IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

PRODUCTION OF BIODIESEL FROM *Calophyllum Inophyllum* L. OIL BY LIPASE ENZYME AS BIOCATALYST

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ABSTRACT
Production of biodiesel from *Calophyllum inophyllum L* oil through transesterification by enzyme as catalyst and methanol as solvent has been done. Determinations of molecular weight of lipase enzyme used SDS - PAGE analysis. While production of biodiesel through transesterification process was conducted with molar ratio of oil to methanol in 1:12 at a temperature of 30 – 45 ºC for 3 hours use 3 % of enzym concentration. Results of analysis SDS - PAGE yielded molecular weight enzyme were26, 31, and 59 kDa . ^{1}H-NMR result showed theoretical biodiesel conversion was 74.84%, while the experimental result was 66.07%. GC-MS spectrums showed that biodiesel contents there are methyl palmitate (3,47%), methyl linoleate (4,57%), methyl oleate (9,93%), and methyl stearate (2,65%).

KEYWORDS: *Biodiesel, calophyllum inophyllum, lipase, SDS-PAGE, transesterification*

INTRODUCTION
Biodiesel is a liquid fuel that can be produced from vegetable oils or animal fats. Biodiesel has advantages such as environmentally friendly, non-toxic, it is essential that the free sulfur and cacinogenic benzene, the results of combustion is CO_2 is consumed by plants for photosynnthesis, can be relatively perfect oxygenated for burned out, decompose naturally. Besides it has the disadvantage of biodiesel is less flammable than diesel, making it easier to stockpiles, and can be blended with diesel [1]. Biodiesel is a mixture of mono-alkyl esters obtained from vegetable oils like soy bean oil, jatropha oil, rapeseed oil, palm oil, sunflower oil, corn oil, peanut oil, canola oil and cottonseed oil [7]. Apart from vegetable oils, biodiesel can also be produced from other sources like animal fat (beef tallow, lard), waste cooking oil [8], greases (trap grease, float grease) and algae [9].
Vegetable oils are promising feedstocks for biodiesel production since they are renewable in nature, and can be produced on a large scale and environmentally friendly [2,20]. Vegetable oils include edible and non-edible oils. More than 95% of biodiesel production feedstocks comes from edible oils since they are mainly produced in many regions and the properties of biodiesel produced from these oils are much suitable to be used as diesel fuel substitute [3]. However, it may cause some problems such as the competition with the edible oil market, which increases both the cost of edible oils and biodiesel [4]. Moreover, it will cause deforestation in some countries because more and more forests have been felled for plantation purposes. In order to overcome these disadvantages, many researchers are interested in non-edible oils which are not suitable for human consumption because of the presence of some toxic components in the oils. Furthermore, nonedible oil crops can be grown in waste lands that are not suitable for food crops and the cost of cultivation is much lower because these crops can still sustain reasonably high yield without intensive care [5,6, 7].
Biodiesel produced from non-edible vegetable oil species such as honge (Pongamia pinnata), jatropha (Jatropha curcas), Mahua (Madhuca indica) and honne (Calophyllum inophyluum linn) etc., could be used as alternative fuel for diesel engine. *Calophyllum inophyluum* plants (Fig.1) is a non-edible oil resources that have been carefully as basic ingredients of biodiesel. Vegetable oil like *Calophyllum inophyluum* has a heating value comparable to that of diesel fuel but its high viscosity and low volatility prohibit it to burn completely [10]. Transesterification can produce ester from vegetable oil. Transesterification is a process of producing a reaction between a triglycerides and alcohol in the presence of a catalyst to produce glycerol and ester (biodiesel). Transesterification makes the viscosity lowered [10,16], in general, there are three categories of catalysts used for biodiesel production are: alkaly, acid and enzymes [14,15,19]. Enzyme catalysts

ISSN: 2277-9655
(I2OR), Publication Impact Factor: 3.785

become more attractive to use because it can avoid the formation of soap and simple purification process [11,17,18].

MATERIALS AND METHODS

Materials

Materials used in this study is potassium phosphate, lipase enzyme [EC.3.1.1.3], aquades, phenolphthalein indicator, KOH, *Calophyllum inophyluum* L from Mollucas island, 95% ethanol, phosphoric acid, methanol, sodium sulfate anhydrous, 10% APS (Amonium persulphate) (Bio Basic Inc), TEMED (N, N, N', N' tetramethy lenediamine) (Bio Basic Inc), 30 % acrylamide (Bio Basic Inc), 0,8 % Bis-acrylamide (Bio Basic Inc), Bufer tris-glisin 10 x (Fermentas), SDS (Sodium dodecyl sulfate) (Bio Basic Inc), 50 % Gliserol (Merck), 0,1 % Bromphenol blue (Wako, Japan), Protein marker (Fermentas), Membran dialisis (MCO:14000) (Wako, Japan)

Estimate of Mass of molecule lipase (SDS-PAGE)

Protein electrophoresis by SDS-PAGE perfomed by using gel seperators 12% polyacrylamide and 5% polyacrylamide gel barrier. Printing gel in the form of two glass plate squeezed and among other things placed spacer of at edge shares, then nipped with the clip and stood the above glass plate. condensation of separating gel entered into glass plate. After the gel ossify later then entered resists gel above. Comb the well printer immediately entered shares of resists gel before gel ossify.

Plate glass that contains a gel that has been hardened incorporated into the electrophoresis apparatus and electrophoresis followed with buffer solution. Protein (20 mL) is suspended with sample buffer (5 mL) and loaded into a polyacrylamide gel as much as 15 mL. As standard proteins used standard proteins of Fermentas were loaded into the wells as much as 5 mL. Then the sample and the standard protein gel electrophoresis was rushed in for 70 minutes at a voltage of 150 V, 400 A. After electrophoresis gels were stained with CBB R-250 for 12 hours. Gel staining results and then soaked in a solution of color remover to remove excess color and protein bands were separated then measured and compared to the migration distance within the standard protein.

Preparasi of Oil of *Calophyllum inophyluum* L **seed**

Calophyllum inophyluum seeds cleaned of dirt by washing with water until clean. Then dried with a dryer and dried in the sun until dry enough, the seeds are separated from the skin danging seeds. Meat seeds that have been separately and then pressed using a pressing machine to separate oil and slag. This stage produces a rough bintanggur oil.

Deguming process

Deguming process is done by adding 20% phosphoric acid 0.5% (w / w) of oil, heated to a temperature of 80ºC for 15 minutes, so it will form compounds that easily separate phosphatides from the oil. The compound then separated by density are compounds phosphatides located at the bottom of the oil. Compounds phosphatides were separated, then the oil is washed with warm water temperature 60ºC until clear. Furthermore, water is evaporated from the oil with a vacuum dryer at a temperature of 80ºC to prevent oxidation reactions that can change the color of the oil becomes dark oil kembali.Selanjutnya characterized using IR spectrophotometer.

Figure 1. *Calophyllum inophyluum*

Analysis of Free Fatty Acy

5 g samples were put into a 250-ml Erlenmeyer flask, add 50 ml of 95% alcohol. Furthermore, the heating for 10 minutes in a water bath until boiling. Then cooled and added a few drops of indicator fenoftalein. After that is done titration with KOH until just pink. Levels of free fatty acids is calculated based on the formula:

$$\text{Free fatty acid} = \frac{a \times M \times 284 \times 100\ \%}{b}$$

$$\text{Acid number} = \frac{\text{mL KOH} \times \text{N KOH} \times 56{,}1}{\text{g sampel}}$$

Wherein:
a = Volume KOH
M = Molaritas KOH
b = Sample weight (gram)
284 = Mr stearate acid (gr/mol)

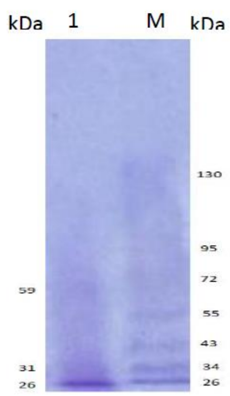
Synthesis of Biodiesel from oil of *Calophyllum inophyluum* seed

Transesterification is done with comparison oil and methanol 1: 12 and added to the lipase enzyme catalyst with variation of 3% by weight of heavy oil and methanol. Refluxing the mixture at a temperature of 30-45 0C for 3 jm. The reaction mixture is cooled and formed three layers, namely a row from top to bottom methyl ester (biodiesel), glycerol, and catalysts. Then the catalyst is separated from the layer of methyl eater and glycerol, then a layer of methyl ester and glycerol is separated by using a separating funnel. Then methyl ester is evaporated to remove the remaining methanol. Methyl ester is then washed with distilled water in a separating funnel to dissolve the rest of the glycerol. The final step is the addition of anhydrous Na2SO4 to tie the remnants of water, then filtered with Whatman filter paper 40. Methyl ester produced from the reaction of trasesterifikasi analyzed by IR spectrophotometer, GS-MS, and ^{1}H-NMR.

RESULTS AND DISCUSSION

Estimate of Molecule Mass Lipase (SDS-PAGE)

The commercial Enzyme Lipase determined molecule mass with the method analyse SDS-PAGE (Sodium of Dodesil Sulfate Polyacrilamide of Gel Electrophoresis). Result of analysis by SDS-PAGE showed at Fig. 2, showing the existence of 3 ribbon from commercial enzyme lipaase at migration distance that is 6.6, 6.4, and 5.6 cm of molecule weighing successively 26, 31, and 59 kDa.



M = Marker; 1= commercial lipase enzyme

Figure 2. Results of a commercial lipase enzyme electrophoresis.

Preparasi of Oil of *Calophyllum inophyluum* L **seed**

Calophyllum inophyluum oil was produced by extracting the seeds bintanggur using a pressing machine. The most appropriate way to separate the oil from the material oil content above 10%. According Bustomi et al. (2008) pulp from the seeds pressing process bintanggur still has a fairly high oil rendamen approximately 48.8%. Oil produced from the pressing process is black or dark, so the oil must be separated before use [12].

***Degumming* process**

Degumming conducted by enhancing phosphoric acid 20% equal to 0,5% (b / b) oil. Then heated at temperature 80ºC during 15 minute, then cleaned by aquades previous have been heated at temperature 60ºC, result deguming will show the very clear difference with the original oil that is from green colour black become the redish clear colour (Fig.3(a, b)). Hereinafter oil bintanggur result of *deguming* done a analysis of free fat. Result analysis free fat obtained equal to 9,52% and its acid number equal to 18,93 mg KOH / g.

Figure 3. Nyamplung Calophyllum inophyllum L Oil before degumming (a) and Calophyllum inophyllum L oil after degumming

Synthesis of biodiesel through transesterification process

Transesterification with lipase enzyme catalyst performed at a temperature of 30-45ºC for 3 hours with a 1:12 ratio of oil and methanol, thus forming three layers successively from top to bottom biodiesel, glycerol, and the catalyst, then separated. Biodiesel and glycerol is separated by using a separating funnel.

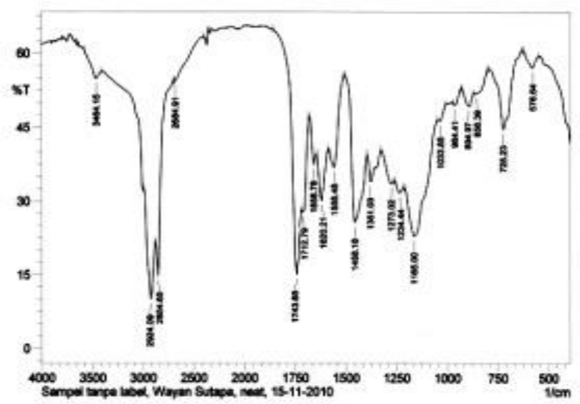
Figure 4. Biodiesel

Biodiesel is formed and then evaporated to remove the remaining methanol. After evaporation process then washed with distilled water that has previously been heated at a temperature of 60 °C, the washing process will form two layers of the top and bottom of the methyl ester is distilled water and residual glycerol. Once separated from the distilled methyl esters was then given Na_2SO_4 anhydrous, further filtering with Whatman filter paper 40 in order to obtain biodiesel by weight 66.07%.

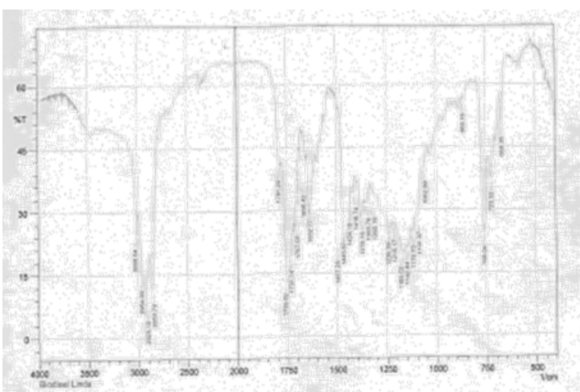
Characterization of Biodiesel

Biodiesel analysis by infrared spectroscopy (FT-IR)

The results of FT-IR spectrophotometer identification of biodiesel from oil bintanggur shows the uptake of CO group at 1165.02 areas, group C = O at 1707.03 to 1739.82 area, the CH at 2853.73 to 2925.10 area, the area in 1434 , a 10-1457.25 absorption $-CH_2-$ groups, group $-CH_3-$ the catchment area from 1350.19 to 1378.16, and the group -CH = CH- in the catchment area from 723.32 to 758.04.



(a)



(b)

Spectroscopic analysis of biodiesel by GC-MS

The results of spectroscopic analysis by GC-MS showed the content of methyl palmitate ($C_{17}H_{34}O_2$) of 3.47%, methyl linoleic ($C_{19}H_{34}O_2$) of 4.57%, methyl oleate ($C_{19}H_{36}O_2$) amounted to 9.93%, and methyl stearate ($C_{19}H_{38}O_2$) of 2.65%.

Analysis of biodiesel with 1 H-NMR spectroscopy

^{1}H-NMR chemical shift seen at 4.1 ppm was methyl ester and shift peak at 5.3 to 5.4 ppm appears protons attached to carbon olefinic in biodiesel and α-CH2 protons in the region of 2.7 ppm. Conversion of biodiesel that is formed is known to use equation [13]:

$$C_{ME=100\times\frac{2\times I_{ME}}{3\times I_{\alpha-CH_2}}}$$

conversion of biodiesel obtained by 74.84%, while the experimentally obtained 66.07% biodiesel conversion.

CONCLUSION

Based on the research that has been done, it can be concluded that the conversion of biodiesel obtained based on the theoretical results of 1H-NMR amounted to 74.84% and amounted to 66.07% experimentally in heavy catalytic transesterification reaction with lipase 3%. GC-MS test results showed the presence of methyl ester content of biodiesel is methyl palmitate 3.47%, 4.57% linoleic methyl, methyl oleate 9.93%, and 2.65% methyl stearate.

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