THE EFFECT OF MICROWAVE RADIATION ON BIODIESEL PRODUCTION

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ABSTRACT

Microwave radiation is believed to have effect toward biodiesel production. Because of that reason, the purpose of this research is to investigate the effect of microwave radiation toward enhancing the biodiesel production. During experimental phase, transesterification process is used in order to produce biodiesel and microwave oven is used in order to expose the reaction to the microwave radiation. Using enzyme as the biocatalyst, the experimental result shows that microwave radiation do affect the enzymatic reaction. The result showed that longer period of exposure to the microwave yields the fastest conversion glycerides into fatty acid methyl ester. The result also showed that higher power, as long as it did not denature the enzyme, will enhance the conversion of the glycerides.

ABSTRAK

Sinaran gelombang mikro diyakini mampu mempengaruhi kadar pengeluaran biodiesel. Oleh kerana itu, kajian ini bertujuan untuk mengetahui kesan radiasi gelombang mikro terhadap peningkatan pengeluaran biodiesel. Semasa membuat kajian,, proses transesterifikasi digunakan untuk menghasilkan biodiesel dan ketuhar gelombang mikro digunakan sebagai sumber sinaran gelombang mikro yang akan didedahkan kepada tindakbalas tersebut. Menggunakan enzim sebagai biokatalis, keputusan menunjukkan bahawa sinaran gelombang mikro ada mempengaruhi tindakbalas enzimatik. Keputusan kajian juga menunjukkan bahawa pendedahan secara berterusan mampu menghasilkan metil dengan lebih pantas.. Keputusan kajian juga menunjukkan bahawa kekuatan sinar radisi yang lebih tinggi, asalkan tidak denaturasi enzim, akan meningkatkan penghasilan biodiesel..

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CHAPTER 1

INTRODUCTION

Research background

The idea of biodiesel is not new and it has been demonstrated as early as 1900 in Paris exposition where the French Otto Company operated a small diesel engine on peanut oil, (G. Knothe et al., 2005) but it was not implemented due to the high viscosity and low volatility of vegetable oils. Recently, with the global shortage of fossil fuels, increased in the crude oil prices and environmental concerns to reduce pollution has resuscitated the interest in biodiesel production.

The idea is to reduce the viscosity of the oil by replacing glycerol with methyl or ethyl alcohol through the transesterification reaction. Catalysis of the transesterification reaction can be broadly classified into two categories, chemical and enzymatic. Chemically transesterification reaction can be acid/base catalyzed. The mixture of oil with excess of ethanol when refluxed at 70 °C for 1 h gave ethyl esters of fatty acids with a yield of 93% (S. Shah et al, 2004). In contrast, biocatalysts allow the synthesis of specific alkyl esters, easy recovery of the glycerol, and the transesterification of triglycerides with high free fatty acid content. In this approach, lipase catalyzed transesterification is carried out in nonaqueous environments.

There are many sources of vegetable oil that have already been used to produce biodiesel. There are corn oil, rapeseed oil, sunflower oil and soy oil and many more.

The world leader in biodiesel production is Germany. They produce more than half of the total amount of biodiesel ever produced in the world. Based on the research, EU members contribute almost 70 percent of biodiesel production. In German, they used rapeseed as oil source due to many rapeseed plants had been plant there.

Even though there is already many country that produce biodiesel and there is already so many technologies developed in order to produce biodiesel, people still believes that biodiesel production can still be optimized in order to increase the amount of biodiesel produced so that the demand of fuel can be fulfill.

In this study, palm oil is used as source of oil due to the abundance amount of palm oil in Malaysia.

1.1. Problem Statement.

The production of biodiesel or more commonly fatty acid methyl esters (FAME) has attracted significant attention lately due to the increasing demand for a cleaner, safer and renewable energy (J. Kansedo, 2009). The high cost of biodiesel, compared to petroleum-based diesel, is a major barrier to its commercialization. It has been reported that 60–90% of biodiesel cost arises from the cost of the feedstock oil. The production and consumption of biofuels continues to increase as more attention is paid to the environment and the depletion of fossil-fuel resources. Furthermore, biodiesel is a fuel from natural oils such as soybean oil, rapeseed oil or animal fats, is a substitute for petroleum-diesel fuel. According to the research, the world is going to start running out of oil and really soon. This is because, petroleum supply is rapidly dwindling yet more and more barrels of oil are being produced each day. For example, U.S. Petroleum production over the course of the years can be graphed in the shape of a bell curve. U.S. production actually reached its peak around 1970 with 3 billion barrels produced each year. Since then, numerous oil fields have dried up, and others have been opened

(namely on the north slope of Alaska), but domestic oil supply continues to fall. Last year the number fell to around 2 billion barrels of oil produced, with only 113 billion barrels left. Following these numbers, the graph indicates that by the year 2060, U.S. oil reserves will be negligible and bordering on economically unfeasible for recovery (Martinez, 2002).

1.2. Objective

The main objective of this research is to study the effect of microwave radiation towards enhancing biodiesel production.

1.3. Scope of study

In order to achieve the objective of this research, this research has been narrowed into two scopes which are, firstly by studying the effect of the duration of the microwave radiation toward biodiesel production. The other scope is to study the effect of the power of microwave radiation toward biodiesel production.

1.4. Benefits and significant of study.

This experiment is hoped to help to preserve the environment by producing greener and cleaner fuel. This is because; it is proven that the biodiesel is emitting less dangerous gas such as carbon monoxide, and etc. This experiment is also hoped to improved the biodiesel by accelerate the rate of converting the fatty acid into ester faster.

CHAPTER 2

LITERATURE REVIEW

2.1. Palm Oil

Palm oil and palm kernel oil are edible plant oils derived from the oil palm *Elaeis guineensis*. Palm oil is extracted from the pulp (Reeves et al, 1979) of the fruit, while palm kernel oil is derived from the kernel (seed) of the oil palm.(Poku and Kwasi, 2002) They should not be confused with coconut oil, which is derived from the kernel of the coconut palm (*Cocos nucifera*). Palm oil is naturally reddish because it contains a high amount of beta-carotene (though boiling palm oil destroys the beta-carotene, rendering the oil colourless.

Because palm oil is one of the few highly saturated vegetable fats, palm oil is semi-solid at room temperatures. Palm oil contains several saturated and unsaturated fats in the forms of glyceryl laurate, myristate, palmitate, stearate, oleate, linoleate, and linolenate (Cottrell, 1991). Palm kernel oil is more highly saturated than palm oil. Like all vegetable oils, palm oil does not contain cholesterol which is always found in unrefined animal fats, although saturated fat intake increases both low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol.

Palm oil is a very common cooking ingredient in Southeast Asia and the tropical belt of Africa. Its increasing use in the commercial food industry in other parts of the

world is buoyed by its cheaper pricing and the high oxidative stability of the refined product when used for frying (United States Department of Agriculture, 2006).

2.1.1. Composition of Palm Oil and Palm Kernel Oil

Palm oil and palm kernel oil are composed of fatty acids, esterified with glycerol just like any ordinary fat. Both are high in saturated fatty acids, about 50% and 80%, respectively. The oil palm gives its name to the 16-carbon saturated fatty acid palmitic acid found in palm oil is also a constituent of palm oil while palm kernel oil contains mainly lauric acid. Palm oil is a large natural source of tocotrienol, part of the vitamin E family.(Ang et al, 1999).

The pie charts below describe the composition of palm oil and palm kernel oil

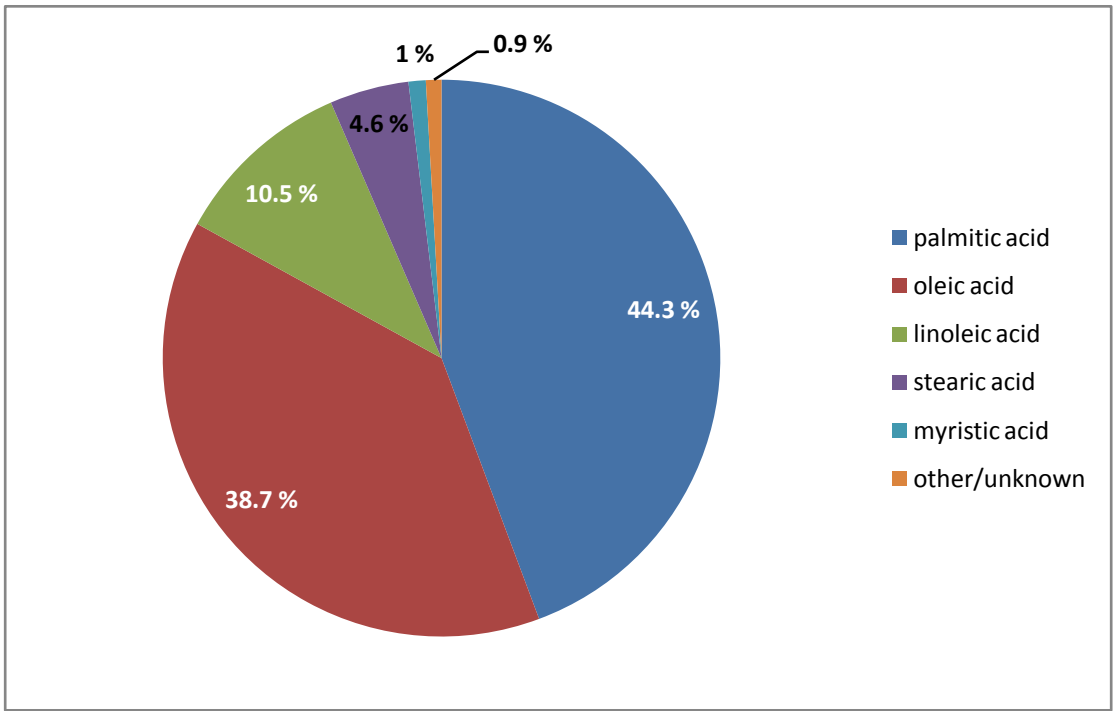

Figure 2.1: Amount of Components in Palm Oil

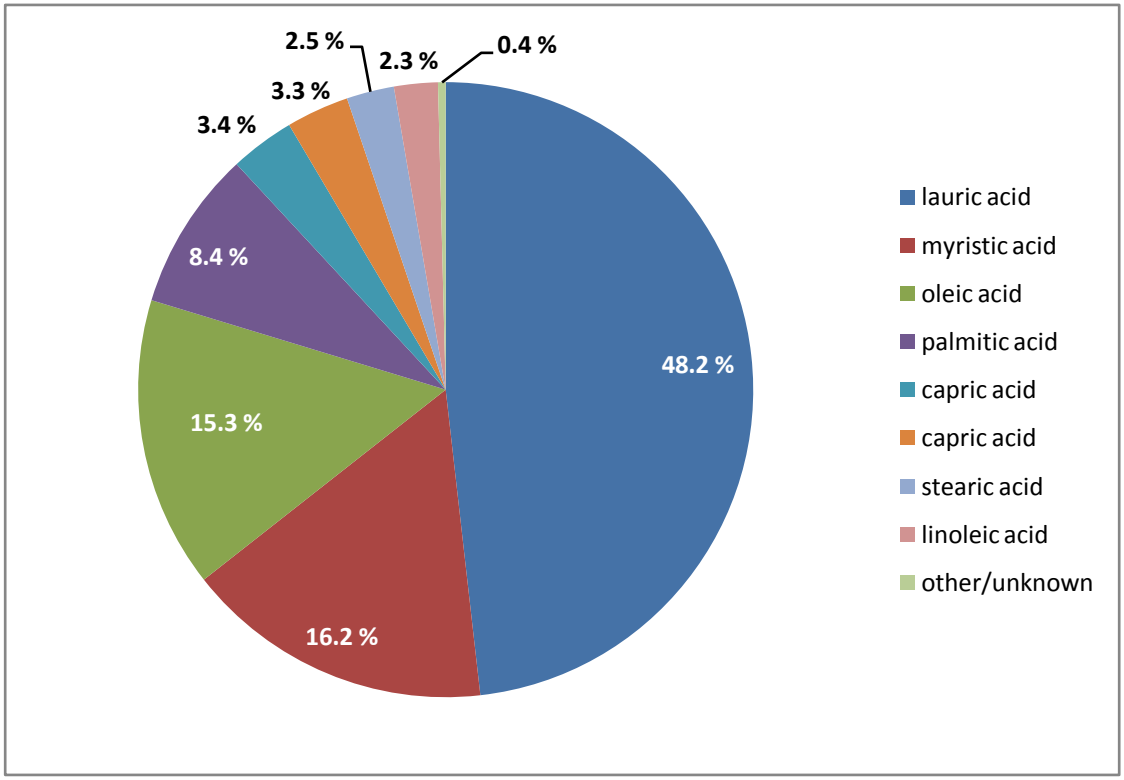

Figure 2.2: Amount of Components in Palm Kernel Oil

2.2. Enzyme

The potential applications of lipases in biotransformations are nowadays well established and fully documented with several of examples, both in hydrolytical (aqueous media) or synthetical (organic media) approaches.

Furthermore, the rapid developments of molecular biology techniques, as well as the availability of more reliable high-throughput-screening methods, have enhanced the utility that enzymes offer for organic synthesis. Currently, biocatalysts of second generation are being produced by adapting a wild-type enzyme to a desired application.

Within the hydrolase-based biocatalysis, lipases from Candida rugosa were firstly described as early as in the sixties, by isolating the yeast from natural soils due to its powerful lipase production capacity. Later on, two isoenzymes which initially called LipA and LipB were identified, purified, and genetically characterized.

Nowadays it is well established that at least seven genes are involved in the *Candida rugosa* lipase producing machinery, being five of them (Lip1–Lip5) fully biochemically characterized. Nowadays the LipA/LipB nomenclature has been practically abandoned, and a new one based on numbers is often used: Lip1, Lip2, Lip3, up to Lip7.

The active site in *Candida rugosa* lipases is covered by an alpha helix structure, composed of aminoacids with amphiphilic properties, namely lid or flap. The aminoacidic composition of such lid has proven to be quite variable among isoenzymes which only 14 residues are conserved in the structures of Lip1, Lip2 and Lip3, and it is crucial for the socalled interfacial activation, and consequently for the catalytical enzymatic activity and/or enantioselectivity. As this region appears to be very diverse among isoenzymes, it is not a surprise that a direct correlation between the variation of

lid composition and some aspects of the different biocatalytical performances among isoenzymes can be found.

In fact, the whole lid structure is fixed by a disulfide bond, and an ionic interaction between Glu96 and Arg37. When the lid is opened, a rotation over two aminoacidic residues is produced, that is, Glu-66 and Pro-92. For such purpose, an isomerisation cis–trans of the peptide bond of Pro-92 is required. In connection with that, it should be noted that the opened lid form is more favored thermodynamically than the closed one, since the hydrophobic aminoacids of the lid interact more efficiently with the lipophilic media. Although the ultimate reasons for the lid movement, as well as their influence in the biocatalytical behaviour, are still a matter of speculation.

Actually, it is well known that parameters like cosolvents, and/or modifications in the pH of the microenvironment or in the dielectric constant of the active site could also play a role in that lid movement, and conclusively in the enzymatic activity. In this sense, recently a different interfacial activation between the lids of Lip1 and Lip3 by polyethylene glycol has been reported (Pablo Domı´nguez de Marı´a, 2006)

2.2.1. Enzyme mechanism.

Enzyme mechanism is also known as enzyme catalysis. Enzyme catalysis is the catalysis of chemical reactions by specialized proteins known as enzymes. Catalysis of biochemical reactions in the cell is vital due to the very low reaction rates of the uncatalysed reactions. The mechanism of enzyme catalysis is similar in principle to other types of chemical catalysis. By providing an alternative reaction route and by stabilizing intermediates the enzyme reduces the energy required to reach the highest energy transition state of the reaction. The reduction of activation energy (*Ea*) increases

the number of reactant molecules with enough energy to reach the activation energy and form the product.

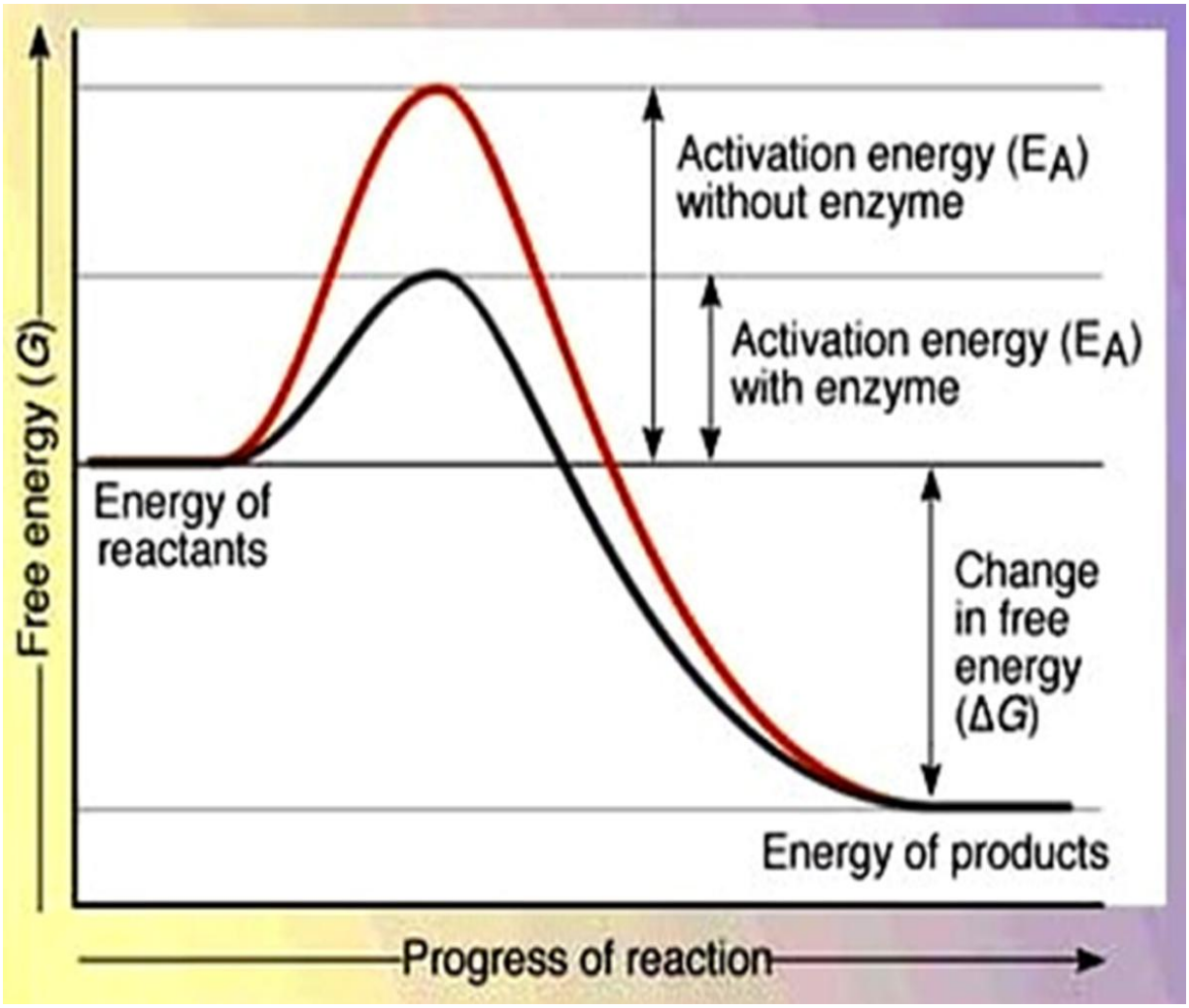

Figure 2.3: Stabilization of the transition state by an enzyme (source: http://www4.nau.edu/meteorite/meteorite/Images/activation-energy.png)

Although there are many type of mechanism suggested, the most favorable type of mechanism is induced fit mechanism (Koshland DE, 1958). This model proposes that the initial interaction between enzyme and substrate is relatively weak, but that these weak interactions rapidly induce conformational changes in the enzyme that strengthen binding.

The advantages of the induced fit mechanism arise due to the stabilising effect of strong enzyme binding. There are two different mechanisms of substrate binding: uniform binding, which has strong substrate binding, and differential binding, which has strong transition state binding. The stabilizing effect of uniform binding increases both substrate and transition state binding affinity, while differential binding increases only transition state binding affinity.

Both are used by enzymes and have been evolutionarily chosen to minimize the Ea of the reaction. Enzymes which are saturated, that is, have a high affinity substrate binding, require differential binding to reduce the Ea, whereas small substrate unbound enzymes may use either differential or uniform binding. These effects have led to most proteins using the differential binding mechanism to reduce the Ea, so most proteins have high affinity of the enzyme to the transition state.

Differential binding is carried out by the induced fit mechanism - the substrate first binds weakly, then the enzyme changes conformation increasing the affinity to the transition state and stabilizing it, so reducing the activation energy to reach it. It is important to clarify, however, that the induced fit concept cannot be used to rationalize catalysis. That is, the chemical catalysis is defined as the reduction of Ea (when the system is already in the ES) relative to Ea in the uncatalyzed reaction in water (without the enzyme). The induced fit only suggests that the barrier is lower in the closed form of the enzyme but does not tell us what the reason for the barrier reduction is.

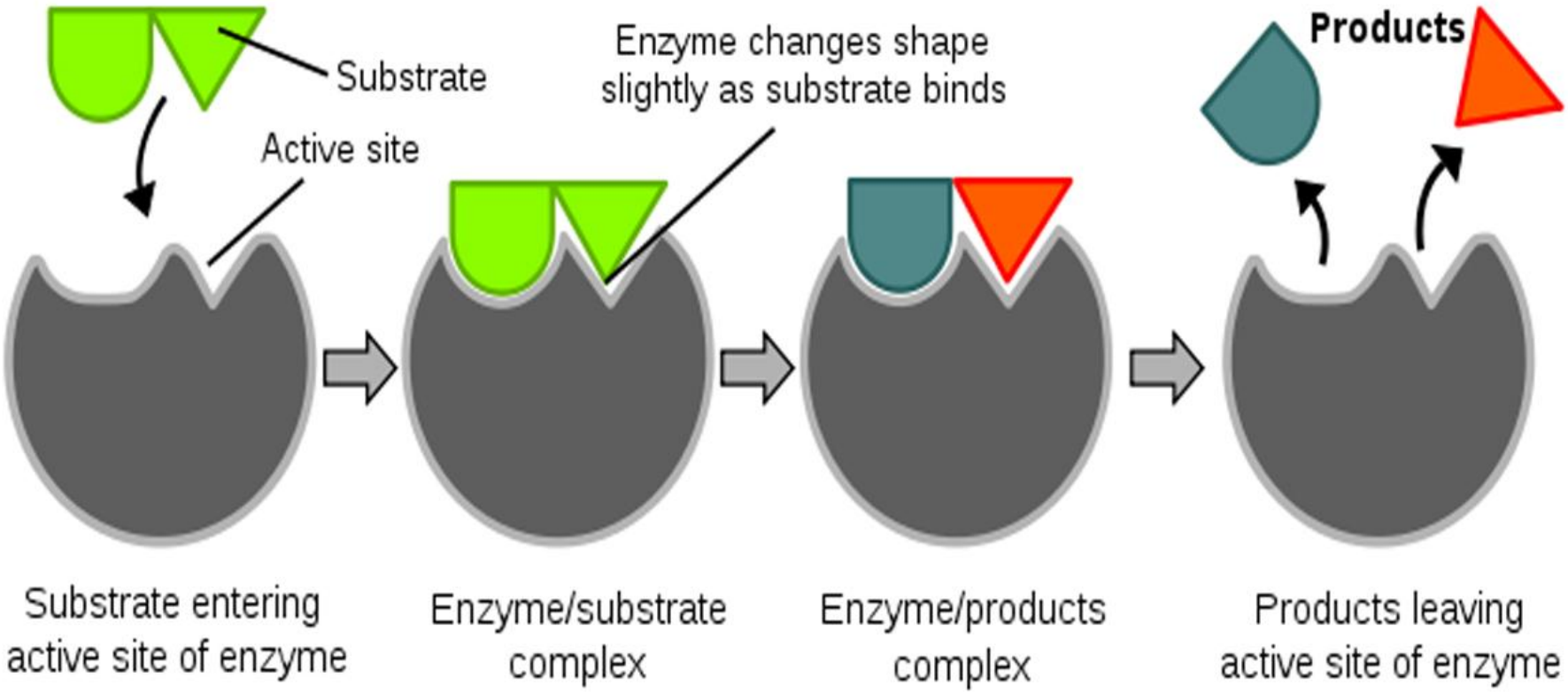

Figure 2.4: Enzyme-substrate Interaction Based on Induce-fit Model (source: http://upload.wikimedia.org/Induced_fit_diagram.svg/648px-Induced_fit_diagram.svg.png)

2.3. Transesterification (methanolysis)

The Transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. A triglyceride has a glycerine molecule as its base with three long chain fatty acids attached. The characteristics of the fat are determined by the nature of the fatty acids attached to the glycerine. The nature of the fatty acids can in turn affect the characteristics of the biodiesel.

During the esterification process, the triglyceride is reacted with alcohol in the presence of a catalyst, usually a strong alkaline like sodium hydroxide. The alcohol reacts with the fatty acids to form the mono-alkyl ester, or biodiesel and crude glycerol. In most production methanol or ethanol is the alcohol used (methanol produces methyl esters, ethanol produces ethyl esters) and is base catalysed by either potassium or sodium hydroxide. Potassium hydroxide has been found to be more suitable for the ethyl ester biodiesel production, either base can be used for the methyl ester. The catalyst used may not be limited to only chemical catalyst.

Nowadays, enzyme, occasionally lipase is used to substitute a chemical catalyst. This way, the producer will save some cost in order to purify product. A common product of the transesterification process is Fatty Acid Methyl Ester (FAME) produced from vegetable oil reacted with methanol. The figure below shows the chemical process for methyl ester biodiesel. The reaction between the fat or oil and the alcohol is a reversible reaction and so the alcohol must be added in excess to drive the reaction towards the right and ensure complete conversion.

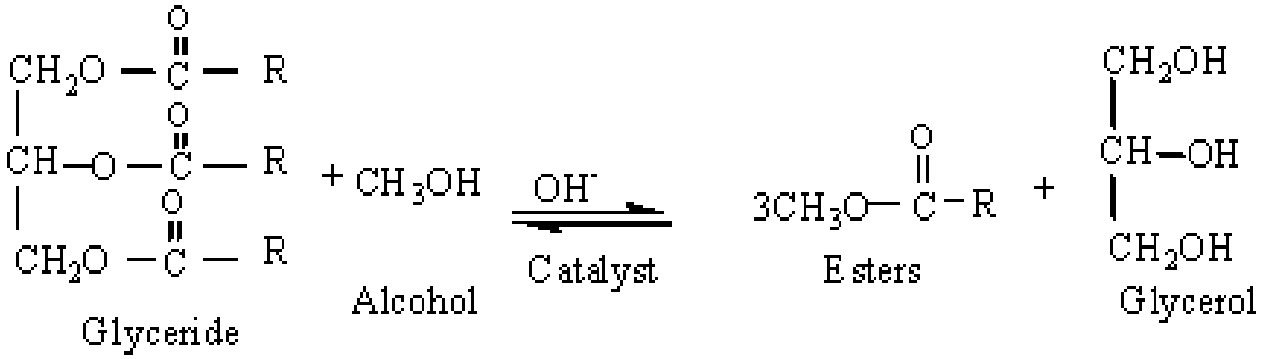

Figure 2.5: Transesterification Process of Glycerides and Alcohol

The products of the reaction are the biodiesel itself and glycerol. A successful transesterification reaction is signified by the separation of the ester and glycerol layers after the reaction time. The heavier, co-product, glycerol settles out and may be sold as it is or it may be purified for use in other industries, such as the pharmaceutical, cosmetics etcetera.

2.4. **Gas Chromatography**

Gas Chromatography (GC) is an advance device invented for the purpose of analyzing volatile compounds. It is a commonly used analytic technique in many research and industrial laboratories. A broad variety of samples can be analyzed as long as the compounds are sufficiently thermal stable and volatile enough.

GC required two types of phases, which is stationary phase, and the other one is mobile phase. The mobile phase (carrier gas) is comprised of an inert gas such as helium, argon, nitrogen, and etcetera. The stationary phase consists of a packed column where the packing or solid support itself acts as stationary phase, or is coated with the liquid stationary phase (high boiling polymer). More commonly used in many

instruments are capillary columns, where the stationary phase coats the walls of a small-diameter tube directly.

GC principal involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

2.4.1. Component on a GC

2.4.1.1.Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependent upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

2.4.1.2.Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a plug of vapour - slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a micro syringe is used to inject sample through a rubber septum into a flash vaporizer port at the head of the column. The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample. For packed columns, sample size ranges from tenths of a microliter up to 20 microliters.

Capillary columns, on the other hand, need much less sample, typically around 10^{-3} mL. For capillary GC, split/splitless injection is used.

The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vaporizes to form a mixture of carrier gas, vaporized solvent and vaporized solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.

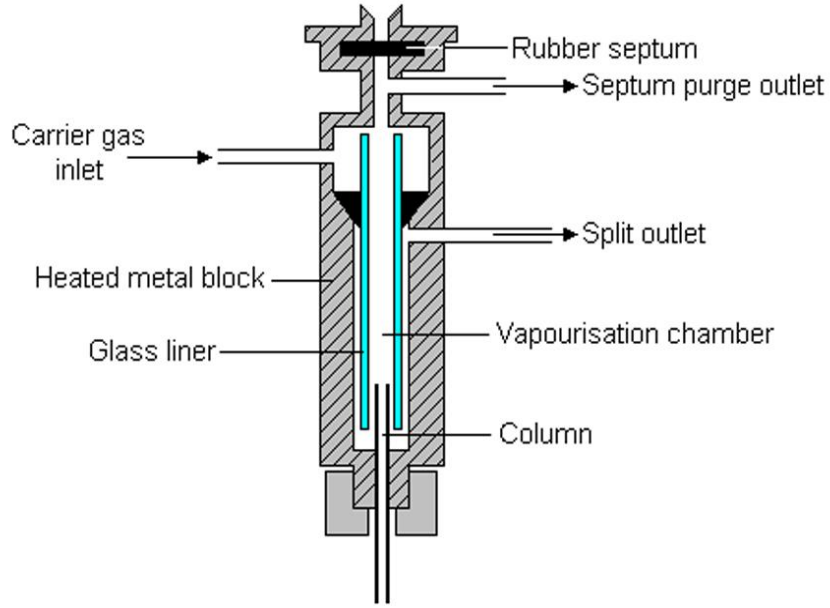

Figure 2.6: Cross Sectional view of a GC Sample Injector

2.4.1.3.Columns

There are two general types of column, *packed* and *capillary* (also known as *open tubular*). Packed columns contain a finely divided, inert, solid support material (commonly based on *diatomaceous earth*) coated with liquid stationary phase. Most