Vol 1 No 4 2024 || E-ISSN xxxx-xxxx

The Journal of Academic Science

journal homepage:
https://thejoas.com/index.php/

Effect Of Time and Catalyst Type On Improved Extraction Yield And Lipid Quality As Biodiesel Feedstock From Marine Microalgae Nannochlropsis Oculata In Situ Transesterification Method Microwave Assisted Extraction

[1]Sapto Raharjo, [2]Iwan Jaya Samudra
Halu Oleo University, Kendari, Indonesia[1,2]
Email: saprjo@yahoo.com, iwanjayasamudra19@gmail.com

KEYWORDS
Nannochloropsis oculata, Biodiesel, GC-MS, SEM, EDS, SNI

ABSTRACT

This study investigates the effect of time and catalyst type on the extraction yield and lipid quality of biodiesel feedstock from marine microalgae Nannochloropsis oculata using the in-situ transesterification method with Microwave Assisted Extraction (MAE). The increasing human population has led to a higher demand for fuel, which is not proportional to the availability of fuel resources, making fuel shortages inevitable. One solution to reduce dependency on fossil fuels is to develop environmentally friendly alternative fuels, such as biodiesel. Biodiesel, derived from plant oils or animal fats, has properties similar to diesel oil. Among Indonesia's vast natural resources, microalgae have significant potential as an alternative raw material for biodiesel production, with Nannochloropsis oculata containing 31-68% lipids. This research explores the transesterification reaction of microalgae oil and methanol using H2SO4 and KOH catalysts, with reaction times of 40, 60, and 80 minutes. The lipid extraction process utilizes MAE, which involves both esterification and transesterification to yield crude biodiesel. Subsequent filtration separates the biodiesel from the formed glycerol. The study aims to determine the extraction yield, Fatty Acid Methyl Ester (FAME) composition, microalgae morphology, and biodiesel quality through % yield calculation, GC-MS analysis, SEM-EDS morphological analysis, and various SNI quality tests. Results indicate that the % yield of biodiesel increases with longer reaction times for both catalysts, with H2SO4 producing higher yields than KOH at all times. GC-MS analysis reveals that the primary FAME components using H2SO4 are methyl hexadecenoate (30.54%) and methyl hexadecenoic (30.08%), while KOH yields are dominated by methyl hexadecenoic (3.83%). SEM-EDS confirms the microalgae as Nannochloropsis oculata, consistent with its cell morphology and elemental composition. SNI tests show that H2SO4 increases density with reaction time (1.0127-1.0341 g/mL), while KOH decreases (0.8213-0.6716 g/mL), with the 40-minute KOH reaction yielding biodiesel closest to SNI standards (0.85-0.89 g/mL). Viscosity tests for both catalysts fall within SNI ranges (2.3-6.0), and acid numbers are well below maximum SNI limits, indicating excellent quality. Total glycerol values for both catalysts (0.1326%-0.2318%) meet SNI 7182:2015 standards, with the highest at H2SO4 40 minutes (0.2318%) and the lowest at H2SO4 60 minutes (0.1326%).

1. INTRODUCTION

The number of human populations is increasing, but it is not proportional to the availability of fuel that exists today. This has led to a world fuel shortage that is difficult to avoid. Global energy sources are currently dominated by fossil fuels which are depleting over time. Many countries are starting to look for and test renewable fuel alternatives to replace or increase the supply of fossil fuels. The development of alternative energy sources must consider economic and ecological factors, namely systems that are capable of producing large amounts of energy at low cost and minimal impact on the environment. One of the alternatives that meets these criteria is the use of vegetable oil as a biofuel.

In Indonesia, some of the sources of biofuel raw materials come from palm oil, sunflower, soybeans, jatropha and corn. However, these materials have disadvantages such as long harvest periods, dependence on large areas of arable land, and the potential for increased food prices due to competition with food needs. Vegetable oils are generally biodegradable and more environmentally friendly than fossil fuels. One of the potential vegetable oils as an alternative fuel is microalgae. Indonesia, with its marine wealth, has great potential to develop microalgae as an alternative energy source.

This research will focus on microalgae of the Nannochloropsis oculata type which has a high lipid content and can be used for biodiesel production. This microalgae was chosen because of its easy-to-cultivate and fast-growing nature, as well as having a high enough lipid content to be processed into biodiesel. This study will explore the influence of catalyst type and reaction time on biodiesel results obtained from the in situ transesterification process using the Microwave Assisted Extraction method.

This study aims to determine the amount of yield produced, the composition of Fatty Acid Methyl Ester (FAME) content, the morphology of the microalgae used, and the quality of the biodiesel produced. The tests include %yield analysis, GC-MS analysis, morphological analysis using SEM-EDS, and several biodiesel quality parameters according to SNI standards.

2. METHOD

Location and Time of Research

This research was carried out from November 2023 to April 2024 at the Integrated Laboratory of Halu Oleo University, Kendari, Southeast Sulawesi.

Tools and Materials

The tools used in this study include:

1. Beakers (Iwaky Pyrex) with capacities of 50 mL, 100 mL, 250 mL, and 500 mL
2. Measuring cup (Iwaky Pyrex) with a capacity of 25 mL
3. Oven (Gallencamp England)
4. Droplet pipette
5. Shaker
6. Vial bottles 250 mL
7. Stirring rod
8. Scales (Eksplorer Ohaus: max 210 g and min 0.001 mg)
9. Spatula
10. Funnel
11. Petri Cups
12. Knife
13. Blender
14. Sieve
15. Jars
16. Scissors
17. Magnetic stirrer
18. Vacuum pump
19. Erlenmeyer
20. Buchner Funnel
21. Separation funnel
22. Sample Bottle
23. Distillation device device
24. Microwave

25. SEM (Scanning Electron Microscope)
26. EDS (Energy Dispersive X-ray Spectroscopy)
27. GC-MS (Gas Chromatography-Mass Spectrometry)

The materials used in this study include:

1. Microalgae Nannochloropsis oculata
2. Aquades
3. Methanol (CH3OH) 96%
4. N-hexane
5. H2SO4 98%
6. KOH 98%
7. Whatman filter paper no. 40

Research Flow

The stages of this research are carried out by following the following flow:

1. Microalgae Sampling: Nannochloropsis oculata microalgae are collected and prepared for the extraction process.

2. Sample Preparation: 100 grams of microalgae powder is put into a beaker, then 500 mL of methanol is added with a ratio of the mass of microalgae
3. 1:5 g/mL. The mixture is homogenized using a magnetic stirrer for 3 hours at a speed of 300 rpm.

4. In situ transesterification using acid catalysts (H2SO4): The process of in situ transesterification with acid catalysts is carried out to convert microalgae lipids into biodiesel.

5. In Situ Transesterification Using Base Catalysts (KOH): In situ transesterification with alkaline catalysts is carried out as an alternative to convert microalgae lipids into biodiesel.

6. Microalgae Oil Yield: Measurement of oil yield resulting from the extraction process using the Microwave Assisted Extraction (MAE) method.

7. FAME (Fatty Acid Methyl Ester) analysis with GC-MS: The analysis was carried out to determine the composition of FAME in the biodiesel produced.

8. Biodiesel Quality Test According to SNI: The biodiesel produced is tested for quality in accordance with SNI standards.

This research process aims to determine the amount of yield produced, the composition of FAME content, the morphology of microalgae used, and the quality of biodiesel produced.

3. RESULT AND DISCUSSION

Biodiesel is a fuel that can be used as a substitute for diesel which is a derivative ester compound from a long chain of fatty acids. Biodiesel is composed of mono alkyl esters from fatty acids derived from renewable lipid sources such as vegetable oils and animal fats, which are used as fuel for one of the plants that can be used as biodiesel, namely microalgae. Microalgae are living things that can photosynthesize, microalgae only need sunlight to grow and several other simple food sources when compared to other biodiesel source plants, microalgae have a fairly high growth rate and productivity and require a relatively low land area (Ahmad et al, 2010). The source of this very promising biodiesel raw material is microalgae and one of the widely studied microalgae species is Nannochloropsis oculate (N. oculate).

The selection of the Nannochlropsis Oculata microalgae because it can produce oil of 46.47% (Purnama et al, 2020), this value is quite large when compared to other types of microalgae. The oil content in N. oculata is quite high, reaching 31-68%. Its existence in nature is very abundant and easy to cultivate en masse. The harvest period of these microalgae is quite short and the cost for cultivation is also relatively cheap (Sukarni et al, 2014). The fatty acid content of N. oculata microalgae is generally dominated by palmitic acid (C16:0), palmitoleic acid (C16:1) and oleic acid (C18:0), making it suitable as a raw material for biodiesel.

The research "The Effect of Time and Catalyst Type for Improving Extraction Yield and Quality of Lipids as Biodiesel Raw Materials from Marine Microalgae Nannochlropsis Oculata Transesterification Method In Situ Microwave Assisted Extraction" was conducted at the Integrated UPT Lab Laboratory, Halu Oleo University. The raw materials used are in the form of Nannochlropsis Oculata microalgae, which are obtained from BBAP Takalar and also Megaplankton Jepara, Central Java. These microalgae are used because they have a high lipid content so they have the potential to be used as raw materials for biodiesel.

The use of technology in the form of microwaves in this study was chosen because of its many advantages, in previous studies using microwaves reported that its use is very simple and also effective for lipid extraction. In an effort to remove lipids contained in cells, there needs to be a breakdown process in the cell wall, where there is a difference between conventional heating or using a microwave. Conventional heating uses excess heat if you want to get oil quickly which has an impact on decreasing the quality of the extracted oil, while using microwaves is utilizing heat through electromagnetic micro waves, so that excessive heat use is not necessary to avoid damage to the extracted oil. In addition to relying on micro electromagnetic waves generated by microwaves, it is also important to use solvents in the extraction process.

This study uses a solvent of methanol compounds, the use of methanol has a role in addition to functioning as an extractor, but also as a reactant. The use of methanol as a reactant is because methanol is a compound that tends to be very polar because it has a hydroxyl group (-OH) although it also has non-polar properties because it has a methyl group (-CH3) (Lazuardi, 2016) and has a shorter alcohol chain.

The extraction results can also be affected by the type of catalyst used, the catalyst plays a fairly important role in improving the extraction yield and also the quality value of the extracted oil, besides that the use of the catalyst can allow the reaction to continue to run under normal operating conditions when compared to not using a catalyst that requires high temperature and pressure. The catalyst in this study is divided into 2 parts, namely the H2SO4 acid catalyst and also the KOH base catalyst.

Extraction and characteristics of Nannochlropsis Oculata microalgae oil using the Microwave Assisted Extraction method

This research has been carried out microalgae oil extraction experiments using the Microwave Assisted Extraction (MAE) method. This MAE method uses microwaves as its energy source, so it has better control over temperature than conventional heating processes. In addition, MAE also has several advantages, including shorter extraction time, less energy and solvency consumption, and higher accuracy and precision

(Barqi, 2015).

In situ Transesterification Process

The first stage before going through the in situ transesterification process, a homogenization process is carried out between the microalgae Nannochlropsis Oculata and methanol with the aim of helping to attract oil that is still trapped in the microalgae cells. After going through the Homogenization process, then the in situ Transesterification process in an effort to produce biodiesel. The in situ transesterification process has several advantages, including that this process can make the extraction time faster and also reduce the use of solvents (Liu et al, 2016), so that biodiesel can be obtained more optimally and economically and also the use of heat is not excessive so as to avoid reducing the quality of the biodiesel oil produced.

The mechanism of In situ transesterification occurs as a stimulant. The oil that has been extracted from the microalgae cells will be directly conjugated to the form of FAME. The use of microwaves is part of the in situ Transesterification process which provides a thermal effect whose source comes from microwaves which can increase alcohol to extract oil from biomass. The heat of microwave radiation heats and evaporates the water on the cell. So that the pressure on the cell wall will increase, as a result of which the cell will undergo swelling. The pressure pushes the cell wall from the inside so that the cell stretches and ruptures, the damage to the material matrix makes it easier for the target compound to come out and be extracted (Jain et al, 2009). The results of transesterification showed that there were 2 layers that did not mix with each other, where methyl ester was in the upper layer and the lower layer was an impurity (a mixture of glycerol and fatty acid salts).

Effect of Reaction Time on H2SO4 catalyst and KOH catalyst on Biodiesel yield

Reaction time and also catalyst differences are factors that have an important role in the conversion of oil into FAME (Biodiesel) products. In this discussion, we will show an effect of the existence of 40, 60, and 80 minute time variables and the use of different catalysts between H2SO4 and KOH catalysts aimed at table 1.

Table 1. Effect of Reaction Time on H2SO4 catalyst and KOH catalyst on Biodiesel yield.

Catalyst	Time (Minutes)	% Yield of Microalgae Oil
$H2SO_4$ 0.35 M	40	18.43
H2SO4 0.35 M	60	21.67
H2SO4 0.35 M	80	25.46
KOH 3%	40	10.78
KOH 3%	60	13.87
KOH 3%	80	15.23

Based on table 1, the results of the in situ transesterification process that occurred in the microwave on the H2SO4 catalyst with a concentration of 0.35 M with a time variation of 40, 60 and 80 minutes at a power of 450 watts and also on the KOH catalyst of 3%, it can be seen from the two catalysts that produce a % yield of biodiesel which is increasingly accompanied by an increase in the time used. This is due to the existence of sufficient time to provide an adequate opportunity between the microwave and the reaction mixture that can produce a more optimal result of a transesterification reaction, but when comparing the yield of %biodiesel between the use of H2SO4 acid catalyst or KOH alkaline catalyst, the best yield value of biodiesel is produced from the use of H2SO4 catalyst at 80 minutes with the yield value produced is 25. 46%.

Research conducted by Bhuana et al (2020) where the highest yield of biodiesel was obtained at 450 watts of power and 60 minutes reaction time, which is 65%. Meanwhile, in this study, at a power of 450 watts and a reaction time of 60 minutes, the yield produced was 21.67%, so that in this study the yield value of biodiesel was lower. Factors that can affect the low %yield value produced are because there may be differences in the amount between the microalgae powder and the solvent used, the type of microalgae used and also the number of catalyst volumes used.

Meanwhile, in the use of acid catalysts, the best yield is produced because in the stirring between methanol and KOH, the formation of water as a result of the reaction between hydroxide and alcohol is inevitable. This can cause hydrolysis of triglycerides or ester products produced so that soap is formed. The results of transesterification showed that there were 2 layers that did not mix with each other, where methyl ester was in the upper layer and the lower layer was an impurity (a mixture of glycerol and fatty acid salts) (Habibi et al, 2010).

Characteristics of FAME Content of Nannochlropsis Oculata Microalgae Oil using GC-MS

Based on this research, after extracting and producing microalgae oil, then FAME content analysis was carried out using Gas Chromatography (GC). The samples tested were H2SO4 catalyst samples with a time of 80 minutes and samples with a KOH catalyst with a time of 80 minutes. The role of the addition of catalysts in an effort to increase the FAME content itself has a different impact on the FAME value produced. GC analysis aims to find out the components contained in lipids, especially fatty acid components and their quantity. The GC-MS results are presented in the figure, chromatography of microalgae oil content with H2SO4 catalyst for 80 minutes as seen in figure 1, while chromatography.

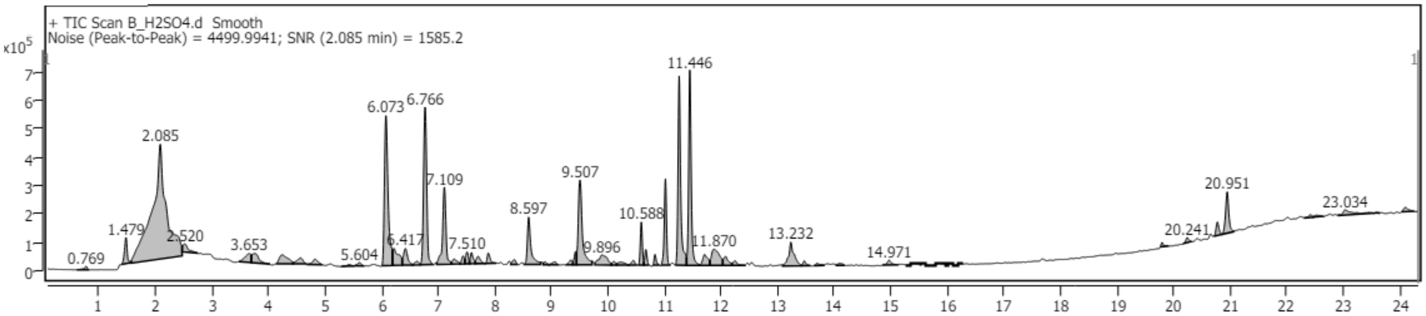

Figure 1. GC-MS Results of N.Oculata Microalgae Content Acid Catalyst

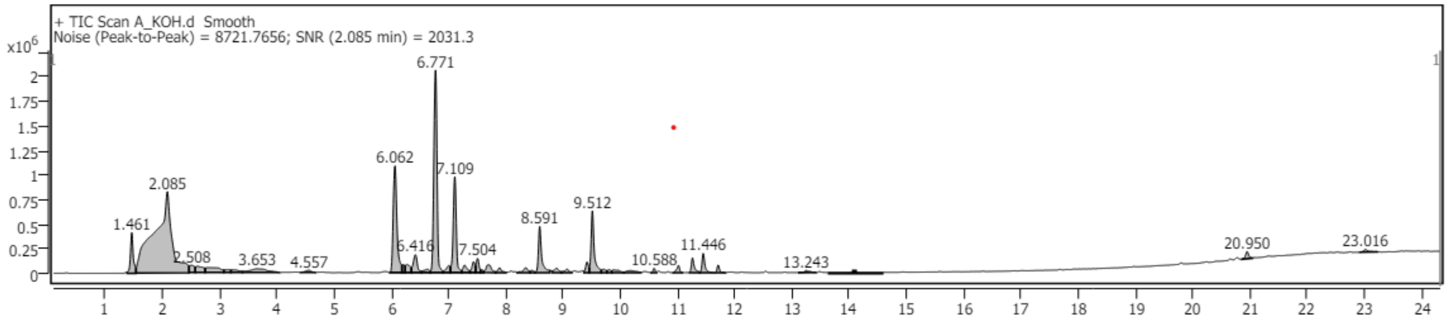

Figure 2. GC-MS Results of N.Oculata Microalgae Content KOH Catalyst

Table 2. Acid catalyst FAME content

No.	Peak	Components Fatty acid methyl ester (FAME)	Chemical formula	Concentration (%)
1	35	Methyl octadecadienoate	$C_{19}H_{34}O_2$	5.23%
2	37	Methyl tetradecadienoate	$C_{15}H_{26}O_2$	1.34%
3	38	Methyl octadecadienoate	$C_{19}H_{34}O_2$	11.49%
4	39	Methyl hexadecenoate	$C_{17}H_{32}O_2$	30.54%
5	40	Methyl hexadecanoic	$C_{17}H_{34}O_2$	30.08%
6	41	Methyl tetradecadienoate	$C_{15}H_{26}O_2$	3.35%
7	43	Methyl tetradecadienoate	$C_{15}H_{26}O_2$	2.59%
8	45	Methyl tetradecadienoate	$C_{15}H_{26}O_2$	7.78%

Table 3. FAME content of alkaline catalysts.

No.	Peak	Components Fatty acid methyl ester (FAME)	Chemical formula	Concentration (%)
1	35	Methyl tetradecadienoate	$C_{15}H_{26}O_2$	0.67%
2	37	Methyl hexadecenoate	$C_{17}H_{32}O_2$	3.06%
3	38	Methyl hexadecanoic	$C_{17}H_{34}O_2$	3.83%

It can be seen from the table The results of the analysis of biodiesel produced using the H_2SO_4 catalyst, show that this biodiesel sample consists of eight identified FAME components. The two main components that dominate the composition are Methyl hexadecenoate ($C_{17}H_{32}O_2$) and Methyl hexadecanoic ($C_{17}H_{34}O_2$), with concentrations of 30.54% and 30.08%, respectively. Together, these two components account for more than 60% of the total composition of FAME. Another significant component is Methyl octadecadienoate ($C_{19}H_{34}O_2$) which appears at two different peaks with a total concentration of 16.72% (5.23% + 11.49%). The dominant presence of these C16 and C18 fatty acid esters is a common characteristic of biodiesel derived from vegetable oils. In addition, there were four peaks identified as Methyl tetradecadienoate ($C_{15}H_{26}O_2$) with a total concentration of 15.06%, this component appearing at four different peaks (37, 41, 43, and 45). The reason for these same components appearing at different peaks may indicate the presence of isomers or differences in the position of their double bonds. The diversity obtained in the composition of FAME can affect the properties possessed by biodiesel, such as flash point, viscosity, and oxidation stability.

(Akhlaghi et al, 2017). In the FAME component results from extraction using KOH, it looks like in table 7 The components detected are Methyl tetradecadienoate ($C_{15}H_{26}O_2$) with a concentration of 0.67%, Methyl hexadecenoate ($C_{17}H_{32}O_2$) with a concentration of 3.06%, and Methyl hexadecanoic ($C_{17}H_{34}O_2$) with the highest concentration of 3.83%. The presence of these three components reflects the diversity of fatty acids in the feedstock used for biodiesel production. Methyl hexadecanoic, which is the methyl ester of palmitic acid, had the highest concentration, indicating the dominance of saturated fatty acids with 16 carbon atoms in this sample.

The results of GC-MS analysis showed a significant difference in the Fatty Acid Methyl Ester (FAME) profile between biodiesel produced using H_2SO_4 and KOH catalysts with a reaction time of 80 minutes. Biodiesel produced with the H_2SO_4 catalyst shows a more diverse FAME profile and higher concentrations. This difference shows that the type of catalyst has a great influence on the efficiency of the conversion of triglycerides to FAME and the type of FAME produced. H_2SO_4 acid catalysts, tend to produce greater amounts of FAME compared to alkaline catalysts such as KOH in the transesterification process for biodiesel production, especially when the condition of the raw material still has a high content of free fatty acids (FFA).

This is due to the ability of acid catalysts to catalyze both in the process of free fatty acid esterification reactions and in the process of simultaneous triglyceride transesterification. Under acidic conditions, protons from acid catalysts can protonate carbonyl groups of free fatty acids and triglycerides, making them more reactive to nucleophilic attack from methanol, thus allowing for more efficient conversion of free fatty acids into FAME, which alkaline catalysts cannot do because alkaline catalysts tend to react with free fatty acids to form soaps, which can reduce biodiesel yield (Lotero et al., 2005). In addition, acid catalysts are more tolerant of the presence of water that may be present in the feedstock. Water can hydrolyze triglycerides into free fatty acids, and if it is in an alkaline condition it will form a soap, but if it is in an acidic state it can still be converted to FAME. Acid catalysts also tend to produce more diverse FAME profiles due to their ability to catalyze reactions in different types of fatty acids, including those with long chains and double bonds. Acid catalysts are effective in converting raw materials with high FFA content into biodiesel (Canakci and Van Gerpen, 1999) while Marchetti and Errazu (2008) affirmed the superiority of acid catalysts in overcoming soap formation problems that often occur with alkaline catalysts in low-quality raw materials.

Morphological test of Nannochlropsis Oculata microalgae using Scanning Electron Microscope (SEM)

The morphological test in this study, to determine the certainty of the type of nannochlopsis oculate microalgae, a Scanning Electron Microscope (SEM) tool was used, where this tool functions to determine the morphology of the microalgae.

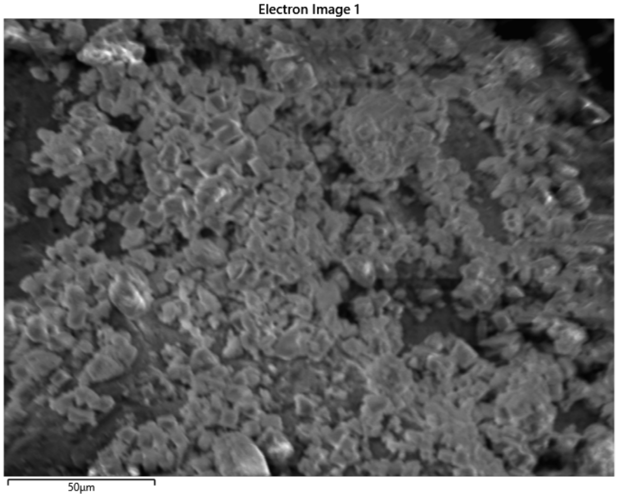

Figure 3. Morphology of N.Oculata microalgae

The result of the morphological description is as seen in figure 16. The similarity of the morphological picture has similarities in research (Dedi et al, 2016) and is also seen in research by (Bajwa, et al 2018), it has similarities in the form of round or slightly oval cell shapes, very small cell sizes ranging from 2-4µm, generally smooth cell surfaces, and also the cells are also separated from each other so that they do not form colonies.

EDX or EDS (Energy Dispersive X – Ray Spectroscopy)

EDS (Energy Dispersive X – Ray Spectroscopy) is an analysis that provides results in the form of the composition of several elements contained in these microalgae. The results of EDS analysis can be seen in the figure below.

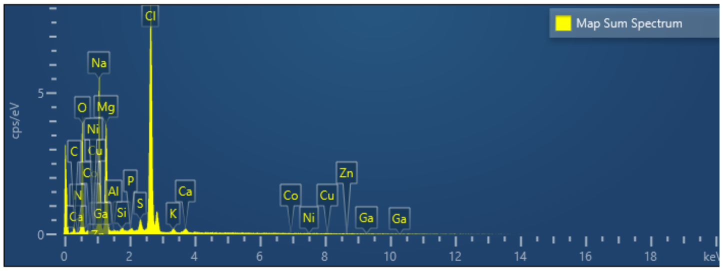

Figure 4. EDS Morphology of N.Oculata

microalgae

The results of EDS (Energy Dispersive X-Ray Spectroscopy) analysis obtained in this study are outlined and can be seen in table 8 showing a diverse composition. The main elements detected are carbon (C), oxygen (O), sodium (Na), and chlorine (Cl), which collectively account for about 90% of the atomic composition. C and O have a large atomic percentage of 25.97% and 38.66%, which are the main organic components in microalgae cells. The high content of Na 12.43% and Cl 13.21% indicates that the sample may have come from a saltwater environment or the influence of salt-containing growth media. Magnesium (Mg) was also detected in significant amounts of 8.02%, which is an important component in the process of photosynthesis. Other macronutrient elements such as phosphorus (P), sulfur (S), and potassium (K) were detected in smaller amounts, 0.14%, 0.61%, and 0.31%, respectively, indicating the presence of nucleic acids and proteins that play a role in cell metabolism.

The analysis also detected some micronutrients and additional elements in small amounts. Silicon (Si) was detected at 0.17%, which may be related to the structure of the cell wall. Calcium (Ca) is present at 0.31%, which can play a role in cellular signaling. Some heavy metals such as copper (Cu) and nickel (Ni) were detected in very small amounts, 0.04% and 0.05%, respectively. The presence of these elements, even in low concentrations, can affect the metabolism and growth of microalgae. some elements normally found in microalgae, such as iron (Fe), were not detected in this analysis, which may be due to the limitations of the instrument's detection or specific growth conditions. The presence of gallium (Ga) in small amounts (0.03%) is unusual and may be a contaminant.

According to a study that has been conducted by Jiang et al (2015), EDX analysis shows that Nannochlropsis oculata has a high content of C and O, reflecting the composition of its organic biomass, besides that this study also reveals the existence of other elements such as N, P and S that play a role in the structure of proteins and nucleic acids. Meanwhile, Mayer et al (2017) in Applied Sciences reported the detection of small amounts of metals such as Mg, Ca, and Fe, which are important for the process of photosynthesis and cell metabolism.

Another study by Benavente-Valdés et al (2016) in Biotechnology Reports used EDX to analyze changes in the elemental composition of Nannochlropsis oculata under various growth conditions. They found that the levels of the C or N ratio would vary depending on the availability of the nutrients obtained by the Nannochlropsis oculata. From the conclusion of some of the studies above, the results of this study provide results that have enough similarity in the amount of element content and on theories related to element levels in Nannochlropsis oculata.

Characteristics of SNI test results of Nannochlropsis Oculata microalgae oil

Density or density indicates the ratio of the union weight of the volume. This characteristic relates to the calorific value and power that will be produced by the type of diesel engine per unit volume of fuel. The higher the density of an object, the greater the mass in each volume. The use of SNI in this quality test aims to produce a good biodiesel value as predetermined, because if biodiesel has a value at the threshold of the provisions that have been set in SNI, then it should not be used in diesel engines, because it will cause worse emission or exhaust gas values, increase engine wear, and will cause damage to the components in the diesel engine. The results of biodiesel analysis of microalgae with different number of catalysts and time variations can be seen in Table 4.

Table 4. Density of biodiesel yield of N.Oculata Microalgae.

Catalyst	Time (Minutes)	Density (g/ml)
H2SO4	40	1,0127
	60	1,0271
	80	1,0341
KOH	40	0,8213
	60	0,7893
	80	0,6716
SNI		0,85-0,89

Based on table 9 For the H2SO4 catalyst, the product density shows an increase in value with increasing reaction time. At 40 minutes, the density obtained was 1.0127 g/ml, increased to 1.0271 g/ml at 60 minutes, and reached 1.0341 g/ml at 80 minutes. All of these values are above the range set by the SNI (0.85-0.89 g/ml). This increase in density may occur because it indicates the presence of several things such as the H2SO4 catalyst can cause the final product to have a high water content or the presence of impurities that have not been completely separated or the formation of by-products as the reaction time increases. On the other hand, for the KOH catalyst, the opposite result is obtained, where the density of the product decreases as the reaction time increases. At 40 minutes, the density obtained was 0.8213 g/ml, decreased to 0.7893 g/ml at 60 minutes, and dropped further to 0.6716 g/ml at 80 minutes. These values are below the SNI range, with the exception of 40 minutes which is close to the lower limit of the standard.

Comparing the two catalysts, it can be seen that H2SO4 produces a product with a higher density than KOH at all reaction times. The value of KOH is better because KOH is a strong base. In the biodiesel transesterification reaction, KOH helps break down triglycerides into methyl esters (biodiesel) and glycerol more efficiently. Strong bases tend to produce purer products with fewer amounts of impurities, as they promote stable ester formation.

The significant difference between the results of these two catalysts suggests that the type of catalyst will have a major influence on the characteristics of the final product produced. In order to optimize the process and achieve density that complies with SNI standards, further adjustments to reaction conditions, such as temperature, reactant molar ratio, or catalyst concentration may be required. In addition, further investigation may be needed to understand why the two catalysts produce opposite data patterns in terms of product density.

Viscosity

Viscosity testing is carried out using a viscometer that functions to test the value of the viscosity of the biodiesel samples that have been produced. A number on the Viscosity value indicates the magnitude of the resistance or resistance in a liquid to flow or the size of the shear resistance in a liquid material. The value of viscosity also affects the performance of the injectors in diesel engines (Riyanti, 2012). Viscosity values that are too high will have an impact on the smoke that will be emitted in diesel engines tends to be dirty and black because the fuel flows slowly and is more difficult to atomize, while if the viscosity value is too low, it will have an impact on lubrication which tends to be bad and worse will result in leakage in the pump (Triana, 2006).

Table 5. Viscosity yield biodiesel Microalgae N.Oculata

Sample code	t-sample	Value cSt/sec	Kinematic Viscosity (cSt)
H2SO4 40 minutes	7.64	0.5	3.82
H2SO4 60 minutes	8.24	0.5	4.12
H2SO4 80 minutes	7.08	0.5	3.54
40-minute KOH	6.47	0.5	3.24
KOH 60 minutes	6.9	0.5	3.45
KOH 80 minutes	8.09	0.5	4.05
SNI : 2.3-6.0			

Based on table 10, it can be seen that the comparison of the results of kinematic viscosity measurement of samples with the SNI 7182:2015 quality standard for biodiesel. According to SNI 7182:2015, the kinematic viscosity of biodiesel at 40°C must be in the range of 2.3-6.0 cSt, so it can be seen that all samples tested have a kinematic viscosity value between 3.24 to 4.12 cSt. This shows that all samples, both treated with H2SO4 and KOH with time variations of 40, 60, and 80 minutes, meet the quality requirements of SNI 7182:2015 in terms of kinematic viscosity. Viscosity in accordance with this standard is important to ensure good performance on diesel engines, including proper fuel atomization and adequate lubrication of engine components.

Determination of Acid Number

The acid number is often defined as the number of milligrams of KOH used to neutralize free fatty acids. The determination of this acid number is directly correlated with the content of free fatty acids (FFA) contained in microalgae oil. Transesterification reactions can take place when the content of free fatty acids is below 5%. The number of acids in the fuel can affect the corrosion properties of the engine. The higher the number of acids, the higher the corrosivity, which can cause damage to the fuel system. Results of analysis of biodiesel quality acid number in H2SO4 and KOH catalysts

Based on the results of the acid number test, it is known that the acid number for all samples (both with H2SO4 and KOH catalysts) is far below the maximum limit of SNI (0.5 mgNaOH/g). This shows that the process with both catalysts results in a product with a very low acid number, which is well in accordance with SNI standards, comparing the two catalysts, KOH produces a lower acid number at a longer reaction time (80 minutes), while H2SO4 is more effective at a shorter reaction time (40 minutes). KOH catalysts are better at producing products with low acid numbers due to their alkaline properties that promote the efficient conversion of free fatty acids into esters, without leaving behind acid residues. More stable reaction conditions and better control in the transesterification process also contribute to the low acid number. In contrast, H2SO4 as a strong acid catalyst tends to leave behind acid residues and other acid by-products, which increases the number of acids in the final product, for both catalysts to be effective in producing products with low acidity. The selection of the optimal catalyst and reaction time will depend on other factors such as product yield, energy efficiency, and economic considerations. For further optimization, testing over a wider time range or different reaction conditions may be required.

Total Glycerol Value

The total glycerol level is carried out as a parameter to show the success rate in the biodiesel manufacturing process. In the results, the total glycerol value in biodiesel is affected by the reaction process and the washing process in biodiesel oil. High glycerol levels can cause problems in the engine, such as injector blockages and deposit formation, while if a low total glycerol value is obtained, then it is identified that in a very efficient transesterification process in converting oil into biodiesel. The value produced in total glycerol varies depending on each policy issued according to the standards that exist in each country. For Indonesia itself, the maximum glycerol value listed in SNI 7182:2015 is 0.24%.

Based on the results of this study as seen in the table. 12 For the H2SO4 catalyst, the highest total glycerol content was found at 40 min reaction time (0.2318%), then decreased significantly at 60 min (0.1326%), and slightly increased at 80 min (0.1574%). This pattern suggests that the transesterification reaction with H2SO4 may be more effective in reducing total glycerol at intermediate reaction time (60 minutes). Meanwhile, for the KOH catalyst, the highest total glycerol content was also found at 40 minutes reaction time (0.2154%), decreased at 60 minutes (0.1736%), and then slightly increased at 80 minutes (0.1986%). This pattern is similar to that observed on the H2SO4 catalyst, suggesting that the optimal reaction time for both catalysts may be around 60 minutes.

Comparing the two catalysts, H2SO4 produced a lower total glycerol content at a 60-minute reaction time compared to KOH. However, at a reaction time of 80 minutes, KOH showed better performance in terms of consistency, with a smaller increase in total glycerol compared to H2SO4. It is important to note that all samples meet the SNI 7182:2015 standard which requires a maximum total glycerol content of 0.24%, except for H2SO4 samples at 40 minutes which slightly exceed the limit (0.2318%). These results show that both catalysts are effective in producing biodiesel with a low total glycerol content, with H2SO4 showing a slight advantage at optimal reaction times. For further optimization, testing at a narrower time range of around 60 minutes or variations in other reaction parameters may be required.

4. CONCLUSION

Based on the results of the research and discussion that has been carried out, several conclusions can be drawn. The production of biodiesel from Nannochloropsis oculata microalgae using the Microwave Assisted Extraction method using 40, 60, and 80 minutes of time variation as well as variations of H2SO4 and KOH catalysts showed that in the transesterification process of microalgae oil using H2SO4 catalyst, the lipid levels produced at 40, 60, and 80 minutes were 18.43%, 21.67%, and 25.46% respectively. Meanwhile, the lipid levels obtained in the KOH catalyst were 10.78%, 13.87%, and 15.23%, respectively. From this data, it can be concluded that the longer the transesterification time, the greater the lipid content obtained.

In the yield percentage value produced, the type of catalyst has a significant influence on the yield percentage of microalgae oil. The H2SO4 catalyst consistently produces higher yields compared to KOH, with H2SO4 achieving the highest yield of 25.46% while KOH only reaches 15.23%. This shows that H2SO4 is more effective in extracting microalgae oil compared to KOH.

The results of the GC-MS analysis showed that the components of FAME content in microalgae oil using H2SO4 and KOH catalysts were different. The FAME content in microalgae oil with H2SO4 catalyst was dominated by methyl hexadecenoate (30.54%) and methyl hexadecenoic (30.08%), while the FAME content in microalgae oil with KOH catalyst was dominated by methyl hexadecenoic (3.83%). Based on these results, it can be concluded that the best FAME content in the transesterification process of microalgae oil was obtained using the H2SO4 catalyst.

The microalgae oil quality test according to SNI standards involves two types of catalysts, namely H2SO4 and KOH, as well as time variations of 40, 60, and 80 minutes. Based on the density test of microalgae oil with the SNI standard (0.85-0.89 g/ml), the results on the H2SO4 catalyst at all time variations showed values above the SNI standard that had been set. However, in the use of the KOH catalyst, all time variations showed values below the SNI standard, with the results closest to the SNI on the KOH catalyst with a time of 40 minutes (0.82 g/mL). Furthermore, the viscosity test showed that in both the H2SO4 and KOH catalysts, the viscosity value was still in the range of SNI values (2.3-6.0).

The microalgae oil acid number test using the H2SO4 catalyst and KOH showed results in accordance with the SNI standard (max 0.5 mgNaOH/g), but the KOH catalyst at 80 minutes gave the best result of 0.0069 mgNaOH/g. The total glycerol test of microalgae oil with H2SO4 and KOH catalysts showed results in accordance with SNI standards (max 0.24%), with the best results in the H2SO4 catalyst at 60 minutes at 0.13% and at the KOH catalyst at 60 minutes at 0.17%.

5. REFERENCES

Azcan N dan Yilmaz O., 2012. Microwave Irradiation Application in Biodiesel Production from Promising Biodiesel Feedstock: Microalgae (Chlorella protothecoides). Proceedings of the World Congress on Engineering and Computer Science. 2

Bacovsky D., Korbitz W., Mittelbach M dan Woegette M., 2017. Biodiesel Production: Technologies and European Providers. Report of Ie a Bioenergy T39-B6.

Barqi W S., 2015. Pengambilan Minyak Mikroalga Chlorella sp. dengan Metode Microwave Assisted Extraction. Jurnal Bahan Alam Terbarukan. 4(1).

Bold, H C dan Wynne, M J., 1985. Introduction to The Algae: Structure and

Reproduction, Second Edition, Prentice-Hall Inc. Englewood Cliffs. New York.

Braunwald, T., Schwemmlein, L., Graeff-Honninger, S., French, WT, Hernandez, R., Holmes, W.E dan Claupein, W. 2013. Effect of different C/N-ratios on carotenoid and lipid production by Rhodotorula glutinis. Appl Microbial Biotechnol, 97 6581-6588.

Chisti,Y. ,2007. Biodiesel from Microalgae. Biotechnology Advances. 11

Energy Technology Essentials. 2007. Biofuel Production. International Energy

Agency.

Fukuda H A., Kondo, H., Noda. 2011. Biodiesel fuel production by transesterification of oils. Journal Bioscience dan Bioenergy. 92 405-416

Gerpen, Van J., 2005. Biodiesel Processing and Production. Fuel

Processing Technology. 86(10)

Halim, R., Michael, K., Danquah, Paul, A dan Webley. 2012. Extraction of Oil from Microalgae For Biodiesel Production: A Riview, Biotechnology Advances. 30(3).

Handayani. S P., 2010. Pembuatan Biodiesel Dari Minyak Ikan Dengan Radiasi Gelombang Mikro. Skripsi. Universitas Sebelas Maret. Surakarta.

Herperian. Kurniawati, E dan Susantiningsih, T, 2014. Pengaruh Pemberian Ekstrak Etanol Biji Jengkol (Pithecellobium lobatum Benth.) Terhadap Kadar Trigliserida Tikus Putih (Rattus norvegicus) Jantan Galur Sprague Dawley yang diinduksi Aloksan. Fakultas Kedokteran Universitas Lampung. Lampung.

Ho, Shih-Hsin., Yi-Di Chen, Ching-Yu Changd. 2017. Biotechnology for Biofuels. Feasibility of CO2 Mitigation and Carbohydrate Production by Microalga Scenedesmus obliquus CNW-N used for Bioethanol Fermentation Under Outdoor Conditions: Effects of Seasonal Changes, 10(27)

Irhamny, Elvitriana, dan Vera, V. 2014. Kultivasi Mikroalga Hijau Pada Sumber Nitrogen Berbeda Untuk Ekstraksi Lipida. Jurnal Purifikasi. 14(2).

Jain, T., V. Jain, R. Pandey, A. Vyas, and S. S. Shukla. 2009. "Microwave Assisted Extraction for Phytoconstituents – An Overview". Asian Journal Research Chemistry, 2(1).

Jordan V dan Gutsche, B., 2011. Development Of an Environmentally Benign Process For The Production Of Fatty Acid Methyl Esters. Journal Of Europe PMC. 43(1).

Kasrina, Sri I dan Wahyu E J. 2012. Ragam Jenis Mikroalga di Air Rawa Kelurahan Bentiring Permai Kota Bengkulu Sebagai Alternatif Sumber Belajar Biologi SMA. Jurnal Exacta 10(1): 36–44

Kawaroe M., Partono T., Sunuddin, A., Sari, S W dan Agustine, D., 2010. Mikroalga: Potensi Dan Pemanfaatannya Untuk Produksi Bio Bahan Bakar. PT. IPB Press: Bogor.

Knothe, G., 2005. Dependence of Biodiesel Fuel Properties on The Structure of

Fatty Acid Alkyl Esters. Fuel Process Technol. 86: 1059-1070.

Liang, H.M., Wu, N., Lan, C.Q dan Dubois-Calero, N. 2012. Biofuels from Microalgae. Biotechnology Progress, 24 (4), 815–820.

Liu, J., Sun, Z dan Gerken, H. 2016. Recent Advances in Microalgal Biotechnology. USA: OMICS Group eBooks.

Li, Y., Horsman, M., Lan, C Q dan Dubois-Calero M., 2008. Biofuels from Microalgae. Biotechnology.

Mahlinda, M., dan Busthan, M. 2018. Transesterifikasi In-Situ Biji Kemiri (Aleurites moluccana L) Menggunakan Metanol Daur Ulang dengan Bantuan Gelombang Ultrasonik. Agritech, 37(3).

Mata, T M., Martins, A A dan Caetano, N S., 2010. Microalgae for Biodiesel Production and other Aplications: A Review, Renew. and Sustainable Energy Revews, 14 (1): 217-232.

Marnelisa E., Azhari, Meriatna, Zainuddin Ginting dan Suryanti., 2022., Pembuatan Biodiesel Dari Minyak Mikroalga Dunaliella Salina Dengan Proses Transesterifikasi Menggunakan Katalis Basa., Jurnal Teknologi Kimia Unimal 11:2

Math MC, Kumar SP, Chetty SV. 2010. Technologies for biodiesel production from used cooking oil—a review. Ener Sustain Dev 14:339–345

Minggang, C., Zhe, L dan Anxiang, Q. 2008. Effects of iron electrovalence and

species on growth and astaxanthin production of Haematococcus pluvialis,

Chin. Journal Oceanol and Limnol. 27(2): 370-375.

Mittle Batch, Remschmidth dan Caludia. 2014. Biodiesel the Comprehensive Handbook. Vienna : Boersedruck Ges.M.Bh.

Mostafa SSM dan El-Gendy NS. 2017. Evaluation of fuel properties for microalgae Spirulina platensis bio-diesel and its blendswith Egyptian petro-diesel. Arab J Chem.

Murtiningrum, M dan Firdaus, A. 2015. Perkembangan Biodiesel di Indonesia Tinjauan Atas Kondisi saat ini, Teknologi Produksi dan Analisis Prospektif. Jurnal PASTI, 9(1): 35–45

Musanif dan Jamil. 2010. Subdit Pengelolaan Lingkungan, Direktorat

Pengolahan Hasil Pertanian, Ditjen Pengolahan dan Pemasaran Hasil

Pertanian. Bio diesel.

Nugroho, A P P., Dwi Fitriyanto dan Achmad R., 2014., Pembuatan Biofuel dari Minyak Kelapa Sawit melalui Proses Hydrocracking dengan Katalis NiMg/γ-Al2O3., Jurnal Teknik Pomits., 3(2).

Orchidea, Lailatul, M. Rachmaniah, Reni, D S.

2011. Pemilihan Metode Ekstraksi Minyak Alga dari Chorella Sp dan Prediksinya Sebagai Biodiesel. Seminar Teknik Kimia Soehadi Reksowardojo.

Purwanti, A. 2014. Pengambilan Lipid dari Mikroalga Basah dengan Cara Ekstraksi Dalam Autoklaf. Jurusan Teknik Kimia. Fakultas Teknologi Industry: Yogyakarta.

Putri P C E, Supriyono. 2019. Transesterifiksi Minyak Kelapa Sawit Menggunakan Katalis Kalsium Oksida (Cao) Menjadi Biodiesel Metana: Media Komunikasi Rekayasa Proses Dan Teknologi Tepat Guna. ISSN: 1858-2907.

Rafaelina M, Rustam Y dan Amini S. 2016. Pertumbuhan dan Aktivitas Antioksidan dari Mikroalga Chlorella sp. Jurnal Biologi Indonesia, 12(1): 12-21

Rahayu, M. 2007. Teknologi Proses Produksi Biodiesel, (Online),

(www.geocities.ws/markal_bppt/publish/biofbbm/biraha.pdf diakses

September 2022).

Rengga P D W, Ade B P, Agus A dan Bayu T. 2019. Ekstraksi Minyak Mikro-Algae Skeletonema costatum dengan Bantuan Gelombang Ultrasonik. Jurnal Rekayasa Bahan Alam dan Energi Berkelanjutan. 3(1).

Rohman, A dan Gandjar, I G. 2007. Kimia Farmasi Analisis, Yogyakarta: Pustaka Belajar.

Setyaningsih I, Salamah E, Rahman D A. 2013. Komposisi Kimia dan Aktivitas Antihiperglikemik Biomassa dan Polisakarida Ekstraseluler dari Mikroalga Porphyridium Cruentum. Jurnal Pengolahan Hasil Perikanan Indonesia.16 (1): 79-85.

Sopiah, N., Mulyanto, A dan Sehabudin, S. 2012. Pengaruh Kelimpahan Sel Mikroalga Air Tawar (Chorella sp.) terhadap Penambatan Karbon Dioksida. Balai Teknologi Lingkungan, Badan Pengkaji dan Penerapan Teknologi, Banten.

Spolaore, P., Joannis-Cassan, C., Duran, E dan Isambet, A. 2006. Commercial applications of microalgae. Journal of Bioscience and Bioengineering. 101 (2), 87–96.

Widianingsih, H., Retno, E., Hadi., M dan Jane. 2013. Fatty Acid

Composition of Marine Microalgae in Indonesia. Journal of

Tropical Biologi and Conservation. 75-82.

Widyastuti dan Ayu, C D. 2014. Sintesis Biodiesel dari Minyak Mikroalga Chorella Vulgaris dengan Reaksi Transesterifikasi Menggunakan Katalis Koh Catur Rini. Jurnal Bahan Alam Terbarukan. 4(1).

Widjaja, Arief., Chao-Chang Chien, dan Yi-Hsu Ju. 2009. Journal of the Taiwan Institute of Chemical Engineers.Study of Increasing Lipid Production from Fresh Water Microalgae Chlorella vulgaris, 40, 13-20.

Wiyarno, B. 2009. Biodisel Microalgae. Islamic international university Malaysia Pahang.