



CHAPTER II

BACKGROUND & LITERATURE REVIEWS

2.1 Biodiesel as engine fuel

The word biodiesel is commonly referred to methyl esters of vegetable oil. It is an alternative diesel fuel that made from renewable biological sources such as vegetable oils and animal fats. It has become more attractive recently because of its characteristic such as non-toxic, biodegradable and renewable. It is a cleaner burning fuel than petroleum diesel with reduced emissions of SO_x, CO, unburnt hydrocarbons, particulate matter and has low emission profiles (Krawczyk et al., 1996).

2.2 The production of biodiesel

2.2.1 Direct use and blending

Beginning in 1980, there was considerable discussion regarding use of vegetable oil as a fuel (Bartholomew et. al., 1981). The most advanced work at that period was with sunflower oil in South Africa. Caterpillar Brazil, in 1980 used pre-combustion chamber engines with mixture of 10% vegetable oil to maintain total power without any alterations or adjustments to the engine. At that point, it was not practical to substitute 100% vegetable oil for diesel fuel, but a blend of 20% vegetable oil and 80% diesel fuel was successful. Some short-term experiments used up to a 50/50 ratio. Nevertheless, the problems appeared after the engine has operating on vegetable oils for long period of time. Schlick et al., (1988) evaluated the performance of a direct injection 2.59 L, 3 cylinder 2600 series Ford diesel engine operating on mechanically expelled-unrefined soybean oil and sunflower oil blended with number 2 diesel fuel on a 25:75 v/v basis. The power remained constant throughout 200 h of operation.

Direct use of vegetable oils and/or the use of blends of the oils has generally been considered to be not satisfactory and impractical for both direct and indirect

diesel engines. The high viscosity, and composition, free fatty acid content, as well as gum formation due to oxidation and polymerization during storage and combustion, carbon deposits and lubricating oil thickening are obvious problems (Schlick et al., 1988).

2.2.2 Microemulsion

To solve the problem of the high viscosity of vegetable oils, microemulsions with solvents such as methanol and 1-butanol have been studied. A microemulsion is defined as a colloidal equilibrium dispersion of optically isotropic fluid microstructures with dimensions generally in the 1-50 nm range formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphiphiles (Schwab et al., 1987). It was found that microemulsion can improve spray characteristics by explosive vaporization of the low boiling constituents in the micelles (Pryde et al., 1984). In short term performances, ionic and non-ionic microemulsion of aqueous ethanol in soybean oil were nearly as good as that of No. 2 diesel, in spite of the lower cetane number and energy content (Goering et al., 1982).

2.2.3 Pyrolysis

Pyrolysis refers to a chemical change caused by the application of thermal energy in the presence of air or nitrogen sparge. Thermal decomposition of triglycerides produces the compounds of classes including alkanes, alkenes, alkadienes, aromatics and carboxylic acids. Different types of vegetable oils produce large differences in the composition of the thermally decomposed oil. Figure 2.1 outlines a schematic that accounts for the formation of alkanes, alkenes, alkadienes, aromatics and carboxylic acids from pyrolysis of triglycerides (Schwab et al., 1988). Mechanisms for the thermal decomposition of triglycerides are likely to be complex because of many structures and multiplicity of possible reactions of mixed triglycerides. Generally, thermal decomposition of these structures proceeds through either a free-radical or carbonium ion mechanism. Formation of homologous series of

alkanes and alkenes is accountable from the generation of the RCOO radical from the triglyceride cleavage and subsequent loss of carbon dioxide. The R radical, upon disproportionation and ethylene elimination, gives the odd-numbered carbon alkanes and alkenes (Anjana et al., 2000). However, while pyrolyzed vegetable oils possess acceptable amounts of sulphur, water, and sediment, as well as giving acceptable copper corrosion values, they are unacceptable in terms of ash, carbon residues, and pour point. In addition, disadvantages of this process include high equipment cost and need for separate distillation equipment for separation of various fractions.

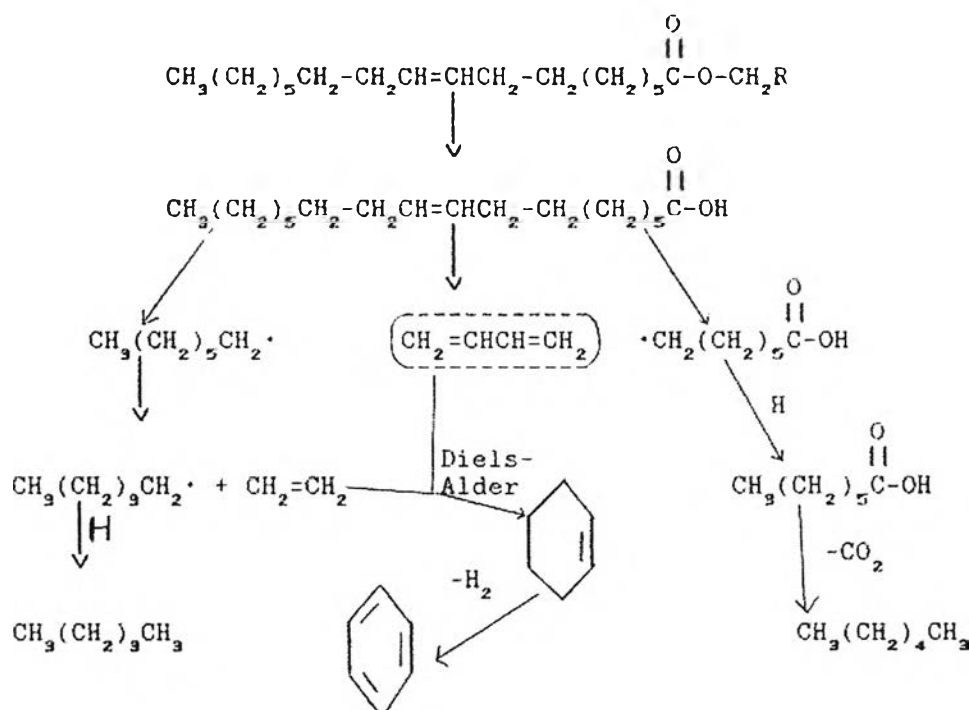


Figure 2.1 The mechanism of pyrolysis of triglycerides (Schwab et al., 1988)

2.2.4 Esterification

Esterification is the formation of ester through a condensation reaction that requires two reactants, carboxylic acids (fatty acid) and alcohol (Solomon et al., 1996). Esterification reactions are proceed slowly in the absence of strong acids such as sulphuric acid, phosphoric acid, organic sulfonic acids and hydrochloric acid. The equation of esterification reaction is shown in Figure 2.2.

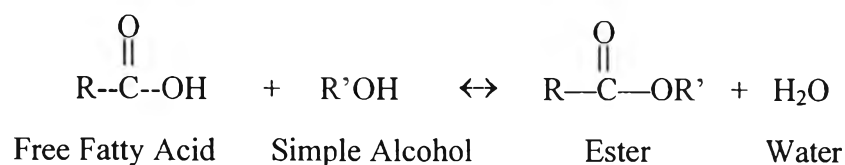


Figure 2.2 Esterification reaction (Khan et al., 2002)

2.2.5 Transesterification

Transesterification is the most popular method of producing biodiesel. This method is the alcoholysis of triglyceric esters resulting in a mixture of mono-alkyl esters and glycerol and the sequence of processes is shown in Figure 2.3

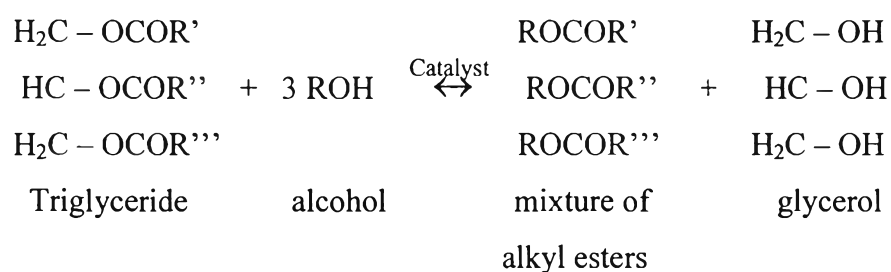


Figure 2.3 Transesterification reaction of triglycerides with alcohol.(Khan et al., 2002)

The first step is the conversion of triglycerides to diglycerides, which is followed by the conversion of diglycerides to monoglycerides and of monoglycerides to glycerol, yielding one methyl ester molecule from each glyceride at each step from the reversible reactions as shown in Figure 2.4. After transesterification of triglycerides, the products are a mixture of esters, glycerol, alcohol, catalyst and tri-, di- and monoglycerides (Freedman et. al., 1986 and Nouredini et al., 1997).

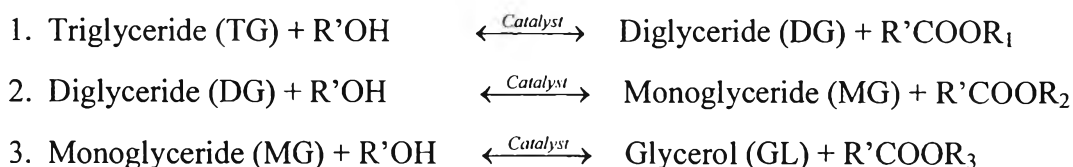
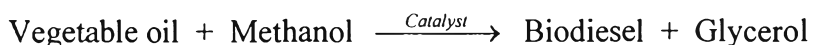
Stepwise Reaction :

Figure 2.4 Three consecutive and reversible reactions. R₁, R₂, R₃ and R' represent alkyl groups.

Alcohols are primary and secondary monohydride aliphatic alcohol having 1-8 carbon atoms (Sprules and Price, 1950). Among the alcohol that can be used in the transesterification process are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are used most frequently, especially methanol because of its low cost and its physical and chemical advantages (polar and shortest chain alcohol). The transesterification process can be done in a number of ways such as using an alkali catalyst, acid catalyst, biocatalyst, heterogeneous catalyst or using alcohols in their supercritical state. The general reaction is shown below



2.2.5.1 Non-Catalytic transesterification method (Supercritical Method)

When a fluid or gas is subjected to temperatures and pressures in excess of its critical point, there are a number of unusual properties exhibited. Firstly, there no longer is a distinct liquid and vapor phase, but a single, fluid phase present. Secondly, solvent containing a hydroxyl (OH) group, such as water or primary alcohol, take on the properties of super-acids. For this reason, transesterification in supercritical alcohol can be achieved without use of catalyst for production of biodiesel. With this process, used oil with high amount of free fatty acids can be used as a raw material. The products are the ester or biodiesel (the upper phase) and glycerol (the lower phase). No soap is produced thus the separation process is simple. In addition, alcohols used in biodiesel production such as methyl or ethyl alcohols are polar

solvents and have hydrogen bonding between OH oxygen and OH hydrogen to which normally form cluster of these molecules, separating them from other non-polar compounds such as glyceride. However, because the degree of hydrogen bonding decreases with increasing temperature, the polarity of the alcohol would decrease in supercritical state. Thus supercritical alcohol has a hydrophobic nature with the lower dielectric constant. As a result, non-polar triglycerides can dissolve in the supercritical alcohol to form a single phase of vegetable oil/methanol mixture (Kusdiana and Saka, 2001).

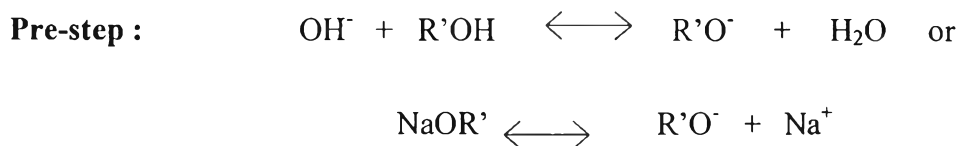
The alcohol molecule attacks the carbonyl atom of the triglyceride because of the high pressure. In the supercritical state, depending on pressure and temperature, hydrogen bonding would be significantly decreased, which would allow methanol to be a free monomer. Transesterification is completed via a methoxide transfer, whereby the fatty acid methyl ester and glycerol are formed.

2.2.5.2 Catalytic transesterification method

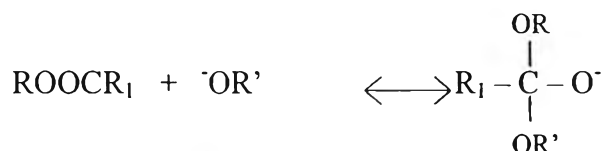
Alkali-catalyzed process

The reaction mechanism for alkali-catalyzed transesterification is formulated as three steps. The first step is an attack on the carbonyl carbon atom of the triglyceride molecule by the anion of the alcohol (methoxide ion) to form a tetrahedral intermediate. In the second step, the tetrahedral intermediate reacts with an alcohol (methanol) to regenerate the anion of the alcohol (methoxide ion). In the last step, rearrangement of the tetrahedral intermediate results in the formation of a fatty acid ester and a diglyceride. When NaOH, KOH, K_2CO_3 or other similar catalysts are mixed with alcohol, the actual catalyst, alkoxide group is formed. A small amount of water, generated in the reaction, may cause soap formation during transesterification. Figure 2.5 summarizes the mechanism of alkali-catalyzed transesterification (Ma and Hanna, 1999). For an alkali-catalyzed transesterification, the glycerides and alcohol must be substantially anhydrous because water makes the reaction partially change to saponification, which produces soap. The soap lowers the yield of esters and renders the separation of ester and glycerol and the water washing difficult. Low free fatty acid content in triglycerides is required for alkali-catalyzed transesterification. The

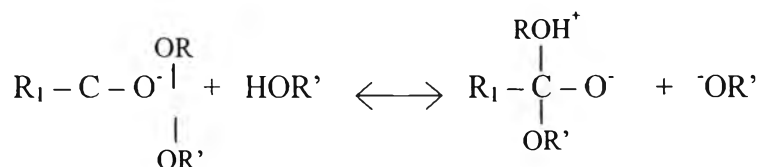
triglycerides can be purified by saponification (known as alkali treating) and then transesterified using an alkali catalyst.



Step 1.



Step 2.



Step 3.

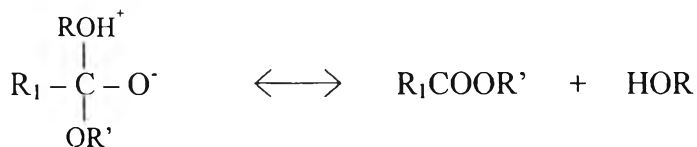


Figure 2.5 The mechanism of alkali-catalyzed transesterification of triglycerides with alcohol (Sridharan and Mathai, 1974; Eckey, 1956).

Acid-catalyzed process

The favorite conventional way of producing biodiesel is using an acid catalyst instead of a base. Any mineral acid can be used to catalyze the process; the most commonly used acids are sulfuric acid and sulfonic acid. Although yield is high, the acids, being corrosive, may cause damage to the equipment and the reaction rate was also observed to be low (Freedman et al., 1984). Although, transesterification by acid catalysis is much slower than that by alkali catalysis (Ma et al., 1999 and Srivastava et al., 2000), acid-catalyzed transesterification is more suitable for glycerides that have relatively high free fatty acid contents and more water (Freedman et al., 1984). The acid could be sulfuric acid, phosphoric acid, hydrochloric acid or

organic sulfonic acid. Acid catalyst transesterification was studied with waste vegetable oil. The reaction was conducted at four different catalyst concentrations, 0.5, 1.0, 1.5, and 2.25 M HCl in presence of 100% excess alcohol and the result was compared with 2.25 M H_2SO_4 . H_2SO_4 was found to have superior catalyst activity in the range of 1.5-2.25 M concentration (Mohamad et al., 2002).

The mechanism of the acid-catalyzed transesterification of vegetable oils is shown in Figure 2.6, for a monoglyceride. However, it can be extended to di- and triglycerides. The protonation of the carbonyl group of the ester leads to the carbocation II which, after a nucleophilic attack of the alcohol, produces the tetrahedral intermediate III, which eliminates glycerol to form the new ester IV, and to regenerate the catalyst H^+ . According to this mechanism, carboxylic acids can be formed by reaction of the carbocation II with water present in the reaction mixture. This suggests that an acid-catalyzed transesterification should be carried out in the absence of water, in order to avoid the competitive formation of carboxylic acids which reduce the yields of alkyl esters (Schuchardt et al., 1997).

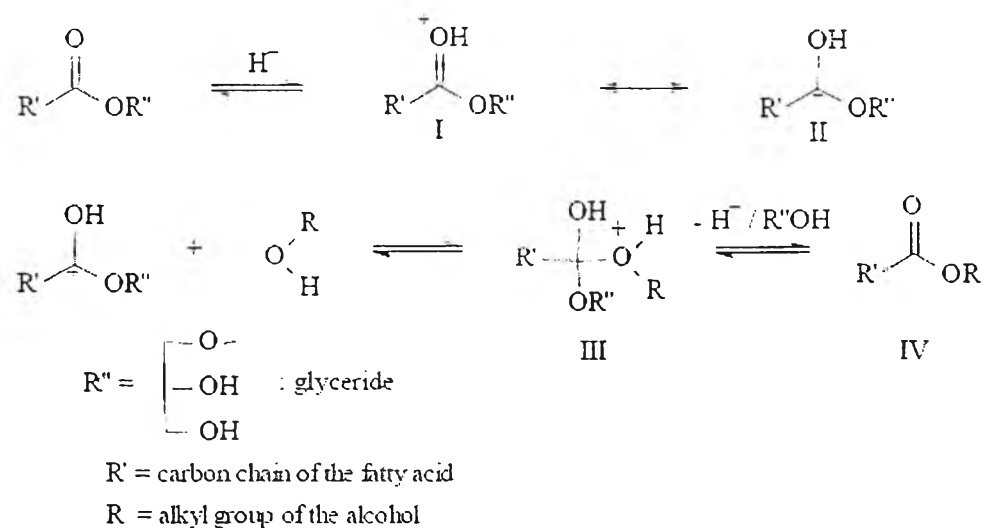


Figure 2.6 Mechanism of the acid-catalyzed transesterification of vegetable oils.

(Sridharan and Mathai, 1974)

Acid-and Alkali- catalysed (two-step Transesterification)

Two step transesterification in which the first acid catalyzed step was followed by the second alkali catalyzed step was developed for the production of biodiesel from oil with high FFA content. Initially, acid catalyst can be used to convert FFA to the ester and to decrease the FFA level. In the second step, alkali catalyst can be performed for the transesterification of oil (two-step). The technique overcomes the problem of a slow reaction rate with acid catalyst and the formation of soap with an alkaline catalyst and increases the ester yield. However, the problem with the removal of the catalysts is still a big issue for the two-step method (Dalai et al., 2006).

Heterogeneous catalysts process

Nowadays, heterogeneous reaction is being considered as a suitable process to replace homogeneous reaction. A solid catalyst would alleviate both the environmental aspects of dealing with sodium methoxide as well as the issues concerning the separation of the catalyst from the reactant for later reuse. Heterogeneous catalysts, usually a solid whose surface is catalyzing reactions of gases and/or liquids, such as amorphous zirconia, titanium-, aluminium-, and potassium-doped zirconias have also become popular for catalyzing the transesterification of vegetable oils (Furuta et al., 2006). The main advantage of a heterogeneous catalyst is that the catalyst is easily separated from the reactants and products. Then, this process could potentially lead to cheaper production costs because it is possible to reuse the catalysts and to carry out both transesterification and esterification simultaneously (Goodwin et al., 2005).

Enzymatic Transesterification

Enzymatic conversion of triglycerides has been suggested as a realistic alternative to the conventional physiochemical methods. Because of difficulty removal of catalyst from biodiesel production that using an inorganic base or acid catalyst at or near the boiling temperatures of the triglyceride/alcohol mixture, it has

been recently found that enzyme such as lipase can be used to catalyze transesterification process by immobilizing them in a suitable support. Comparison between alkali-catalysis and lipase-catalysis methods for biodiesel fuel production is shown in Table 2.1 (Fukuda et al., 2001). Also, the operating temperature of the process is low (50°C) compared to other techniques (Nelson et al., 1996) and receive a higher quality of the products due to stereoselective and regioselective transformations of substrates. Although, the using of lipase as catalyst has some of the disadvantage such as the lose of initial activity due to volume of the oil molecule, the inconsistent of the support and the high cost of natural enzyme.

Table 2.1 Comparison between alkali-catalysis and lipase-catalysis methods for biodiesel fuel production (Ma et al., 1999)

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

For this reason, the achievement in enzymatic process lies in successful immobilization of the enzyme. Immobilization is the most widely used method to attain lipase stability using in biodiesel production. Moreover, immobilization provides enzyme reuse and eliminates the costly processes of enzyme recovery and enzymes recycle. Nelson et al., (1996) studied the lipase-catalyzed synthesis of normal and branched-chain alkyl esters of agriculturally derived triglycerides (TG): vegetable oils, tallow, and restaurant grease with using free and immobilized lipase from varies of source of lipase. From this study, the lipase from *M. miehei* was the most efficient for converting triglycerides to alkyl esters with primary alcohols,

whereas that from *C. antarctica* was the most efficient for transesterifying triglycerides with secondary alcohols to give branched alkyl esters. The reaction generally requires the following conditions: 0.34 M tallow in hexane, 45°C, 200 rpm, 4-8 h, 12.5–25% enzyme by weight of tallow, alcohol-to-TG ratio of 3:1, give over 90% conversion (maximum 94.8-98.5%), and over 90% for secondary alcohols with minimum production of other glycerides. These conditions also gave conversion between 75–85% when applied to soybean and rapeseed oils. The immobilized lipase also proved to be stable and lost little activity when was subjected to repeated uses.

2.3 Variables affecting transesterification and esterification

The process of transesterification and esterification are affected by various factors depending upon the reaction condition used. The effects of these factors are described below.

2.3.1 Ratio of alcohol to oil or fatty acids

For transesterification, it is generally known that one of the most important variables affecting the yield of ester is molar ratio of alcohol to triglyceride. The stoichiometric ratio for transesterification requires three moles of alcohol and one mole triglyceride to yield three moles of fatty acid alkyl esters and one mole of glycerol. However, transesterification is an equilibrium reaction in which a large excess of alcohol is required to drive the reaction to the right. For maximum conversion, a molar ratio of alcohol to oil at 6:1 should be used. However, the high molar ratio of alcohol to vegetable oil interferes with separation of glycerin because there is an increase in solubility. When glycerin remains in solution, it drives the equilibrium back to the left, lowering the yield of esters.

For esterification, the molar ratio of alcohol to fatty acids is also of importance. In this case, the stoichiometric ratio requires 1 to 1 mole ratio of the two reactants. Furthermore, no glycerol is produced. As a result, it is expected that lower alcohol to fatty acids molar ratio would be needed compared with triglyceride transesterification.

2.3.2 Reaction temperatures

The rate of reaction is strongly influenced by the reaction temperature. However, given enough time, the reaction will proceed to near completion even at room temperature. Generally, the catalytic reactions are conducted close to the boiling point of alcohol (60°C to 70°C), under atmospheric pressure. For supercritical conditions, the reaction is carried out under high pressure (9000 kPa) and high temperature (above 240°C).

2.3.3 Reaction time

The conversion increases with reaction time. For example, Freedman et. al., (1984) studied the transesterification of peanut, cotton-seed, sunflower and soybean oil under the condition of methanol to oil molar ratio of 6:1, 0.5% sodium methoxide catalyst, and at 60°C. An approximate yield of 80% was observed after 1 min for soybean and sunflower oils. After 1 h, the conversion was almost the same (93-98%) for all four oils.

2.3.4 Use of organic co-solvent

An improved process was investigated by Krisnangkura and Simamaharnnop (1992) for methanolysis and ethanolysis of fatty acid glycerides such as those found in naturally occurring fats and oils derived from plant and animals. The processes comprise solubilizing oil or fat in methanol or ethanol by addition of toluene as co-solvent in order to form a one phase reaction mixture, and addition an esterification catalyst. The processes proceed quickly, usually in less than 20 min, at ambient temperature, atmospheric pressure, and without agitation. The co-solvent increases the rate of reaction by making the oil soluble in methanol, thus increasing contact of the reactant. The lower alkyl fatty acid monoesters produced by the process can be used as biofuels and are suitable as diesel replacement or additives.

2.3.5 Purity of reactant

Impurities present in the oil also affect conversion levels. Under the same condition, 67% to 84% conversion into ester using crude vegetable oils can be obtained, compared with 94% to 97 % when using refined oils. The free fatty acids in the original oils interfere with catalyst, however, under conditions of high temperature and pressure this problem can be overcome (Freedman et. al., 1984).

2.3.6 Catalyst type and concentration

Catalysts used for transesterification of triglyceride are classified as alkaline, acid, heterogeneous catalyst, among which alkaline is the most effective transesterification catalyst compared to acidic catalyst. Sodium alkoxides are among the most efficient catalyst used, although KOH and NaOH can also be used. The alkaline catalyst concentration in range of 0.5 to 1% by weight yields 94% to 99%. The acid could be sulfuric acid, phosphoric acid, hydrochloric acid or organic sulfonic acid. In addition, lipases are enzyme used to catalyze some reaction such as hydrolysis of glycerol, alcoholysis and acidolysis. It has been discovered that lipase can be used as catalyst for transesterification and esterification reactions too. The extra cellular and the intracellular lipases are also able to catalyze the transesterification of triglycerides effectively (Marchetti J.M. et. al., 2007).

2.3.7 Presence of water

In biodiesel production, it is well known that the vegetable oils used as raw material for the transesterification should be water free since water has a negative effect on the reaction. Water can consume the catalyst and reduce catalyst efficiency and it is believed that the presence of water has a greater negative effect than that of the free fatty acids. Therefore, it is generally recommended that for typical transesterification of vegetable oil, the water content should be kept below 0.06% (Ma et al., 1998). For the enzymatic transesterification, lipases possess the unique feature of acting at the interface between an aqueous and an organic phase. Lipase interfacial

action is due to the fact that their catalytic activity generally depends on the aggregation of the substrates. Activation of the enzyme involves unmasking and restructuring of the active site through conformational changes of the lipase molecule, which requires the presence of oil-water interface. Lipase activity generally depends on the available interfacial area. With the increased addition of water, the amount of water available for oil to form oil-water droplets increases, thereby, increasing the available interfacial area. However, since lipases usually catalyze hydrolysis in aqueous media, excess water may also stimulate the competing hydrolysis reaction. The optimum water content is a compromise between minimizing hydrolysis and maximizing enzyme activity for the transesterification reaction (Noureddini et al., 2005).

2.4 Enzymatic Immobilization

In recent year, immobilization is the most widely used method for achieving stability in lipases that to make them more attractive for using in biodiesel production. Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are enzyme that used to catalyze some reaction such as hydrolysis of glycerol, alcoholysis and acidolysis, but it has been discovered that they can be used as catalyst for transesterification and esterification reactions too. Therefore, the use of pure enzymes is recommended. In order to use lipases more economically and efficiently immobilization techniques can be applied.

Alcoholysis :

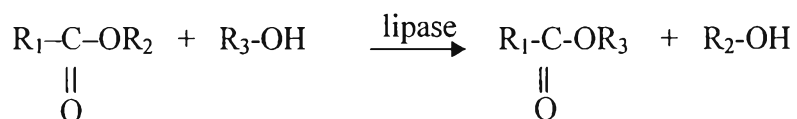


Figure 2.7 The reactions catalyzed by lipase in aqueous and non-aqueous solutions.

Common immobilization techniques that mostly applied to use with lipase immobilization include physical adsorption onto a solid support (e.g. Bosley and Pielow, 1997), physical entrapment within a polymer matrix support (e.g. Pizarro et

al., 1997) and covalent bonding to a solid support (e.g. Walt and Agayn, 1994). Entrapment of lipase within an inorganic polymer matrix such as alginate is one method that has received a considerable attention in recent years. The lipase from *Candida cylindracea*, after immobilization in calcium alginate beads, catalysed transesterification in n-hexane under both batch and continuous-flow conditions. The operational stability of the lipase was markedly enhanced by alginate entrapment (Hertzberg et al., 1992).

The formation of alginate gels in the presence of divalent cations is one of the best understood biopolymer gelling systems, and the functional properties of calcium alginate beads in aqueous systems have been thoroughly investigated. However, there have been comparatively few studies of alginate beads in organic solvents. It is known that the water in alginate gels, in contrast to other ionic hydrogels, can be exchanged with nonaqueous solvents such as ethanol without major changes in the volume or structure of the gel (Grasdalen et al., 1974).

2.5 Literature review

More than decade, a number of research works on the production of biodiesel have been reported using a variety of oils, different catalysts, alcohols and reaction conditions.

Aracil et al., (2004) used different basic catalysts such as sodium methoxide, potassium methoxide, sodium hydroxide and potassium hydroxide for transesterification of sunflower oil. They found that biodiesel purity near 100 wt.% was obtained for all catalysts. Although all the transesterification reactions were quite rapid and the product achieved nearly 100% methyl ester concentrations, the reactions using sodium hydroxide turned out the fastest.

Mcleod et al., (2007) studied the effects of the alkali metal doped (Li, Na, K), the support (CaO, MgO, Al₂O₃), and preparation method (calcined at 600°C or heated to 110°C) on the catalyst activities in transesterification of rapeseed oil to biodiesel. Of those evaluated, LiNO₃/CaO, NaNO₃/CaO, KNO₃/CaO and LiNO₃/MgO exhibited >90% conversion in a standard 3 h test. The alkali-metal

doped CaO catalysts (calcined and uncalcined) show higher base strength than CaO alone ($8.2 < \text{pKBH}^+ < 10.1$).

Although high purity and yield of biodiesel can be achieved in a short time with the alkali process. However, it is very sensitive to the purity of reactants and the amount of FFA and moisture causing soap formation, which results in the lower yield of ester and renders the separation of ester and glycerol by water washing difficult. Zheng et al., (2006) studied the reaction kinetic of acid catalyzed (H_2SO_4) transesterification of waste frying oil in excess methanol to form fatty acid methyl esters for possible use as biodiesel. The results demonstrated that the oil:methanol:acid molar ratios and temperature were the most significant factors affecting the yield of methyl ester. The optimal conditions were methyl ester at 70°C with oil:methanol:acid molar ratios of 1:245:3.8 and at 80°C with oil:methanol:acid molar ratios in range 1:74:1.9-1:245:3.8. As a result of the large excess of methanol, the reaction completed (99.1% conversion) in 4 hrs.

The production of biodiesel using a biocatalyst such as lipase can eliminate the disadvantages of the alkali process by producing product of very high purity with less downstream operations (Fukuda et al., 2001). Nouredini et al., (2001) used methanol and ethanol for the transesterification of soybean oil in the presence of immobilized enzyme obtained from *Pseudomonas fluorescens* and reported conversions of 67% and 65% for methanol and ethanol respectively. Modi et al., (2007) reported the use of Novozyme 435, to achieve conversions above 90% for oils such as jatropha, karanj and sunflower oil. However ethanolysis activity could not be maintained for more than 6 cycles.

Various techniques and even more support materials have been studied and consequently many immobilized preparations with a wide range of efficiency, stability and activity have been offered. Ghamgui et al. (2004) reported that CaCO_3 is the most suitable adsorbent preserving the catalytic activity almost intact, offers maximum adsorption capacity and achieves the yield more than 95% during 30 min with the loading of 2570 IU/g support. The optimum temperature for both free and immobilized lipase activities was 37°C . After 24 h of incubation at 50°C , the

immobilized maintained 67% of its initial activity, while the free enzyme was completely inactivated.

Ganapati et. al., (2005) has proposed that pre-immobilization of *Candida antarctica* lipase B (CAL B) on hexagonal mesoporous silica follow by encapsulation in calcium alginate beads was found to be the most active for transesterification reaction and they founded that the pre-immobilization of CAL B on hexagonal mesoporous silica was carried out by simple physical adsorption with an immobilization yield of 62% and the maximum loading of the enzyme was 100 mg per gram support. The pre-immobilized enzyme when encapsulated in calcium alginate beads yields a highly reusable biocatalyst with no leaching even after the fourth reuse. The activities of all lipases in different physical forms were evaluated in the transesterification of *p*-chlorobenzyl alcohol with vinyl acetate to give *p*-chlorobenzyl acetate at 30°C (Ganapati et al., 2005). They founded that a conversion 68% with 100% selectivity for *p*-chlorobenzyl acetate was obtained at 30°C in 120 min.

Table 2.2 Review studies production of biodiesel by heterogeneous, acid or alkali catalyzed methods.

Reference	Material	Method	Condition			Conversion	Objective
			Temp. (°C)	Rx Time (hr)	Molar ratio (Alc:oil)		
Gryglewicz et al., 1999	1.rape seed oil 2.methanol 3.CaO 4.Ca(CH ₃ O) ₂ 5.Ba(OH) ₂	Conventional (heterogeneous catalyst)	62	2	4.5:1	90%	To compare three heterogeneous catalyst,CaO,Ca(CH ₃ O) ₂ , Ba(OH) ₂ for biodiesel production.
Granados et al., 2006	1.sunflower oil 2.methanol 3.CaO	Conventional (heterogeneous catalyst)	600	2	13:1	92%	To investigate how important the homogeneous contribution that occurring at the surface of the solid (heterogeneous)
Issariyakul et al., 2006	1. Waste fryer Grease 2. methanol 3. ethanol 4. KOH	Conventional (heterogeneous-base catalyst)	25	0.5	6:1	90%	To study the transesterification of WFG with methanol, ethanol and various mixtures of methanol and ethanol by using KOH as a catalyst.

Reference	Material	Method	Condition			Conversion	Objective
			Temp (°C)	Rx Time (hr)	Molar ratio (Al:oil)		
Cheryan et al., 2000	1. palm oil 2. methanol 3. potassium hydroxide	Conventional (base catalyst)	40-50	50-60	3:1-15:1	85%	To investigate the reaction variables effecting the transesterification such as concentration of catalyst, oil/alcohol ratio, and temperature.
Ramadhas et al., 2005	1. rubber seed oil 2. methanol 3. sulfuric acid 4. NaOH	Conventional (base catalyst)	60	20	6:1	76.9%	To develop a process for producing biodiesel from a low-cost feedstock like crude rubber seed oil.
Zheng et al., 2006	1. waste frying oil 2. methanol 3. H ₂ SO ₄	Conventional (acid catalyst)	70	4	6:1-24:1	99%	To develop a process for continuous transesterification and determine the optimal residence time for maximum production of methyl esters from palm oil.

Reference	Material	Method	Condition			Conversion	Objective
			Temp (°C)	Rx Time (s)	Molar ratio (Alc:oil)		
Saka et al., 2001	1. rape seed oil 2. methanol	Conventional (supercritical method)	300- 450	10-240	42:1	95%	To study effect of free fatty acids and water in triglycerides on biodiesel fuel production.
Cao et al., 2005	1. soybean oil 2. methanol	Conventional (supercritical method)	200- 310	5-30	6:1-42:1	98%	To study the transesterification of soybean oil in methanol under supercritical conditions in the presence of propane as co-solvent.

Table 2.3 Review studies production of biodiesel by enzymatic catalyzed methods.

Reference	Material	Source of lipase, method (Support)	Condition				Conversion	Objective
			Volume reactor (ml)	Temp (°C)	Time (hr)	Molar ratio (Alc:oil)		
Chulalaksananukul et al., 1990	1. Oleic acid 2. Ethanol 3. n-hexane	<i>Mucor miehei</i> , Adsorption (macroporous anionic resin beads)	-	40	2	1:1	95%	To investigate the kinetics of esterification catalyzed by immobilized lipase and determine kinetic constants.
Nelson et al., 1996	1.Soybean oil 2.Tallow 3.Olive oil 4.Greases 5.Rapeseed oil 6.Ethanol	Crude lipase (<i>C. antarctica</i>)	125	45	5	3:1	90%	To report the lipase-catalyzed synthesis of normal and branched-chain alkyl esters of agriculturally derived triglycerides (TG): vegetable oils, tallow, and restaurant grease.

Reference	Material	Source of lipase, method (Support)	Condition				Conversion	Objective
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Dossat et al., 1999	1. Sunflower oil 2. butanol 3. n-hexane	Lipozyme [®] (<i>Rhizomucor miehei</i>), Adsorption (macroporous anionic resin beads, Duolite A568)	-	40	48	3:1	80%	To propose an efficient process to produce butyl oleate using direct transesterification of high oleic sunflower oil with butanol.
Samukawa et al., 2000	1. Soybean oil 2. ethanol 3. hexane	<i>Candida antarctica</i> (Novozyme 435), Adsorption (Solid support material)	30-50	35	12	1:1	97%	To describe the effects of the preincubation of the enzyme and various reaction conditions on the reaction kinetics of ethanolysis of soybean oil catalyzed by the enzyme

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Iso et al., 2001	1. Trioleic 2. 1-butanol	<i>Pseudomonas fluorescens</i> , Adsorption (inorganic porous kaolinite, Toyonite 200-M)	-	50	25	3:1	90%	To study the transesterification reaction for the production of biodiesel fuel that was performed from triglycerides and short-chain alcohol using immobilized lipase obtained by effective method employing an inorganic porous particle as a carrier in non-aqueous condition.
Soumanou et al., 2003	1. sunflower Oil 2. methanol 3. n-hexane	<i>Pseudomonas fluorescens</i> , Adsorption (Celite 545 or polypropylene EP100)	-	40	24	4.5:1	>90%	To investigate the biodiesel production by using the enzymatic of sunflower oil with methanol by various crude and immobilized lipases from different microorganisms in organic solvent and to study the effect of temperature and stability of immobilized lipases.

Reference	Material	Source of lipase, method (Support)	Condition				Conversion	Objective
			Volume reactor (ml)	Temp (°C)	Time (hr)	Molar ratio (Alc:oil)		
Noueddini et al., 2004	1. soybean oil 2. ethanol 3. methanol	<i>Pseudomonas cepacia</i> , Entrapment (Hydrophobic sol-gel)	-	35	1	15.2:1* 7.5:1**	65%* 67%**	To study the lipase PS from <i>Pseudomonas cepacia</i> that was entrapped within a sol-gel polymer matrix for using in the transesterification of soybean oil with methanol and ethanol.
Du et al., 2005	1. soybean oil 2. methanol	<i>Thermomyces lanuginosus</i> , Adsorption (Silica gel)	50	40	72	1:1	66%	To study the factors including lipase protein, water and the immobilized materials-silica gel that may influence the acyl migration.

* The molar ratio for using ethanol.

** The molar ratio for using methanol.

Reference	Material	Source of lipase, method (Support)	Condition				Conversion	Objective
			Volume reactor (ml)	Temp (°C)	Time (hr)	Molar ratio (Alc:oil)		
Royon et al., 2006	1. cotton seed oil 2. methanol 3. t-butanol	<i>Candida antarctica</i> (Novozyme 435), Adsorption	-	50	24	3.6:1	95%	To study the methanolysis of cotton seed oil using t-butanol as a solvent and immobilized <i>Candida Antarctica</i> lipase (Novozym 435) as catalysts.
Shah et al., 2006	1. jatropha oil 2. ethanol	<i>Pseudomonas cepacia</i> , Adsorption (Celite 545)	-	50	8	4:1	98%	To study the economic factor being an important consideration for biodiesel production with using free and immobilized lipase on celite.
Orcaire et al., 2006	1. sunflower oil 2. methyl acetate	<i>Burkholderia cepacia</i> , Encapsulation (Silica aerogels resin forced with silica quartz fibre felt)	-	30	-	3:1	56%	To study the synthesis of biodiesel from vegetable oils with using immobilized lipase that was encapsulated in sol-gel materials.

Reference	Material	Source of lipase, method (Support)	Condition				Conversion	Objective
			Volume reactor (ml)	Temp (°C)	Time (hr)	Molar ratio (Alc : oil)		
Yang et al., 2006	1. soybean oil 2. methanol 3. hexane	<i>Candida</i> sp. 99-125, Adsorption (nonpolar resin NKA)	50	40	24	-	97.3%	To study the factors influencing synthesis utility of immobilized lipases for biodiesel production such as enzyme loading, reaction temperature, pH, water content
Yagiz et al., 2007	1. waste cooking oil 2. methanol	Lipozyme-TL IM, Adsorption (hydrotalcite and zeolite)	-	45	105	3:1	95%	To investigate the immobilization conditions such as temperature, pH, time and particle sizes on enzyme activity. The effects of different types of support used in immobilization on biodiesel production.