# Influence of 1,4-Dioxane in Enzymatic Process for Biodiesel Production using Crude Palm Oil (CPO) as Substrate

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Nowadays, mass biodiesel production usually using chemical reaction method. However, since last decade there are many biodiesel researches using enzymatic method. In this enzymatic process, esterification and transesterification can happen concurrently. Besides, there are many advantages using enzymatic method compared to chemical method. As Indonesia is being on of the largest producers of Crude Palm Oil (CPO) in the world, producing renewable energy bases on plant oil is a huge potential for this country. In this report, we show the effort to increase the yield of biodiesel production using enzymatic process with CPO was raw material by using emulsifier. We have demonstrated biodiesel (FAME) production by enzymatic process using *Pseudomonas cepacia* lipase and Crude Palm Oil (CPO) as substrate. In this experiment, the yield of biodiesel was still very low. Introducing of 1,4-dioxane as emulsifier in producing biodiesel using CPO as substrate did not affected significantly on the yield of biodiesel.

Key words: CPO, *Pseudomonas cepacia*, enzymatic process, biodiesel

## Introduction

Nowadays, mass biodiesel production usually using chemical reaction method. However, since last decade there are many biodiesel researches using enzymatic method. In this enzymatic process, esterification and transesterification can happen concurrently. Besides, there are many advantages using enzymatic method compared to chemical method. Since the end this decade, Indonesia is being the largest producers of Crude Palm Oil (CPO) in the world. This is a huge potential for producing a renewable energy bases on plant oil. In this report, we show biodiesel production process using CPO and methanol as raw material catalysis by lipase from *Pseudomonas cepacia.*

## Materials and Methods

CPO as fatty acids source was obtained from PTPN XIII in Paser Regency, East Kalimantan Province, absolute methanol as alkyl source, sodium sulfate anhydrate, and hexane were obtained from Merck, *Pseudomonas cepacia* lipase as biocatalystaor was obtained from Sigma, kaolin as amobile enzyme carrier, alkyl esther for C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3, potassium dihidrogen phosphate and potassium hidrogen phosphate was obtained from Merck.

**FAME production**

Thirty (30) grams (33,9 mmol) CPO was mixed with methanol in molar ratio of 1:9 in the present of constructed amobile enzyme using kaolin as matrix, which was already incubated in phosphate buffer of pH 7.0. The mixture was incubated at 50 °C and the FAME was assayed at 4-10 h.

**Esterase activity assay**

FAME produced was assayed by GC using capillay coloum of Innowax dan FID. Sample of 1 mL was picked up from the mixture, centrifuged to separate between glycerol and methyl esther. Hundred µL of methyl esther phase was pippeted and added by sodium sulphate anhydrate and then 1.0 mL of hexane was added. The hexane phase of 1 µL then subjected to GC following vortexed and centrifuged (inlet temp. of 220 °C, detector temp. of 275 °C, and gradient program of coloumn temp. of 150 °C for 1.0 min, then increased to 240 °C by 15 °C per min, then increased to 260 °C by 5 oC per min and holded for 8 min.

## Results and Discussion

Previous study showed that Pseudomonas cepacia lipase having esterase activity using palm kernel oil (Abigor *et al.*, 2000), and we have also demonstrated that this lipase was also active on Crude Palm Oil (CPO) as substrate in producing biodiesel (FAME). In this production, the esterase activity still increased until molar ratio of CPO and methanol of 1:9.

Kaolin gave showed better as matrixes compared to celite in constructing immoblized lipase from *P.cepacia.* The methanolysis was optimum at 50 °C, which are very suitable for CPO. CPO has melting point between 31-41 °C, so that it become liquid at the process.

In this experiment, the yield of biodiesel was still very low, around 6.5 % (Figure 1). To overcome this problem, some emulsifier are being tried to optimize the enzyme activity as described by Iso *et al.* (2001), however even using of 1,4-dioxane as emulsifier could increase the yield to 100 %, it still could not be compared to chemichal production method, which can achieved a yie3ld of 80-90 %. Some other emulsifiers will be studied in this effort to increase the yield of FAME production using CPO as substrate, and other incubation method is still being developed to increase the yield of biodiesel, including isolating indigenous lipase bacteria from waste treatment of CPO manufactured, which has been being in progress.

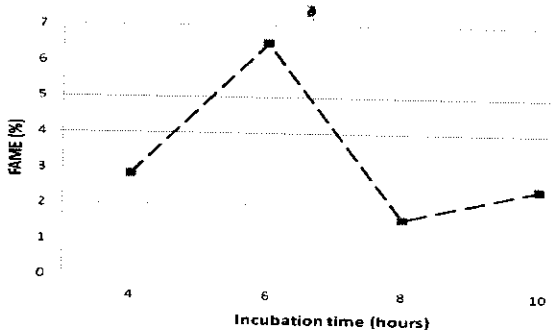


**Figure 1. Enzymatically production of FAME using CPO as substrate.** *Incubation temperature was at 50 °C*

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## Parallel Session 3
## Room A (Bidadari Room)

1. **Krishna Purnawan Chandra**
   Influence of 1,4-Dioxane in Enzymatic Process for Biodiesel Production Using Crude Palm Oil (CPO) as Substrate

2. **Rita Arbianti**
   Interesterification of Fried Palm Oil with Methyl Acetate using *Porcine pancreatic* Lipase to Produce Biodiesel

3. **Setijo Bismo**
   Synthesis and Analysis of Ozonated Methyl Ester from RBD and Waste Palm Oil for Diesel Fuel

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## Abstract

Nowadays, mass biodiesel production usually using chemical reaction method. However, since last decade there are many biodiesel researches using enzymatic method. In this enzymatic process, esterification and transesterification can happen concurrently. Besides, there are many advantages using enzymatic method compared to chemical method. Nowadays, Indonesia is being the largest producers of Crude Palm Oil (CPO) in the world after Malaysia. This is a huge potential for producing a renewable energy bases on plant oil. In this report, we show the effort to increase the yield of biodiesel production using enzymatic process with CPO was raw material by using emulsifier. We have demonstrated biodiesel (FAME) production by enzymatic process using *Pseudomonas cepacia* lipase and CPO as substrate. In this experiment, the yield of biodiesel was still very low. To overcome this problem, we introduced 1,4-dioxane as emulsifier to optimize the catalytic process of the enzyme as described in previous report. However, the addition of 1,4-dioxane gave no influence in the producton of biodiesel using CPO as substrate. A better technique is still being developed to increase the yield of biodiesel, including isolating indigenous lipase bacteria from waste treatment of CPO manufactured.

**Keywords:** CPO, *Pseudomonas cepacia*, enzymatic process, biodiesel



# Interesterification of Fried Palm Oil with Methyl Acetate Using *Porcine pancreatic* Lipase to Produce Biodiesel

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## Abstract

A new method on biodiesel synthesis via non-alcohol route with biocatalyst was developed. This method replaces conventional method using alkali catalyst by replacing alcohol with alkyl acetate as alkyl acceptor in the reaction. Interesterification of triglyceride from used cooking oil with alkyl acetate can produce biodiesel. Alcohol substitution with alkyl acetate may enhance the biocatalyst stability during reaction process significantly. The application of waste as triglyceride sources expected to enhance the economic feasibility of biodiesel synthesis using biocatalyst. Methyl acetate was reacted with triglyceride from fried palm oil using *Porcine pancreatic* lipase in batch reactor. The reactants and products were analyzed using HPLC. The effect of operating factors such as enzyme concentration, subastrates ratio, operating temperature and addition of inhibitor using free and immobilized enzyme were investigated. The biodiesel yield increased and tends to be constants after 50 h reaction. The results showed that 62.78 and 53.26% of triglyceride was converted to its corresponding methyl esters under the condition of 4% wt lipase based on substrate weight, 1/12 mol rasio of oil/methyl acetate using free and immobilized lipase. A large excess of methyl acetate was required to shift the interesterification reaction in forward direction. The effect of reaction temperatures from 25 to 50 °C on biodiesel yield were investigated. The highest yield of biodiesel was obtained at the temperature of 37 °C. In order to investigate the effect of inhibition, 1% palmitic acid (% wt/wt of oil) was added into the substrate fried palm oil using free and immobilized lipase.These results showed that free fatty acid inhibited the interesterification reaction. Biodiesel yield for both of free and immobilized *Porcine pancreatic* lipase was 23.97 and 20.76%. Stability test of the immobilized *Porcine pancreatic* lipase indicated that the activity of the immobilized lipase still remained after three reaction cycles.

**Keywords:** interesterification, fried palm oil, *Porcine pancreatic*, biodiesel

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