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Utilization of n-Hexane as Co-Solvent to Increase Biodiesel Yield on Direct Transesterification Reaction from Marine Microalgae

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Abstract

Direct transesterification reaction requires optimization of reaction conditions due to both the lipid extraction and lipid transesterification reactions that occur at the same place and time. Co-solvent utilization is considered as one of some ways to improve the yield in the direct transesterification reaction. In this study, transesterification was done directly on the marine microalgae biomass *Nannochloropsis* sp. Design variations in this research including the volume ratio of methanol: n-hexane, the molar ratio of lipid: methanol, and reaction time. From these variations, the volume ratio of methanol : n-hexane 1:1, molar ratio of lipid:methanol 1:400, and reaction time 4 hours can increase biodiesel yield until 90.9% for *Nannochloropsis* sp. FAME contents were analyzed by Gas Chromatography and Mass Spectroscopy (GCMS). Saturated fatty acid content is more dominant on biodiesel reached 52.72%.

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Keywords: Biodiesel, co-solvent, direct transesterification, *Nannochloropsis* sp, n-hexane

1. Introduction

Microalgae is autotrophs microorganisms which has three main components, those are carbohydrates, proteins and lipids¹. Microalgal biomass has been developed into food, animal feed, and high value bioactive substances because of the potential content of carbohydrate and protein². Meanwhile, the lipid content in microalgae can be developed into biodiesel. This study will use a species of chlorophyta marine microalgae that is *Nannochloropsis* sp. with lipid content that can reach 32%². Microalgae class of chlorophyta accumulate oils generally higher than other algal taxa such as red algae or brown algae³.

The method for microalgae biodiesel production usually uses transesterification reaction that initiated by lipid extraction. But, this method can reduce lipid content along the extraction and separation process prior to the transesterification reaction. Therefore, to reduce the possibility of lipids loss during this process, transesterification will be done directly on the biomass. Direct transesterification is able to increase biodiesel yield of *Chlorella* sp.

from 63% to 83%⁴. On large scale, this alternative reaction is expected to reduce unit production operations and reduce the biodiesel production cost needed.

Direct transesterification reaction requires optimization of reaction conditions due to lipid extraction and transesterification reactions that occur at the same place and time. Utilization of co-solvent is considered as one way to increase biodiesel yield in the direct transesterification reaction. This is happened because co-solvent will be able to increase the solubility of alcohol on lipid and accelerates direct transesterification⁵. This utilization of co-solvent is also expected to extract lipids before finally react with alcohol. The use of molar ratio of 1:52 pentane and diethyl ether with 1:79 molar ratio increases the yield until 99%⁶. The use of n-hexane with volume ratio to methanol 1:1 also increases FAME conversion up to 94%⁷. This research will use n-hexane as co-solvent and the effects of its use in the conversion of microalgae biomass to biodiesel will be examined.

The molar ratio of alcohol, either methanol or ethanol, determine the conversion of triglycerides into biodiesel. In direct transesterification reaction, the alcohol required would be greater because it has both the function of extracting lipid out of the cell and converting lipid into biodiesel. In some studies, the use of lipid to methanol molar ratio of 1:300 to 1:370 increase biodiesel yield up to 93.2 and 98.2%⁵. In this research, effect of molar ratio of lipid to methanol will be examined.

Direct transesterification reaction variables such as reaction time will also be examined in this study. Optimization of time on direct transesterification reaction is able to convert lipids into biodiesel with the percentage reached 91.3%⁸. In the production of biodiesel from microalgae, different reaction time affect the yield of biodiesel if done on reaction with different catalysts. At 45 minutes, the largest biodiesel conversion occurs in the reaction with the lipid to catalyst ratio of 0.35. While at the time of 75 minutes, there was no significant difference in the rate of biodiesel conversion with H₂SO₄ catalyst ratio of 0.15, 0.25, or 0.35, where the ratio of the smallest catalyst was chosen to minimize the consumption of catalyst⁹. Based on these studies, then these factors need to be investigated to determine the appropriate operating conditions for the production of biodiesel with high conversion from *Nannochloropsis* sp. biomass.

The results of this direct transesterification reaction study will be compared to transesterification that initiated by lipid extraction with the optimum operating conditions of direct transesterification. Response variables that will be considered is the mass of biodiesel obtained gravimetrically. Fatty acid alkyl esters content will then be analyzed using gas chromatography and mass spectroscopy (GCMS).

2. Materials and Method

2.1. Materials

Initial cultures of microalgae *Nannochloropsis* sp. obtained from LIPI. The materials for medium walne including metal solution: ZnCl₂, CoCl₂.6H₂O, (NH₄)₆Mo₇O₂₄.4H₂O, CuSO₄.5H₂O (Merck); Nutrition solution: FeCl₃.6H₂O, MnCl₂.4H₂O, H₃BO₃, EDTA, NaH₂PO₄.2H₂O, NaNO₃ (Merck); and vitamin solution: cyanokolobamin, thiamin, biotin (Sigma-Aldrich). For transesterification reaction, n-hexane, methanol, aquadest (Bratachem) and KOH (Merck) as catalyst were used.

2.2. Equipment Sterilization

Equipment sterilization procedures performed by washing the utensils with soap and rinsed with water, drying the equipment, and then wrapping the equipment with plastic wrap to avoid contamination after sterilization. Next, the equipments were rinsed by alcohol 70% for 5 minutes and then rinsed by water. Sterilized equipments then stored in the storage closet.

2.3. Medium Preparation

The solution is made by mixing ingredients with sea water. Materials stocks were mixed in sea water and cooled before being inserted into the photobioreactor. Medium used in this study was walne medium.

2.4. Equipment Assembling

This study uses 18 L photobioreactor. Photobioreactor to be used are put in a position parallel to and facing the halogen lamp as the illumination source. Flowmeter calibration is done so that the scale of each flowmeter can be known exactly. This is important because the gas stream containing CO₂ must be kept constant. Each hose connection pipe is coated with a seal so that no connection leaking and prevents contaminants enter the circuit equipment. Illumination sources used are two halogen lamps with the power of the light intensity to 5000 lx. The series of equipment used is illustrated in Fig.1.

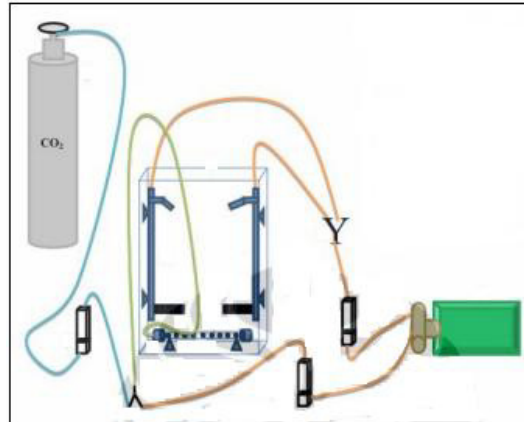


Fig. 1. Cultivation System¹⁰

2.5. Cultivation, Dewatering and Biomass Recovery

Microalgae cultivation is done by entering the medium into the 18 L photobioreactor. The temperature is maintained at room temperature and neutral pH is maintained with the addition of low concentrations of HCl or CO₂. Light intensity of 3000-5000 lx is used. Dewatering is done by using a ceramic membrane which is inserted in watering house. After 204 hours, microalgae were flowed into the watering house for about 30 hours and the contents are allowed to settle in the bottom of watering house. The precipitate was taken and dried at room temperature for 1 day or with the help of heating with temperatures up to 70°C.

2.6. Optical Density Measurement

Measurement of optical density (OD) conducted to determine the cell growth. By knowing the value of OD, the growth of microalgae cultivated will be known. Microalgae growth is marked with the increasing value of measurable OD. OD value was measured every 6 hour until total time of cultivation during 204 hours. This measurement utilize the spectrophotometer which regulated at a wavelength of 540 nm. To see the value OD on this research spectrophotometer single beam is used, and visible light (VIS) becomes light source which will be absorbed by microalgae.

2.7. Direct Transesterification Reaction

Biomass that has been obtained is mixed with n-hexane according to predetermined volume variations in the boiling flask that was connected to the heater and condenser. Methanol was mixed with KOH and then inserted to the flask. Temperature of 60°C is maintained. After the reaction is complete, the reaction product was cooled for 10 minutes. The mixture was centrifuged to separate biodiesel phase with glycerol phase. Distilled water added as many as 10% of the biodiesel phase obtained and biodiesel is washed until the water phase is clear. Biodiesel phase

is poured into an empty vial bottles and solvent remained is allowed to evaporate. Biodiesel yield was calculated gravimetrically using Equation 1.

$$\text{Biodiesel yield} = \text{biodiesel dry weight (g)} / \text{lipid dry weight (g)} \times 100\% \quad (1)$$

2.8. Transesterification Initiated by Lipid Extraction

Biomass that has been obtained is mixed with n-hexane and sonicated for 20 minutes. Lipid phase then distilled at temperature of 60°C. Methanol was mixed with KOH and then mixed with lipids. Temperature of 60°C is maintained. Furthermore, the acquisition of biodiesel and yield calculation was done as outlined before.

2.9. FAME Content Analysis

In this study, the measured parameter is the yield of biodiesel obtained. The content of the fatty acid alkyl ester was then analyzed to determine if the biodiesel contains general fatty acid alkyl esters of biodiesel. The content of fatty acid alkyl esters were analyzed using Gas Chromatography and Mass Spectrophotometry (GCMS) in Central Forensic Laboratory, the Indonesian Police Headquarters (Police Headquarters Forensic Laboratory).

3. Result and Discussion

3.1. Effect of Volume Ratio of Methanol to N-hexane on Biodiesel Yield

In direct transesterification reaction studied, dried microalgae biomass is used directly in the reaction. Effect of the addition of n-hexane as co-solvent and how much of the volume ratio to methanol used can be seen in Fig. 2. In this variation the constant variables are reaction time of 2 hours, the ratio of lipid:methanol 1:200, KOH 2% mass of biomass mass, biomass mass of 1 g, and reaction temperature of 60°C. The lipid content of the biomass dry basis is 3.4%. The addition of n-hexane with 1:1 volume ratio to methanol increase the yield of biodiesel up to 5% compared to the ratio of 1:0 or without the use of n-hexane. N-hexane causes lipid to contact first with n-hexane which then reacts with methanol. However, the addition of n-hexane to 1:1.5 did not increase the yield of biodiesel. The excess n-hexane is estimated to reduce lipid contact with methanol so that the resulting product is reduced. Another thing

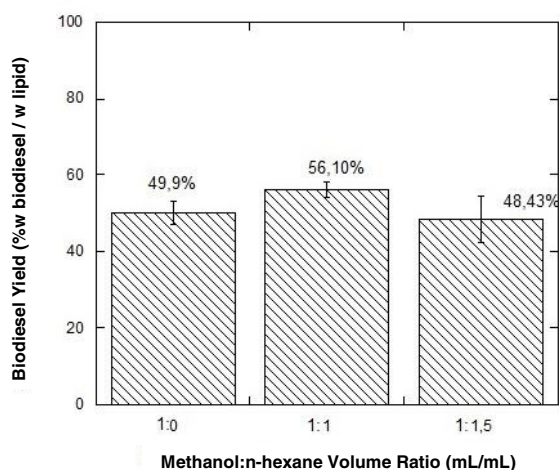


Fig. 2. Effect of volume ratio of methanol to n-hexane on Biodiesel Yield

that can be predicted is n-hexane excess reduces the mass transfer between lipid and methanol also reduces transfers heat so that the transesterification reaction is not running optimally.

In other studies using ethanol as a reactant in the direct transesterification of *Chlorella* sp., the addition of co-solvent with the volume ratio of 1:1 ethanol also increased the yield up to 94.3%⁷. The use of co-solvent diethyl ether in a direct transesterification of *Chlorella* sp. with methanol increases the yield to 79% with the molar ratio of methanol and diethyl ether, respectively for oil 1:79⁶. The addition of co-solvent capable of increasing the yield on a certain amount of usage. The addition of co-solvent is also expected to help methanol in contact with the lipid. Polar methanol has a poor ability to extract the lipids that tend to be non-polar because of its CH.

3.2. Effect of Lipid to Methanol Molar Ratio on Biodiesel Yield

On lipid:methanol molar ratio variation, constant variables are kept microalgae mass 1 g, KOH mass 2% microalgae mass, volume ratio of methanol: n-hexane 1:1, reaction time 2 hours, and the temperature of 60°C. The addition of methanol molar ratio of 1:400 increase the yield of biodiesel by nearly 80% as shown in Fig. 3. Excess reactants such as methanol continue to shift the reaction equilibrium to the product side so that the reaction products will increase. Increased yield can also occurred due to the increasing number of methanol used make more extensive contact between lipids and methanol. In this variation, the volume ratio of methanol: n-hexane 1:1 is maintained. With the addition of methanol in a molar ratio of up to 1:400 this means that n-hexane used also increased. This means the addition of n-hexane, for the same volume ratio with methanol, give no big impact on reducing the yield of biodiesel as the use of n-hexane with a volume ratio of 1:1.5. The profile is consistent with the notion that with the addition of n-hexane then lipids were successfully drawn more and with the addition of methanol the reaction equilibrium shifts towards products. Overall, the addition of a molar ratio of methanol to volume ratio of 1:400 with methanol:n-hexane volume ratio 1:1 was also successful in increasing the yield of biodiesel significantly.

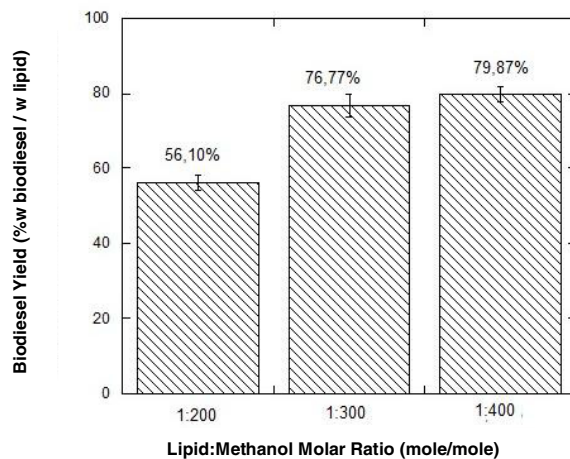


Fig 3. Effect of Lipid Molar Ratio on Methanol in Biodiesel Yield

3.3. Effect of Reaction Time on Biodiesel Yield

In this variation, the variables kept constant are microalgae mass of 1 gram, KOH mass 2% microalgae mass, molar ratio lipid: methanol 1:200, and the volume ratio of methanol:n-hexane 1:1. Effect of reaction time variations can be seen in Fig. 4. Reaction time up to 4 hours can increase biodiesel yields approaching 70%. However, the addition of up to 6 hours of reaction time did not increase the yield of biodiesel. Transesterification reaction is a

reversible reaction. This yield reduction can occur causing the reverse reaction, thereby reducing the resulting product. It is also in line with studies on *Chlorella* sp direct transesterification with room temperature where the reaction time up to 10 hours increases the yield to 65% biodiesel. However, the yield decreased when the reaction was continued for up to 12 hours⁴. This decrease can also occur because when the transesterification reaction has lasted long enough, then the fewer catalysts used in the reaction⁴. The remaining catalyst was able to react with the free fatty acids causing saponification and reduce the conversion of reactants into biodiesel.

We also examined the effect of time by using a molar ratio of lipid:methanol 1:400 to see trends on the molar ratio of greater methanol. This influence is examined at the time of 2 hours and 4 hours as shown in Fig. 5. With the molar ratio of lipid:methanol 1:400, volume ratio of methanol:n-hexane 1:1 can increase yield by 90% within 4 hours. This means that 4 hours is enough to extract most of the lipids contained in the biomass and the reaction with methanol without significant backlash in reducing the yield of biodiesel.

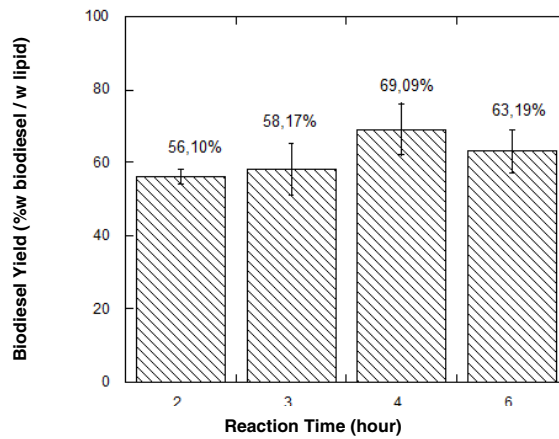


Fig. 4. Effect of Reaction Time on Biodiesel Yield

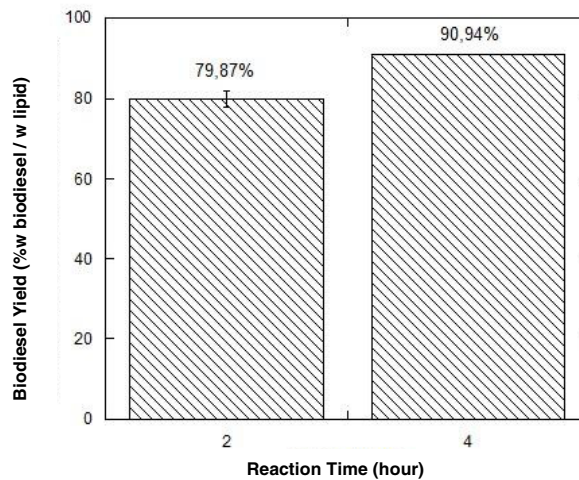


Fig. 5. Effect of Reaction Time on Biodiesel Yield (molar ratio of lipid: methanol 1:400)

3.4. Comparison with the transesterification reaction initiated by Extraction

Direct transesterification reaction results of microalgae then compared to the transesterification reaction initiated by extraction. Transesterification initiated by extraction given the same treatment such as molar ratio of lipid: methanol, volume ratio of methanol: n-hexane, and reaction time. N-hexane used in the reaction initiated by extraction not added to the direct transesterification reaction. The results of this comparison can be seen in Fig. 7. on *Nannochloropsis* sp. biodiesel yield increased from 78% to 90%. From this it can be estimated that there is possible loss of lipids during the extraction process to transesterification.

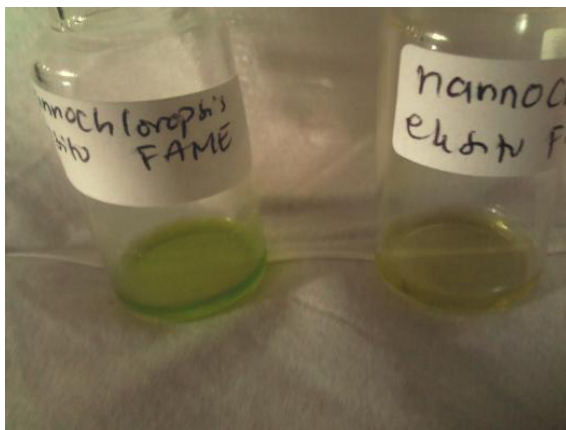


Fig. 6. Biodiesel from *Nannochloropsis* sp. by direct transesterification (left) and transesterification initiated by extraction (right)

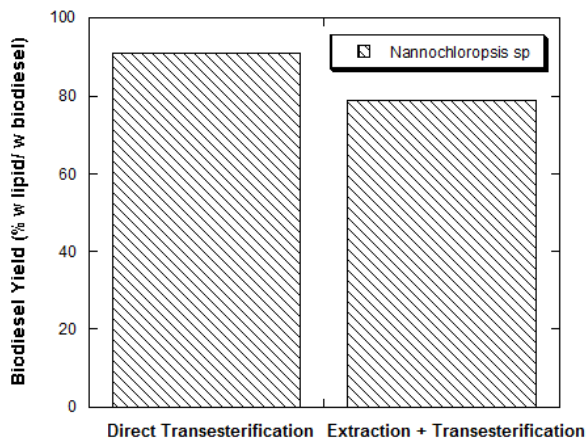


Fig. 7. Comparison of Biodiesel Yields on Direct transesterification and transesterification initiated by extraction

3.5. Analysis of Fatty Acid Methyl Esters by GCMS

The comparison of FAME from direct transesterification and transesterification initiated by extraction can be seen in Table 1. In general, biodiesel from *Nannochloropsis* sp. has similar composition of saturated and unsaturated

FAME. The only difference that can be seen is the percent area in the direct transesterification of microalgae is greater. It shows that this method produces greater FAME biodiesel.

Table 1. Fatty Acid Methyl Esters Detected in *Nannochloropsis* sp. by Direct Transesterification and extraction + Transesterification

FAME (%)		Direct Transesterification	extraction +Transesterification
Methyl dodecanoat	C12:0	0,56	-
Methyl Myristate	C14:0	5,82	10,91
Methyl Myristoleic	C14:1	-	-
Metil pentadecanoic	C15:0	0,59	-
Methyl Palmitic	C16:0	42,02	48,31
Methyl palmitoleic	C16:1	28,05	26,77
Methyl heptadecanoic	C17:0	0,61	-
Methyl stearic	C18:0	3,12	-
Methyl Oleic	C18:1	10,49	14,01
Methyl Linoleic	C18:2	1,58	-
Methyl Linolenic	C18:3	-	-
Methyl Eicosenoic	C20:1	-	-
Methyl arachidonate	C20:4	2,01	-
Methyl Behenate & Eicosapentanoic	C22:0 C20:5	5,15	-
Methyl Ester Total		100,00	100,00
Total Saturated Fatty Acids Methyl Ester		52,72	59,22
Total Unsaturated Fatty Acid Methyl Ester		47,28	40,78
The % total area		83,57	56,9

4. Conclusion

From this study it can be concluded that the addition of n-hexane as co-solvent in the transesterification reaction able to increase the yield of biodiesel from marine microalgae *Nannochloropsis* sp. with the following conditions:

1. The volume ratio of methanol: n-hexane 1:1 reaction time up to 4 hours with the addition of 1:400 molar ratio of methanol increased the yield of biodiesel up to 90.94%
2. GCMS test results show that the content of saturated FAME is not much different with the unsaturated FAME. The direct transesterification give more biodiesel with % total area Of 83.57%.

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References

1. I. Rawat, R. Ranjith Kumar, T. Mutanda, and F. Bux. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Appl. Energy*. Mar. 2013. **103**: 444–467.
2. Y. Chisti. Biodiesel from microalgae. *Biotechnol. Adv.* 2007. **25**: 294–306.
3. Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, and A. Darzins. Microalgal triacylglycerols as feedstocks for biofuel production : perspectives and advances. 2008. pp. 621–639.
4. X. Ma. Biodiesel Production from Algae through In Situ Transesterification Technology. 2012. University of Minnesota.

5. O. D. Samuel, M. Eng, O. U. Dairo, and D. Ph. A Critical Review of In-situ Transesterification Process for Biodiesel Production. 2012. **13**: 72–79.
6. E. a. Ehimen, Z. Sun, and G. C. Carrington. Use of Ultrasound and Co-Solvents to Improve the In-Situ Transesterification of Microalgae Biomass. *Procedia Environ. Sci.*. Jan. 2012. **15**: 47–55.
7. E. Martinez-guerra, V. G. Gude, and A. Mondala. Extractive-Transesterification of Algal Lipids under Microwave Irradiation. *Bioresour. Technol.* 2014.
8. E. a. Ehimen, Z. F. Sun, and C. G. Carrington. Variables affecting the in situ transesterification of microalgae lipids. *Fuel*. Mar. 2010. **89**: 677–684.
9. S. B. Velasquez-Orta, J. G. M. Lee, and a. Harvey. Alkaline in situ transesterification of *Chlorella vulgaris*. *Fuel*. Apr. 2012. **94**: 544–550.
10. I. Margaret. Optimasi Rasio Polaritas Pelarut dan Waktu Perendaman dalam Metode Perkolasi untuk Ekstraksi Lipid *Nannochloropsis* sp. Universitas Indonesia. 2013.