# Biodiesel Production from Crude Palm Oil under Different Free Fatty Acid Content using Eversa® Transform 2.0 Enzyme

Obie Farobie*‡, Qatrinada Radiyatul Jannah**, Edy Hartulistiyoso*

*Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University (IPB University), PO Box 220, 16002 Bogor, Indonesia

**Surfactant and Bioenergy Research Center, Bogor Agricultural University, Jalan Pajajaran No. 1, Kampus IPB Baranangsiang, 16144 Bogor, Indonesia

(obiefarobie@apps.ipb.ac.id, qatrinada2603@gmail.com, edyhartulistiyoso@apps.ipb.ac.id)

‡Corresponding Author; Obie Farobie, Department of Mechanical and Biosystem Engineering, IPB Darmaga Campus, PO Box 220, 16002 Bogor, Indonesia, Tel: +62 812 898 11381, obiefarobie@apps.ac.id

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**Abstract-** Low-cost yet viable feedstock, i.e., crude palm oil (CPO) is one of the potential sources to be utilized in the biodiesel industry. However, CPO mainly contains a high free fatty acid (FFA) content. One of the promising methods to treat feedstock containing high FFA such as CPO is an enzyme-catalyzed transesterification owing to no sensitivity to the presence of FFA. Hence, this study aims to investigate biodiesel production from CPO under different FFA content using enzyme-catalyzed transesterification. Eversa® Transform 2.0 lipase which is a low-cost enzyme was used as a liquid enzyme. Experiments were conducted by changing the temperature (30–60 °C), the methanol-to-CPO molar ratio (3:1–8:1), and enzyme concentration (0.1–0.4 wt%). Intriguingly, the higher the FFA content of CPO, the higher the biodiesel yield. The result showed that biodiesel yield as high as 97.91% could be attained at a milder temperature of 40 °C, CPO with FFA content of 19.33%, and a methanol-to-CPO molar ratio of 7:1.

**Keywords** Biodiesel; CPO; enzyme; Eversa; transesterification.

## 1. Introduction

Nowadays, the world is facing challenges due to global warming and environmental damages as a result of fossil fuel burning. This issue has encouraged worldwide researchers to search for renewable and sustainable energy [1]-[5]. Biodiesel is considered alternative renewable energy to replace fossil fuels since it helps to reduce environmental problems due to greenhouse gas emissions. Moreover, biodiesel has superiority in terms of lower particulate matter, carbon monoxide (CO) emission, aromatic and sulfuric content, as well as better biodegradability over petroleum-based fuel [6]–[9].

To make biodiesel production more sustainable, the selection of appropriate feedstock is important. There are several feedstocks predominantly utilized for the production of biodiesel worldwide including soybean, rapeseed, and palm oil. Compared to the other terrestrial plants, palm oil has the highest yield of oil concerning oil production per hectare of plantation. Nowadays, Indonesia is the largest palm oil producer in the world with the production capacity reaching 47 million tons in 2020 [10]. However, biodiesel production in Indonesia generally uses refined palm oil which is more expensive than crude palm oil. It is because biodiesel production from CPO is challenging due to its high FFA contents and other impurities. Therefore, the appropriate approach to generate biodiesel from CPO is currently being sought to maintain the sustainability of biodiesel.

There are many studies on biodiesel production from CPO via either acid- or alkali-catalyzed esterification-transesterification processes. The acid-catalyzed biodiesel production (with HCl or $H_2SO_4$) has drawbacks due to the need for a long reaction time and the corrosion in the reactor [11], [12]. Meanwhile, the alkali-catalyzed transesterification (with KOH or NaOH) remains the major drawback including the sensitivity to the presence of FFA and generation of wastewater after the washing step [13], [14]. Hence, it

prolongs the biodiesel production process, leading to further complications and increasing the biodiesel production cost.

Besides chemical-catalyzed esterification and transesterification processes, biodiesel production from CPO can also be generated using biocatalyst through an enzyme-catalyzed process. The green process of biodiesel using enzyme has superiority over the chemical-catalyzed biodiesel production owing to low energy consumption, mild reaction condition, and tolerance to the FFA and water [14]–[18]. Moreover, the generated glycerol as a co-product of biodiesel is of high purity [19]–[23]. Nevertheless, biodiesel production using the enzymatic process has not been widely used in industrial applications due to the overpriced lipase. Several efforts have been evolved to reduce the cost by immobilizing the enzyme on supporting materials to improve the enzyme's reusability and stability [24]–[29]. However, the immobilized enzymes also still have drawbacks since the support materials can cause mass transfer limitation, thus, decreasing the reaction rate [30], [31]. To circumvent the above issue, the low-cost enzyme with the commercial name of Eversa® Transform 2.0 for the production of biodiesel is interesting to be investigated. Fraga et al. reported that Eversa® Transform 2.0 exhibited excellent catalytic performance in terms of high activity and specificity [32]. Nevertheless, to the best of the authors' knowledge, the catalytic performance of a liquid Eversa® Transform 2.0 on biodiesel production from real CPO under different FFA content has not been studied well. Hence, this study aims to examine the production of biodiesel from CPO under different FFA contents using a liquid Eversa® Transform 2.0 lipase. The catalytic performance of Eversa® Transform 2.0 was assessed by considering the effect of reaction conditions including reaction time, concentration of enzyme, temperature, and methanol-to-CPO molar ratio.

## 2. Materials and Methods

### *2.1. Experimental*

Production of biodiesel via enzymatic process was performed in a 500-mL batch-mode reactor submerged in a water bath shaker (Baths WSB Shaking water bath, Germany). Figure 1 shows the experimental apparatus of biodiesel production using an enzymatic process. The enzymatic process to produce biodiesel from CPO was investigated in the temperature range of 30–60 °C, methanol-to-CPO molar ratios of 3:1–8:1, and catalyst loading of 0.1–0.4 wt.%.

Prior to the reaction process, the pH of the feedstock was adjusted in the range of 4.5-5.0 by dosing 16% NaOH as much as 20 ppm. The moisture content of the CPO was also adjusted by adding the distilled water to ensure a total moisture content of 2%. As much as 200 g of CPO was put inside the glass reactor. The esterification-transesterification process was carried out by dosing methanol using the dosing pump into the glass reactor. To prevent the inhibition of an enzyme, the flow rate of methanol was set constantly from the initial time until the 4 h reaction time. Please note that the flow rates of 9.15, 7.92, 5.72, 3.43 mL/h were used for methanol-to-CPO molar ratios of 3:1, 5:1, 7:1, and 8:1, respectively. At 4 h, the methanol dosages were changed to the lower flow rates of 1.41, 1.22, 0.88, and 0.53 mL/h for methanol-to-CPO molar ratios of 3:1, 5:1, 7:1, and 8:1, respectively. At 30 h, the methanol dosing was stopped but stirring was continued till 60 h. The collection of samples was carried out at 4, 24, 30, and 48 h reaction times to monitor the FFA and methyl ester contents. After 60 h reaction time, the reaction was stopped and the glass reactor containing samples was submerged in a water bath at 100 °C for 30 min to deactivate the enzyme. To separate the biodiesel from the glycerol fraction, the samples were centrifuged at 12,000 rpm within 5 min. The upper layer of the sample was subjected to FFA and methyl ester analyses.

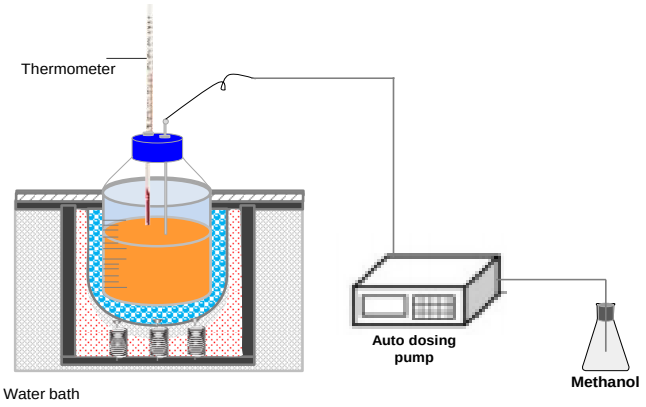


**Fig. 1.** Experimental apparatus for enzymatic biodiesel production from CPO.

### *2.2. Analytical Methods*

Production of biodiesel via enzymatic process was performed in a 500-mL batch-mode reactor submerged in water bath shaker (Baths WSB Shaking water bath, Germany). Figure 1 shows the experimental apparatus of biodiesel production using enzymatic process.

The properties of biodiesel, i.e., specific density, kinematic viscosity, and FFA were determined according to Indonesia National Standard for biodiesel called SNI 7182:2015. Meanwhile, the biodiesel yield was analyzed using a gas chromatograph (GC) (HP-6890 series) equipped with a flame-ionization detector and a MET-Biodiesel column (Sigma Aldrich, 28668-U), following ASTM D6584-17. Helium was employed as the carrier gas. The temperature program in the oven was initially set at 50 °C for 1 min. The temperature was then increased to 250 °C using a heating rate of 15 °C/min for 15 min. The concentrations of biodiesel were calculated using a calibration curve based on peak areas. The yields of biodiesel were determined by considering the moles of biodiesel product and moles of initial CPO as shown in the following formula.

$$(\text{Biodiesel yield}) = \frac{(\text{Molar amount of biodiesel product})}{(\text{Molar amount of initial CPO})} \quad (1)$$

### *2.3. Reagents and Materials*

All chemicals used in this study were used without further treatment. CPO feedstock with low FFA content (3.83) used in the experiments was kindly provided by PT. Dharma Satya Nusantara, Indonesia. Meanwhile, CPO with high FFA contents (6.74-19.33) was obtained from PT. Perkebunan Nusantara III, Bogor, Indonesia. The liquid lipase enzyme which is a genetically modified *Aspergillus oryzae* with the commercial name Eversa® Transform 2.0 was obtained from Novozymes, Malaysia. The analytical grade of methanol (99%), phenolphthalein, KOH, and oxalic acid dihydrate (Merck) was used. To prepare the GC standard solution, the standard compound of methyl palmitate (≥99%) was purchased from Sigma-Aldrich (Indonesia).

## 3. Results and Discussion

### *3.1. Effect of time*

Reaction time plays a vital role in enzymatic biodiesel production. The details of the reaction time effect on FFA content and biodiesel yield are presented in Figure 2. As observed, for all CPO investigated, the FFA content was dramatically decreased with a longer reaction time. It can be explained because Eversa® Transform 2.0 lipase has excellent catalytic activity and good solubility in CPO. The FFA content decreased from 3.83 to 2.27%, from 6.74 to 4.39%, from 8.97 to 3.80%, and from 19.33 to 3.23% with prolonging reaction time from 0 to 4 h for the CPO1, CPO2, CPO3, and CPO4, respectively.

Furthermore, biodiesel yield was found to increase with a longer residence time until the equilibrium was achieved. It is believed that the reaction time always depicts the reaction rate. As observed, the highest biodiesel yield was found at CPO4 which has an FFA content of 19.33% at 48 h reaction time, resulting in biodiesel yield as high as 97.91%. It is interesting to note that higher FFA content of CPO results in higher biodiesel yield. It could be attributed to the fact that FFA has higher reactivity than triglycerides.

### *3.2. Effect of temperature*

Besides reaction time, temperature also has a significant effect on biodiesel yield. To investigate the temperature effect, a series of experiments were conducted by ranging the reaction temperatures from 30 to 60 °C at a fixed reaction time of 48 h and a methanol-to-CPO molar ratio of 7:1. The effect of temperature on enzyme-catalyzed biodiesel production of CPO is presented in Figure 3. It can be noticed that the enzyme-catalyzed biodiesel production of CPO was remarkably affected by the reaction temperature.

As shown in Figure 3, the yield of biodiesel increased significantly as the temperature increased from 30 to 40 °C. The highest biodiesel yield of 97.91 was achieved at 40 °C within 48 h. This could be explained that as the temperature raised from 30 to 40 °C, the mass transfer limitation was decreased, generating the complete reaction process. Furthermore, the increasing temperature from 30 to 40 °C could also enhance the collision frequency between substrates and enzymes. However, biodiesel yield decreased from 87.91% to 76.3% by increasing the temperature from 40 to 50 °C for CPO1. Furthermore, as the temperature continues to increase gradually to 60 °C, biodiesel yield decreased dramatically to 29.30%. The same trend was observed for CPO2, CPO3, and CPO4. This could be attributed to the breakage of hydrogen bonding and weak ions of Eversa® Transform 2.0 lipase at high temperature (above 40 °C), leading to the thermal denaturation of lipase [15], [33]. This finding confirms that 40 °C is the optimum temperature for the production of biodiesel from CPO using Eversa® Transform 2.0 lipase. The optimum temperature of CPO catalyzed by Eversa® Transform 2.0 lipase was comparable with biodiesel production from palm oil using *Thermomyces lanuginosus* (45 °C) [34].

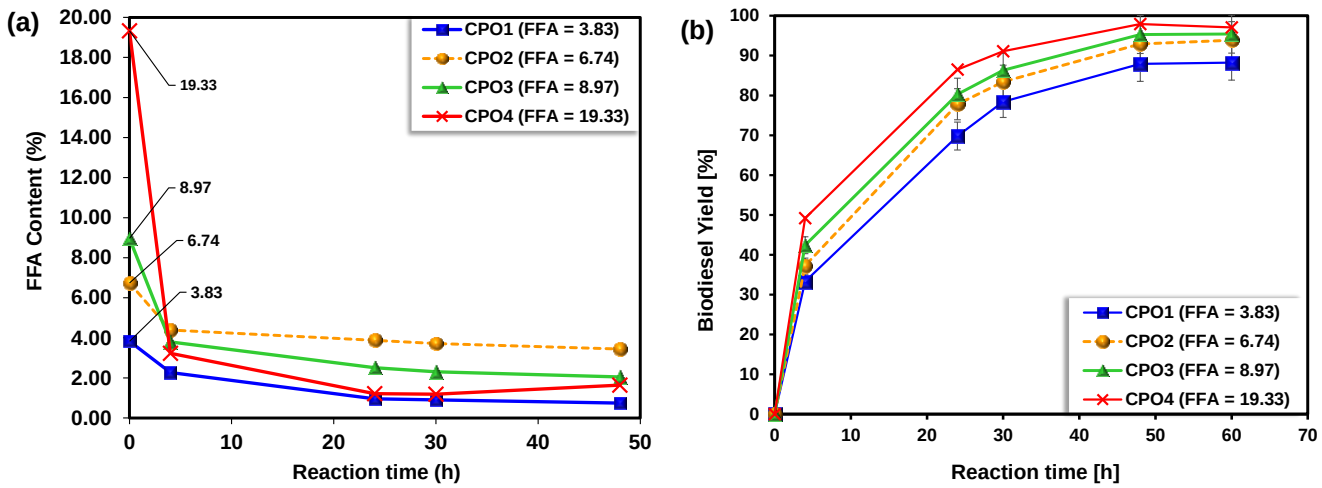


**Fig. 2**. Effect of reaction time on (a) FFA content and (b) biodiesel yield of CPO catalyzed by Eversa® Transform 2.0 lipase.

**Fig. 3.** Effect of temperature on biodiesel yield of CPO catalyzed by Eversa® Transform 2.0 lipase.

*3.3. Effect of enzyme concentration*

Next, the effect of enzyme loading was also investigated herein. The feedstock of CPO4 was employed to examine the effect of enzyme loading since it showed a significant yield of biodiesel. The effect of enzyme concentration on biodiesel yield of the enzymatic process is presented in Figure 4. As observed, the concentration of enzyme had a significant impact on the enzymatic catalysis of CPO. Overall, the yield of biodiesel raised from 59.61 to 97.91% with the increment of the enzyme loading from 0.1 to 0.3 wt.% for 48 h. This finding is in good agreement with the previous studies by Abdulla and Ravindra and Sun et al. [15], [35].

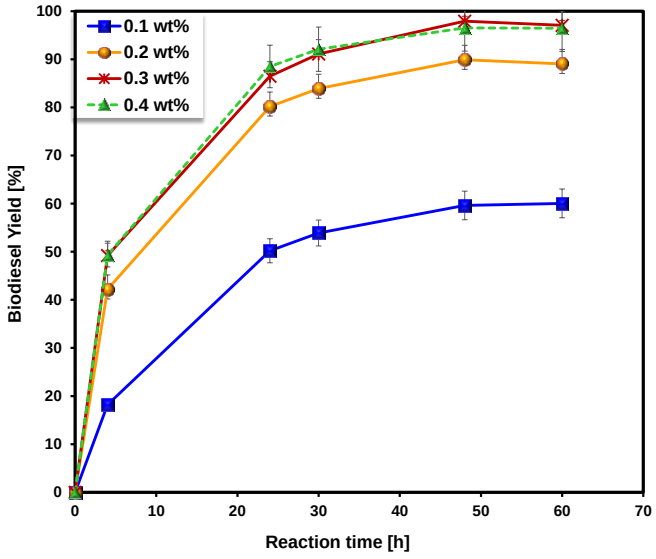


**Fig. 4.** Effect of enzyme concentration on biodiesel yield of CPO4 using Eversa® Transform 2.0 lipase.

They reported that increasing enzyme loading could enhance the arrangement of substrate-enzyme complex owing to the emergence of more active sites, enabling increasing biodiesel yield. However, as the enzyme loading was further raised from 0.3 to 0.4 wt.%, no significant increase in the biodiesel yield was observed, confirming that 0.3 wt.% is the optimum concentration for CPO catalyzed Eversa® Transform 2.0 lipase. This could be due to the hindrance of interaction between enzyme-substrate in the excess amount of Eversa® Transform 2.0 lipase.

*3.4. Effect of methanol-to-CPO molar ratio*

Finally, the effect of the reactant-to-CPO molar ratio was investigated. The methanol-to-CPO molar ratio is an important factor used to confirm the transformation of CPO to biodiesel. According to the stoichiometric, to move forward the reaction to the final product, an excess amount of methanol is required due to the reversible reaction. However, the use of a too high amount of methanol is not beneficial since it will increase the cost of biodiesel production. Hence, the effect of methanol-to-CPO molar ratio on biodiesel yield was examined by changing the molar ratio from 3:1 to 8:1 at a fixed temperature of 40 °C, enzyme concentration of 0.3 wt.%, and reaction time of 48 h, as shown in Figure 5.

**Fig. 5.** Effect of methanol-to-CPO molar ratio on biodiesel yield using Eversa® Transform 2.0 lipase.

As observed, biodiesel yield significantly increased as an increase in a methanol-to-CPO molar ratio from 3:1 to 7:1. The highest biodiesel yields were achieved at the methanol-to-CPO molar ratio of 7:1 for all CPO investigated, i.e., 87.91, 92.91, 95.30, and 97.91% for CPO1, CPO2, CPO3, and CPO4, respectively. However, increasing a methanol-to-CPO molar ratio gradually from 7:1 to 8:1 revealed an insignificant increase in biodiesel yield. This can be explained because the folding structure of the enzyme could be possibly altered since the excess amount of methanol (methanol-to-molar ratio > 7:1) in the reaction system could adhere to the enzyme and strip water from the surface of the enzyme, resulting in a decrease in the catalytic activity of the enzyme [36], [37]. Nevertheless, it is interesting to note that the methanol-to-CPO molar ratio in the biodiesel production process of CPO catalyzed Eversa® Transform 2.0 lipase was higher (7:1) than that of some other enzymes of lipozyme RMIM (3:1) and *Thermomyces*

*lanuginosus* lipase (4:1), confirming that Eversa® Transform 2.0 lipase exhibited superior resistance to the methanol [36], [38], [39].

### *3.5. Characteristics of Biodiesel from CPO*

The physicochemical properties of biodiesel produced from CPO using Eversa® Transform 2.0 was investigated. Table 1 shows the physicochemical properties of biodiesel from CPO in comparison with the Indonesian National Standard (SNI 7182:2015). All parameter of biodiesel from CPO met the standard of SNI 7180:2015. It is interesting to note that cetane number of the biodiesel from CPO has excellent property compared to the diesel fuel, confirming that biodiesel from CPO is applicable for diesel fuel substitute.

**Table 1**. The physicochemical properties of biodiesel from CPO

| **Properties** | **Biodiesel from CPO** | **SNI 7182:2015** |
|---|---|---|
| Ester content (% mass) | 97.9 | 96.5 (min) |
| Density at 15 °C (kg/L) | 0.88 | 0.85–0.89 |
| Kinematic viscosity at 40 °C ($mm^2/s$) | 4.3 | 2.3–6.0 |
| Flash point (°C) | 172 | 130 (min) |
| Cetane number | 60.3 | 51 (min) |
| Acid value (mg KOH/g) | 0.12 | 0.5 (max) |

## 4. Conclusion

Production of biodiesel from low-quality feedstock (CPO) catalyzed by the low-cost enzyme of Eversa® Transform 2.0 lipase was successfully investigated under different FFA contents for the first time. The enzymatic biodiesel production from CPO using Eversa® Transform 2.0 was significantly affected by reaction time, catalyst loading, temperature, and methanol-to-CPO molar ratio. The highest biodiesel yield of 97.91% was found in CPO containing high FFA content (CPO4, FFA content of 19.33%), confirming that enzymatic biodiesel production is insensitive to the FFA. The optimum yield of biodiesel could be attained at 40 °C, 0.3 wt.% enzyme loading, and the methanol-to-CPO molar ratio of 7:1 within 48 h reaction time. This finding confirms that enzyme-catalyzed transesterification using Eversa® Transform 2.0 Enzyme is a viable process to produce biodiesel from low-cost feedstock (CPO) containing high FFA content.

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