CHAPTER I

INTRODUCTION

1.1 Motivation

According to the diminishing petroleum reserves and environmental consequences of exhaust gases from petroleum-fueled engines, biodiesel becomes more attractive and promising alternative energy sources. Biodiesel has proved to be eco-friendly far more than fossil fuels because biodiesel is non-toxic, biodegradable, and excellent replacement for petroleum diesel (Noureddini et al., 2004). The most popular method of producing biodiesel is the transesterification of vegetable oils or animal fat with short chain alcohol. The transesterification process can be done in a number of ways such as using a homogeneous base or acid catalyst. Alkaline acid, sodium hydroxide or potassium hydroxide are used as a catalyst along with methanol or ethanol. This process is the most efficient reaction rate is reasonably high even at low temperature of 60 °C (Ranganathan et al., 2007). Nevertheless, this process causes a reaction change to saponification, which produces soap that reduces the catalytic efficiency and causes difficulty in separation of glycerol (Ma et al., 1998). The alternative is the acid catalyzed process using acid catalyst such as sulfuric acid and sulfonic acid. Although yield is high and soap is eliminated, the acids, being corrosive, may cause damage to the equipment and the reaction rate is also observed to be low (Freedman et al., 1984). Therefore to replace homogeneous catalysts, many different heterogeneous catalysts have been developed to catalyze the trans-esterification of vegetable oils with methanol (Xie et al.,2005). Suppes et al. achieved conversion of 78% at 513 K and > 95% at 533 K for transesterification of vegetable oils using calcium carbonate as catalyst. Alternatively, transesterification using alcohol in the supercritical state at temperature between 350 – 400°C and pressure of 45 – 65 MPa can be carried out even without any catalysts (Scott et al., 1972). However, the supercritical reaction requires high temperature and pressure resulting in high production cost.

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(Fukuda et al., 2001). Enzymatic transesterification of triglycerides offers an environmentally more attractive option to the conventional process. However, the high cost of enzymes often makes the enzymatic processes economically unattractive. The key step in enzymatic processes lies in immobilization of the enzyme, which allows for its recovery and reuse (Balcao et al., 1996). One of the most widely methods to immobilize enzyme is adsorption onto a solid support that remains the most simple and cost-effective method. Ghamgui et al., (2004) reported that, $CaCO_3$ was one of the most suitable adsorbents preserving the catalytic activity almost intact and offering maximum adsorption capacity, the yield more than 95% during 30 min with the enzyme loading of 2570 IU/g support, the conversion yield more than 95% was achieved after 24 h of incubation at 50 °C, the immobilized maintained 67% of its initial activity, while the free enzyme was completely inactivated. However, in stirred tank reactor, leaching of enzyme from mesoporous support is almost unavoidable. It would be worthwhile to study the effect of encapsulation on this pre-immobilized biocatalyst. Encapsulation in calcium alginate matrix offers a shield against the shear involved in the stirred reactor and also allows easy passage of reactant and products through the matrix (Yadav et al., 2005). Ganapati et al., (2005) has proposed that the pre-immobilization of *Candida antarctica* lipase B (CAL B) on hexagonal mesoporous silica followed by entrapment in calcium alginate beads was found to be the most active for transesterification reaction. This study exploits this idea with the pre-immobilization of lipase on heterogeneous base by adsorption on $CaCO_3$ followed by entrapment in calcium alginate beads to give an efficient reusable biocatalyst. Furthermore, the suitable condition for production of biodiesel by using this immobilized lipase is investigated.

1.2 Objectives

1. To develop $CaCO_3$ doped alginate gel for lipase immobilization.
2. To investigate the important parameters for ethyl ester production by immobilized lipase in $CaCO_3$ doped alginate gel.

1.3 Working Scope

In this study, *Candida rugosa* lipase was used as a biocatalyst. The enzyme was adsorbed on $CaCO_3$ followed by entrapment in calcium alginate gel. Then, the immobilized enzyme was used for ethyl ester production from palm oil and palm fatty acid in batch system. The performance index was included enzyme activity, ethyl ester yield and stability. The working scopes are as follows:

1.3.1 The study of the controlled parameters for *C. rugosa* lipase immobilization;

- The effect of lipase quantity: 1%, 2%, 3%, 5% and 10% (based on weight of oil)
- The effect of adsorption time: 30, 60, 90, 120, 150 and 180 minutes
- The effect of enzyme/$CaCO_3$ ratio
- The effect of bead diameter: 1.7 mm, 2 mm, 4 mm
- The effect of Na-alginate concentration: 1.0%, 1.5% and 2.0% w/v
- The effect of temperature: 37 °C, 45 °C, 50 °C and 60 °C

1.3.2 The study of the operating condition for enzymatic ethyl ester production;

- The effect of shaking speed: 150, 200, 250 and 300 rpm
- The effect of molar ratio of ethanol to reactants: 3:1-15:1 for palm oil to ethanol and 1:1-1:9 for palm fatty acid to ethanol
- The effect of percentage of mass ratio of substrate mixture (palm oil:palm fatty acid): 90:10, 80:20, 70:30, 50:50
- The effect of addition time of palm fatty acid: 6, 12, 24 h
- The ethyl ester production by using immobilized *C. rugosa* lipase (CRL);

  CRLA = *C. rugosa* lipase adsorbed on calcium carbonate

  CRLE = *C. rugosa* lipase entrapped in Ca-alginate matrix

  CRLAE = *C. rugosa* lipase adsorbed on calcium carbonate following By entrapment in Ca-alginate matrix
- Repeated use of the immobilized *C. rugosa* lipase

1.4 Expected benefits

1. Invention of novel lipase immobilization carrier for ethyl ester production.
2. Useful information for a better understanding of immobilized enzyme technology.