

**FINAL REPORT  
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اَوْبُو سَيِّدِي تَكْوَلُو كِي مَارَا  
UNIVERSITI  
TEKNOLOGI  
MARA

**ULTRASOUND-ASSISTED TRANSESTERIFICATION OF NON-EDIBLE OILS USING  
IMMOBILIZED LIPASE FOR BIODIESEL PRODUCTION**

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3. Research Cluster/ Theme : Energy/ New and Renewable Energy

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Using Immobilized Lipase for Biodiesel Production

### Abstract

Biodiesel is fatty acid methyl ester derived from vegetable oils and/or animal fats. Biodiesel is beneficial in terms of environmental aspect because of its biodegradable properties, renewability, and nontoxicity. Biodiesel can be produced by transesterification using alcohols. Compared to the inorganic-catalyzed process, transesterification via enzymatic catalysis is superior since it provides an advantage in high product purity, easy recovery, and green technology process. However, enzymatic catalysis is a time consuming process, where it is the slowest pathway in biodiesel production. The application of ultrasound device in enzymatic transesterification is helping in reducing the reaction time. In this work, free-cell lipase and immobilized lipase (Lypozyme from *Rhizomucor miehei*) was used as biocatalyst to enhance the transesterification reaction of non-edible oils to produce biodiesel. The reaction was performed in an ultrasound-enhanced stirred batch reactor. This research aims at evaluating the effects of main parameters of reaction, including the enzyme loading, time and temperature of reaction, and conducting the kinetic study of the reaction. Kinetics model of the enzymatic reaction will be carried out based on the ping-pong bi bi with reserved inhibition of methanol. Kinetics study is important for equipment design and scaling-up the process at industrial scale. This research is expected to provide the renewable energy using green and environmental friendly technology.

**Keyword:** Enzymatic, kinetic study, renewable energy, biodiesel, biocatalyst

## CHAPTER I. INTRODUCTION

Most of current industrial production of biodiesel from vegetable oils is achieved through transesterification (Aboelazayem et al., 2018; Demibas, 2006). Theoretically, in transesterification, to achieve complete conversion of one mol of triglycerides to alkyl esters; at least three moles of alcohol are required (Islam, 2014). In general, there are two types of catalyst that have been used industrially, namely chemical catalysts (such as alkali, acid and silica) and biocatalysts (i.e., lipase). The drawbacks of using chemical catalyst in biodiesel industry due to glycerol recovery and removal of inorganic salt in chemical transesterification, will not be found when using lipase as biocatalyst as stated by Surendhiran and Vijay (2013). Lipase is more environmental-friendly, able to esterify both acyglycerol-linked and free fatty acid (FFA) in one step, able to produce glycerol side stream with minimum water content and free of inorganic material. This enzyme is commercially available and it enables conversion under mild temperature, pressure and pH (Duarte, 2015).

There are several problems associated with the application of lipases as catalysts in transesterification. Most lipases are expensive and they can be inactivated by short-chain alcohols due to its immiscibility with oil and by glycerol adsorption onto the support of immobilized lipases. The key to these problems is using immobilized lipases, in transesterification with techniques of solvent and solvent-free systems (Costa-Silva, 2017; Yu et al., 2013). In a solvent system, organic solvents can be used as diluents to enhance the miscibility of alcohol and oil and as washing media for regeneration of lipase (Gusniah et al. 2019). For a solvent-free system, the inhibition by short chain alcohols can be overcome by lowering the concentration of alcohol through stepwise additions and replacing the short chain alcohols with other alternative acyl acceptors (Caetano et al., 2012).

The advantages of enzymatic transesterification are easy purification of the byproduct, a high level production, mild operating conditions, a high degree of purity of the product, and the ability to process the feedstock with FFA content. However, the enzyme is very expensive and has a longer reaction time. Thus, an immobilized enzyme has been used as a solution to the high purchase cost of the enzyme, and the enzyme can be recycled in the process. However, enzymatic catalysis is a time consuming process, where it is the slowest pathway in biodiesel production (Gusniah et al., 2019).

Hence, process intensification using advanced reactor technology is necessary to work continuously at high reaction rates, with fewer post reaction cleaning operations and less separation for product and reactant. The application of ultrasound device in enzymatic transesterification to help in reducing the reaction time is prospective. In this work, immobilized lipase (Lypozyme from *Rhizomucor miehei*) will be used as biocatalyst to enhance the transesterification reaction of non-edible oils (waste cooking oil, kapok seed oil and nyamplung seed oil) to produce biodiesel. The kinetics study will also be performed.

In this work, the reaction will be carried out in an ultrasound-enhanced stirred batch reactor. There were several work on the ultrasound technology application for biodiesel production. However, the research on the ultrasound-assisted enzymatic transesterification using

immobilized lipase from *Rhizomucor miehei* with the biodiesel local feedstocks of waste cooking oil and nyamplung seed oil (*Callophyllum inophyllum L.*) have never been reported.

Kinetics model of the enzymatic reaction will be carried out based on the ping-pong bi bi with reserved inhibition of methanol. It is important to comprehend the kinetic of enzymatic transesterification for ideal immobilized enzymatic reactor. Various models for describing the rate of enzymatic transesterification have been proposed. Nonetheless, most of researchers on enzyme kinetic in biodiesel synthesis concluded that the transesterification reaction of lipase follows the ping pong bi bi mechanism (Babaki et al., 2016). Though the solutions for drawbacks in lipase catalysis applications and the rate models of enzyme kinetic have been recognized, it is important to study the effect of inhibition and the kinetic of each lipase as each lipase behaves differently in different reactions. Hence, using this model the level of inhibition, the technique of the solvent system, and the stability of each lipase will be diverse.

Thus, this research offers significant **novelty** in the area of biodiesel synthesis, especially in terms of:

1. The application of ultrasound-assisted reactor of transesterification using immobilized lipase catalyst for local Indonesian feedstocks.
2. The kinetics study approach for enzymatic transesterification using the ping pong bi bi mechanism.

## CHAPTER II. LITERATURE REVIEW

### Previous Research

World is facing serious issue of limited supply of fossil energy leading to exploration into alternative renewable energy. Currently, harvesting electricity from renewable energy sources such as solar, wind and biomass have been proposed as the alternative technologies and commercially well-established in the latest years (Veney, 2014). Rapid depletion of fossil energy and the limited resources as well as un-sustainability of fossil energy are among major world concerns in modern life, especially in terms of transportation. In addition, the deteriorated atmospheric greenhouse effect and global warming due to burning fossil fuels becomes a huge obstacle of environmental sustainability (Abdullah, 2017). The depletion of petroleum energy sources will affect numerous aspects of the consumer life and economy, and hence concentrating on renewable alternative is now more substantial.

Generally, biofuels are renewable fuels produced from biomass and are categorized as biodiesel, bioethanol, biogas and biohydrogen which are currently studied extensively as renewable energy resources. To date, biodiesel has been accepted as a promising fuel because of being highly biodegradable and containing low toxicity to be replaced by diesel fuel in devices like boilers and internal combustion engines with a low decrease in performances and no huge alteration (Ondul et al., 2015). Biodiesel can also be blended with the conventional petroleum diesel fuel in order to reduce CO<sub>2</sub> emissions problems. Furthermore, biodiesel is estimated to be 66% more efficient as a lubricant in diesel engines compared with petro-diesel. Biodiesel contains properties like diesel fuel leading to make it a suitable alternative to diesel fuel. The overall strategy of generation of hydrocarbon fuels from renewable resources is to decrease the oxygen content of the feedstock to energy density and to create CAC bonds between biomass-derived intermediates, and to increase the molecular weight of the produced hydrocarbons. Hence, the physicochemical properties and qualities of biodiesel produced from any resource and through any chemical reaction must follow international specification standards such as European (Issariyakul, 2014).

Union Standards for Biodiesel (EN 14214) and the American Standards for Testing Materials (ASTM 6751-3). Technically, biodiesel is described as fatty acids monoesters derived from long chain renewable sources including vegetable oils and animal fats. It consists of a mixture of esters chiefly produced through transesterification reaction of triglycerides by short chain alcohols such as methanol and ethanol while the main by-product is glycerol (Dermibas, 2006). Vegetable oils are not used directly as fuels in diesel engines, particularly due to their high viscosity, Fatty Acid (FA) composition and free fatty acids (FFAs) content. To overcome this issue, conversion of FA and FFA to their respective Fatty Acid Alkyl Esters (FAAE) is widely investigated. Transesterification of vegetable oils has been broadly known and applied since the 19th century (Veney et al., 2014). This reaction is a simple, prominent method which proceeds well in the presence of a catalyst. The reaction is carried or by either catalytic or non-catalytic or methods. In supercritical conditions, alcohol is both a reactant and an acid catalyst (Aboelazayem et al., 2018). For non-catalytic reactions it is very rapid by supercritical fluids compared with the

conventional catalytic biodiesel production. In the production of biodiesel, any triglyceride of the feedstock is transesterified where the feedstocks are mainly bearing crops, animal fats, and algal lipids and the alcohol is methanol or ethanol (Dermibas, 2006).

The choice of catalyst is critical in order to design an efficient process capable of maximizing the value of starting materials while minimizing waste generation and energy. So far, a broad range of catalysts has been used to catalyze biodiesel production reactions including acids and bases, sugars, ion exchange resins, zeolites, enzymes (lipases) and other materials (Abdullah, 2017). Three types of most applied and common catalysts are homogenous chemical catalysts, heterogeneous solid catalysts and biocatalysts. NaOH and KOH, carbonates, and the corresponding sodium and potassium alkoxides are the major chemical catalysts used in the reaction. In general, transesterification via chemical catalysts results in shorter reaction times with higher yield production. However, there are some drawbacks to the use of homogeneous catalysts in the biodiesel production.

Currently, there is a favorable trend of the use of heterogeneous catalysts, especially biocatalysts to reduce problems associated with chemical homogeneous catalyzed reaction (Mehrasbi, 2017). Lipases are isolated from several biological resources which usually go through immobilization process prior to the application. The use of microorganism as lipase resources is advantageous since most of them are capable of producing enzyme at high production rate and relatively low cost. Currently, Novozym 435, which is isolated and purified from fungal resources of *Candida Antarctica* and *Rhizopus oryzae*, is the most common lipase currently employed to catalyze enzymatic transesterification with really high efficiency (above 90%). However, commercial lipases are limited to their cost. Moreover, compared to chemical catalysis, lipase requires longer reaction time for transesterification. It may also get inactivated by methanol and glycerol during transesterification (Yu, 2013). A large number of researches focused on the use of whole cell lipases from the wild-type microorganisms containing lipase or genetically modified microorganisms with high lipase activity to reduce the cost. It must be economically feasible and several aspects of lipase catalyzed biodiesel production needs to be neatly and wisely studied to address the limitation for industry used in the future scale-up. In this case, the correct catalyst, feedstock and acyl acceptor, adoption of feasible methods, proper design of bioreactors and the reaction modelling are of importance.

However, there are some disadvantages of lipase enzyme. Some people can develop allergies to the enzymes (e.g. biological washing powders). Besides, Enzymes can be denatured by even a small increase in temperature. It is also expensive to produce and contamination of the enzyme with other substances can affect the reaction. To overcome this problem, immobilized lipase is applied. Immobilized lipase provides benefits, i.e. increased enzyme stability, reduced enzyme costs, greater ease of enzyme separation and recovery for reutilization, possibility of operating continuously, easy product separation, reduced effluent problems, in some cases, increased activity. Enzymatic catalysis also has a drawback since it is a time consuming process, where it is the slowest pathway in biodiesel production. Hence, intensification of the reactor technology is crucial. Ultrasound technology is among the



prospective alternative. The application of ultrasound device in enzymatic transesterification is helping in reducing the reaction time.

Enzymatic biodiesel production is prospective to be scaled-up to a large production capacity. For this purpose, modeling and kinetics study of enzymatic transesterification is essential to obtain the kinetics data required for process design of bioreactor.

### **This proposed Research**

This research will focus on the application of immobilized lipase as biocatalyst for transesterification reaction in biodiesel production using ultrasound-assisted reaction. The reaction will be performed in an ultrasound-enhanced stirred batch reactor. Ultrasound is a vibration of the same physical nature as sound but with frequencies above the range of human hearing (Gusniah, 2019). The transesterification process is involved in the interface of two phases, such as liquid-liquid, which are immiscible to each other and thus create the same lag time in achieving the stability of the mixture before the reaction begins. Therefore, the function of ultrasound is to provide mechanical energy for mixing and the required activation energy for initiating the transesterification (Adewale, 2016 et al., 2017), so it will help to cut the created lag phase as demonstrated in Figure 2.1.

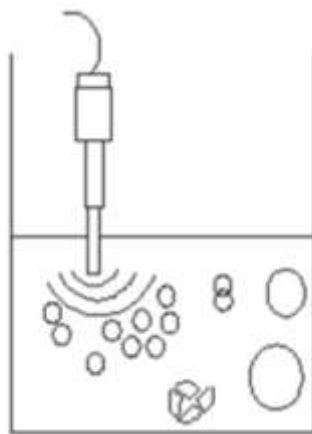


Figure 2.1. Collision of Bubbles in Cavitation by Ultrasonic Device

Basically, ultrasonics can be divided into two groups based upon frequency and power, which is high frequency (1000-10000 kHz) with low power and low frequency (20-1000 kHz) with high power. For the type of high frequency with low power, it is mostly used in the medical field, while the other one is applied in the chemistry field. In this work, the low frequency ultrasound device will be used (Subhedar and Gogate, 2016).

The reaction of ultrasonics is assisted by the presence of cavitation and can be divided into four broad categories, namely, particle-induced, optical, acoustic, and hydrodynamic. This phenomenon was discovered by Leonhard Euler in 1754. Cavitation can be defined as the formation, growth, and collapse of a bubble within a liquid, releasing a large amount of energy in a small surrounding area. The energy result of the collapsed bubbles in the solution. The

advantage of ultrasonics is elevating the temperature and pressure under ambient conditions and helping to eliminate the mass transfer problem with a high intensity of microlevel turbulence generated by oscillating cavities with high interfacial area in an ultrasonic reactor. The most common problem in the enzymatic transesterification reaction is the slowest reaction of biodiesel production rate, where it is significantly dependent on the yield of biodiesel with respect to the time. It was determined that the slow reaction rate and low yield of biodiesel are attributed to poor mixing of alcohol and oil, as they are not completely miscible with one another. Hence, poor mixing of the substrate is the major problem in the enzymatic transesterification reaction. Therefore, the ultrasonic-assisted method has been explored in overcoming the hindrance in transesterification through the enzymatic reaction.

In this research, **experimental laboratory work** and **kinetics study** of enzymatic transesterification of non-edible oil (waste cooking oil and nyamplung seed oil) using immobilized lipase from *R. miehei* (Lipozyme IM) will be conducted. This work aims at evaluating the effects of main parameters of reaction, including the enzyme loading, types of feedstock, time and temperature of reaction, as well as the ultrasound reactor frequency and conducting the kinetic study of the reaction. Kinetics model of the enzymatic reaction will be carried out based on the ping-pong bi bi with reserved inhibition of methanol. The best kinetics model will best fit with the experimental data. Kinetics study is important for equipment design and scaling-up the process at industrial scale. This research is expected to provide the alternative of biodiesel development as renewable energy using green and environmental friendly technology. Road-map of this research is presented in Figure 2.2.

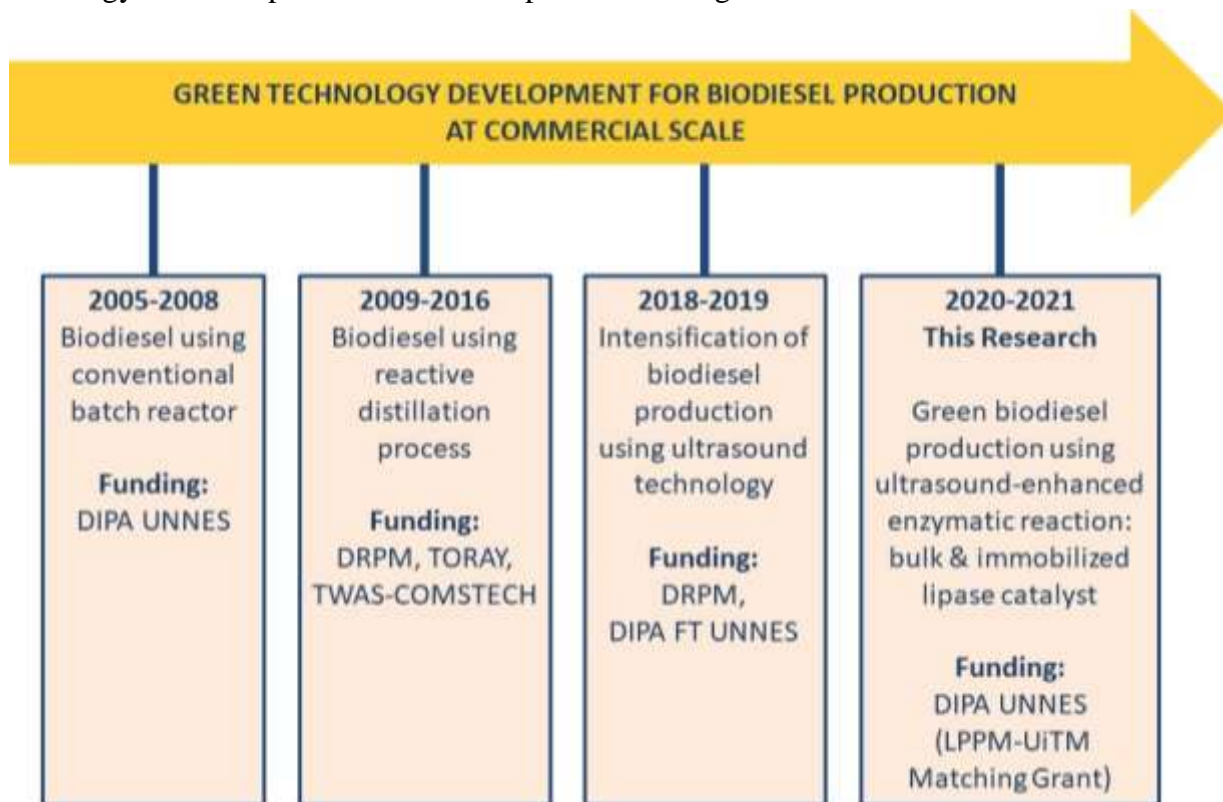


Figure 2.1. Road-Map of This Research

### CHAPTER III. OBJECTIVES AND SIGNIFICANT CONTRIBUTION

**Objectives of this research are:**

1. Evaluating the effects of main parameters of reaction, including the enzyme loading, types of feedstock, time and temperature of reaction, as well as the ultrasound reactor frequency
2. Conducting the kinetic study of the reaction.

**Urgency and the significant contribution** of this work can be stated as follows. This work offer green technology of biodiesel production using ultrasound-assisted enzymatic reaction which is environmentally friendly, mild operation condition, fast, and economical. It also contribute on providing kinetics data which is important for process equipment design, plant design, and scaling-up the process into industrial-scale capacity.

## CHAPTER IV. METHOD

**This research consists of 4 steps:**

- a. Pretreatment: Degumming and Drying to reduce the water content
- b. Experimental work of ultrasound-assisted enzymatic reaction for biodiesel production  
Experimental work of biodiesel production through enzymatic transesterification reaction is conducted to study the effects of the variables (enzyme loading, types of feedstock, time and temperature of reaction, as well as the ultrasound reactor frequency) on biodiesel yield.
- c. Kinetics study  
An enzymatic kinetics model is developed. The kinetics model is validated using the experimental data. The proximity between the model calculation with the experimental data indicates that the model fits the reaction system. The reaction constant can also be obtain, which is important for process equipment design and scaling-up.
- d. Biodiesel characterization  
Biodiesel properties as fuel are tested using ASTM standard test to determine density, viscosity, FTIR, methyl ester content, and calorie value.

This research is continuation of the previous work applying ultrasound technology for biodiesel production as presented in Figure 4.1 (Research Flowchart)

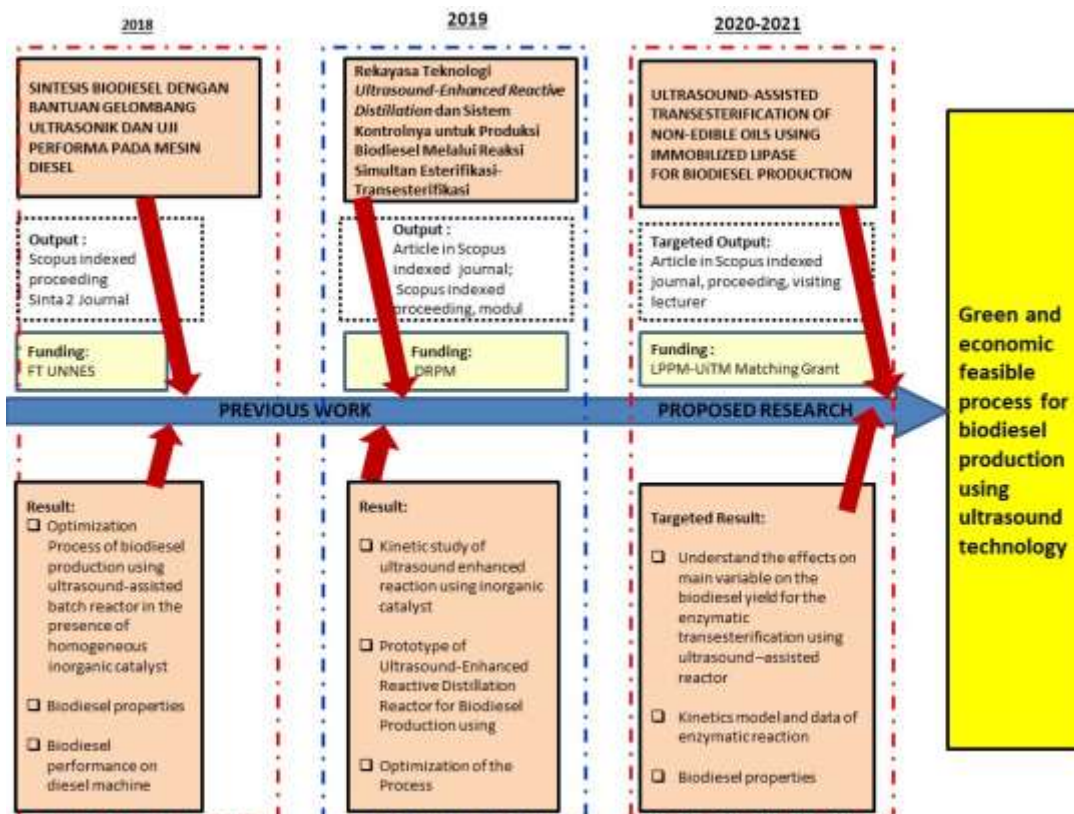


Figure 4.1. Research Flowchart

**Research Location:**

Laboratory of Chemical Engineering Department of UNNES, Semarang, Indonesia

## Materials

The materials used in this research are:

1. Whole cell enzyme from Novozym
2. Immobilized lipase from *R. miehei* (Lipozyme IM), from Sigma Aldrich Malaysia, which is Registered Trade mark of Novozymes. It is a 1,3-specific lipase immobilized on macro porous ion exchange resin with particle diameter distribution in the range of 0.3–0.6 mm
3. Reference standards for FAMES that is a mixture of methyl oleate, methyl palmitate, methyl linoleate, methyl stearate, methyl linolenate and methyl myristate of >99% purity are supplied from Sigma–Aldrich.
4. Pure methanol, n-hexane, isopropanol, ethanol, tert-butanol and diethyl ether are from Merck.
5. Ethyl acetate is from BDH.
6. Waste cooking oil and nyamplung seed oil are from UMKM Indonesia
7. Sulfuric acid

## Equipment and Experimental Set-Up

The main equipment of ultrasound-assisted enzymatic reactor is presented in Figure 4. Lipozyme IM is used as biocatalyst. Raw materials used are waste cooking oil and nyamplung seed oil. Vegetable oil is prepared by removing the solid residue. A pretreatment process to decrease the free fatty acid (FFA) will be carried out via esterification reaction. The main enzymatic transesterification is conducted in ultrasound-enhanced batch reactor (300 mL volume) which equipped with the stirrer, thermocouple, transducer, and condenser. Initially, a certain amount of vegetable oil is introduced into the reactor with the certain oil: methanol ratio (1:6). The temperature is set at a certain value (30°C, 40°C, 50°C, 60°C). Biocatalyst loading is varied at 0,5 gr, 1 gr, 1,5 gr, 2 gr. The reaction is conducted for 8 hr and biodiesels samples are periodically taken at 0, 10, 20, 30, 40, 50, 60 minutes, and 2, 4, 6, 8 hr. The ultrasound frequency is set at 20, 40, and 60 kHz. Biodiesel yields and composition are analyzed using Gas Chromatography.

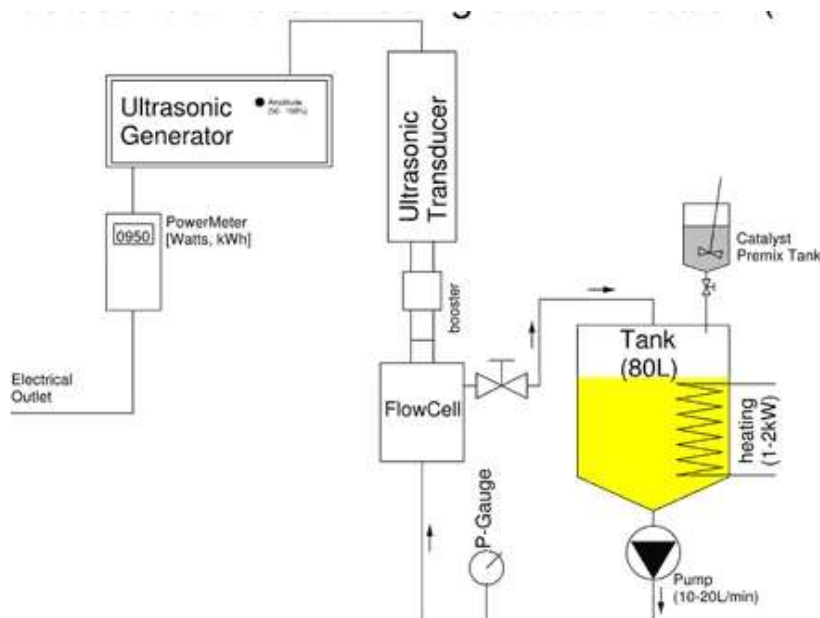


Figure 4.2. Ultrasound-Assisted Reactor for Enzymatic Transesterification

### Analysis of Biodiesel Properties

The methyl esters mixture is analyzed with Gas Chromatograph Mass Spectrophotometer (GC–MS) HP 6890A connected to a DB-23capillary column (60 m x 0.250 mm x 0.15  $\mu$ m, J&W Scientific, USA) with helium as carrier gas. The inlet temperature is 250  $^{\circ}$ C with oven temperature program as follows: Oven is heated to 50 $^{\circ}$ C for 1 min, increase 25  $^{\circ}$ C/min to 175  $^{\circ}$ C, ramping 4  $^{\circ}$ C/min to 235  $^{\circ}$ C and hold for 5 min. The yield percentage of biodiesel was calculated based on weight of total methyl esters gained over unit weight of oil. Biodiesel properties (density, viscosity, and caloric value) are determined using ASTM package tests.

### Role of the Research Team

No.	Name of Researcher	Institution	Duty
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2	Dhoni Hartanto, S.T., M.T., M.Sc.	UNNES	Thermodynamic behaviour
3	Haniif Prasetiawan, M.Eng.	UNNES	Process optimization
4	Ariyani Widyastuti, S.E. (Staf)	UNNES	Administration
5	Sarah Ayatillah	UNNES	Laboratory observation
6	Nur Hayati	UNNES	Laboratory observation
7	Elva Dianis Novi Anisa	UNNES	Property analysis
8	Dr. Harumi Veny	UiTM	Mass transfer study
9	Dr. Fazlena Hamzah	UiTM	Ultrasound development
10	Miradatul Najwa Muhd Rodhi, M.Sc.	UiTM	Enzyme catalyst preparation

## CHAPTER V. RESULT AND DISCUSSION

In this research, an experimental study of biodiesel synthesis was carried out through the transesterification reaction of nyamplung seed oil with a mole ratio of oil: ethyl acetate of 1:3, a catalyst concentration of 0.2% (w/v) lipase at 30oC, 40oC, 50oC and 60oC with a time variable. 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. Before transesterification of nyamplung seed oil, pre-treatment was carried out, namely degumming and washing processes.

### **5.1 Degumming**

The research begins with degumming first with the aim of removing latex in the oil. Oils and fats that have been separated from their tissues of origin must contain small amounts of components other than triglycerides, namely phospholipids, sterols, free fatty acids, waxes, oil-soluble pigments and hydrocarbons (Ketaren, 1986). The substance added in the degumming process is phosphoric acid ( $H_3PO_4$ ). Nyamplung seed oil is an oil obtained from nyamplung seeds which contains gum, so a degumming process is necessary.

The degumming process was carried out by adding 0.3% (w/w) phosphoric acid from nyamplung seed oil and then washing it by adding 5% distilled water by weight of the oil. This washing aims to remove the acid contained in the oil (Hew, 2019). The distilled water was heated at 40oC and put into a separatory funnel then homogenized and allowed to stand for 24 hours. After the degumming process, the color of the nyamplung seed oil changed to reddish yellow due to the loss of the natural chlorophyll pigment in the nyamplung oil (Nurhidayanti, 2017) and after the addition of warm distilled water, the oil color became yellow-black. After being left for 24 hours there are two layers, the top brownish yellow layer is oil while the black lower layer is gum and impurities which dissolve in water to be separated.

Furthermore, the oil is heated in the oven at 105°C to reduce the water content contained in the oil until a constant oil weight is obtained (Adhytia, 2017). After the oven the color of the oil becomes yellowish brown and clear, or darker than the previous color. This is because the oil oven process takes a long time, causing the oil to take too long to go through the heating process (Nurhidayanti, 2017). After that, the analysis of density, viscosity, and acid number was carried out.

## 5.2. Raw Material Characteristic

Nyamplung seed oil is analyzed first using the GC-MS tool to determine the fatty acid content in the oil, density test to determine the density of the oil, viscosity test to determine the thickness of the oil, and acid number test to determine the initial acid number in the oil prior to processing. transesterification. Comparison of the specifications of nyamplung seed oil is shown in Table 5.1 below:

Table 5.1 Nyamplung Oil Characteristic Before and After Degumming

Quantitative Test	<i>Before Degumming</i>	<i>After Degumming</i>
Density (kg/m <sup>3</sup> )	926	924
Viscosity (mm <sup>2</sup> /s)	62,9	58,25
Acid Number (mg KOH/kg)	17,76	11,41

Analysis of the molecular weight of nyamplung seed oil using GC-MS is shown in Figure 5.1

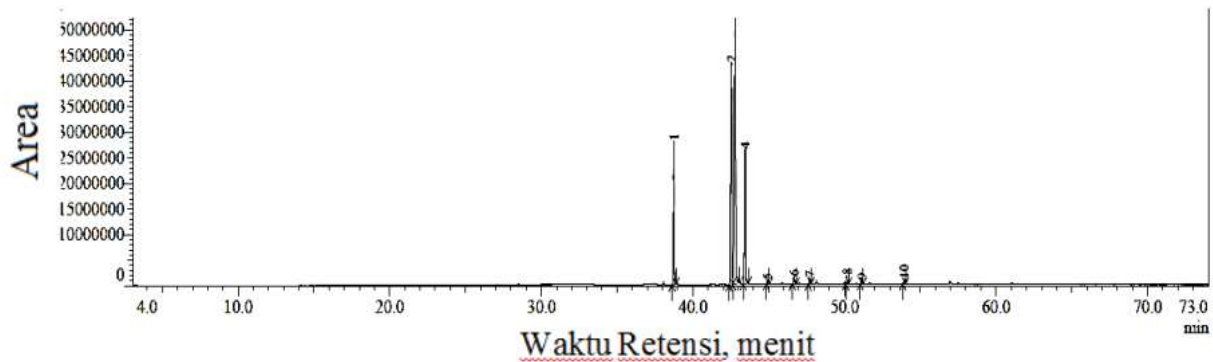


Figure 5.1 Chromatogram GC-MS of Nyamplung Oil

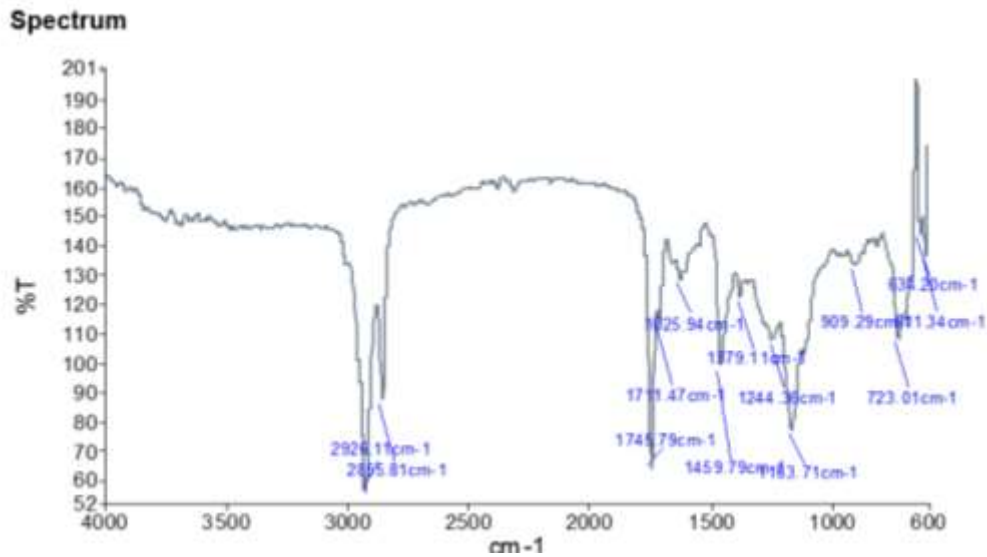
Based on the GC-MS test results, data on the composition of nyamplung seed oil were obtained as shown in Table 5.2 as follows:

Table 5.2 Composition of Nyamplung Oil

Fatty Acid	Molecular Weight (g/mol)	Area (%)
Palmitic acid	256,2228	15,51
Linoleic acid	280,45	28,94
Oleic acid	282,52	40,55
Stearic acid	284,47	14,39
Arachatic acid	312,54	0,60



The presence of fatty acid molecules is proven by the FTIR test as shown in Figure 5.2 below.

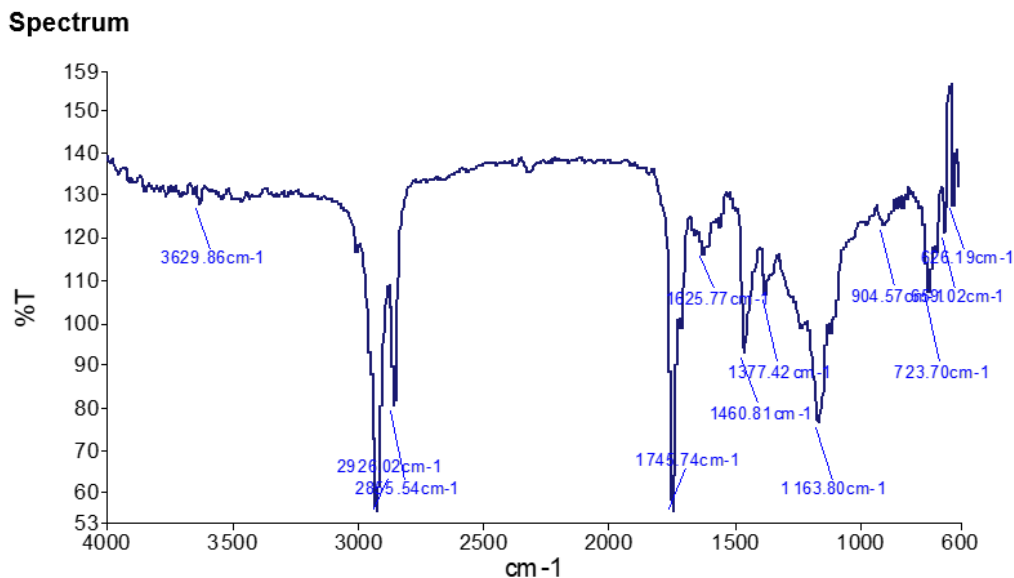


**Figure 5.2** Chromatogram of FTIR of Nyamplung Oil

From the results of the analysis using the FTIR spectrophotometer, the presence of fatty acids in nyamplung seed oil is proven by the absorption of a double bonded C atom (C=C) at 1625.94 cm-1, reinforced by the presence of =CH absorption at 909.29 cm-1 and 723.01 cm-1. C=O absorption is at 1745.79 cm-1 and 1711.47 cm-1 while C-O absorption is at 1244.36 cm-1 and 1163.71 cm-1 which indicates the carboxylic group present in unsaturated fatty acids in oil nyamplung seeds. At wave numbers 2926.11 cm-1, 2855.81 cm-1, 1459.79 cm-1, and 1379.11 cm-1 there are C-H absorptions from the CH<sub>2</sub> and CH<sub>3</sub> groups. Based on data on the compounds that make up nyamplung seed oil, the Molecular Weight (MW) of nyamplung seed oil can be obtained using equation (5.1) as follows:

$$MW_{\text{minyak}} = (3 \times MW_{\text{total}} - 3 \times AW_{\text{H}}) + (3 \times AW_{\text{C}} + 5 \times AW_{\text{H}}) \quad (5.1)$$

From the results of calculations using Equation 5.1, the molecular weight of nyamplung oil is 872.956 g/mol. These results are close to the molecular weight values reported by Haryono et al. (2016) with a molecular weight value of 869.74 g/mol. The FTIR test of nyamplung seed oil after degumming was carried out with the aim of comparing the effect of degumming on changes in compounds contained in nyamplung seed oil as presented in Figure 5.3



**Figure 5.3** Chromatogram FTIR Nyamplung Oil after Degumming

Based on the results of analysis using the FTIR spectrophotometer, the presence of fatty acids in nyamplung seed oil is evidenced by the absorption of double bonded C atoms (C=C) at 1625.77 cm<sup>-1</sup>, reinforced by the presence of =CH absorption at 904.57 cm<sup>-1</sup> and 723.70 cm<sup>-1</sup>. OH absorption occurs at 3629.86 cm<sup>-1</sup> while C=O absorption occurs at 1745.74 cm<sup>-1</sup> and C-O absorption occurs at 1163.80 cm<sup>-1</sup> which indicates a carboxylic group present in unsaturated fatty acids in nyamplung seed oil after degumming. At wave numbers 2926.02 cm<sup>-1</sup>, 2855.54 cm<sup>-1</sup>, 1460.81 cm<sup>-1</sup>, and 1377.42 cm<sup>-1</sup> there are C-H absorptions from the CH<sub>2</sub> and CH<sub>3</sub> groups. From the tests that have been carried out, there are differences in compound groups in nyamplung seed oil before and after degumming, namely the formation of OH groups and the reduction of C=O and C-O groups. This happens because the C=O and C-O groups, which are long-chain carboxylic groups, react with phosphoric acid to cause bond breaking to form OH groups, which are carboxylic group monomers.

The density of nyamplung seed oil decreased after degumming and washing. This is due to the latex or gum content in the seed oil nyamplung has decreased or decreased (Nurhidayanti, 2018). Based on the results of raw material analysis, the density of nyamplung seed oil was 926 kg/m<sup>3</sup> for crude or before degumming and 924 kg/m<sup>3</sup> for nyamplung seed oil after degumming and washing.

Based on the data in Table 4.1, the viscosity before degumming was 62.9 mm<sup>2</sup>/s and after degumming it was 58.25 mm<sup>2</sup>/s. The viscosity value of nyamplung seed oil has decreased

because during the degumming process there is a change in temperature, if the temperature of an oil increases, the viscosity will decrease. The viscosity value is also affected by the density value, the greater the density value, the greater the viscosity value (Budiman et al, 2014). The fatty acid composition of the oil has a close relationship with the viscosity value, the longer a fatty acid chain or alcohol group, the higher the viscosity value (Khaidir et al, 2015).

The acid number value is directly proportional to the free fatty acid content in the oil, the greater the acid number value, the greater the free fatty acid content. The high acid number value makes the quality of the oil produced lower (Sanjiwani et al., 2015). Based on Table 4.1, the acid number in nyamplung seed oil is 17.76 mg KOH/kg oil and Haryono et al. (2016) reported an acid number value of nyamplung seed oil of 0.47 mg KOH/kg oil. Based on these data, in this study, nyamplung seed oil has a higher acid number value than previous studies, however, these results are still classified as high acid number values, so degumming and washing is necessary. Degumming aims to reduce the value of the acid number by removing the gum, while washing using warm aims to remove impurities and residual use of phosphoric acid from degumming. After degumming and washing, the acid number value becomes 11.41 mg KOH/kg oil. In this study the esterification process was not carried out because it could affect the optimization of the lipase biocatalyst at the transesterification stage, due to the influence of the use of an acid catalyst in the esterification process. This is because the lipase enzyme is not resistant to environments that are too acidic and optimum at pH 6 (Wardoyo, 2015).

### **5.3. Synthesis of Biodiesel using Lipase Catalyzed Transesterification**

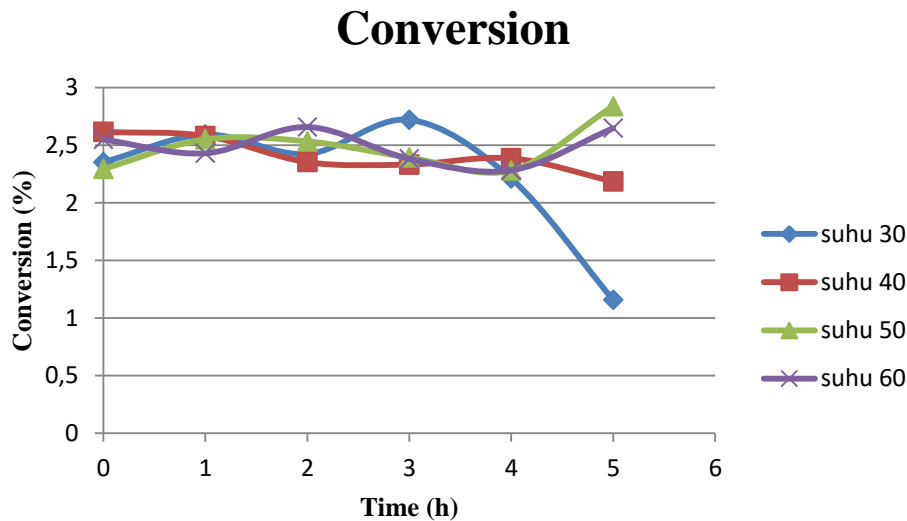
Transesterification was carried out with a ratio of oil and ethyl acetate (1:3) with 0.2% (w/v) lipase biocatalyst. The transesterification process was carried out for 5 hours with each variable 1 hour, 2 hours, 3 hours, 4 hours and 5 hours. The transesterification process has advantages compared to the conventional method, which requires a shorter time in the process of making biodiesel (Fitriani, 2016). In addition, the synthesis of biodiesel uses an environmentally friendly lipase biocatalyst, mild operating conditions, high purity of biodiesel, no pretreatment required before transesterification and high quality of triacetin (Moazeni, 2019). Several studies have shown differences in the conversion of biodiesel using lipase.

The solvent used in the transesterification process is ethyl acetate. The use of ethyl acetate has been used in various previous studies. In Park's 2017 study, biodiesel with microalgae as raw material used ethyl acetate solvent with an operating temperature of 115°C and 2 hours of

time obtained a biodiesel conversion of 97.8%, and Sootchiewcharn's research in 2015 with ethyl acetate solvent working at 350 oC and 2 hours of time obtained biodiesel conversion of 78.3%. The use of ethyl acetate will react with triglycerides to produce a side product in the form of triacetin, so it has a high value quality (Sootchiewcharn, 2015).

### 5.3.1 Effects of Temperature and Reaction Time on the Biodiesel Conversion

The experiment was carried out at 30oC, 40oC, 50oC and 60oC with 0.2% (w/v) lipase catalyst and nyamplung oil. The transesterification reaction lasted for 5 hours. Samples were taken every 1 hour within 5 hours during the transesterification process. then the GCMS test was carried out to determine the components contained in the biodiesel. Compounds that are converted from oil to biodiesel include: Acetic acid-ethyl ester, Hexadecanoic acid-methyl ester, Hexadecanoic acid-ethyl ester, Heptadecanoic acid-methyl ester, Octadecanoic acid-methyl ester, 9-Octadecenoic acid-methyl ester, Octadecanoic acid- ethyl ester, 9-Octadecenoic acid (Z)-ethyl ester. From the GCMS data, the conversion of oil into biodiesel can be obtained which can be seen in Figure 5.4.



**Figure 5.4. Correlation of Temperature and Reaction Time to the Biodiesel Conversion**

In this study, the temperature variables were 30, 40, 50, 60°C and time variables 0, 1, 2, 3, 4, 5 hours at a molar ratio of oil:ethyl acetate 1:3 with 0.2% (w/ v). Each temperature variable is used for sampling at intervals of 1 hour up to 5 hours. In Table 5.3 the temperature and reaction time can affect the conversion of the resulting fatty acid ethyl ester (FAEE).

Table 5.3. Correlation of Reaction Time and Temperature on the Reaction Conversion i

Time	T 30	T 40	T 50	T 60
0	2.35	2.61	2.29	2.55
1	2.59	2.58	2.55	2.43
2	2.427	2.35	2.53	2.66
3	2.72	2.33	2.39	2.38
4	2.21	2.38	2.28	2.28
5	1.16	2.18	2.84	2.65

Table 5.3 shows that the optimal conversion at 30oC for 3 hours is 2.72%, at 40oC for 0 hours a conversion is 2.61%, at 50oC for 5 hours a conversion is 2.84%, and at a temperature of 60oC with a time of 2 hours a conversion of 2.66% was obtained. This study did not show that the higher the temperature and time, the higher the conversions obtained (Guo, 2020).

According to Hidayat (2008) the optimal conversion value is obtained at a temperature of 50oC because lipase enzyme activity works at a temperature of 30oC – 60oC. This is according to this study because the highest conversion was obtained at 50oC for 5 hours. Santaraite (2020) also states that the optimal temperature for biodiesel conversion is at 50oC with a reaction time of 24 hours, a conversion of 97.0% is obtained. Kumar (2019) also stated that the optimal temperature for biodiesel conversion was at 50oC with a reaction time of 24 hours to obtain a conversion of 78%.

However, the conversion value obtained in this study is low due to the relatively short reaction time. In addition, the process of separating biodiesel from by-products (triacetin) is not optimal, causing low ethyl esters to be formed and impurities still attached to biodiesel. In addition, it causes the conversion to increase and decrease at a certain temperature and time. Because the more impurities in the biodiesel product, the smaller the conversion produced (Wahyuni et al, 2016). Using one sample pipette to take each product sample at different times can also affect biodiesel conversion. The sample pipette used to take samples at the next time may still have sample remaining at the previous time, although it has little effect on the resulting bodysel conversion.

In this study, the conversion of the ethyl ester produced was still low. This can be caused because the lipase used is not immobilized beforehand, so that lipase will act as an impurity in the final biodiesel product. One of the immobilization of lipase, namely the zeolite

adsorption method, has been shown to increase conversion, because the lipase will be attached to the zeolite substrate and can be taken and used as a catalyst for reuse, so that the lipase will not become an impurity in the final biodiesel product.

### 5.3.2. Effects of Temperature and Reaction Time on the Biodiesel Selectivity

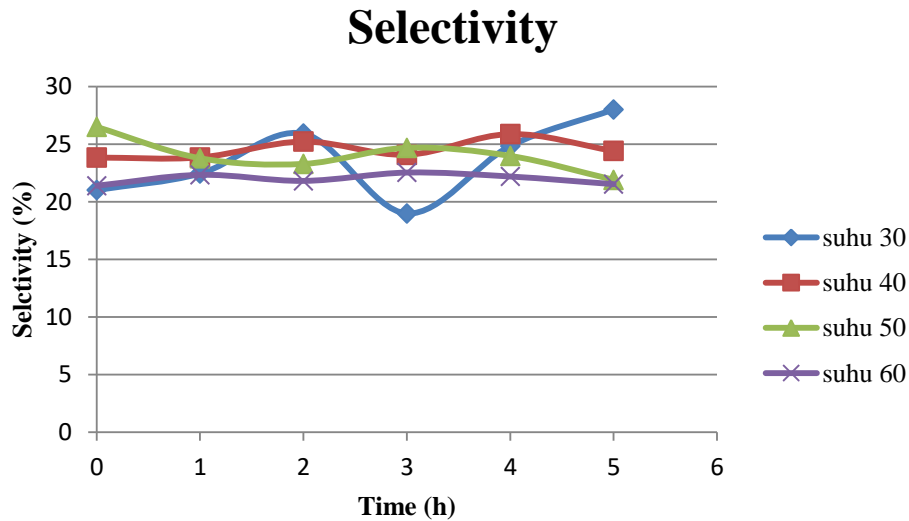


Figure 5.5. Effect of Temperature and Reaction Time on Selectivity

In Table 5.4, the optimal selectivity at 30oC for 5 hours is 28%, at 40oC for 4 hours a conversion is 25.87%, at 50oC for 0 hours a conversion is 26.49%, and at a temperature 60oC with a time of 3 hours obtained a conversion of 22.53%.

Table. 5.4. Effect of Temperature and Reaction Time on Selectivity

Time	T 30	T 40	T 50	T 60
0	21.01	23.83	26.49	21.39
1	22.44	23.84	23.77	22.33
2	25.90	25.22	23.28	21.81
3	18.98	24.07	24.68	22.53
4	24.83	25.87	23.97	22.19
5	28.00	24.41	21.90	21.52

According to Cheng (2019), the increase in selectivity is affected by temperature and time. In addition, the higher the conversion, the higher the selectivity (Febriyanti, 2020). This is not in accordance with the research that has been done, at 50oC the highest conversion was

obtained while the selectivity obtained was not optimum but at 30oC. The possibility that can occur is due to the presence of inhibitors that inhibit the action of the lipase enzyme. These inhibitors can attach to the active site or the free side of the enzyme or enzymes that have been bound to the substrate. Inhibitors that often inhibit the work of lipase enzymes can come from side products produced during the reaction. So it can be said that after the product is formed in the early hours, side products are also formed. The by-product in the form of triacetin binds to the free side of the enzyme or the enzyme-substrate complex so that in the following hour the enzyme is unable to produce more reaction products than in the previous hour. One of the ways to increase selectivity is by prior immobilization of the enzyme (Kahveci, 2011).

### 5.3.3. Characteristic of Biodiesel Product

The yield characteristics of the transesterified biodiesel were carried out by several tests including density, viscosity and FFA analysis which can be seen in Table 5.4.

#### a. Density

Density is one parameter of the success of the transesterification reaction. Density is also one of the determinants of biodiesel because it is related to the calorific value and power produced by diesel engines. The lower the density value, the higher the heating or combustion value (Hartono et al, 2016).

Table 5.4 Density of Biodiesel

T (°C)	Density (kg/m <sup>3</sup> )
30	922
40	918
50	916
60	908

The resulting biodiesel has a lower density value than the raw material for nyamplung seed oil before and after degumming. Table 5.4 shows that the lowest density value is 922 kg/m<sup>3</sup> at 30oC and the highest density is 908 kg/m<sup>3</sup> at 60oC. It can be seen that with every increase in temperature, the density of biodiesel decreases. In accordance with Sinaga's research (2014) which stated that the lower the temperature, the higher the density of biodiesel obtained, and vice versa. This is caused by the presence of a by-product (triacetin) in biodiesel which can affect the density of biodiesel because triacetin has a relatively high density (1.16 g/cm<sup>3</sup>), so that if the

triacetin is not properly separated from biodiesel, the density of biodiesel will increase. . In addition, the decreased density value is caused by the chain bonds contained in the compound and unsaturation. The shorter the fatty acid chain, the density will decrease (Mikah, 2017). Unsaturation also affects the density, where the more number of double bonds contained in the product, the density will decrease. Widyawati (2017) states that the factor affecting density is the molecular weight of the fatty acids, the higher the density, the greater the molecular weight of the fatty acids in biodiesel. In this study, a density of 916 was obtained at 50oC and did not meet the biodiesel density standard from SNI 7182: 2015, which is between 850-890 kg/m3 (BSN, 2015).

b. Viscosity

Nyamplung oil is a vegetable oil that has a viscosity that is too high to be used directly in a diesel engine, so a process is needed to reduce the viscosity. The enzymatic transesterification process is a way to reduce the viscosity value of the oil (Nenobahan et al, 2020). According to Musta et al. (2017), a fluid that has a low viscosity will be more dilute and flow easily, while a fluid that has a high viscosity value will be more viscous and difficult to flow. Sarwono et al. (2017) also reported that the higher the viscosity, the greater the resistance to flow. If the viscosity is too high, there will be a large loss of friction in the pipe, the work of the pump will be heavy, the filtering will be difficult and it is possible that dirt will settle in large quantities, and it will be difficult to fog up the fuel.

Table 5.5. Viscosity of Biodiesel

Biodiesel	Viscosity (mm <sup>2</sup> /s)
T 30°C	10,73
T 40°C	11,30
T 50°C	10,61
T60°C	11,40

The resulting biodiesel has a lower viscosity value than the viscosity of the raw material for nyamplung seed oil before and after degumming. One of the reasons for the decrease in viscosity that occurs is due to the continuous heating process (Nurhidayanti, 2017). Based on table 4.4, the viscosity of biodiesel increased from 30oC to 40oC, then decreased at 50oC and increased again at 60°C. The lowest viscosity was found in biodiesel with a temperature variable of 50°C, this result indicates that the best viscosity is produced in biodiesel which has optimal



conversion and selectivity. This is not in accordance with Wahyuni (2015) who reported that the higher the temperature, the lower the viscosity, because the movement of molecules is faster so that the collisions between reacting molecules increase. There is a possibility of a decrease in viscosity at 40°C and an increase at 60°C due to the less than optimal transesterification process. At 40°C, it can be caused by the incomplete mixing or homogenization process between nyamplung seed oil, ethyl acetate, and lipase enzyme catalyst. While the increase in viscosity at 60°C can be caused by the lipase enzyme catalyst which begins to experience denaturation so that the lipase enzyme cannot accelerate the transesterification reaction and eventually only becomes an impurity in the biodiesel produced. Another possibility could be due to the incomplete pretreatment and separation of triacetin (Raharjo, 2019) and the presence of glycerol carried in the methyl ester phase (Cahyaningtyas, 2019). The biodiesel produced does not meet the biodiesel viscosity standard according to SNI 7182: 2015 at a temperature of 40°C in the range of 2.3-6.0 mm<sup>2</sup>/s (BSN, 2015), so it cannot be used as an alternative fuel to replace diesel in diesel engines. Due to the high viscosity, more fuel is needed to be injected into the combustion chamber to get the same engine power. So a lower viscosity is better for use as fuel so it doesn't require a lot of biodiesel to burn in the engine so it can save fuel consumption (Wahyuni, 2015).

### c. Acid Number

The acid number in the fuel can affect the corrosion properties of the engine, because the higher the acid number in biodiesel, the higher its corrosivity (Haryanto et al, 2015). Fuel with high corrosivity will quickly cause scale which will affect the diesel engine combustion chamber (Nugraheni, 2017). Handayani (2015) also stated that a high acid number can result in lower biodiesel quality.

Table 5.6. Acid Value of Biodiesel

Biodiesel	Acid Value (mg KOH/g oil)
T 30°C	37,8791
T 40°C	37,2143
T 50°C	36,644
T 60°C	37,3569

Based on the results of the acid number test, it was found that biodiesel with a transesterification temperature of 30oC to 50oC had a decreasing acid number, then at 60oC the acid number value increased again. This is not in accordance with Sinaga, et al. (2013) which states that the higher the transesterification temperature, the lower the acid number value. The increase in acid number at 60oC was caused by the lipase enzyme starting to denature so that the lipase enzyme could not accelerate the transesterification reaction, and as a result there were still many acid numbers in biodiesel. The lowest acid number value was obtained for biodiesel with transesterification at 50oC, which was 36.644 mg KOH/g oil, indicating that the quality of biodiesel was better than the others. This happens because the optimal conversion of biodiesel is obtained at a temperature of 50 oC. The lower the value of the acid number, the higher the biodiesel conversion obtained (Udyani, 2014).

In this study, the biodiesel obtained had a high acid number value and did not meet the 2015 SNI standard of 0.5 mg KOH/g oil (BSN, 2015). This is because biodiesel still contains high free fatty acids, making it difficult to separate (Handayani, 2015). In addition, the high acid number is also caused by several factors including the type of raw material, the level of purity of the raw material, the biodiesel production process, the biodiesel storage process and the transesterification process which lasts less time (Kasim, 2011). Efforts that can be made to reduce the number of free fatty acids in oil are by carrying out pretreatment so that it makes it easier to separate biodiesel from by-products (Hadrah, 2018).

#### 5.3.4. On Progress Improvement

The research result has not been satisfying. Hence, improvement is still on progress regarding the addition of catalyst amount, the longer reaction time, and optimization using Response Surface methodology using Bohn-Behnken Method

## CHAPTER VI. CONCLUSION AND RECOMMENDATION

### 6.1. Conclusion

1. The use of the transesterification method using a lipase biocatalyst produces an optimal conversion of 2.84% at 50oC for 5 hours
2. The use of the transesterification method using a lipase biocatalyst produces an optimal selectivity of 21.9% at 50oC for 5 hours

### 6.2. Suggestion

Further research is needed regarding the effect of time and catalyst concentration in order to obtain high biodiesel conversion and selectivity. Hence, the research is still in the continuation of improving the experimental method to obtain higher conversion and optimization in term of increasing the catalyst amount and conduting optimization using Response Surface Methodology (Box-Behnken Method)

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## ATTACHMENT

### DOCUMENTATION OF THE BIODIESEL MAKING

#### 1. Tahap *Pre-treatment* Bahan Baku



Proses degumming



Pencucian



Pemisahan



Pengovenan

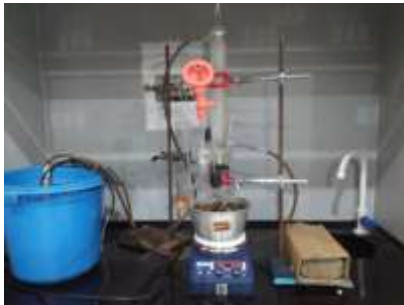


Penimbangan



Minyak sebelum dan sesudah *degumming*

#### 2. Transesterifikasi



Transesterifikasi



Sampel sebelum sentrifugasi



Sampel sesudah sentrifugasi

### 3. Analisa karakteristik Biodisel



Piknometer



Viskometer batch KV-6



GC-MS



KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN  
UNIVERSITAS NEGERI SEMARANG  
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT

Gedung Prof. Dr. Retno Sriningsih Satmoko, Kampus Sekaran, Gunungpati, Semarang 50229

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**SURAT PERJANJIAN**  
**PELAKSANAAN PENELITIAN KERJASAMA**  
**DANA DIPA UNNES TAHUN 2021**  
**Nomor: 247.26.4/UN37/PPK.3.1/2021**

Pada hari ini Senin tanggal Dua puluh enam bulan April tahun Dua ribu dua puluh satu, kami yang bertandatangan di bawah ini:

- 1. Dr. Suwito Eko Pramono, M. Pd.** : **Pejabat Pembuat Komitmen** Lembaga Penelitian dan Pengabdian Kepada Masyarakat Universitas Negeri Semarang yang berkedudukan di Semarang, berdasarkan Keputusan Rektor Universitas Negeri Semarang Nomor : B/3/UN37/HK/2021 tanggal 4 Januari 2021, dalam hal ini bertindak untuk dan atas nama KPA Universitas Negeri Semarang, untuk selanjutnya disebut **PIHAK PERTAMA**;
- 2. Dr Ratna Dewi Kusumaningtyas, S. T., M. T** : Dosen pada FT Universitas Negeri Semarang, dalam hal ini bertindak sebagai Pengusul dan Ketua Pelaksana Penelitian Kerjasama Tahun Anggaran 2021 untuk selanjutnya disebut **PIHAK KEDUA**

**PIHAK PERTAMA** dan **PIHAK KEDUA** secara bersama-sama bersepakat mengikatkan diri dalam suatu Perjanjian Pelaksanaan Penelitian Kerjasama dengan ketentuan dan syarat-syarat yang diatur dalam pasal-pasal sebagai berikut.

**PASAL 1**  
**Dasar Hukum**

Perjanjian penugasan ini berdasarkan kepada:

1. Peraturan Menteri Riset, Teknologi dan Pendidikan Tinggi Republik Indonesia Nomor 23 Tahun 2015 tentang Organisasi dan Tata Kerja Universitas Negeri Semarang.
2. Peraturan Menteri Keuangan Nomor 32/PMK.02/2018 tentang Standar Biaya Masukan Tahun Anggaran 2018 Nomor 511.
3. Keputusan Rektor Universitas Negeri Semarang Nomor : 302/P/2018 tanggal 26 Juni 2018, tentang Pemberhentian dan Pengangkatan Pimpinan Lembaga dan Pimpinan Pascasarjana Antar waktu Universitas Negeri Semarang.
4. Keputusan Rektor Universitas Negeri Semarang Nomor B/3/UN37/HK/2021 tanggal 4 Januari 2021, tentang Pengangkatan Pejabat Perbendaharaan/Pengelola Keuangan Tahun Anggaran 2021 Universitas Negeri Semarang.
5. Surat Keputusan Rektor Universitas Negeri Semarang Nomor : B/335/UN37/HK/2021 tanggal 12 April 2021 tentang Penetapan Pelaksanaan Penelitian dan Pengabdian Kepada Masyarakat Universitas Negeri Semarang Tahun 2021.
6. Daftar Isian Pelaksanaan Anggaran (DIPA) Universitas Negeri Semarang (UNNES) Nomor DIPA : SP DIPA-023.17.2.677507/2021, tanggal 23 November 2020.

**PASAL 2**  
**Ruang Lingkup Perjanjian**

- (1) **PIHAK PERTAMA** memberi tugas kepada **PIHAK KEDUA**, dan **PIHAK KEDUA** menerima tugas tersebut untuk melaksanakan Penelitian Kerjasama tahun 2021 dengan judul "ULTRASOUND-ASSISTED TRANSESTERIFICATION OF NON-EDIBLE OILS USING IMMOBILIZED LIPASE FOR BIODIESEL PRODUCTION"
- (2) **PIHAK KEDUA** bertanggungjawab penuh atas pelaksanaan, administrasi dan keuangan atas pekerjaan sebagaimana dimaksud pada ayat (1) dan berkewajiban menyerahkan semua bukti-bukti pengeluaran serta dokumen pelaksanaan lainnya dalam hal diperlukan oleh **PIHAK PERTAMA**.

**PASAL 3**  
**Dana Penelitian**

- (1) Besarnya dana untuk melaksanakan penelitian dengan judul sebagaimana dimaksud pada Pasal 2 adalah sebesar Rp. 100.000.000,00 (seratus juta Rupiah) sudah termasuk pajak.
- (2) Dana Penelitian sebagaimana dimaksud pada ayat (1) dibebankan pada Daftar Isian Pelaksanaan Anggaran UNNES Nomor SP DIPA-023.17.2.677507/2021, tanggal 23 November 2020.

**PASAL 4**  
**Tata Cara Pembayaran Dana Penelitian**

- (1) **PIHAK PERTAMA** akan membayarkan Dana Penelitian kepada **PIHAK KEDUA** secara bertahap dengan ketentuan sebagai berikut:
  - a. Pembayaran Tahap Pertama sebesar 70% dari total dana penelitian yaitu  $70\% \times \text{Rp. } 100.000.000,00 = \text{Rp. } 70.000.000,00$  (tujuh puluh juta Rupiah), yang akan dibayarkan oleh **PIHAK PERTAMA** kepada **PIHAK KEDUA** setelah mengunggah hasil revisi proposal yang sudah disahkan oleh Pejabat yang berwenang, RAB, dan instrumen penelitian ke SIPP
  - b. Pembayaran Tahap Kedua sebesar 30% dari total dana penelitian yaitu  $30\% \times \text{Rp. } 100.000.000,00 = \text{Rp. } 30.000.000,00$  (tiga puluh juta Rupiah), dibayarkan oleh **PIHAK PERTAMA** kepada **PIHAK KEDUA** setelah mengunggah Laporan Kemajuan, Laporan Akhir yang sudah disahkan oleh Pejabat yang berwenang, Catatan Harian, SPTB dan Laporan Penggunaan Anggaran pada SIPP **paling lambat tanggal 13 November 2021**
- (2) Dana Penelitian sebagaimana dimaksud pada ayat (1) akan disalurkan oleh **PIHAK PERTAMA** kepada **PIHAK KEDUA** melalui rekening BNI atas nama Dr. Ratna Dewi Kusumaningtyas, S. T., M. T dengan nomor rekening 0246582443

**Pasal 5**  
**Jangka Waktu**

Jangka waktu pelaksanaan penelitian sebagaimana dimaksud dalam Pasal 2 sampai selesai 100%, adalah terhitung sejak **Tanggal 26 April** dan berakhir pada **Tanggal 13 November 2021**.

**Pasal 6**  
**Target Luaran**

- (1) **PIHAK KEDUA** berkewajiban untuk mencapai target luaran wajib seperti tersebut di bawah:  
**Luaran Wajib :**
  - a. Implementation Arrangement (IA) (Sudah Jadi)
  - b. Publikasi di Jurnal internasional bereputasi terindeks pada database internasional bereputasi (Accepted)
  - c. Sertifikat Visiting Profesor/Researcher/Guest Lecture (Sudah Jadi)
  - d. Presentasi di Seminar/Simposium/Lokakarya tingkat Internasional/Nasional tetapi tidak dimuat dalam p (Accepted)

- (2) **Semua anggota peneliti** harus dimasukkan ke luaran wajib penelitian dan pada artikel **disebutkan nomor kontrak pada bagian “ucapan terimakasih”**.
- (3) **PIHAK KEDUA** berkewajiban untuk melaporkan perkembangan pencapaian target luaran sebagaimana dimaksud pada ayat (1) kepada **PIHAK PERTAMA**.

## **Pasal 7 Hak dan Kewajiban Para Pihak**

- (1) Hak dan Kewajiban **PIHAK PERTAMA**:
  - a. **PIHAK PERTAMA** berkewajiban untuk memberikan dana penelitian kepada **PIHAK KEDUA** dengan jumlah sebagaimana dimaksud dalam Pasal 3 dan dengan tata cara pembayaran sebagaimana dimaksud dalam Pasal 4;
  - b. **PIHAK PERTAMA** berhak untuk mendapatkan dari **PIHAK KEDUA** luaran penelitian sebagaimana dimaksud dalam Pasal 6.
- (2) Hak dan Kewajiban **PIHAK KEDUA**:
  - a. **PIHAK KEDUA** berhak menerima dana penelitian dari **PIHAK PERTAMA** dengan jumlah sebagaimana dimaksud dalam Pasal 3 dan dengan tata cara pembayaran sebagaimana dimaksud dalam Pasal 4.
  - b. **PIHAK KEDUA** berkewajiban menyerahkan kepada **PIHAK PERTAMA** luaran wajib sebagaimana pada pasal 6

## **Pasal 8 Pelaksanaan Penelitian**

- (1) **PIHAK KEDUA** berkewajiban mengunggah hasil revisi proposal yang disahkan oleh Pejabat yang berwenang, RAB, dan instrumen penelitian ke SIPP dan menyerahkan *hardcopy* dokumen masing-masing 1 (satu) eksemplar **paling lambat tanggal 8 Mei 2021**
- (2) **PIHAK KEDUA** berkewajiban mengisi Catatan Harian beserta mengunggah bukti-bukti kegiatan atau pengeluaran dana, laporan penggunaan anggaran, SPTB (70%), dan Laporan kemajuan ke SIPP serta menyerahkan *hardcopy* dokumen masing-masing 1 (satu) eksemplar **paling lambat 7 Oktober 2021**
- (3) **PIHAK KEDUA** berkewajiban mengisi Catatan Harian beserta mengunggah bukti-bukti kegiatan atau pengeluaran anggaran 100%, Laporan Akhir, Poster, Artikel Ilmiah, Profil dan SPTB (100%) pada SIPP **paling lambat 13 November 2021**
- (4) **PIHAK KEDUA** berkewajiban menyerahkan *Hardcopy* Catatan Harian, Laporan Akhir, Laporan Penggunaan Anggaran beserta bukti-bukti pengeluaran, artikel ilmiah masing-masing satu eksemplar kepada **PIHAK PERTAMA** paling lambat **31 Desember 2021**
- (5) **PIHAK KEDUA** berkewajiban mengunggah bukti luaran wajib sebagaimana pada Pasal 6 paling lambat pada Tanggal **31 Agustus Tahun 2022** dengan status **PUBLISHED**
- (6) Laporan hasil Penelitian sebagaimana tersebut pada ayat (4) harus memenuhi ketentuan sebagai berikut:
  - a. Format font Times New Romans Ukuran 12 spasi 1,5
  - b. Bentuk/ukuran kertas A4;
  - c. Warna sampul (disesuaikan dengan ketentuan di panduan penelitian dan pengabdian kepada masyarakat tahun 2021)
  - d. Di bawah bagian sampul ditulis:

Dibiayai oleh:

Daftar Isian Pelaksanaan Anggaran (DIPA) Universitas Negeri Semarang  
Nomor : SP DIPA-023.17.2.677507/2021, tanggal 23 November 2020, sesuai dengan  
Surat Perjanjian Pelaksanaan Penelitian Dana DIPA UNNES Tahun 2021  
Nomor 247.26.4/UN37/PPK.3.1/2021, tanggal 26 April 2021

**Pasal 9**  
**Monitoring dan Evaluasi**

- (1) **PIHAK PERTAMA** dalam rangka pengawasan akan melakukan Monitoring dan Evaluasi internal terhadap kemajuan pelaksanaan Penelitian Tahun Anggaran 2021
- (2) **PIHAK KEDUA** selaku Ketua Pelaksana **wajib hadir** dalam kegiatan Monitoring dan Evaluasi internal, jika berhalangan wajib memberikan kuasa kepada anggota tim peneliti dalam judul yang sama.

**Pasal 10**  
**Penilaian Luaran**

Penilaian luaran penelitian dilakukan oleh Komite Penilai/*Reviewer* Luaran sesuai dengan ketentuan yang berlaku.

**Pasal 11**  
**Penggantian Ketua Pelaksana**

- (1) Apabila **PIHAK KEDUA** selaku ketua pelaksana tidak dapat melaksanakan penelitian ini, maka **PIHAK KEDUA** wajib mengusulkan pengganti ketua pelaksana yang merupakan salah satu anggota tim kepada **PIHAK PERTAMA**.
- (2) Perubahan terhadap susunan tim pelaksana dan substansi pelaksanaan penelitian ini dapat dibenarkan apa bila telah mendapat persetujuan tertulis dari **PIHAK PERTAMA**.
- (3) Apabila **PIHAK KEDUA** tidak dapat melaksanakan tugas dan tidak ada pengganti ketua sebagaimana dimaksud pada ayat (1), maka **PIHAK KEDUA** harus mengembalikan dana penelitian kepada **PIHAK PERTAMA** yang selanjutnya disetor ke Kas BLU.
- (4) Bukti setor sebagaimana dimaksud pada ayat (3) disimpan oleh **PIHAK PERTAMA**.

**Pasal 12**  
**Sanksi**

- (1) Apabila sampai dengan batas waktu yang telah ditetapkan untuk melaksanakan Kontrak Penelitian telah berakhir, **PIHAK KEDUA belum menyelesaikan** tugasnya dan atau **terlambat** mengirim dan mengunggah laporan Kemajuan, catatan harian, Surat Pernyataan Tanggungjawab Belanja (SPTB) dan Laporan akhir, maka **PIHAK KEDUA** dikenakan **sanksi denda sebesar 1‰ (satu permil)** untuk setiap hari keterlambatan sampai dengan **setinggi-tingginya 5% (lima persen)** dihitung dari tanggal jatuh tempo (13 November s.d. 31 Desember 2021)
- (2) Apabila sampai dengan batas waktu tanggal **31 Desember 2021**, **PIHAK KEDUA tidak melaksanakan kewajiban** sebagaimana dimaksud dalam Pasal 8, maka **PIHAK KEDUA** dikenai **sanksi denda** berupa **mengembalikan dana 30% dari dana penelitiannya** ke Kas BLU dan **sanksi administratif tidak dapat mengajukan proposal penelitian dalam kurun waktu 2 (dua) tahun berturut-turut.**
- (3) Apabila **PIHAK KEDUA** tidak dapat memenuhi luaran yang telah dijanjikan sebagaimana dimaksud dalam Pasal 6 ayat (1) sampai dengan tanggal **31 Agustus 2022** maka:
  - a. **PIHAK KEDUA** dikenakan **sanksi denda** berupa **mengembalikan dana biaya publikasi sebesar 5%** dari total dana penelitian ke kas BLU
  - b. **PIHAK KEDUA tidak dapat mengajukan proposal penelitian** pendanaan LPPM UNNES dalam kurun waktu **2 (dua) tahun berturut-turut baik sebagai Ketua maupun Anggota**
- (4) Apabila **PIHAK KEDUA** tidak hadir dalam kegiatan Monitoring dan Evaluasi tanpa pemberitahuan sebelumnya kepada **PIHAK PERTAMA**, maka **PIHAK KEDUA tidak berhak menerima dana Tahap Kedua** sebesar 30%.

### **Pasal 13**

#### **Pembatalan Perjanjian**

- (1) Apabila dikemudian hari terhadap judul Penelitian sebagaimana dimaksud dalam Pasal 2 ditemukan adanya duplikasi dengan Penelitian lain dan/atau ditemukan adanya ketidakjujuran, itikad tidak baik, dan/atau perbuatan yang tidak sesuai dengan kaidah ilmiah dari atau dilakukan oleh **PIHAK KEDUA**, maka perjanjian Penelitian ini dinyatakan batal dan **PIHAK KEDUA** wajib mengembalikan dana penelitian yang telah diterima dari **PIHAK PERTAMA** yang selanjutnya akan disetor ke Kas BLU.
- (2) Bukti setor sebagaimana dimaksud pada ayat (1) disimpan oleh **PIHAK PERTAMA**

### **Pasal 14**

#### **Pajak-pajak**

- (1) **PIHAK KEDUA** berkewajiban memungut dan menyetor pajak ke kantor pelayanan pajak setempat sesuai dengan ketentuan yang berlaku
- (2) **PIHAK KEDUA** berkewajiban menyerahkan bukti pembayaran pajak kepada **PIHAK PERTAMA**

### **Pasal 15**

#### **Peralatan dan/alat Hasil Penelitian**

- (1) Hak kekayaan intelektual yang dihasilkan dari Pelaksana Penelitian diatur dan dikelola sesuai dengan peraturan dan perundang-undangan.
- (2) Setiap publikasi, makalah dan/atau ekspos dalam bentuk apa pun yang berkaitan dengan hasil penelitian ini wajib mencantumkan **PIHAK PERTAMA** sebagai pemberi dana.
- (3) Pencantuman nama **PIHAK PERTAMA** sebagaimana dimaksud pada ayat (2), paling sedikit mencantumkan nama Lembaga Penelitian dan Pengabdian kepada Masyarakat UNNES.
- (4) Hasil penelitian berupa peralatan dan/atau peralatan yang dibeli dari kegiatan ini adalah milik negara, dan dapat dihibahkan kepada institusi/lembaga melalui Berita Acara Serah Terima (BAST)

### **Pasal 16**

#### **Integritas Akademik**

- (1) Pelaksana penelitian wajib menjunjung tinggi integritas akademik yaitu komitmen dalam bentuk perbuatan yang berdasarkan pada nilai kejujuran, kredibilitas, kewajaran, kehormatan, dan tanggung jawab dalam kegiatan penelitian yang dilaksanakan.
- (2) Penelitian dilakukan sesuai dengan kerangka etika, humum dan profesionalitas, serta kewajiban sesuai dengan peraturan yang berlaku
- (3) Penelitian dilakukan dengan menjunjung tinggi standar ketelitian dan integritas tertinggi dalam semua aspek penelitian.

### **Pasal 17**

#### **Keadaan Memaksa (*force majeure*)**

- (1) **PARA PIHAK** dibebaskan dari tanggung jawab atas keterlambatan atau kegagalan dalam memenuhi kewajiban yang dimaksud dalam Perjanjian Penugasan Pelaksanaan Penelitian disebabkan atau diakibatkan oleh kejadian di luar kekuasaan **PARA PIHAK** yang dapat digolongkan sebagai keadaan memaksa (*force majeure*).
- (2) Peristiwa atau kejadian yang dapat digolongkan keadaan memaksa (*force majeure*) dalam Perjanjian Penugasan Pelaksanaan Penelitian ini adalah bencana alam, wabah penyakit, kebakaran, perang, blokade, peledakan, sabotase, revolusi, pemberontakan, huru-hara, serta adanya tindakan pemerintah dalam bidang ekonomi dan moneter yang secara nyata berpengaruh terhadap Perjanjian Penugasan Pelaksanaan Penelitian.
- (3) Apabila terjadi keadaan memaksa (*force majeure*) maka pihak yang mengalami wajib memberitahukan kepada pihak lainnya secara tertulis, selambat-lambatnya dalam waktu 7 (tujuh)



hari kerja sejak terjadinya keadaan memaksa (*force majeure*), disertai dengan bukti-bukti yang sah dari pihak berwajib dan **PARA PIHAK** dengan etiket baik akan segera membicarakan penyelesaiannya.

### **Pasal 18 Penyelesaian Sengketa**

Apabila terjadi perselisihan antara **PIHAK PERTAMA** dan **PIHAK KEDUA** dalam pelaksanaan perjanjian ini akan dilakukan penyelesaian secara musyawarah dan mufakat, dan apabila tidak tercapai penyelesaian secara musyawarah dan mufakat maka penyelesaian dilakukan melalui proses hukum yang berlaku dengan memilih domisili hukum di Pengadilan Tinggi Semarang



### **Pasal 19 Lain-Lain**

- (1) **PIHAK KEDUA** menjamin bahwa penelitian dengan judul tersebut di atas belum pernah dibiayai dan/atau diikutsertakan pada Pendanaan Penelitian lainnya, baik yang diselenggarakan oleh instansi, lembaga, perusahaan atau yayasan, baik di dalam maupun di luar negeri.
- (2) Segala sesuatu yang belum cukup diatur dalam Perjanjian ini dan dipandang perlu diatur lebih lanjut dan dilakukan perubahan oleh **PARA PIHAK**, maka perubahan-perubahannya akan diatur dalam perjanjian tambahan atau perubahan yang merupakan satu kesatuan dan bagian yang tidak terpisahkan dari Perjanjian ini.

### **Pasal 20 Penutup**

Perjanjian ini dibuat dan ditandatangani oleh **PARA PIHAK** pada hari dan tanggal tersebut di atas, dibuat dalam rangkap 3 (tiga) dan bermeterai cukup sesuai dengan ketentuan yang berlaku, yang masing-masing mempunyai kekuatan hukum yang sama.

**PIHAK PERTAMA**

  
  
**Dr. Suwito Eko Pramono M. Pd.**  
NIP. 195809201985031003

**PIHAK KEDUA**

  
  
**Dr Ratna Dewi Kusumaningtyas S. T., M. T**  
NIP. 197603112000122001



KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN  
UNIVERSITAS NEGERI SEMARANG  
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT

Gedung Prof. Dr. Retno Sriningsih Satmoko, Kampus Sekaran, Gunungpati, Semarang 50229

Telp/Fax (024) 8508087, (024) 8508089

Laman: <http://lppm.unnes.ac.id> Email: [lppm@mail.unnes.ac.id](mailto:lppm@mail.unnes.ac.id)

## SURAT PERNYATAAN

Yang bertanda tangan dibawah ini:

Nama : Dr Ratna Dewi Kusumaningtyas S. T., M. T  
NIP : 197603112000122001  
Unit Kerja : FT  
Universitas Negeri Semarang

Dengan ini menyatakan bahwa Penelitian saya berjudul:

“ULTRASOUND-ASSISTED TRANSESTERIFICATION OF NON-EDIBLE OILS USING IMMOBILIZED LIPASE FOR BIODIESEL PRODUCTION”

yang dibiayai oleh DIPA (Daftar Isian Pelaksanaan Anggaran) Universitas Negeri Semarang Nomor: SP DIPA-023.17.2.677507/2021, tanggal 23 November 2020, dengan Surat Perjanjian Penugasan Pelaksanaan Penelitian Dana DIPA UNNES Tahun 2021 Nomor 247.26.4/UN37/PPK.3.1/2021, tanggal 26 April 2021, adalah **bersifat original dan belum pernah dibiayai oleh lembaga/sumber dana lain.**

Bilamana dikemudian hari ditemukan ketidak sesuaian dengan pernyataan ini, maka saya bersedia dituntut dan diproses sesuai dengan ketentuan yang berlaku dan mengembalikan seluruh biaya penelitian yang sudah diterima ke kas BLU.

Demikian pernyataan ini dibuat dengan sesungguhnya dan dengan sebenar-benarnya.

Semarang, 26 April 2021

Mengetahui,  
Ketua LPPM UNNES

Yang menyatakan,  
Ketua Pelaksana



**Dr. Suwito Eko Pramono M. Pd.**  
NIP. 195809201985031003



**Dr Ratna Dewi Kusumaningtyas S. T., M. T**  
NIP. 197603112000122001



**KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN**  
**UNIVERSITAS NEGERI SEMARANG**  
**LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT**

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**PERNYATAAN KESANGGUPAN PELAKSANAAN PENELITIAN**

NOMOR: B/2336/UN37.3.1/PG/2021

Saya yang bertanda tangan di bawah ini :

Nama : Dr Ratna Dewi Kusumaningtyas S. T., M. T

Alamat : Jl Cempakasari Timur 26 RT 04 RW 01, Sekaran, Gunungpati, Semarang

Sehubungan dengan pembayaran uang yang diterima dari Kuasa Pengguna Anggaran Universitas Negeri Semarang (UNNES) Kode Satker (677507) sebesar Rp. 100.000.000,00 (seratus juta Rupiah), berdasarkan Kontrak Penelitian:

Tanggal : 26 April 2021

Nomor : 247.26.4/UN37/PPK.3.1/2021

Pekerjaan : Penelitian Penelitian Kerjasama Dana DIPA UNNES Tahun 2021  
ULTRASOUND-ASSISTED TRANSESTERIFICATION OF NON-EDIBLE OILS USING  
IMMOBILIZED LIPASE FOR BIODIESEL PRODUCTION

Nilai Kontrak : Rp. 100.000.000,00

Dengan ini menyatakan bahwa Saya bertanggungjawab penuh untuk menyelesaikan prestasi pekerjaan sebagaimana diatur dalam Kontrak Penelitian tersebut di atas.

Apabila sampai dengan masa penyelesaian pekerjaan sebagaimana diatur dalam Kontrak Penelitian tersebut di atas saya lalai / cidera janji / wanprestasi dan / atau terjadi pemutusan Kontrak Penelitian, saya bersedia untuk mengembalikan / menyetorkan Kembali uang ke kas BLU sebesar nilai sisa pekerjaan yang belum ada prestasinya.

Demikian surat pernyataan ini dibuat dengan sebenarnya.

Semarang, 29 April 2021

Mengetahui,  
Ketua LPPM UNNES

Yang menyatakan  
Ketua Pelaksana,



Dr. Suwito Eko Pramono M. Pd.  
NIP. 195809201985031003



Dr Ratna Dewi Kusumaningtyas S. T., M. T  
NIP : 197603112000122001