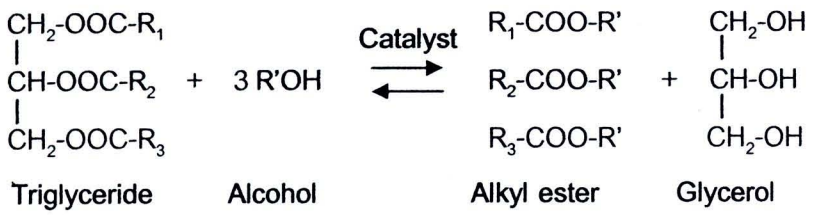
CHAPTER I

INTRODUCTION

1.1 Statement of purpose

The growth in consumption of petroleum oil throughout the world has caused urgent economic, security, and environmental problems. One of the best ways to reduce our dependence on petroleum oil is to develop renewable fuels such as biodiesel (Guan *et al.*, 2008). Biodiesel is a natural substitute of diesel fuel that comes from renewable sources (Rosa *et al.*, 2009), that can be produced from a range of organic feedstock including fresh or waste vegetable oils, animal fats, and oilseed plants (Jeong *et al.*, 2009 and Patil and Deng, 2009). Biodiesel has significantly lower emissions than petroleum-based diesel when it is burned, whether used in its pure form or blended with petroleum diesel. It does not contribute to a net rise in the level of carbon dioxide in the atmosphere and leads to minimize the intensity of greenhouse effect (Antolín *et al.*, 2002 and Vicente *et al.*, 2004). In addition, biodiesel is better than diesel fuel in terms of sulfur content, flash point, aromatic content and biodegradability. Vegetable oils are becoming promising alternative to diesel fuel because they are renewable in nature and can be produced locally and environmental friendly as well. Many researchers have been searching for cheaper plant oils to be used as alternative feedstock for biodiesel production. Few sources have been identified such as waste cooking oil (Wang *et al.*, 2006) and oils from non-edible oil-producing plants such as physic nut (Modi *et al.*, 2007 and Berchmans and Hirata, 2008), cotton seed (Kose *et al.*, 2002, Royon *et al.*, 2007 and Demirbas A., 2008), rubber seeds (Ramadhas *et al.*, 2005) and pumpkin (Schinas *et al.*, 2009).

The general method to produce biodiesel fuel is by transesterification of vegetable oil with methanol in the presence of either alkaline or strong acid catalysts. The transesterification reaction can be represented as



The production of biodiesel by transesterification process employing alkali catalyst has been industrially accepted for its high conversion and reaction rate (Lu *et al.*, 2007 and Shao *et al.*, 2008). However, the reaction has several drawbacks. It is energy intensive and recovery of glycerol is difficult. The acidic or alkaline catalyst has to be removed from the product, alkaline wastewater requires treatment (Zeng *et al.*, 2006), and free fatty acid and water interfere with the reaction. Recently, enzymatic transesterification has attracted much attention for biodiesel production as it produces high purity product and enables easy separation from the byproduct, glycerol (Ranganathan *et al.*, 2007 and Dizge and Keskinler., 2009)

Lipases, known as glycerol ester hydrolases (EC. 3.1.1.3), is one of the most extensively used enzymes that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids (Chang *et al.*, 2008 and Lei *et al.*, 2009). Depending on the nature of substrate and reaction conditions, lipases can catalyze a wide range of enantio- and regioselective reactions such as hydrolysis, esterifications, transesterifications, aminolysis and ammoniolysis (Deng *et al.*, 2005, Chang *et al.*, 2008, Dizge *et al.*, 2008 and Lei *et al.*, 2009). Lipases are widely spread in plants, animals and microorganisms (Cihangir and Sarikaya, 2004 and Deng *et al.*, 2005). Microbial lipases are most interesting and more useful than lipase derived from plants or animals because of the greater and available varieties of catalytic activities, the possible high yields, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations, and rapid growth of microorganisms on inexpensive media (Ibrahim C.O., 2008 and Fang *et al.*, 2009). Lipases are produced by a widespread number of microorganisms, including bacteria such as *Bacillus* sp. *Pseudomonas* sp. fungi such as *Aspergillus* sp. *Rhizopus* sp. and yeast such as *Candida* sp. Among them, *Candida rugosa* lipase (CRL) is one of the most commonly used (Rahman

et al., 2005) enzyme in organic solvent owing to its high activity in hydrolysis, esterification, transesterification and aminolysis. Due to the wide variety of environmental conditions, lipases are often easily inactivated, difficult to be separated from the reaction system for reuse and high cost. Utilization of lipase as a catalyst for biodiesel production is a clean technology due to its non-toxic and environmental friendly nature. Byproduct glycerol, can be easily recovered without complex processing and free fatty acid contained in waste oils and fats can be completely converted to methyl ester (Mamoru *et al.*, 2001 and Kösa *et al.*, 2002) Only mild operating conditions are required compared with chemical method (Wang *et al.*, 2006). However, due to the wide variety of environmental conditions, lipases are often easily inactivated, difficult to be separated from the reaction system for reuse resulting in higher cost than alkaline or acidic catalysts. Consequently, the further industrial applications of lipases are limited. The synthetic utility of lipase can be greatly improved by immobilization which has become a widely used technique to overcome practical problem in the use of crude lipase (Lei *et al.*, 2009). Many methods have been developed for immobilization of enzymes onto supports. Physical methods, especially adsorption may have a higher commercial potential than other methods because adsorption is simple, less expensive, and high catalytic activity can still be retained (Gitlesen *et al.*, 1997, Xu *et al.*, 2006 and Chang *et al.*, 2007). From transesterification process, by product glycerol is hydrophilic and insoluble in the oil so it is easily adsorbed on to the surface of the immobilized lipase leading to negative effect on lipase activity and operational stability (Wang *et al.*, 2006). Therefore, one way to address this problem, the enzyme can be applied on hydrophobic carriers such as macroporous polymers by adsorption due to the special characteristics of lipases, for example, avoids product contamination and allows biocatalyst recovery, reuse and continuous operation, enzyme activity stability, thermal stability and operational lifetime of lipase can be enhanced (Yang *et al.*, 2006). One account of the relatively high surface hydrophobicity of lipase, simple adsorption of lipase on suitable hydrophobic support has been the more popular strategy over covalent conjugation methods (Petkar *et al.*, 2006). There are varieties of support materials such as chitosan (Amorim *et al.*, 2003, Feresti and Ferreira, 2007 and Ye *et al.*, 2007), silica (Blanco

et al., 2004 and Blanco *et al.*, 2007), $CaCO_3$ (Rosu *et al.*, 1998 and Ghamgui *et al.*, 2004) that can be used for lipase immobilization and greatly improved the stability of enzyme. They also showed great potential for the production of biodiesel with the 70.2% conversion rate remained after 19 consecutive batches of reusages (Yang *et al.*, 2006). When an immobilization of *Candida rugosa* lipase by adsorption on bentonite was studied (Yeşiloğlu Y, 2005), the half –life of the immobilized enzyme was about 45 min, whereas for the soluble free lipase was 17 min at 50°C. Moreover, previous studies have shown that many factors can affect the activity recovery and reusability of enzymes in immobilization process. Some of the most important factors are the properties of the enzyme molecule, concentration of enzyme, temperature, ionic strength, pH, choice of support, the selection of an immobilization strategy, water and solutes present (Cruz *et al.*, 2009).

Since the immobilization efficiency of the enzyme depends on various factors, this study focussed on the optimal conditions for immobilization of lipase from *Candida rugosa* on hydrophobic supports for the transesterication of palm oil with methanol. Subsequently, the optimal conditions (addition mode, molar ratio of oil:methanol, enzyme loading, water content, time and temperature) by the optimized immobilized lipases for the conversion to biodiesel were investigated.

1.2 Objectives of this research

The aim of this study was to investigate the optimal conditions for immobilization of lipase from *Candida rugosa* on hydrophobic support for the production of biodiesel.

1.3 Scopes of the investigation

1.3.1 To select the appropriate hydrophobic support for the immobilization

1.3.2 To determine the optimal conditions for the immobilization

1.3.3 To select the potential feedstocks from non-edible and waste plant oils to be used as the optimal substrates for the production of biodiesel by transesterification.

1.3.4 To determine the optimal conditions for transesterification catalyzed by obtained immobilized lipase for the production of biodiesel

1.3.5 To compare the yield of biodiesel from transesterification catalyzed by 2 commercial lipases, Novozyme435 and Lipozyme RMIM with the obtained immobilized *Candida rugosa* lipase

1.3.6 To determine the stability of immobilized lipase

1.4 Expected results

This research will provide potential feedstocks from non-edible and waste plant oils to be used as the optimal substrates for the production of biodiesel by transesterification. The present work also offers the type of hydrophobic support suitable for the optimal immobilization of lipase from *Candida rugosa*. The yield of biodiesel from optimized transesterification will also be compared with the commercial lipases.

1.5 Thesis organization

This thesis consists of five chapters as follows: Chapter 1 is the introduction. Chapter 2 gives the theoretical and literature reviews. Chapter 3 comprises material and methods. The results can be found in Chapter 4 and the final chapter contains the discussion and conclusion.