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The Optimum Yield of Nannochloropsis sp Microalgae from the Lipid Cultivation and Extraction Process with Soxhlet Method

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Abstract

The new renewable energy source, namely microalgae biomass, which is the third generation in its utilization derivative, has a lipid content productivity that has great potential to be used as raw material for biodiesel production. One species of microalgae, Nannochloropsis sp., is ideal for cultivation and has a higher photosynthetic efficiency than land plants. Methods to obtain microalgae oil from dry raw materials can be carried out from several processes, but the most effective and easy method is extraction with solvents using a Soxhlet device. The purpose of this study was to determine the optimal growth of Nannochloropsis sp microalgae cell density observations in the cultivation process and to see the percent yield of lipids from microalgae Nannochloropsis sp. through the extraction process with the Soxhlet method using n-hexane and ethanol solvents at a ratio of 1:1, 1:2, 1:3, 1:4 and 1:5. The optimal amount of cell density during the cultivation process has obtained an average of 32.206 x 10^4 cells/ml on day of $9^{th,}$ and the optimal lipid yield was in the ratio of hexane: ethanol 1:1 with a value of 32.13%. These results show that the optimal conditions for yield can be obtained and also has the potential as a raw material biodiesel production.

Keywords: Microalgae Nanochloropsis sp, cultivation, lipid extraction, biomass, biodiesel

Abstrak (Indonesian)

Sumber Energi baru terbarukan yaitu biomassa mikroalga yang merupakan generasi ketiga dalam turunan pemanfaatannya memiliki produktivitas kandungan lipid yang berpotensi besar untuk digunakan sebagai bahan baku pembuatan biodiesel. Salah satu spesies mikro alga, *Nannochloropsis sp.*, sangat ideal untuk dibudidayakan dan memiliki efisiensi fotosintesis yang lebih tinggi dibandingkan dengan tanaman darat. Metode untuk memperoleh minyak mikro alga dari bahan baku kering dapat dilakukan dari beberapa proses, namun metode yang paling efektif dan mudah dilakukan melalui ektraksi dengan pelarut menggunakan alat *Soxhlet*. Tujuan penelitian ini adalah mengetahui pertumbuhan optimal dari pengamatan kepadatan sel mikroalga Nannochloropsis sp pada proses cultivasi dan melihat perolehan persen yield lipid dari mikroalga *Nannochloropsis sp.* melalui proses ekstraksi dengan metode soxhlet menggunakan pelarut n-heksana dan etanol pada perbandingan 1:1, 1:2, 1:3, 1:4 dan 1:5. Perolehan jumlah kepadatan sel yang optimal saat proses kultivasi didapatkan sebesar rata-rata 32.206 x 10^4 sel/ml pada hari ke-9 dan perolehan yield lipid optimal pada perbandingan heksana:etanol 1:1 dengan nilai 32,13 %. Hal ini menunjukan kodisi optimal untuk perolehan yield bisa didapatkan dan juga memiliki potensi sebagai bahan baku pembuatan biodiesel.

Kata kunci: Mikroalga nannochloropsis sp, kultivasi, ekstraksi lipid, biomassa, biodisel

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INTRODUCTION

Indonesia is an archipelagic country with fairly high population growth, economic growth and energy consumption level so that it has a considerable influence on the consumption of fossil fuels. As a result of this, increasing growth impacts supply and demand, which has resulted in the price of fuel oil continues to increase. Therefore it is necessary to develop alternative fuels to minimize the occurrence of a sustainable energy crisis.

The new and renewable energy (NRE) that is being applied is the use of geothermal energy power plants, solar power, bioenergy, hydropower, and wind power [1]. Not only that, but the government has also implemented a policy of using biofuel (B-20), which is mixing diesel engine fuel with palm oil to reduce the use of fossil energy [1]. Indonesia's NRE energy mix target reaches 31%.

The target of the new and renewable energy mix in 2025 is at least 23% and 31% in 2050. Indonesia has an enormous enough potential for new and renewable energy to achieve the primary energy mix target [2].

The minimal use of renewable energy for electricity is due to the relatively high production price of NRE-based power plants making it difficult to compete with fossil plants, especially coal [3]. In addition, the lack of support from the domestic industry related to renewable energy generation components and the difficulty of obtaining low-interest funding are also the causes of impediments to the development of renewable energy.

One of the renewable energy sources that can be used as an alternative energy source is biomass [4]. The advantages of biomass energy sources are renewable so that they can provide a sustainable energy source, can reduce dependence on fossil fuels, reduce pollution, are carbon neutral, abundant availability, versatile and low cost compared to fossil fuels. Other energy sources can come from the use of solar energy, geothermal energy, wind, ocean waves and mini hydro. For the application of biomass energy, among others, it is used as biogas, bioethanol and biodiesel [1].

In general, biomass is a material obtained from plants either directly or indirectly and used as energy or materials in large quantities. Biomass is also known as "phytomass" and is often translated as bioresource or resources obtained from biological resources [5]. Biomass is organic materials of a relatively young age and originates from plants or animals, industrial products and waste, agriculture, water, plantations, forestry, livestock, and fisheries. Many bioenergy products can be produced from biomass. For example, food, fiber, and wood as residues from the industrial sector, energy and short rotations of crops and agricultural waste, and agricultural forests (agroforestry) as residues from the forestry sector, all of which can be used to generate electricity, heat, combined heat and energy, and other forms of bioenergy [1,3]. Bioenergy resources that can be used as a renewable alternative are biodiesel [4].

Biodiesel is alternative energy from biofuel from vegetable oil/biomass, which is being introduced and implemented by the government [5]. This fuel is expected to gradually reduce the role of diesel (fossil) [7]. Through the National Energy Management Blue Print compiled by the Ministry of Energy and Mineral Resources (ESDM), the government has determined that the national biodiesel needs in 2025 will be met from 5% of New and Renewable Energy sources, equivalent to 4.7 million Kiloliters. [8]. This data also goes hand in hand with government policies to increase the energy mix that is proclaimed, to anticipate the increasingly limited national fossil energy reserves and the increasing energy needs of the government community to promote the use of Renewable Energy.

Renewable energy development can be a promising alternative in the future. One of the biomass sources that still need to be further developed comes from microalgae, the third generation of biomass utilization derivatives. Micro algal biomass will be the best choice in the future with the existing potential because Indonesia is a country with the largest water area [9].

Microalgae are micro-sized plants found in water, including sea, brackish water, freshwater, and are the most efficient in capturing and utilizing solar energy and CO_2 for photosynthesis [10]. Microalgae are organisms with a diameter of 2 µm. Microalgae contain much more oil than macro algae. In addition, microalgae are also easier and faster to grow than microalgae [11]. Microalgae can be used as natural food for zooplankton, foods with high energy and fiber content, herbal medicinal ingredients, and a substitute for fossil fuels currently being developed [12].

The oil content in these microalgae has excellent potential to be used as a raw material for making biodiesel [13]. In Indonesia, there are no private and state-owned enterprises that process microalgae as raw material for biodiesel both on a small scale, as is done by BUMN in processing Fatty Acid Mill Effluent (FAME) into biodiesel. Several countries in Europe, which are located close to marine waters,

such as Norway and Italy, have developed microalgae for biodiesel on a small scale [14].

Microalgae contain abundant lipids as raw material for biodiesel, carbohydrates as raw material for bioethanol, and valuable nutrients, such as protein and chlorophyll [14]. One of the microalgae species, Nannochloropsis sp., is ideal for biodiesel production because it is easier to cultivate and the efficiency of photosynthesis is higher than land plants [15, 16]. Although several other types of microalgae, such as Botryococcus brauni., contain more lipids 25 - 80% dry weight. Nannochloropsis sp. still able to compete in lipid productivity of 31 - 68% dry weight. What's more, it can be used for high concentrations of CO_2, making it suitable for commercial cultivation with exhaust gas as a source of CO_2. Research on alternative fuels has been extensively carried out. In this study, developing the application of renewable energy derived from micro algae Nannochloropsis sp. as an alternative energy potential of biodiesel in terms of the results of the extraction process using a mixture of N-Hexane and Ethanol solvents with certain ratios to obtain optimal and high lipid content. Biodiesel is an alternative energy source that can be synthesized from various kinds of vegetable oil. Biodiesel/fatty acid methyl ester can be synthesized through several methods, one of which is the transesterification reaction. In this study, the application of renewable energy derived from microalgae Nannochloropsis sp. as an alternative energy potential of biodiesel in terms of the results of the extraction process using a mixture of N-Hexane and Ethanol solvents with certain ratios to obtain optimal and high lipid content. Biodiesel is an alternative energy source that can be synthesized from various vegetable oils. Biodiesel/fatty acid methyl esters can be synthesized through several methods, one of which is the transesterification reaction. Transesterification is a reaction for the formation of esters and glycerol from triglycerin (fat/oil) with bio-alcohol (methanol or ethanol). Transesterification is a type of equilibrium reaction (reversible), where the addition of a base catalyst (chemical catalyst) can accelerate the achievement of the equilibrium state.

The alga micro-biomass of this alga species is dried, and the oil is extracted by the Soxhlet extractor using hexane: ethanol as a solvent. The solvents used for the extract are ethanol and n-hexane, because ethanol has a low boiling point and tends to be safe. Ethanol is also non-toxic and dangerous, besides that ethanol also has a high polarity making it easy to dissolve resin compounds, fats, oils, fatty acids, carbohydrates and other compounds. Solvent n-hexane is a non-polar solvent that is stable and volatile, selectively dissolves and extracts large amounts of fragrance. The extracted oil is converted into biodiesel through a transesterification process using Sodium Methoxide (NaOH) or Potassium Hydroxide (KOH) as a catalyst.

MATERIALS AND METHODS

Cultivation of Nannochloropsis sp

The raw material for the microalgae Nannochloropsis sp was obtained from the Center for Brackish Water Cultivation Development (BBPBAP) in the Lampung area. Based on references from BBPBAP, this research begins with the cultivation stage. According to the reference from the center, the process of cultivating microalgae is the microalgae nursery Nanochloropsis sp. with seawater at a ratio of 1:4 where the salinity content is conditioned to 28-32%, salinity during the study is salinity suitable for Nannochloropsis sp. to grow optimally. High or low salinity can affect the growth of Nannochloropsis sp. because it can make photosynthetic depression which inhibit the growth of Nannochloropsis sp. Cultivation of microalgae Nannochloropsis sp. was carried out in an Erlenmeyer volume of 1000 mL with the addition of 1 ml of Walne nutrient media in a culture equipped with an aerator. The process of planting in Erlenmeyer culture was carried out indoors with the help of a 20 watt TL lamp or about 4000 lux with a lighting time of 12 hours of light and 12 hours of darkness. During the cultivation stage, cell count calculations were carried out every day during the culture period by calculating cell density using a hemocytometer and a microscope as a tool to view the microalgae Nannochloropsis sp. Prior to cultivation in a photobioreactor, it must be ensured that the equipment used is sterile. The microalgae seeds and nutrient medium are inserted into the photobioreactor. Air and CO_2 are also blown into the photobioreactor with a CO_2 concentration of 10% of the air exhaled [17]. After 10 days, microalgae harvesting will be carried out, then the harvested microalgae are dried and their lipids extracted.

In the cultivation process, tests are also carried out related to the condition of the cultivation water. The parameters tested were salinity, temperature, pH, NO_2, NO_3, NH_3, and PO_4 with the methods of pH meter, SCT meter and Spectrophotometer in Laboratory. The method of water quality analysis refers to the APHA (2012). The optimal growth rate was harvested using 125 ppm NaOH. The 125 ppm NaOH dose is the optimal dose for the manufacture of Nannochloropsis sp. paste.

Extraction of Nannochloropsis sp

The microalgae Nannochloropsis sp. as a result of the cultivation process, is carried out by the drying process in an oven at 60ºC in the oven. A number of 10 grams of dry microalgae are placed in the lead, then placed in a soxhlet tube that has been connected to the distillation flask, heated at 60 °C, and condenser. The hexane: ethanol solvent was varied in ratios of 1:1, 2:1, 3:1, 4:1, and 5:1 [16]. Furthermore, the largest yield percentage of the variation was carried out by the free fatty acid analysis process (FFA).

Determination of free fatty acid (FFA) levels is one of the parameters of quality testing of oil from microalgae using the volumetric titration method. Samples were weighed as much as ± 3 g were put into Erlenmeyer. Added 25 ml of 95% methanol and 3 drops of PP (Phenolphthalein) indicator. Then it was titrated with standardized 0.1 N NaOH, until a pink color was reached, and did not disappear for 30 seconds. The percentage of free fatty acids is expressed as oleic in most oils and fats. Microalgae oil is expressed as palmitate. Free fatty acids are expressed as % FFA.

RESULTS AND DISCUSSION

Characteristics of Nannochloropsis sp Cultivation

In the results stage, after testing the microalgae cultivation of Nannochloropsis sp. with the parameter determination of cell density to determine the rate of microalgae growth using a hemacytometer.

Data from observations of the density of microalgae growth cells of Nannochloropsis sp can be seen in Table 1.

Table 1. Observation Results of Cell Density Nannochloropsis sp

Time Observation (Day)	Average Cell Density (x 10^4 cell/ml)
1	500
2	997
3	1311
4	1535
5	1858
6	2005
7	2397
8	2548
9	32066
10	27559

* The research was conducted in October 2020

Visually observing the change in color density during the cultivation process can be seen in Figure 1.

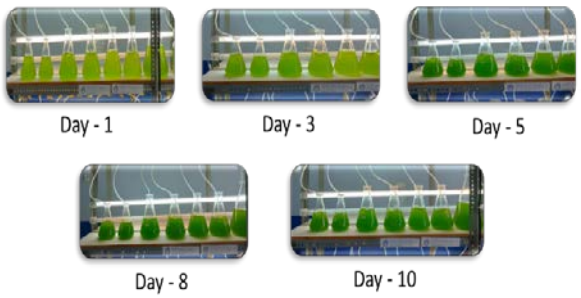

Figure 1. Observations of the Cultivation Process

The color change that occurs indicates the number of increased cell densities, and observations are made every day to calculate the total cell density. Table 1 results from observing the density of Nannochloropsis sp cells that can be converted into graphical form as in Figure 2.

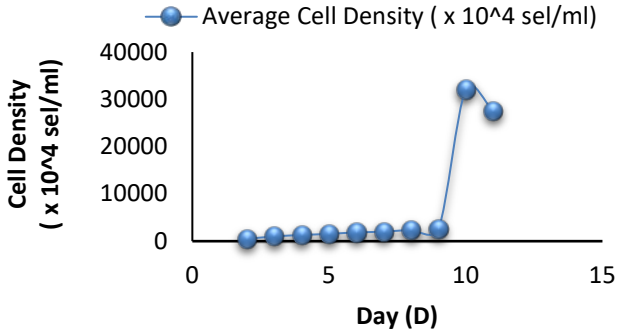

Figure 2. Cell Density Graph of Microalgae Nannochloropsis sp

The results showed that the largest number of micro-cell densities of Nannochloropsis sp was obtained on the 9th day of cultivation, namely at an average of 32,206 x 10^4 cells/ml. The system in microalgae cultivation used in this study is a closed system or photobioreactor, which is cultivated using simple glass equipment and is integrated with lighting and aeration settings in the laboratory. This system provides better optimization than open systems. In an open system, the risk of contamination and evaporation is relatively higher. In addition, the closed system is more flexible so that it can be specially conditioned according to the characteristics of certain microalgae. Four characteristics are used to distinguish the division of micro algae, namely; the type of cell tissue, the presence or absence of flagella, the type of photosynthetic components, and the type of cell pigment. In addition, cell morphology and how the nature of the cells attached in the form of colonies/filaments are important information in distinguishing each group.

The growth phase of microalgae is divided into five phases: the lag phase, the logarithmic phase, the

stationary phase, the decline phase, and the death phase. From day 1 to day 8, the microalgae Nannochloropsis sp experienced a lag phase. Cell division occurs, but the resulting cells do not show a significant increase in numbers. This result happened because the microalgae Nannochloropsis sp is in the adjustment stage with the media in the photobioreactor. From the 8th day to the 9th day, the microalgae Nannochloropsis sp experienced a logarithmic phase. In this phase, the cells divide rapidly and the number of cells increases drastically from 2,548 x 10^4 cells/ml to 32,066 x 10^4 cells/ml. In this phase, the microalgae Nannochloropsis sp has

been optimally adapted to the media, increasing its growth rate. From the 9th day to the 10th day, the microalgae Nannochloropsis sp experienced a decline phase or decreased growth rate. This phase happened because the microalgae cells that tend to divide rapidly are not proportional to the nutrients available in the media, so there is competition in each algal micro cell for nutrients. Some of the microalgae cells undergo a death phase, so that the cell number decreased from 32.066 x 10^4 cells/ml to 27.559 x 10^4 cells/ml.

The optimal cell number on the 9th day is used for the next process, namely the extraction process. Ni Kadek E. Juniantari et al. [18], the results of his research concluded that Nannochloropsis sp. Cultivated on various types of media have different growth and optimum harvest times. Guillard media has optimum harvest time and is the best medium for the growth of Nannochloropsis sp. with the highest amount of biomass of 1.4 x 10^7 ± 2.0 cells/ml on the 11th day of cultivation. Researcher M.B. Hermanto et al. [19], gave the highest yield of Nannochloropsis oculata which reached 3,293 x 10^4 cells/ml of water on the 11th day of culture, but decreased on the 12th to 15th day of culture by 655 x 10^4 cells/ml. This is due to the depletion of the nutrient content in the culture media. The largest fat content was on the 11th day, which was 17.89%. When compared to the two previous researchers, the abundance of microalgae cells was still relatively higher and faster than the cultivation time, which was 32,066 x 10^4 cells/ml and the 9th day.

Boni, et al. [20], in his research extract lipids from the microalgae Botryococcus Braunii using the Soxhlet method. At the time of cultivation, microalgae experienced growth which slowly increased until the 7th day. This shows that the optimal cell abundance is 5,208 x 10^4 cells/ml, when compared with the results, the researchers have different sources of raw materials and the speed of cell abundance is relatively faster, the method used to obtain lipids is the same as that used by researchers, namely Soxhlet extraction.

The microalgae harvesting process generally includes mechanical, chemical, biological, and electrical methods and a combination of these processes. However, mechanical processes such as filtration and centrifugation require a lot of energy, and the operating costs are too expensive. The harvesting process by coagulation and flocculation generally does not cause contaminants, is non-toxic, more efficient, and can be reused, thereby reducing environmental impact [21].

One of the microalgae harvesting processes is auto flocculation through the addition of alkaline. This process is inexpensive, requires little energy, and is non-toxic to microalgae cells. When the pH of a solution increases above 10, microalgae undergo auto flocculation caused by electrostatic interactions between the anions of the microalgae and divalent cations to form calcium or magnesium deposits. One of the solutions used to increase pH is NaOH. Flocculation using NaOH is more effective for biomass and results in a deposition efficiency of more than 90%. In this study, the results of the optimal number of cells, namely on the 9th day, were harvested using NaOH solution. NaOH solution interacts with the biomass, binds to the cell membrane or cell wall, then forms a “layer” to prevent solvent activity and extraction of fat membranes [21].

Borges, et al. [21] carried out the process of harvesting microalgae Nannochloropsis sp using various methods. As a result, the harvest process with flocculation using NaOH, then washing using ammonium formate obtained the highest percent yield among other methods.

Data from the observation of the water quality of Nannochloropsis sp microalgae culture can be seen in Table 2. Table 2 is the result of observations that the culture water of Nannochloropsis sp can be converted into a graph, as shown in Figure 3.

Table 2. Results of Data Observation of Culture Water Quality Nannochloropsis sp

No	Time Observe. (Day)	Salinity	Temp.	pH	Nitrite (NO_2)	Nitrate (NO_3)	Ammonia (NH_3)	O-Phosphate (PO_4)
					mg/L			
1.	D1	28	28.3	7.99	1.212	0.857	9.478	1.759
2.	D4	28	28.7		-	-	-	-
3.	D7	29	28.9		-	-	-	-
4.	D10	30	29.8	8.02	1.237	0.863	8.762	1.396

* The test result was carried out at the BBPBAP Laboratory in Lampung

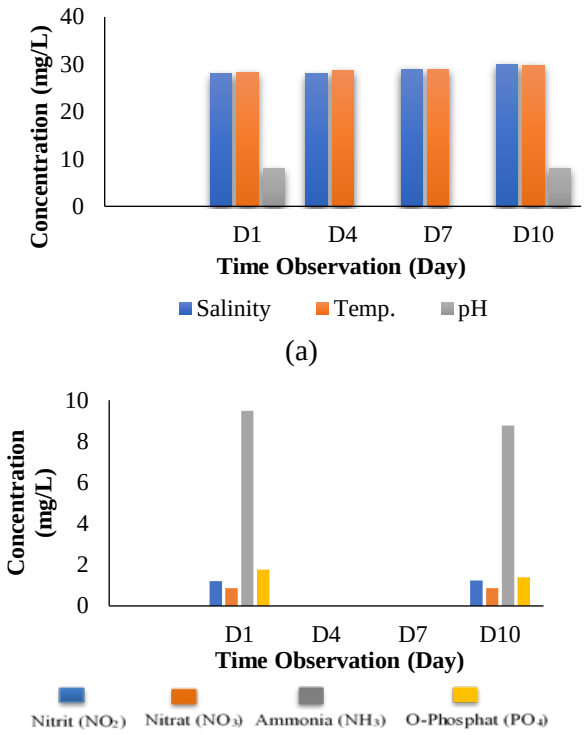

Figure 3. Graph of Culture Water Observation from Nannochloropsis sp: Graph of Corelation Salinity, Temperature and Ph, Graph of Correlation Nitrite (NO_2), Nitrate (NO_3), Ammonia (NH_3) and O-Phosphate (PO_4)

During the cultivation process, the researchers carried out tests related to the conditions of the culture water. The parameters tested were salinity, temperature, pH, NO_2, NO_3, NH_3, and PO_4. This test proceeded to find out what effects can be observed when cultivation occurs. Based on the test results, it can be seen that changes related to salinity, temperature, and pH tend to be relatively stable, although there is a delta difference of 1-2 points. The possibility can be influenced by the rate of evaporation that occurs and the influence of room humidity when cultivation is carried out.

The microalgae culture systems are influenced by different factors, such as temperature, light intensity, carbon dioxide, pH, and the nutrient composition of the culture medium [22]. Among the environmental factors, light and temperature are two of the most important affecting algal growth and biomass production [23], with the optimal temperature and the requirements in light varying among the different algal species. When cultivated in stress conditions, such as inadequate nutrients in the culture medium, and/or very high or low light intensities and temperatures [24], a decrease in biomass production and in growth rate is observed [25]. The macronutrient composition, namely lipids, proteins, and carbohydrates of the microalgae biomass produced, is affected by the environmental and cultural condition variations, such as temperature, light intensity, pH, and nutrient composition of the culture medium [22, 26].

Furthermore, the results of the analysis of the NO_2, NO_3, NH_3, and PO_4 tests were only carried out at the beginning and end of the cultivation process, namely Day 1 and Day 10. Oxygen content can affect the yield of culture water and also ammonia. (NH_3), when compared with the test results for the content of NH_3 & PO_4, the levels decreased. This still has a correlation, decreased levels of NH_3 ± 0.8 mg/L and PO_4 ± 0.5 mg/L. The changes that occur are probably due to the dissolution of ammonia into a base and phosphate into phosphoric acid. This affects the increase in nitrite and nitrate levels, where the levels increase by ± 0.025 and 0.006 mg/L. The test results can be seen in the graph in Figure 3.

Influence of Organic Solvents on Extraction Process

In this study, the process of separating the lipid content of microalgae using the Soxhlet method. Where this process is a liquid-solid extraction process using a mixture of hexane and ethanol. Hexane is considered because it has a low boiling point, so it is easy to separate. In addition, hexane is inert, so it does not allow a reaction to the extracted biomass. The use of ethanol is considered because ethanol is a type of liquid that is volatile, flammable, colorless, and is the alcohol most often used in everyday life, more environmentally friendly.

The extracted microalgae weight is a fixed variable in this study, which is 10 grams. The control variable in this study was the ratio of n-hexane: ethanol with a ratio of 1:1, 2:1, 3:1, 4:1, and 5:1. The total volume of solvent in the overall ratio was 250 ml. The extraction lasts for 3-4 hours, then the extracted oil and the solvent are separated by distillation.

Figure 4. Extraction Process & Distillation

The microalgae used as much as 10 grams are microalgae that have been dried in the oven. The use of dry microalgae tends to be easier and more efficient than wet microalgae in the extraction process. Wet microalgae require higher amounts of organic solvents which can increase operating and material handling costs [27]. The results of research on microalgae extraction using n-hexane: ethanol is presented in Table 3.

Table 3. Observation Data of Soxhlet Extraction Microalgae Nannochloropsis sp

No	n-Hexane: Ethanol Ratios	%yield
1	1:1	32.13
2	2:1	29.50
3	3:1	26.87
4	4:1	15.23
5	5:1	13.26

The data in Table can be presented with a graph as shown in Figure 5.

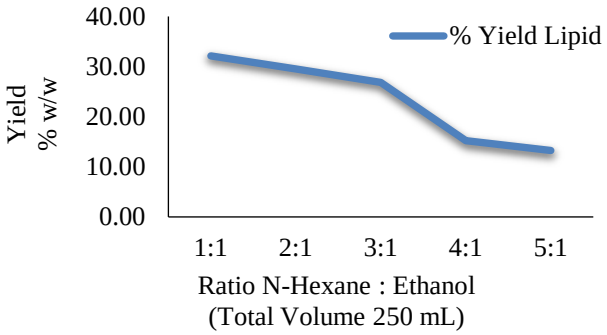

Figure 5. Graph of Ratio n-hexane: ethanol and % yield

From Figure 5, it can be observed that the greater the N-Hexane: Ethanol ratio, the lower the % yield. The decrease in % yield indicated that less oil from microalgae Nannochloropsis sp was absorbed. Non-polar organic solvents can break down membrane-protein-lipids in microalgae while polar organic solvents can break down protein-lipids, lipid uptake mechanism through 5 steps namely penetration of organic solvents through cell membranes, interactions of organic solvents with complex lipids, during the interaction of non-polar organic solvents surrounding complex lipids and forming van der Waals bonds with neutral lipids in complexes, while organic solvents form hydrogen bonds with polar lipids in complexes. This hydrogen bond is quite strong to replace lipid-protein couplings that bind lipid complexes to the cell membrane. The third stage is the formation of a lipid-solvent complex and its release from the cell membrane. Furthermore, the complex lipid-solvent diffusion out of the cell membrane. Finally, the mixing of the complex lipid-solvent mixture with the solvent body outside the microalgae [28].

The mixture of n-hexane and ethanol is closely related to the degree of polarity of the solvent because it is one of the factors in the oil extraction process from microalgae to determine the optimal yield obtained. n-hexane is a non-polar solvent, while ethanol is a polar solvent. The polarity in both can make alternative mixtures to obtain optimal yields.

Therefore, mixing polar and non-polar solvents has the potential to get a higher percentage yield. As for getting more optimal results, further research can be done. The use of n-hexane, a non-polar solvent, is based on the fundamental characteristic of oil that is soluble in non-polar solvents [29]. The yield percentage obtained at the N-Hexane: Ethanol ratio of 1:1 is the most optimal yield percentage in this study. These results indicate that the physical

properties of polarity the solvent has a dominant influence on the extraction yield with a ratio of 1:1 mixture of hexane and ethanol, if the test is carried out on the effectiveness of physical properties the effect of boiling point will be more visible. Where the mixing of two polar and non-polar solvents has the effect of changing the boiling point. The resulting oil extracted from the ratio of the solvent mixture can be used to make biodiesel.

The Soxhlet method's extraction process resulted in a higher yield of 8.5% than other extraction processes. The maceration process resulted in 6% yield lipid, 5% from percolation, 2.5% from the autoclave, and 0.8% from osmotic, according to J Boni et al. [20], Soxhlet extraction method can be used as a method of choice in producing higher lipids than other processes.

Free Fatty Acid Contain in Lipid of Nannochloropsis sp

Determining which microalgae species are suitable for biodiesel requires a high percentage of yield and a suitable fatty acid composition, which consists of saturated fatty acids and unsaturated fatty acids [30].

The lipids produced from the ratio of n-hexane: ethanol with an amount of 1:1 were analyzed for free fatty acid content (FFA) with volumetric Titration method. Analyzing the FFA content in Nanno-chloropsis sp microalgae oil is very important to determine the next process, whether to go through the transesterification stage directly or to do the esterification stage first.

The FFA content in Nannochloropsis sp microalgae oil in this study was 32.81%. These results indicate that there is still 32.81% free fatty acid that must be converted to methyl esters through the esterification stage, then other fatty acids can be converted into methyl esters through the transesterification stage.

Pena et al. [31], extracted the microalgae Nannochloropsis gaditana for biodiesel production process. The content of free fatty acids in the extracted lipids in the process is converted into methyl esters through the esterification process using an acid catalyst, the content of free fatty acids is 93%. The transesterification process can obtain an optimum reaction if the FFA content is less than 2%. The formation of FFA in the extraction process can be influenced by the length of time the microalgae is in the form of a wet paste and the increase in temperature during the extraction process takes place [32].

Another study used n-hexane as a solvent in the extraction of Botryococcus Braunii microalgae using the Soxhlet method. By extracting 10 grams of microalgae using 175 ml of n-hexane, the extraction resulted in a 24%% lipid yield [29].

Leila Kalsum, et al. [7], based on the research results, it can be concluded that in cultivation studies, the optimum pH in microalgae cell growth of *Spirulina platensis* is 9, while the growth peak of *Spirulina platensis* microalgae occurs on the 7th day for pH of 8.9 and 10 with the number of cells at pH of 3,125x10^4 cells/ml; 3,472x10^4 cells/ml ,and 3,298x10^4 cells/ml. As the result of dry extraction lipid, the maceration extraction method produces a higher lipid yield of 5.5%, while the osmotic extraction method only produces lipid of 0.6%. However, compared to the Soxhlet method, the result is higher, which is 9% lipid.

Balduyck, et al. [32], researched the effect of High Pressure Homogenization (HPH) on FFA formation in the extraction of Nannochloropsis sp. The FFA resulting from this study was relatively high, which is 13.2 ± 0.4 % using chloroform: ethanol solvent with a ratio of 1:17. In his research, he hypothesized that FFA is formed from the lipolysis reaction during harvesting and after harvesting. Storage of microalgae Nannochloropsis sp at 2ºC before the extraction process is also suspected of having a lipolysis reaction to form FFA. Then the lipolysis reaction can also be triggered by damage to cells or some cell parts of the Nannochloropsis sp microalgae during the extraction process by the HPH method [32].

CONCLUSION

To achieve an optimal biodiesel production process from microalgae Nannochloropsis sp, the selection of the extraction process is a very important aspect in determining the resulting lipid yield and fatty acid content.

The optimal growth of microalgae was on the 9th day of the cultivation process with an average cell density of 32,206 x 10^4 cells/ml. The optimal yield of microalgae cells can be harvested and the lipid obtained by soxhlet extraction with the best ratio in the lipid extraction process is a mixture of n-hexane: ethanol 1:1, where the lipid yield obtained is 32.13%. so that the microalgae Nannochloropsis sp has the potential to be used as raw material for biodiesel.

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