CHAPTER II
BACKGROUND AND LITERATURE REVIEWS

2.1 The Function of Lipase as An Enzyme

2.1.1 Enzyme

Enzymes are known as the biological catalyst controlling biochemical reactions at the molecular level, where they play important role in inducing and storing the energy for living cells. In general, enzymes are classified into six main classes based on their functions:

1. Oxidoreductases: for catalyzation of the oxidation/reduction reactions
2. Transferases: for catalyzation the group transfer reactions
3. Hydrolases: for catalyzation the hydrolytic reactions
4. Lyases: for catalyzation the reactions involving the removal/addition of a double bond
5. Isomerases: for catalyzation the reactions of isomerization
6. Synthases: for catalyzation the reactions involving the molecules coupling/breakdown of pyrophosphate of adenosine triphosphate (ATP)

2.1.2 Lipase

Lipases are used interchangeably as these enzymes catalyze the hydrolysis of carboxylic ester bonds and are classified among the hydrolases. The most common sources of lipase are microorganism followed by mammalian cell and plants with the molecular weight range 20,000-60,000.

Candida antractica lipase B (CALB) is the atypical lipase from yeast called "Candida antarctica". The crystal structure of CALB appears to be in an open conformation with an accessible active site. It does not have a typical lid domain, but it has a short helix with high mobility, which might act as a lid.

Thermomyces lanuginosa lipase is obtained from fungus called "*Themomyces lanuginosus*". TLL is a typical lipase having a lid domain around 15 amino acids long.

This lid will open upon contact of the lipase with an interface and thus lead to restructuring of the lipase

2.2 Lipase-Catalyzed Reaction

The catalytic reactions of lipase normally occur at the interface between hydrophobic and hydrophilic regions, which are necessary for lipases to exhibit their activity due to their existence of hydrophobic lid in their structure, which cover the catalytic site.

2.2.1 Lipase-Catalyzed Esterification and Hydrolysis

Lipase usually use for transformation of water insoluble substrate not only in hydrolysis of triglycerides to glycerol and free fatty acid but also used in esterification reaction in low water-content-media (Stamatis *et al.*, 1993). In Figure 2.1 showed two examples of esterification reaction.

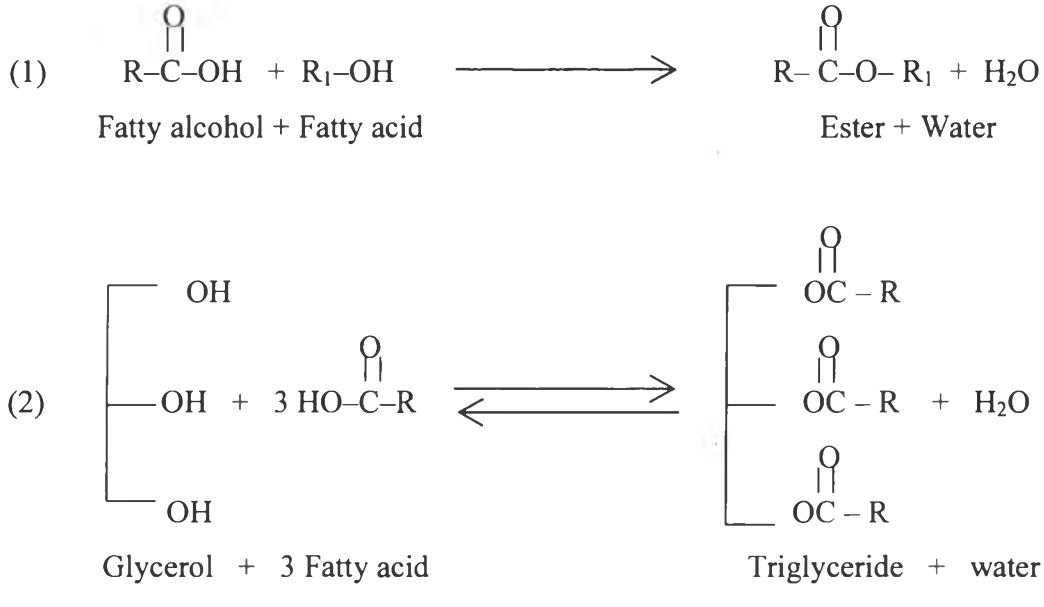

Figure 2.1 Two examples of esterification reactions with lipase
(1) fatty acid-alcohol esterification (2) glyceride synthesis

For glyceride synthesis, reaction media must have low water content to hinder the reverse reaction: hydrolysis, from occurring. Because of this reason, the most of investigation of esterification have been focused on reverse micellar system.

In 1996, Manoj and Swaminathan studied on the hydrolysis of fatty acyl-alcohol ester by using four fungal lipases in the reverse micellar system.

Salis *et al.* (2002) evaluated the catalytic activity of *Candida antarctica* lipase B (CALB) and *Thermomyces lanuginosa* lipase (TLL) in the hydrolysis of tributyrin in oil-in-water microemulsion (o/w emulsions). They reported that CALB has higher efficient in tributyrin hydrolysis than TLL due to the difference in the structure of these two lipases.

2.2.2 Lipase-Catalyzed Tranesterification

Transesterification is the reaction used to exchange of carboxyl group between ester (i.e., replacement of one acyl group in triglyceride). This is a commercial reaction to produce biodiesel or methyl ester, as shown in Figure 2.2.

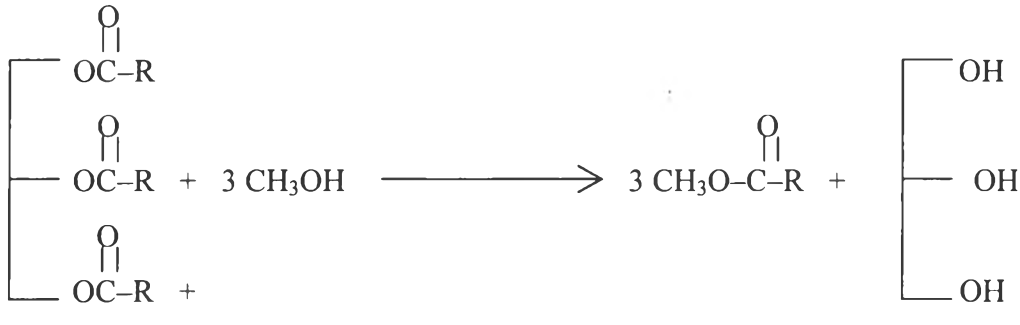

Figure 2.2 The production of biodiesel or methyl ester by tranesterification.

The majority of metyl ester in present day is produced by lipase-catalyzed transesterification because this is the most economic reaction in several reasons such as operating at low pressure and temperature, high conversion and direct conversion to metyl ester with on intermediate steps.

2.2.3 Lipase-Catalyzed Interesteriofication

The lipase-catalyzed reaction allows production of various triglycerides by a process called interesterification. Enzyme acts as acidolyases (carboxyl group exchange between esters and alcohols). Cocoa butter production, a commercial process to produce high value triglycerides from more plentiful and cheaper raw material, is the outstanding example as shown in Figure 2.3

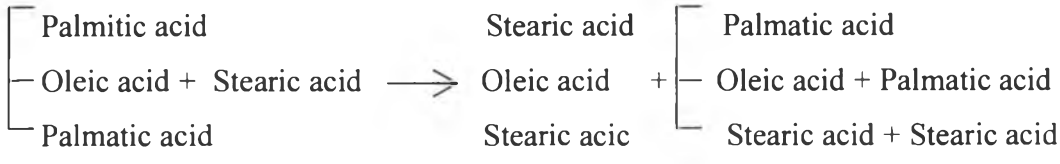

Figure 2.3 Conversion of palm oil into cocoa butter fat.

2.3 Microemulsion and Microemulsion-based Biocatalysis

Microemulsion is transparent dispersions containing two immiscible liquids with particles of 10-100 nm diameters and these dispersed phases are stabilized by an interfacial film of surfactant. Microemulsions may be water-external (O/W) or oil-external (W/O), or both (Rosen, 1989).

There have been widely interested in microemulsion over the past decade because of their unique properties, such as ultra low interfacial tension, large interface area and high solubility capacity for both oil- and water-soluble compounds.

2.3.1 Formation of Microemulsion

Microemulsions are generally prepared with more than one surfactant or with a mixture of surfactant and a co-surfactant (e.g., a polar compound of intermediate chain length). Microemulsions are thermodynamically stable because the interfacial tension between oil and water is low enough to be compensated by the entropy of dispersion. Surfactants with well-balance hydrophile-lipophile (H-L) properties have the ability to reduce the interfacial tension to the value required for

microemulsion formation. In the system that containing surfactants with unbalanced H-L properties that unable to reduce the oil-water interfacial tension to value lower than about 1 mN/m is often require co-surfactant to form microemulsion (Solans *et al.*, 1997).

2.3.2 Type of Microemulsion

There are three types of microemulsion discrete by the solubilization capacity of water relative to oil: 1) O/W microemulsion (oil droplets dispersed in water), 2) W/O microemulsion or reverse micelle (water disperse in oil) and 3) bicontinuous structure. To modify these three types can do by changing the temperature of the system and adding a co-surfactant or electrolyte.

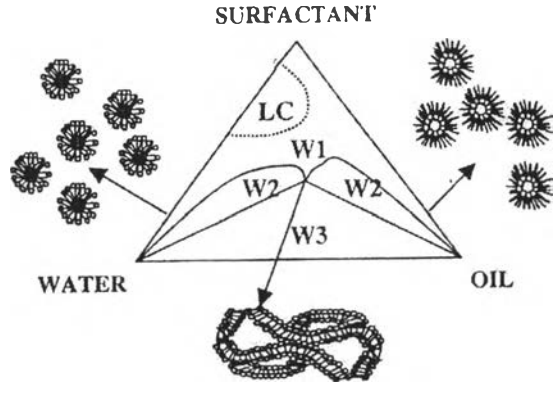

Figure 3.1 Schematic of ternary phase diagram for typical water/nonionic surfactant / oil system at HBL temperature. Microemulsion structure is shown in the normal regions of occurrence; left to right: O/W globular microemulsion (L1), bicontinuous microemulsion (L3) and W/O microemulsion (L2).

2.3.3 Encapsulation of Enzyme in Reverse Micelle

Water in oil microemulsions or reverse micelles are the nanometer-sized water droplets dispersed in organic media by action of surfactant, amphiphilic molecules arrange themselves in such a way that their hydrophobic part are in contact with the bulk solution of organic solvent, while the polar (hydrophilic) head groups surround the water core, water pool. In these water regions, enzyme

molecule can be entrapped, avoiding direct contact with the unfavorable organic medium and retaining their catalytic activity. The substrates are solubilized in the aqueous or organic phase, depending on their nature, undergo an enzymatic conversion and products diffuse to the phase corresponding to their polarity. Using microemulsion to encapsulated enzyme provides not only low water environment to maintain high activity of lipase but also large interfacial area of water and oil catalyze esterification reactions.

2.3.4 Lipase-Catalyzed Esterifications in Reverse Micelles

Lipase-catalyzed esterification in reverse micelles had been the important investigation topic for past decade. In 1989, Hayes and Gulari studied the activity of lipase from *Canadida cylindracea* and *Rhizopus delemar* in water/AOT (sodium bis(2-ethyhexyl)sulfosuccinate)/isooctane reverse micellar media. They compared the specific activity between lipase in reverse micellar media to the same reaction in O/W emulsion system. The result showed that the specific activity of lipase to fatty acid esterification in both media is same. Thus the specific activity of enzyme is not altered by encapsulation in reverse micelle. Lipase unlike other enzymes such as crymotrypsin because has not exhibited catalytic enhancement in reverse micellar systems (Freeman *et al.*, 2000). They also found that the type and concentration of the substrates and products can used for dictating the structure of the media or diameter of micelle (Rh) and the water/AOT ratio (*Wo)* has a strong effect on enzyme activity as dictated. In addition, the activity of enzyme also depends on pH and temperature of the system. Moreover, the studied also focusing on the effect of water content (defined by the *Wo* parameter) on lipase activity. It is commonly found that the rate of reaction as a function of W_0 gives rise to a bell-shape profile with a maximum in rate at *Wo* 5-10. The role of enzyme localization was also studied; lipases show a remarkable selectivity regarding the chain length and the structure of the substrates. This selectivity appears to be related to the micellar microstructure due to hydrophobic/hydrophilic character of protein (Stamatis *et al.*, 1993). Moreover, Stamatis and his group (1995) investigated the lipase kinetics behavior in fatty acid-alcohol esterification and suggested that the kinetic of this synthesis follow a Ping-Pong Bi-Bi mechanism.

Although lipase-catalyzed reaction in organic media (e.g., isooctane) has been an area of major research activity, supercritical fluid and liquefied gas have also been used. The esterification by lipase has been analyzed in reverse micelle of all anionic, AOT, (Sebastião *et al.*, 1993), cationic, CTAB, (Chaudhuri *et al.*, 1999) and nonionic, Marlipal 013-60, (Orlich *et al.*, 2001) surfactant. NaDEHP or sodium bis(2-ethyhexyl) phosphate, another anionic surfactant, has also been used. For example, in 2001, Zhou and his group studied the spectroscopic characterization of solubilized water in water /NaDEHP/n-heptane microemulsion. NaDEHP reverse micelles also have been used for protein extraction (Hu and Gulari, 1996). Recently, Anukunprasert (2001) investigated the esterification by *R. delemar* lipase encapsulated in NaDEHP reverse micelle.

To date there are the number of reactions catalyzed by lipase in reverse micelles (Cavalho and Cabral, 2000). Many studies aim to improve the activity and stability of lipase such as the study conducted by Freeman and his group (2000); bile salt co-surfactants generally increase the reaction rate and stability of the enzyme. Recently, it has been shown that the enzymatic activity for esterification is affected by a choice of hydrocarbon/water interface and a type of lipase (Maruyama *et al.*, 2001).