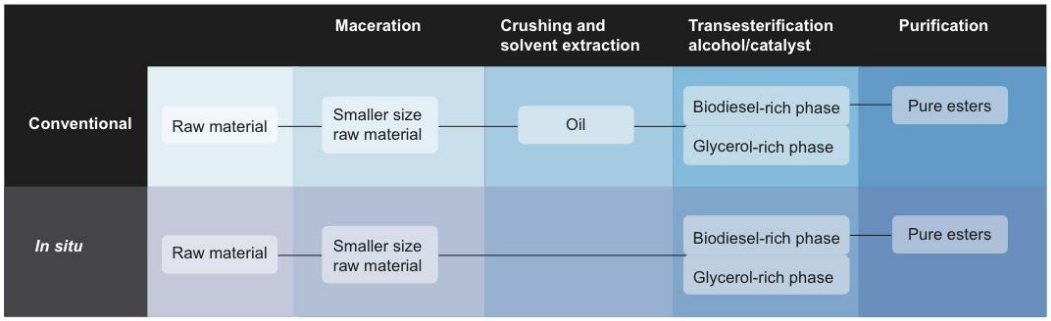

Figure 1. Conventional versus *in situ* transesterification.

To measure the efficiency of *in situ* transesterification, most publications used the terms yield and purity. The majority of researchers define yield as the percentage of biodiesel-rich phase over oil content in raw materials, which is determined by hexane Soxhlet extraction. Purity is defined as the percentage of methyl esters in product obtained from the purification stage. This percentage is usually calculated from a gas chromatogram result.

Variables in *in situ* transesterification

▪ Raw materials

Traditional oil-bearing seeds, such as rapeseed and sunflower seed, or even materials such as distiller's dried grains with solubles (DDGS) [22] and jatropha seed [23,24], have been studied by researchers. The fatty acid profiles of the oils produced by these materials vary substantially and, consequently, process parameters differ. Even though fatty acid profiles are known to influence biodiesel properties such as cetane number and cold filter plugging point [25], no researchers have studied this with respect to *in situ* transesterification. The *in situ* approach, however, can be applied to almost any lipid-bearing material (Table 1) [14,22,23,26–32].

▪ Catalyst

It is well-documented that *in situ* transesterification is unable to proceed without a catalyst [26,33]. Short-chain alcohols, particularly methanol, are poor solvents for lipids. Zeng *et al.* observed that methanol alone was capable of extracting only 4.5% (seed mass) of oil from 20 g of soybean, compared with 45% when using n-hexane [34]. Acid or alkali catalysts in *in situ* transesterification help to break oilseeds' cell walls, thereby allowing methanol to access the oil in cotyledon cells.

Table 1. Different raw materials used by researchers in *in situ* transesterification.

Raw material	Oil (%)	Fatty acid composition (%)	Ref.
Sunflower seed	38	16:0(6.8), 18:0(5.0), 18:1(19.6), 18:2(68.6)	[14]
Soybean	23	16:0(12.0), 18:0(5.0), 18:1(25.0), 18:2(52.0), 18:3(6.0)	[26]
Distiller's dried grains with solubles	8.8	16:0(12.9), 18:0(1.6), 18:1(28.5), 18:2(55.5), 18:3(1.4)	[22]
Meat and bone meal	9.1	16:0(25.2), 18:0(19.7), 18:1(35.6), 18:2(1.9), 18:3(0.3)	[22]
Palm oil pulp	80	12:0(0.3), 14:0(0.8), 16:0(44.3), 18:0(5), 16:1(0.2), 18:1(39.1), 18:2(10.1), UK(0.2)	[40]
Cottonseed	31.6	16:0(28.7), 18:0(0.9), 18:1(13.0), 18:2(57.4)	[27]
Rapeseed	42	16:0(4.0), 18:0(1.9), 18:1(62.1), 18:2(32.0)	[28]
Jatropha curcas	54	16:0(16.0), 18:0(7.0), 18:1(45.0), 18:2(32.0)	[23]
Wastewater sludge (primary sludge)	2	16:0(42.0), 18:0(14.0), 18:1(28.0), 18:2(10.0), 20:1(6.0)	[29,30]
Microalga (*Schizochytrium limacinum*)	51‡	14:0(2.06), 16:0(35.5), 18:0(0.81), 22:5(8.58), 22:6(53.05)	[31]
Microbial biomass (*Lipomyces starkeyi*)	50†	14:0(0.4), 16:0(33.0), 17:0(0.4), 18:0(4.7), 16:1(4.8), 18:1(55.1), 18:2(1.6)	[32]
Microbial biomass (*Rhodosporidium toruloides*)	58‡	14:0(0.7), 16:0(24.3), 17:0(0.6), 18:0(7.7), 16:1(1.1), 18:1(54.6), 18:2(2.1), uk(8.9)	[32]
Microbial biomass (*Mortierella isabellina*)	53‡	14:0(1.2), 16:0(28.2), 18:0(1.0), 16:1(5.8), 18:1(55.5), 18:2(5.8), 18:3(2.4), uk(0.1)	[32]

†Total lipid extraction.
uk: Unknown.

Ren *et al.* investigated the *in situ* transesterification of canola using scanning electron microscopy (SEM) and light microscopy [35]. Lipid staining showed that this reactive extraction followed a 'shrinking core' model, as the mass of cells containing lipid within the cell could clearly be seen to shrink as the extraction progressed. Sodium hydroxide can dissolve cell walls, but they did not find any evidence for this for cells inside the particles when examining sections via light microscopy.

The total mass yield of extract by *in situ* transesterification (40.9%) was found to be greater than that obtained from transesterification (30.3%) [14]. It was claimed that this was due to the capability of *in situ* transesterification to extract materials that were not extracted from the seed by hexane [14]. As a nonpolar solvent, hexane can only extract nonpolar substances, such as triglycerides. When acidified/alkaline methanol was used instead of hexane, both polar and nonpolar substances were extracted from the seed. Dufreche *et al.* also claimed that a higher percentage of material was extracted (19.39%) when using methanol rather than when using hexane (1.94%) when they extracted sewage sludge [29]. The sharp increase in methanol extraction in this case was due to the presence of large amounts of phospholipids, in the form of microorganism cell membranes, in the sewage.

Acid catalysts

Acids, in particular sulfuric acid, were the preferred catalysts in earlier (1980s) research into *in situ* transesterification for biodiesel production. Harrington and D'arcy-Evans were the first researchers working on sulfuric acid as a catalyst in *in situ* transesterification [14,15]. Acid catalysis has often been investigated for the treatment of raw materials with high levels of free fatty acids (FFAs). Alkaline catalysts will react with FFAs to produce soap and glycerol, decreasing the amount of the catalyst, or consuming it altogether. Furthermore, soap acts to emulsify the product, rendering the separation between alkyl esters and glycerol more difficult. Acid catalysis, by contrast, does not promote saponification. Mondala *et al.*, for instance, used sulfuric acid as the catalyst for conversion of their raw material, municipal sewage sludge, as it contained 65 wt% FFA [30]. Turkay *et al.*, who investigated extraction from rice bran, used acid catalysts, because the acidity of rice bran oil was unpredictable, and usually high [36–38].

Most researchers report high conversion of methyl esters when using acid catalysts. Harrington and D'arcy-Evans achieved 98% conversion of sunflower seed oil to fatty acid methyl ester (FAME) using a methanol/sulfuric acid mixture [14,15]. Siler-Marinkovic and Tomasevic, who also worked with a sunflower seed/methanol/sulfuric acid system, observed conversion over 90% over a wide range of experimental condition [39]. Shuit *et al.* reported that 90% of the oil was extracted from *J. curcas* seed when using acid-catalyzed *in situ* transesterification and 100% of it was converted to FAME [23]. Obibuzor *et al.* also reported high conversion (97%) of oil to FAME from reactive extraction of oil palm waste pulp using a methanol/sulfuric acid mixture [40]. Acid catalysis also worked efficiently in reactive extraction of oleaginous microbial biomass. Lipid contents from three different oleaginous biomass, *Lipomyces starkeyi*, *Mortierella isabellina* and *Rhodosporidium toruloides*, were successfully converted to FAME at 97, 91 and 98%, respectively. Liu *et al.* investigated the *in situ* transesterification of cellular biomass of yeast and fungi using an acid catalyst and methanol [32]. They found that both sulfuric acid and hydrochloric acid could produce moderate ester yields, of 60 and 53%, respectively. Significantly lower yields (10%) were achieved when phosphoric acid was used, but the authors do not offer an explanation for this.

Researchers also observed that the reaction time was longer when using an acid catalyst rather than an alkaline catalyst. Shuit *et al.*, for instance, found that 90% conversion of *J. curcas* using sulfuric acid required 24 h [23]. Obibuzor *et al.* obtained more than 90% conversion in 12 h when using reactively extracted palm oil pulp waste with sulfuric acid [40].

Alkaline catalysts

The first *in situ* transesterification using an alkaline catalyst was reported by Haas *et al.* [41]. The experiment was conducted using soybean flakes as a raw material. The combination that produced the highest percentage of methyl ester was 12.5 ml of methanol, 0.18 N of sodium hydroxide. This was equivalent to 226:1:1.6 molar ratio of methanol/oil/NaOH. Comparing this value with the conventional transesterification experiments (6:1:0.22) by Freedman *et al.* [42], it is clear that *in situ* transesterification requires more methanol and more catalyst.

When comparing the effectiveness of both acid and alkali catalysts, Haas *et al.* listed three advantages in favor of using alkaline catalysts [41]:

- Instead of using pulverized beans as described in previous literature [14,15,26], *in situ* transesterification with an alkali catalyst only requires soybeans to be flaked;
- The amount of reagent required is reduced and milder process condition are required;
- Higher yields of methyl ester are observed.

The advantages of the first two options can be clearly seen in the literature. For instance, a molar ratio of 553:1 methanol to oil was used by Harrington and

D'Arcy-Evans [14] (sunflower seed/methanol/sulfuric acid) to achieve 97% conversion, whereas using an alkaline catalyst, Georgogianni *et al.* used 163:1 molar ratio (sunflower seed/methanol/sodium hydroxide) and achieved 95–97% conversion [43]. However, for the third option, it seems that both types of catalysts produced a comparable yield of FAME, but not at the same rate. The reaction time required when using sulfuric acid to produce 97% yield was 4 h, while sodium hydroxide only needed 2 h to produce the same yield. Furthermore, at 40 min, 94% of oil had already been converted to methyl ester.

The conversion of oil to methyl esters was typically very high when using methanol and sodium hydroxide. Among researchers who successfully achieved high yield of conversion were Georgogianni *et al.* [27,43] on sunflower seed/methanol/sodium hydroxide (97%) and cottonseed/methanol/sodium hydroxide (97%), and Haas *et al.* [41] on soybean/methanol/sodium hydroxide (88%). Qian *et al.* also achieved over 95% conversion in reactive extraction of cottonseed using methanol and sodium hydroxide [33]. Alkaline catalyst reactive extraction has also been applied to a number of non-oilseed feedstocks. To investigate whether *in situ* transesterification was applicable to all lipid-bearing materials, Haas' group performed experiments on DDGS and meat and bone meal (MBM). Both raw materials contained low percentages of oil, but via alkaline methanolysis, the oil fractions of DDGS and MBM were successfully converted to methyl ester at 91 and 93% conversion, respectively. Dufreche *et al.*, using acid catalysis, noted that *in situ* transesterification of sewage sludge achieved 6.23% conversion compared with 0.38% when hexane extraction/acid transesterification was used [29]. Even the second highest conversion (3.44% – achieved when a mixture of hexane, methanol and acetone used to extract the oil) was 2.79% lower than *in situ* transesterification. Clearly, this is a significant difference, and could make a difference in determining the economic viability of low oil content feedstocks.

Table 2 lists different raw materials, catalyst and solvents used by researchers to produce biodiesel through *in situ* transesterification. The selection of catalyst very much depends on the feedstock properties, especially the content of FFA.

Table 2. Different raw materials, catalysts and solvents used by researchers to produce biodiesel through *in situ* transesterification.

Raw material	Solvent	Catalyst (mol/l)	Molar ratio solvent:oil	Reaction time (h)	Temp. (°C)	Conversion (oil basis) (%)	Notes	Ref.
Sunflower	Methanol	H_2SO_4 (0.75)	532:1	5	65	93		[15]
Sunflower	Methanol	H_2SO_4 (0.7)	300:1	4	64.5	98.2		[39]
Soybean	Methanol	H_2SO_4 (0.75)	281:1	10	65	23.3		[26]
Soybean	Methanol	H_2SO_4 (0.75)	150.1	3	121	83	CO_2 cosolvent	[44]
Jatropha curcas	Methanol	H_2SO_4 (0.2)	300:1	24	60	99.8	Hexane cosolvent	[23]
Microbial biomass	Methanol	H_2SO_4 (0.2)	830:1	20	70	96.8 (*Lipomyces starkeyi*) 91.0 (*Mortierella isabellina*) 98.1 (*Rhodosporidium toruloides*))		[32]
Primary sewage sludge	Methanol	H_2SO_4 (0.9)	1400:1	24	75	66		[30]
Soybean	Methanol	NaOH (0.09)	543:1	8	23	88		[41]
DDGS	Methanol	NaOH (0.4)	655:1	1.2	35	91.1		[22]
MBM	Methanol	NaOH (2.0)	550:1	0.2	35	93.3		[22]
Cottonseed	Methanol	NaOH (0.4)	673:1	0.3	60	95	Ultrasound	[27]
Cottonseed	Ethanol	NaOH (0.4)	613:1	0.7	80	98	Ultrasound	[27]
Sunflower	Methanol	NaOH (0.4)	476:1	0.7	60	97	Ultrasonic	[43]
Sunflower	Ethanol	NaOH (0.4)	434:1	0.7	80	98	Ultrasonic	[43]
Sunflower	Methanol	NaOH (0.2)	101:1	13	20	98	DEM cosolvent	[34]
Jatropha curcas	Methanol/ ethanol mix	NaOH (0.02)	512:1	1	60	87		[24]
Jatropha curcas	Methanol	NaOH (0.04)	100:1	1	60	70		[45]

DDGS: Distiller's dried grains with solubles; DEM: Diethoxymethane; MBM: Meat and bone meal; Temp.: Temperature.

▪ Moisture content

In conventional transesterification, the presence of water in the process would cause soap formation and frothing. This would result in increased viscosity, gel formation and difficulty in separation between the glycerol and alkyl ester-rich phases [13]. In addition, the saponification process will consume triglyceride, thereby reducing the potential yield of the methyl ester. In reducing the moisture content prior to the reaction, Haas *et al.* found that the amount of alcohol required for the process was lessened significantly [46]. They reported 60% reduction of methanol and 56% reduction of sodium hydroxide when soybean flakes were dried in a convection oven until they had a water content of 0%. Experiments at 2.6% water content samples reduced the methanol and sodium hydroxide requirement by 40 and 33%, respectively.

In situ transesterification has been shown to require higher alcohol to oil ratio than conventional transesterification. Even though the application of *in situ* transesterification eliminates the need for pre-extracted oil, it is asserted by Haas that it is more expensive than biodiesel produced by conventional transesterification [20]. The reduction of water however, was able to reduce the estimated biodiesel production cost from US$3.14 to 1.02 per gallon [19]. The same trend was also reported by Qian *et al.* [33]. Methyl ester conversion was found to increase significantly from 80 to 98% when the moisture content was reduced from 8.7 to 1.9%. Further reduction of moisture content, however, had very little effect on the conversion.

By contrast, research at Newcastle on *in situ* transesterification of ground rapeseed using methanol/sodium hydroxide has shown that drying the seeds from 6.7 to 0 wt% water does not reduce the solvent requirement, nor increase the yield of ester [28]. It was found that the ester yield only reduced when there was more than 2 wt% water in the solvent. This indicates that, for rapeseed at least, the drying step may be unnecessary, which should reduce the cost of biodiesel produced by this method, as drying can incur substantial running costs. The mechanism is simply that the methanol extracts the water rapidly, followed by reaction within the seed, thereby separating the water from the reaction. Saponification could still occur in the bulk liquid phase, but the water concentration is greatly reduced.

▪ Mixing intensity

Georgogianni *et al.* observed the effect of low-frequency ultrasound on *in situ* transesterification reaction [27,43]. In both their studies, Georgogianni *et al.* compared the difference between the use of a mechanical stirrer (600 rpm) and low-frequency ultrasound (24 kHz) as a means of agitation. When the experiments were conducted on *in situ* transesterification using methanol, no significant difference was noticed. Both agitation methods gave high conversions of methyl ester after 20 min of reaction. However, when ethanol was used, the application of ultrasound produced high conversions more rapidly than mechanical stirring. At 40 min, 98% conversion was achieved with ultrasound, whereas mechanical stirring resulted in a lower yield (88%). Both works on ethanol reported the same results, despite using different raw materials (sunflower and cottonseed). They asserted that the reason behind this phenomenon was that ultrasound produced less soap because no stirring action was required. No further experiments were conducted to confirm the hypothesis. However, saponification occurs as a result of the reaction between sodium hydroxide and FFA and, as is the case for any reaction, its occurrence will depend to some extent upon the degree of mixing, but is unlikely to be dependent on the form of mixing, so this point is debatable. It may be that, as ethanol is a better solvent for triglycerides than methanol, more of the reaction takes place in the liquid phase, rather than in the seed, leading to a sonochemical enhancement for ethanol, but not for methanol.

Zeng *et al.* studied *in situ* transesterification of sunflower seeds with diethoxymethane (DEM) as co-solvent [34]. They found that agitation speed had no influence on biodiesel yield or FAME purity, which is as would be expected for an effective co-solvent.

▪ Molar ratio of alcohol to oil

All researchers agree that the required molar ratio of alcohol to oil in *in situ* transesterification was extremely high compared with the conventional transesterification of vegetable oil. For example, Siler-Marinkovic and Tomasevic [39] used a 300:1 ratio in their experiments with sulfuric acid catalyst, while Haas *et al.* [41] applied a 543:1 ratio for sodium hydroxide. The typical conditions used for conventional transesterification are 6:1 [42]. Calculations performed by Haas' group indicated that the amount of methanol involved in this process was the main reason for the high price of biodiesel produced by this method [19]. This increase is mainly due to the fact that the purification of the biodiesel became more complicated and costly.

As in transesterification, insufficient alcohol leads to incomplete reaction. Kildiran and coworkers achieved only 23.3% (oil basis) conversion from total oil dissolved in methanol when they used a 281:1 molar ratio of methanol to oil [26]. On the contrary, works by Harrington and D'arcy Evans [15] and Siler-Marinkovic and Tomasevic [39] reported conversions of 93.2 and 98.2%, respectively, when they used methanol-to-oil ratios of 370 and 300:1. Distinctively, conversion also decreases above a certain molar ratio. Siler-Marinkovic and Tomasevic [39] used

1.81-times less alcohol than Harrington and D'arcy Evans [15], but maintained all other parameters, and observed that the conversion increased from 93 to 98%.

Interestingly, researchers are now trying to find ways of reducing the amount of alcohol required. The use of co-solvent in conventional transesterification is known to improve the solubility of alcohol, thereby increasing the rate of reaction [47]. Qian *et al.* discussed the feasibility of using petroleum ether as a co-solvent in the process [33]. The amount of oil extracted from seed and dissolved in methanol increased from 95% in 1 h without co-solvent, to 98% with petroleum ether/ methanol mixture. However, petroleum ether/methanol was only effective below a volume ratio of 1:3. The concentration of oil was reported to be diluted when the ratio exceeded 1:3.

The application of co-solvent in the *in situ* transesterification has also been investigated at length by Zeng *et al.* [34]. They demonstrated that using DEM as a co-solvent reduces the amount of methanol required. At a 58:1 molar ratio of DEM/oil, only 101:1 molar ratio of methanol/oil was required to produce a 96% yield of crude biodiesel. For comparison, the highest yield achieved by researchers working with sunflower seeds was 97%, but the methanol/oil molar ratio was 476:1 [43].

The most recently reported attempt to lower the alcohol/oil ratio was by using CO_2 as a co-solvent [44] at temperatures and pressures at which methanol acts as a less polar solvent, which should increase the rate of triglyceride extraction, and therefore the overall reaction rate. However, the addition of CO_2 only gave a positive result when it was being used with an acid catalyst (in this case sulfuric acid) rather than an alkali. Sodium carbonate was detected in the system, suggesting that the methoxide was converted to carbonate in the presence of CO_2, thereby reducing the amount of catalyst and therefore rate of reaction. Using sulfuric acid, the authors claimed that the total volume of methanol can be lowered by a third without adversely affecting methyl ester yield. Not only did it lower the methanol volume, but the rate of reaction increased by as much as 2.5-fold.

The reason for the requirement for a large molar excess of alcohol in *in situ* transesterification may be that the rate-determing step is the diffusion of the alcohol into the particles. A high molar ratio would be required to overcome substantial mass transfer resistances for the reaction to proceed at an appreciable rate. Further evidence for this is the increase in rate with decreasing particle size (see later section on particle size).

▪ Temperature

Haas *et al.* compared *in situ* transesterification (soybeans/methanol/sodium hydroxide) reaction performance at two different temperatures: 60°C and room temperature (23°C) [41]. Both temperatures were sufficient to yield high percentages of methyl ester. However, the reaction at room temperature required more methanol than the reaction at 60°C. At the lower reaction temperature, the optimal molar alcohol-to-oil ratio was 2.4-times higher than at the higher temperature. The same pattern was also observed in the study in Newcastle. Increasing the temperature from 30 to 60°C increased the initial rate of ester formation, but the time needed to reach equilibrium (60 min) was comparable (rapeseed/methanol/sodium hydroxide) [28].

In conventional transesterification, Noureddini and Zhu observed that the temperature influenced mass transfer as well as conversion [48]. The mass transfer region period became shorter (from 55 to 20 min) when the temperature was increased (from 30 to 60°C). This effect was very obvious when the reaction was conducted at low mixing intensity (Re = 3100) but became insignificant at high mixing intensity (Re = 6200). This indicates that, at higher mixing intensities, the external mass transfer resistance is removed and the rate is no longer dependent on temperature. Temperature should not have a strong effect on *in situ* transesterification, as the reaction is believed to be largely mass transfer controlled. This is in agreement with the results reported by Haas *et al.* [46]. The conversions achieved with the reaction (DDGS/methanol/sodium hydroxide) at three different temperatures (35, 45 and 55°C) were almost the same and the reactions completed at the same time (180 min).

Liu and Zhao [32], on the other hand, reported a considerable increase in reaction rate with increasing temperature when an acid catalyst was used (biomass/methanol/sulfuric acid). For a 20-h reaction using 0.2 M sulfuric acid, the yield of ester increased progressively from 44.8 to 74.5 to 85.1 to 96.8% when the temperature was increased from 40 to 50 to 60 to 70°C, respectively. As transesterification with an acid catalyst is generally much slower than with an alkaline catalyst, it is conceivable that an increase in temperature will produce a more significant effect in an acid catalyst than an alkaline catalyst.

It should be noted that it is very likely that optimal temperature is a function of feedstock. Different feedstocks will have different internal structures and, therefore, different effective diffusivities, and this may explain some of the apparent contradictions in findings in the literature.

▪ Catalyst concentration

Catalyst concentration has been identified as the most important factor in determining the rate of conventional transesterification by some researchers [49,50]. However, in *in situ* transesterification, Zeng *et al.* found that while the catalyst concentration did not affect methyl ester yield, it did influence the purity of the methyl ester [34]. For instance, reactive extraction (sunflower

seed/methanol/sodium hydroxide) at low catalyst concentration (molar ratio of oil/catalyst = 0.3:1) achieved 93% conversion with 30% purity while at high concentration (molar ratio of oil/catalyst = 0.4:1), the conversion was 95% with 98% purity.

This is however, in contrast with Qian *et al.*, who reported that the conversion of oil to methyl ester was increased from 33 to 97% when the concentration of sodium hydroxide was increased from 0.05 to 0.1 mol/l (cottonseed/methanol/sodium hydroxide) [33]. Nonetheless, 0.05 mol/l in this case is equivalent to a 0.2:1 molar ratio of catalyst/oil, which is low compared with the levels in Zeng *et al.*'s experiments. The smaller amount of catalyst used by Qian *et al.* [33] may be the reason that they have observed the opposite result from Zeng and coworkers. In addition, the rate-determining step is the diffusion of the alcohol into the particles. Therefore, the fact that different feedstocks were used may contribute to the contradiction between these findings, as different oilseeds have different internal structures.

▪ Particle size

As the particle size of the seeds plays a very important factor in conventional solvent extraction [51,52], it should be similarly important in *in situ* reactive extraction. Kildiran *et al.* investigated two sizes of soybeans seed (<1 and <0.5 mm) at three different reaction times [26]. At 1 h reaction time, a particle size greater than 1 mm gave the highest percentage of oil dissolved in ethanol. However, when the reaction time became longer (i.e., at 3 and 5 h), smaller particle sizes (<0.5 mm) produced better yields. Ren *et al.* investigated the effect of particle size in rapeseed *in situ* transesterification [35]. Light microscopy and SEM analysis of seed samples showed that at the smallest particle size all the lipids were removed from the seed particle in 1 h. At larger particle sizes, some lipids remained in the center of the particles at this time, and it was evident from experiments with light microscopy and lipid staining that there was a 'shrinking core' of oil-bearing material. As the particle size of the rapeseed fragments increased from 300–500, to 500–850, to 1000–1400 µm, the 1-h conversion decreased from 86 to 65 to 43%, respectively. The results clearly suggest that, for rapeseed at least, the transport of the methanol into the seed particles was the rate-determining step.

▪ Alcohol type

At least five types of monohydroxy alcohols have been evaluated as reagents in *in situ* transesterification. Ozgul and Turkay investigated the possibility of changing the reagent [38]. They evaluated methanol, ethanol, propanol and butanol as a reagent in the *in situ* transesterification of rice bran oil. The solubility of the oil increased with the alcohol chain length. However, they noted that, even though the amount of oil dissolved increased, the alkyl ester content decreased. The reduction in polarity of the alcohol molecule as the chain length increases enables it to increasingly stabilize emulsions formed during the course of the reaction. The emulsion formed can persist and adversely affect conversion.

▪ Biodiesel quality

One of the most important factors to be considered in the development of *in situ* transesterification is whether the process can provide the market with quality biodiesel and meet the requirement of governing bodies. Two of the most accepted standards are ASTM D6751 and EN 14214.

Haas and Scott examined methyl ester produced from soybean flakes via *in situ* transesterification and compared it with the ASTM D6751 standard [46]. The methyl ester passed all the tests except for the acid number test, which required additional washing before it passed the test a second time. Table 3 shows the comparison reported by Haas and Scott against another standard, EN 14214 [146].

Table 3. Comparison of soybean flake methyl ester obtained via *in situ* transesterification against ASTM D6751 and EN 14214.

Property	Soybean methyl ester	ASTM D6751	EN 14214
Flash point (°C)	160	>130	>101
Water and sediment (vol%)	0	0.05	0.05
Carbon residue (wt%)	<0.010	0.05	0.3
Sulfated ash (mass%)	0.000	0.020	0.02
Kinematic viscosity (cSt, at 40°C)	4.017	1.9–6.0	3.5–5.0
Sulfur (wt%)	0.00035	0.05	0.001
Cloud point (°C)	0.0	Report	Not specified
Cetane number		>47	>51
Copper corrosion	Class 1a	Class 3	Class 1
Acid number (mg KOH/g)	0.04	0.80	0.50
Free glycerine (wt%)	0.000	0.02	0.02
Total glycerine (wt%)	0.071	0.240	0.25
Phosphorus (wt%)	0.000	0.001	0.001
Reduced pressure distillation (temperature at 90% recovery,°C)	350	360	NA

NA: Not applicable.
Data from [146].

Future perspective

As the world will always depend on transport, the search for alternative and greener fuel sources is poised to become more intense.

'Second-generation' biodiesel, and biofuels in general, should have fewer conflicts with food supplies and more negative lifecycle carbon balances.

In situ transesterification has developed significantly since first reports in 1985 [14]. Technical feasibility has been demonstrated for a range of feedstocks, catalysts and alcohols. The challenges for researchers in this area now are to find methods of rendering this process profitable. This process should, in principle, be implemented on a large scale: biodiesel is after all a bulk commodity and should be produced at these scales to realize economies of scale. However, certain features of the process may allow producers in rural areas to produce their own fuel. The relative simplicity of the process may allow oilseed growers to move along the complete length of the value chain of the product, by removing their dependence on often large-scale centralized crushing and solvent extraction facilities. Process economics change with scale, feedstock, time and geography, and there may well already be niche applications for this process. One obvious niche is as a 'bolt-on' to existing biofuel plants, or plants producing oil-bearing waste, which have very different process economics from purpose-built processes.

In situ transesterification presents researchers with huge challenges in order to make it profitable. The most important challenges must be to reduce the volume of alcohol in the reaction. To overcome this obstacle,

Executive summary
***In situ* transesterification**
▪ *In situ* transesterification is an alternative method of producing methyl ester transport fuels.
▪ The process directly uses oil-bearing materials rather than pre-extracted oil, as in conventional transesterification.
▪ The process eliminates processes such as crushing, solvent extraction and degumming, and perhaps drying, which are key processes in conventional biodiesel production.
Raw materials
▪ Any lipid-bearing material can be a substrate for biodiesel production via the *in situ* transesterification approach, but there are likely to be significant changes in rate and conversion due to varying internal structures of the feedstock.
Catalysts
▪ The process does not proceed in the absence of a catalyst.
▪ Both acid and alkali catalysts can be used for *in situ* transesterification.
▪ Selection of a catalyst depends on the properties of the raw materials, especially the free fatty acid content (acid catalysts should be used for higher free fatty acid contents).
Moisture content
▪ Extraction of some oilseeds is strongly affected by moisture content. Reducing moisture content can reduce the methanol requirement.
▪ Other oilseeds do not seem to require drying prior to *in situ* transesterification.
Mixing intensity
▪ The mixing intensity has been shown to have little effect on the process, probably because the rate-limiting step is intraseed diffusion, rather than external mass transfer. Further clear evidence of this being the rate-limiting step is that: reducing particle size clearly increases the rate of extraction; and for rapeseed, a 'shrinking core' of oil has been shown to exist in the seed fragments as the reaction progresses.
Molar ratio of alcohol to oil
▪ The current greatest obstacle to realization of this process is that the higher methanol/oil molar ratios that seem to be required militate against the economic viability of this process, due principally to the energy costs involved in recovering the methanol for reuse, and to some extent the increased equipment size. Various methods of reducing the methanol requirement or reducing its impact on the process economics are currently under investigation.
Temperature
▪ It is feasible to perform *in situ* transesterification either at room temperature or at high temperature without compromising on the reaction rate and conversion.
▪ It is possible that optimal temperature is a function of feedstock.
Catalyst concentration
▪ The effect of catalyst concentration on *in situ* transesterification is unclear. There must be a catalyst, otherwise the reaction will not proceed at all, but the literature gives conflicting results as to the dependence of the rate upon catalyst concentration at realistic conditions.
Particle size
▪ Smaller seed particle size increases rate of reaction, indicating that the rate is controlled by internal mass transfer (studies on rapeseed).
Alcohol type
▪ Various monohydroxy alcohols can be used in the process, but the usage of higher alcohols reduces the biodiesel's purity, and these alcohols tend to be more expensive. However, when using the most usual alcohol, methanol, the quality is comparable to that of conventional biodiesel.
Biodiesel quality
▪ Biodiesel produced by this method can meet the ASTM D6751 and EN 14214 biodiesel standards.

future research on *in situ* transesterification should concentrate on three areas. The first will be to study a wide range of chemicals as possible co-solvents, in order to reduce the ratio of reactant to oil. The second area is to investigate the process path itself, to involve more intensified unit operations, such oscillatory baffle reactors (already proven for conventional transesterification [53] and being investigated at Newcastle for reactive extraction) and microreactors. The discovery of novel processes or unit operations might make the process more economically viable. The third area that needs more understanding is the *in situ* transesterification reaction mechanism. The mechanism of the process is crucial in predicting the various effects of process parameters on the system and in providing insight into possible further improvements. For example, if the reaction occurs inside the seeds, parameters such as mixing intensity in bulk solvent will not cause significant improvement to the kinetics of the process, and the seed particle size becomes critical.

For the process to be acceptable and profitable, all output streams from the plant must be used. These plants should not simply be biofuel production facilities, rather they should be biorefineries. Studies on the meal left after the process also must be intensified to provide another source of income. The meal can be sold as animal feed, if it still contains nutritional materials. Ren *et al.* found that, for rapeseed subjected to *in situ* transesterification, the carbohydrates and protein remained unaffected by the process and were intact inside the meal [35]. Barrows *et al.*, who conducted a field trial on soybean meal from *in situ* transesterification as fish food, observed that the rainbow trout fed with this meal gained weight in a normal manner, as with control fish (fed with industrial hexane-extracted soybean meal) [54].

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Influence of various parameters on reactive extraction of *Jatropha curcas* L. for biodiesel production

Farizul H. Kasim*, Adam P. Harvey

School of Chemical Engineering and Advanced Materials, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom

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ABSTRACT

Reactive extraction (*in situ* transesterification) of *Jatropha curcas* L. (Jatropha) seeds for biodiesel production is influenced by a variety of parameters, including seeds size, agitation speed, reaction temperature, reaction time, catalyst concentration and molar ratio of alcohol compare to the oil. In this study, these parameters were studied in the ranges of <0.5–4 mm seeds particle size, 200–300 rpm, agitation speed, 30–60 °C reaction temperature, 10–60 min reaction time, 0.1–0.2 N NaOH concentration and 100–600 molar ratio of methanol-to-oil. It was established that the smallest particle size (below 0.71 mm) resulted in the highest yield of biodiesel production. The biodiesel yield was found to be independent of intensity of the mixing once it reached 300 rpm, whereas reaction temperature did not exhibit any significant effect on the yield. It was also demonstrated that alkaline reactive extraction was complete in 20–30 min. The concentration of NaOH can affect biodiesel yield in both positive and negative way. Low concentration of NaOH (0.05 N) resulted in low yield, but at higher concentrations (0.2 N), emulsions form, due to a saponification side reaction, adversely affecting the yield. In this case, a NaOH concentration of 0.15 N produced the highest yield. It was also discovered that when the methanol-to-oil ratio reached 400, the biodiesel yield reached a constant state. The optimal conditions in this study are approximately <0.71 mm seeds particle size, 300 rpm mixing speed, 30 °C reaction temperature, 30 min reaction time, 0.15 N NaOH concentration and methanol:oil molar ratio of 400.

1. Introduction

Reactive extraction (or "*in situ* transesterification") is an alternative method of producing biodiesel from oil-bearing materials. In reactive extraction, oil-bearing materials are brought directly into contact with alkaline/acidic alcohol. The method has been applied to various oil-bearing substances, including rapeseed [1], soya [2], sewage [3] and algae [4]. Reactive extraction from oilseeds for biodiesel production is reviewed in Kasim et al. [5], including the breadth of oilseeds studied the history of the process and a discussion of the possible processing advantages. In brief, the process has advantages in reducing capital cost, but running costs due to the large excess of alcohol required, must be reduced.

Jatropha has been a subject of interest by many researchers, particularly in the biodiesel area. It is a promising raw material for biodiesel production, because the seed oil content is potentially high, at 35–55% of the seed dry weight, and it has been shown to grow on marginal, arid land [6], which is not usable for food production, so may not compete for land. However, it does require water to develop [7], so may compete for water. It was claimed that jatropha had low nutrient requirements for growth, but recent studies have shown that insufficient supply of nutrients will lead to reduced growth and crop production [8,9]. Jatropha is harmful to digest [10], and is not browsed by any animal. Despite all the potential advantages surrounding the plant, more studies are needed and it still cannot be considered as an established domestic crop [11].

Apart from agronomic uncertainties, there are also processing challenges when working with jatropha. The oil contains high levels of free fatty acids [12–14] and phorbol esters [10]. Free fatty acids in the oil cause saponification reaction when alkaline catalysts employed in the reaction. In conventional processing this necessitates additional stages, usually to esterify the free fatty acids to biodiesel prior to transesterification [15]. Phorbol esters, on the other hand are responsible for the toxicity of jatropha. The concentration of phorbol esters in jatropha oil ranges from 3.10 to 3.77 mg/g [8] and ingestion of low concentrations of phorbol esters has been shown to adversely affect animals [16]. In conventional transesterification process, the need to press the oil out from the seeds exposes workers to the toxic compounds, whereas in reactive extraction, this step is bypassed.

The main objective of this study is to investigate the sensitivity of the reactive extraction of jatropha seeds to various processing parameters. Six key parameters have been studied in this project:

* Corresponding author. Tel.: +44 0191 222 7266; fax: +44 0191 222 5292.
E-mail addresses: f.h.kasim@ncl.ac.uk, farizulhafiz@gmail.com (F.H. Kasim), a.p.harvey@ncl.ac.uk (A.P. Harvey).

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seed particle size, agitation speed, reaction temperature, reaction time, catalyst concentration and molar ratio of methanol to oil. This systematic study will lead to greater understanding of the advantages and disadvantages of the use of jatropha as a raw material in reactive extraction.

2. Materials and methods

2.1. Jatropha seeds

Jatropha seeds were provided by Indian Institute of Petroleum (IIP), Dehradun, India. The seeds were stored in dark air-tight containers to prevent photo-oxidation, as well as to minimize moisture adsorption.

2.1.1. Preparation of jatropha seeds

The whole jatropha seeds (shell and kernel) were utilised in the experiments. The seeds were ground using a blender (Kenwood BL440, UK) to reduce the particle size. A vibrator sieve-shaker (Retsch, Germany) was then employed to separate the ground seeds into five different sizes (2–4 mm, 1–2 mm, 1–0.71 mm, 0.71–0.5 mm and <0.5 mm). These seeds then were placed in a drying pan and were dried in a drying oven (Memmert, Germany), at 105 °C for an hour.

2.2. Chemicals and reagents

Methanol (99.8% purity) and NaOH were purchased from Fisher Scientific, UK and Merck, Germany, respectively. Hexane and acetic acid (glacial), which were used in post-reaction steps, were acquired from Fisher Scientific, UK. Heptane and methyl heptadecanoate, were purchased from Sigma Aldrich, UK.

2.3. Reactive extraction

Reactive extraction experiments were conducted in sealed, chemically resistant 250 ml Schott bottles. The bottle was filled with methanol to achieve the required pre-determined molar ratio. The relevant amount of catalyst (NaOH) then was dissolved in the methanol using magnetic stirrer/hot plate (Bibby, UK). When the catalyst was completely dissolved, the mixture was pre-heated to the reaction temperature in a programmable incubator shaker (IKA, Germany). Jatropha seeds (10 g) were put into the bottles after the mixture reached previously set temperature. The reaction was conducted in an incubator shaker with controlled temperature, agitation speed and time.

After the reaction, the bottle was taken out from the incubator and vacuum-filtered with vacuum pump (KNF, Germany). This will separate the meal and the liquid mixture. The liquid mixture then was weighed. To ensure that the transesterification reaction stopped, the catalyst was neutralised using glacial acetic acid. The sample was then taken and analysed by gas chromatography (GC) to determine the yield. The remaining mixture was placed in an evaporator flask, to evaporate the methanol. The rotary evaporator (Buchi, Switzerland), which was set at 55–60 °C evaporated the methanol and collected it in another connecting flask 10 ml of hexane was then added to the remaining mixture. The solution then was transferred to separating funnel and was allowed to separate gravitationally. Two distinct layers, the biodiesel-rich upper layer, and the glycerol-rich lower layer appeared. The mixture was then washed with warm water to remove impurities from the upper layer. After the glycerol layer was drained out, the upper hexane/biodiesel layer was put on an evaporating disc and heated at 60 °C using hot plate in fume hood. This is to evaporate the hexane from the mixture. The mass of the purified biodiesel was then recorded, and analysed by GC to determine percentage of methyl ester.

2.4. Biodiesel analysis

2.4.1. Gas chromatography (GC)

Flame ionisation detector (FID) HP5890 Series II (Hewlett Packard, USA) gas chromatograph fitted with BPX70 column, 30 m long × 0.32 mm ID × 0.25 μm film thickness (SGE, Australia) was used to analyse the samples. Helium was used as carrier gas at a pressure of 7 psi. The oven was maintained at 230 °C for 30 min.

The data was acquired and processed using Clarity Chromatography Station for Windows (DataApex, Czech Republic). The software allowed integration of peaks to be performed on the chromatogram.

2.4.2. Sample preparation

The internal standard, methyl heptadecanoate solution was prepared in 10 mg/ml concentration by dissolving 500 mg methyl heptadecanoate in 50 ml heptane/methanol.

250 mg of sample was weighed and placed in the vial and, 5 ml of methyl heptadecanoate solution was added to the sample. The mixture was mixed thoroughly using the MS1 Minishaker (IKA, Germany). 1 μL of sample was then injected to the GC using a 5 μL microsyringe (SGE, Australia).

2.4.3. Yield calculation

The yield was calculated by modifying the standard for calculating FAME (BS EN 14103:2003). Firstly, the mass of the liquid mixture after the separation of solid and liquid was taken and recorded (w). The internal standard was prepared by mixing methanol with methyl heptadecanoate, instead of heptane. After adding 5 ml internal standard to the 250 mg sample, the sample was injected to the GC. The integration of the peaks from chromatogram obtained was performed in order to eliminate the solvent peak (methanol) from the calculation. The ester content in the sample, C, expressed as a mass fraction in percent, can be calculated using Eq. (1).

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

where ΣA is the total peak area from the methyl ester C_{14}–$C_{24:1}$; A_{EI} is the peak area corresponding to methyl heptadecanoate; C_{EI} is the concentration, in mg/ml of the methyl heptadecanoate solution; V_{EI} is the volume, in ml of the methyl heptadecanoate solution being used; m is the mass of the sample (mg).

The mass of methyl ester then was calculated by multiplying C with mass of the sample, w, as in Eq. (2).

$$\text{Mass of methyl ester (g)} = C(\%) \times w(\text{g}) \quad (2)$$

Yield, in mass percentage, was determined by comparing the mass of methyl ester obtained from the experiment with the initial triglyceride mass.

$$\text{Yield}(\%) = \frac{\text{Mass of methyl ester from experiments (g)}}{\text{Mass of triglycerides in Jurcas seeds used (g)}} \times 100\% \quad (3)$$

2.4.4. FAME content calculation

FAME content is the percentage of methyl ester in the biodiesel rich phase of the sample. Because it is possible for other compounds such as mono-, di- and triglycerides to presence in the sample, it is very important to determine this parameter. As in yield calculation, the method and calculation was based on the standard by British Standard Institution (BSI) BS EN 14103:2003.

Table 1
List of experiments conducted with respective parameters setting. Colour boxes indicate the parameter of interest.

Experiment	Parameters					
	Seed size (mm)	Mixing speed (rpm)	Reaction temperature (°C)	Reaction time (min)	Catalyst concentration (N)	Methanol:oil molar ratio
Effect of particle size	<0.5-4	400	60	60	0.1	400
Effect of mixing speed	<0.71	100-400	60	60	0.1	400
Effect of reaction temperature	<0.71	400	30-60	60	0.1	400
Effect of reaction time	<0.71	400	60	10-60	0.1	400
Effect of catalyst concentration	<0.71	400	60	60	0.1-0.2	400
Effect of methanol to oil molar ratio	<0.71	400	60	60	0.1	200:1-600:1

In the sample preparation, heptane was used as a solvent to dissolved methyl heptadecanoate, the internal standard. As in Section 2.4.3, the chromatogram was processed before it can be used in Eq. (1).

2.5. Parameter study

The parameter study experiments were done by changing the parameter of interest whilst other parameters were in constant. Table 1 summarised the setting of all parameters used in the experiments.

3. Results and discussion

3.1. Particle size

Fig. 1 shows yield of methyl ester decreases with increasing particle size, when the particle size is larger than some threshold value of around 0.71 mm. No significant difference was determined between the yield for the smallest two particle size ranges, <0.5 mm and 0.5–0.71 mm, with the former yielding 86.1% yield and the latter 83.7%. The largest particle size range (2–4 mm) produced the lowest yield, at 35.5%. This finding is consistent with those reported in literature [17].

In terms of methyl ester content, only the two smallest particle sizes produced more than 80% of methyl ester in the sample. The other particle sizes produced lower methyl ester contents; with size 0.71–1 mm has 70.5% methyl ester, size 1–2 mm; 77.0% and 2–4 mm, 64.0%. No further analysis was done to determine the other compounds in the sample, but the most likely suggestion would be the presence of unreacted glyceride in the form of mono-, di- and triglycerides [15,18]. Because of the settling and hexane washing, the chances of other compounds such as glycerol and polar lipids being present in the sample are small.

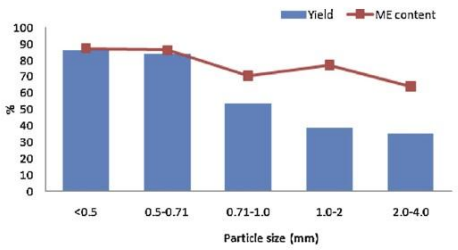

Fig. 1. Percentage of seeds' mass extracted, methyl ester yield and methyl ester content for various particle sizes. Alcohol to oil ratio = 400:1; NaOH concentration = 0.1 N, reaction temperature = 60 °C; mixing speed = 400 rpm; reaction time = 1 h.

3.2. Mixing speed

From Fig. 2, it is noticeable that the mixing intensity is not a mass transfer limiting factor once it reaches 300 rpm. The finding suggests that there is no point increasing the agitation beyond this level, as it will not lead to a significant improvement in yield. However, decreasing the speed to 200 rpm, and further to 100 rpm, will

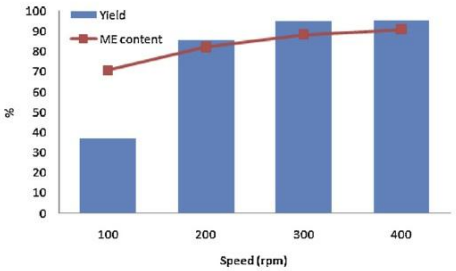

Fig. 2. Plotted data for the effect of mixing intensity to reactive extraction of Jatropha. Other parameters: alcohol to oil = 400:1, NaOH concentration = 0.1 N, reaction temperature = 60 °C, reaction time = 1 h, seeds size = <0.71 mm.

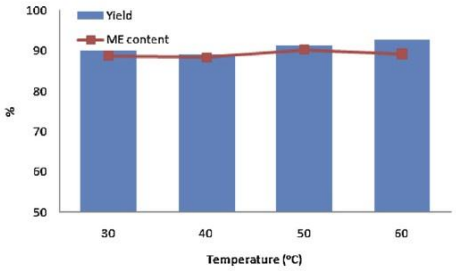

Fig. 3. Methyl ester yield, methyl ester content and mass extracted of jatropha seed at four different temperature (30, 40, 50, 60 °C). Other parameters: alcohol to oil = 400:1, NaOH concentration = 0.1 N, mixing speed = 400 rpm, reaction time = 1 h, seeds size = <0.71 mm.

decreased the yield from 94.8% to 85.7% and further down to 37.2%. At low agitation speeds, the distribution of seeds was not as uniform as at higher agitation speeds. The seeds settled on the bottom of the reaction vessel and this reduced the biodiesel yield.

3.3. Reaction temperature

Four different temperatures (30, 40, 50 and 60 °C) were used in the experiment to study the influence of temperature towards reactive extraction of jatropha seeds. The data obtained are plotted in Fig. 3.

In this process, temperature did not have a significant effect on biodiesel yield. This result is in agreement with the work by Haas et al., where they observed that triglyceride can be converted to biodiesel at both low and high temperature [2].

3.4. Reaction time

The reaction rate of plant oil seeds reactive extraction has been claimed to be very high [2]. The result on the effect of reaction time is in agreed with this finding. However, this is only true with alkali-based catalyst. Reactive extraction of jatropha seeds with acid catalyst reportedly needs more time [19].

The results obtained from the experiments are presented in Fig. 4. The methyl ester yield showed minimal changed after 30 min. It is therefore very likely that the reactive extraction itself completed between 20 and 30 min. It also can be observed from Fig. 4 that reactions less than 20 min did not achieve high yields. This is in agreement with other findings, which report rapid increases in yield within the first 30 min of reaction [2,17].

Table 2
Comparison between reactive extraction with hexane extraction (8 h) and methanol extraction. The condition of *in situ* transesterification as follow: alcohol to oil = 400:1, mixing speed = 400 rpm, reaction temperature = 60 °C, seeds size = <0.71 mm, reaction time = 60 min.

Extraction method	Mass of oil extracted (g)	Extraction efficiency (%)	Methyl ester yield (%)
Hexane–soxhlet	5.53	100	0.0
Methanol–NaOH	4.66	84.3	81.9
Methanol only	0.8	14.5	0.0

3.5. Catalyst concentration

The transesterification reaction does not proceed at all without catalyst. The comparison between reactive extractions with catalyst and without catalyst is tabulated in Table 2.

Solvent extraction with methanol yielded some extract, but no methyl ester was detected in the samples. The extract probably consists of polar components such as phospholipids [20]. The methanol–NaOH extraction (reactive extraction) extracted 4.66 g oil of a possible 5.53 g. 3.8 g (89.1%) was converted to biodiesel. In conventional transesterification, Tapanes et al. achieved a 96.3% yield using 9:1 methanol alcohol ratio [21], meaning that if they started with the same amount of oil (5.53%), they will get 5.3 g of biodiesel. However, refined, bleached, deodorized jatropha oil was used, rather than jatropha seed itself, and each of the preliminary stages would have an associated loss of yield, and associated capital and running costs. These effects must be weighed against one another to determine the economic viability of this process.

The table also shows that adding sodium hydroxide to methanol significantly increases its extraction efficiency. Ren et al. [1] studied lipid content in rapeseeds during reactive extraction with methanol, with and without sodium hydroxide. Without sodium hydroxide, lipid staining and microscopy clearly demonstrated that the lipids were still present and the morphology of the seed unchanged. With sodium hydroxide present, almost all of the lipids in the seeds were removed: the presence of catalyst is essential for reactive extraction to take place. Furthermore, the oil-containing part of the seed clearly shrank as the reaction progressed, indicating that this is a diffusion-controlled process, and that reaction takes place largely within the seed.

Fig. 5 represents the data from the study of NaOH concentration. Three different catalyst concentrations were subjected to the experiments.

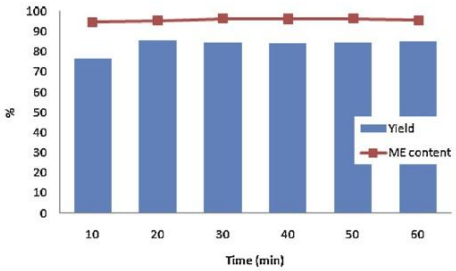

Fig. 4. Methyl ester yield, methyl ester content and mass extracted on various reaction time. Other parameters: alcohol to oil = 400:1, NaOH concentration = 0.1 N, mixing speed = 400 rpm, reaction temperature = 60 °C, seeds size = <0.71 mm.

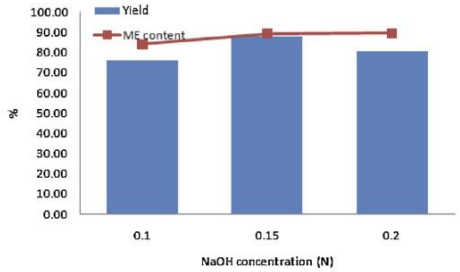

Fig. 5. Methyl ester yield, methyl ester content and mass extracted on various NaOH concentration. Other parameters: alcohol to oil = 400:1, reaction time = 1 h, mixing speed = 400 rpm, reaction temperature = 60 °C, seeds size = <0.71 mm.

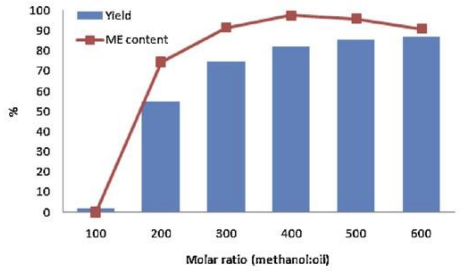

Fig. 6. Methyl ester yield, methyl ester content and mass extracted on various molar ratio of alcohol to oil. Other parameters: NaOH concentration = 0.1 N, reaction time = 1 h, mixing speed = 400 rpm, reaction temperature = 60 °C, seeds size = <0.71 mm.

From the methyl ester yield data, the addition of NaOH, although in small quantity (0.1 N) give a significant effect converting oil to methyl ester. Increment in NaOH concentration to 0.15 N increased the methyl ester yield from 76.2% to 87.8%. However further increment to the concentration (0.2 N) decrease the yield to 80.8%. It is also interesting to note that, with a further increase in NaOH concentration, an emulsion started to form, consequently reducing the yield. The most likely cause of the emulsion is the formation of soap that is a competing reaction in alkali catalysed transesterification process. The formation of soap occurs through two different mechanisms: saponification and hydrolysis [15]. In conventional transesterification with alkali catalyst, the formation of emulsion (soap) occurs as a result of substantial amount of free fatty acids [22]. To overcome this problem, usually the feedstock is pre-treated with acid catalyst, to esterify the free fatty acids, prior to transesterification process with alkali catalyst [23]. Generally, jatropha oil has a high acid value number, which is why the majority of researchers adopted this route to produce biodiesel from its oil [12,13,24], although there were also reports by various other means of reaction [14,19,25,26]. Oliveira et al. [14] in particular, reported that when jatropha oil was transesterified using sodium hydroxide as catalyst, a stable emulsion formation was observed in the sample, and limited the final yield to 68% only. Interestingly, it is apparent from Fig. 5 that through reactive extraction, the high free fatty acids content of jatropha oil had no negative effect to the yield, until the NaOH concentration was more than 0.2 N.

Fig. 5 also shows that increasing catalyst concentration from 0.1 N to 0.15 N had a positive impact on FAME conversion. However, further increase from 0.15 N to 0.2 N did not have any impact on methyl ester content, presumably due to the formation of more soap.

3.6. Methanol–oil molar ratio

The methanol volume requirement in reactive extraction is very high compared to the conventional process [2]. In this study, the molar ratio of methanol to oil was varied from 100 to 600. The results are as in Fig. 6:

No methyl ester was produced at a molar ratio of 100, even though 18.1% of mass was extracted from initial 10 g of seeds. 52% yield of methyl ester was obtained at 200 ratio and then increases steadily as the ratio increases. The yield at a molar ratio of 300 is 74.7% and the yield at 400, 500 and 600 are 81.9%, 85.7% and 86.9%, respectively.

The results suggested that the amount of methanol must be very high to achieve an appreciable rate. This was presumably to drive the penetration of alkaline methanol into the seed, as observed in Ren et al. [1]. Further excess of methanol (e.g. 600) does not greatly increase the yield, but is undesired, as it will increase the load on downstream separation processes.

4. Conclusion

This study was designed to determine the effect of various parameters on the reactive extraction of jatropha. Particle size and molar ratio of methanol to oil strongly affect the yield of methyl ester. Investigation of the effect of mixing speed indicated that a certain minimum agitation must be provided for the process to occur. The reaction was shown to be complete after 20–30 min. This study also demonstrated that for jatropha, the catalyst is required to achieve a meaningful yield. An important observation is that the optimum conditions are not the same for jatropha as for other oilseeds, such as rapeseed, soybeans, sunflower and others.

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