

The Determination Of Salinity Profile And Nutrition (NaH_2PO_4) Profile In Utilizing *Nannochloropsis oculata* To Gain Maximum Lipid

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Abstract :

The determination of salinity profile and nutrition (NaH_2PO_4) profile in utilizing *Nannochloropsis oculata* to gain maximum lipid was investigated. The purpose of carrying out this research is to determine the optimum salinity and the optimum nutrition (NaH_2PO_4) to gain maximum lipid contained in *Nannochloropsis oculata*. Phosphor as a nutrition can be obtained by adding NaH_2PO_4 and a salinity enhancement can be applied by adding NaCl to the culture. This research was run by using variable of salinity (33, 34, 35, and 36 ppt) and variable of NaH_2PO_4 (5, 10 and 15 ppm). The results show that the percentage of maximum lipid in *Nannochloropsis oculata* was obtained when the salinity of 35 ppt and 5 ppm NaH_2PO_4 is 37,68%. However, this maximum lipid percentage is low and it can be caused by many factors, such as the drying temperature for preparing the dry microalgae to extract, the cell disruption method and the extraction solvent used in the research. By seeing the result, salinity can affect the total lipid contained in *Nannochloropsis oculata*. The higher the salinity contained in culture the higher the lipid contained in *Nannochloropsis oculata*. Otherwise, a different respond was obtained by adding NaH_2PO_4 . If NaH_2PO_4 concentration is higher, the lipid contained *Nannochloropsis oculata* will be lower.

Keywords: *Nannochloropsis oculata*, maximum lipid, nutrition (NaH_2PO_4), salinity

1. Introduction

Microalgae is a kind of microorganism which capable of utilizing light to react water with CO_2 then the biomass produced (photosynthesis). Microalgae has a big potential to be converted to some beneficial products for human, such as food materials, feed for animals, medicines, and energy [1].

The concept of material selection in producing biodiesel is intended to fulfill a lack of materials. Microalgae is observed as one of the materials for producing biodiesel. Furthermore microalgae is one of the natural resources in Indonesia. Microalgae contains of high lipid content, even some of this microorganism have a lipid content more than 50%. A high lipid content identify how high the fatty acid in microalgae. The higher a fatty acid content in a material, the higher the potential of the material converted to a biodiesel.

Lipid is one of the microalgal components which depends on the a kind of microalgae and a growth condition. Lipid content is ranged between 2-60% of dry weight [2]. Some of them which able to be converted to vegetal oil are *Chlorella* (32%), *Dunaliella* (23%), *Isochrysis galbana* (35%), dan *Nannochloropsis oculata* (68%). Lipid can be utilized as a material for producing a liquid fuel. Triglyceride and fatty acid which is a lipid component can be converted to methyl ester. Methyl ester produced has some excellences if it is compared with fossil energy. The excellences are it's renewable, biodegradable, and low pollution. A lipid production is influenced by the nutrition availability and light intensity. Some microalgae can produce lipid in a big amount when it lacks of nutrition. One of the nutrition that usually used is urea [3].

Nannochloropsis oculata has a high lipid content (31-68%) [1] and it's been cultivated in Lampung province. It's more often utilized as feed to rotifier *Brachionus plicatilis*, a zooplankton which is cultivated massively for feeding fish larvae and the other marines [4]. *Nannochloropsis oculata* is classified as *Chrysophyta*. *Chrysophyta* is a division of unicellular marine organisms or freshwater. This division consists of diatoms (*Bacillariophyceae*), gold or gold brown (*Chrysophyceae*), yellow-green algae (*Xanthophyceae*). *Chrysophyta* has a thread form physically and its cell wall consists of cellulose with a big amount of silica. The food storage in *Chrysophyta* is chrysolaminarin. The main materials are oil and leucosin.

There are many researches proved that the quantity and quality of lipid contained in microalgae varies, as the result of the influence of a culture condition. Environment can influence the lipid content in microalgae [5]. Furthermore a lipid content in microalgae can be influenced by some nutritions (phosphor, nitrogen, sulphur, iron, ammonia, etc.) [3] and a salinity change. Phosphor as a nutrition can be obtained by adding NaH_2PO_4 and a salinity enhancement can be applied by adding NaCl to the culture. Lipid contained in microalgae is potential as a raw material of producing biodiesel. The lipid enhancement can be influenced by some environments, such as salinity and nutrition.

Some factors that influence a cultivation and lipid contained in *Nannochloropsis oculata* are light, CO_2 concentration, nutrition, salinity, pH and temperature. To produce a maximum lipid in *Nannochloropsis oculata* it needs a light intensity of 4000 lux [6], CO_2 concentration of 2% [7], 40 ppm urea [8], temperature of 25°C [9]. Nevertheless the former researches haven't proved that salinity and nutrition of NaH_2PO_4 can influence a lipid content in *Nannochloropsis oculata* yet.

Therefore it's needed to investigate and determine the optimum salinity and optimum nutrition of NaH_2PO_4 to gain the maximum lipid in *Nannochloropsis oculata*.

2. Materials and Methods

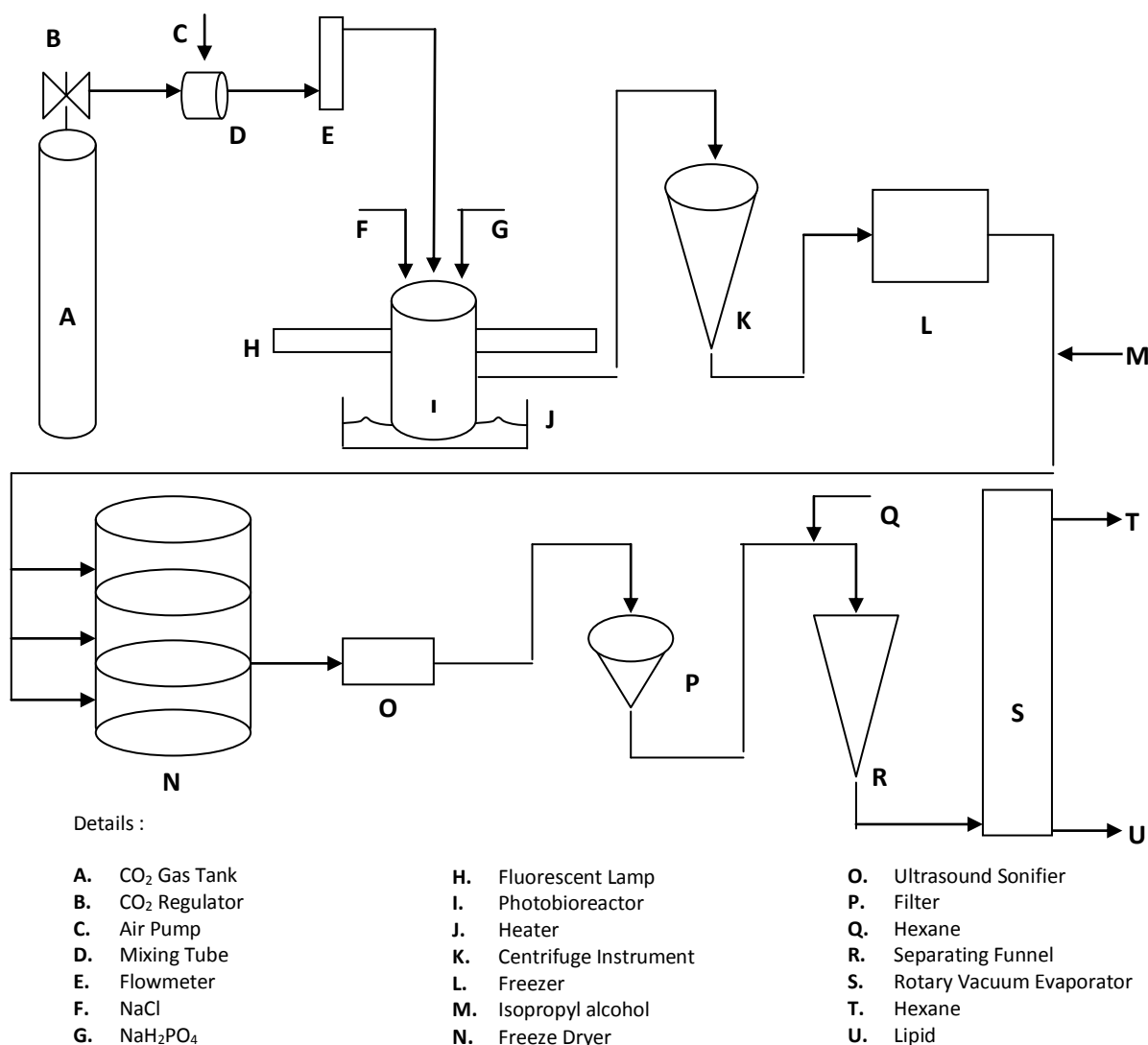


Figure 1. Scheme of Instrument Series

The materials used are *Nannochloropsis oculata*, an ozonised sea, NaH_2PO_4 , urea ($\text{NH}_2\text{COONH}_2$), NaCl , CO_2 , air, isopropyl alcohol and hexane. The instruments used in this research are photobioreactor (diameter of 10 cm and height 23,5 cm), refractometer (ATAGO S/Mill-E), air pump, CO_2 gas tank, CO_2 regulator, gas flowmeter, fluorescent lamp of 20 Watt, digital scale, luxmeter (TENMARS TM-204), mixing tube, heater, haemocytometer, microscope (OLYMPUS CX31), ultrasound sonifier (BANDELIN Sonorex Technik), centrifuge instrument, rotary vacuum evaporator (BUCHI R 210), freezer, freeze dryer (SCANVAC) and separating funnel. The instrument series can be seen in Figure 1.

When starting to cultivate *Nannochloropsis oculata*, 40 ppm urea and 5 ppm NaH_2PO_4 is added to the culture and cultivation is applied at salinity of 33 ppt. The cultivation is applied until the cell density of *Nannochloropsis oculata* reach exponential phase then the cultivation time is obtained. Afterward the determination of cultivation time is applied for the other 11 variations. The cultivations for every variation is repeated by applying the cultivation time. After the culture is centrifuged, it is freezeed by using a freezer then water is separated by using freeze dryer until a dry biomass is obtained. The dry biomass is dissolved in 20 ml of isopropanol and it is put in ultrasound sonifier to disrupt the cells. The mixture of dry biomass and isopropanol is separated by screening it. The result is mixed with 20 ml of hexane and then hexane is separated from isopropanol. The addition of hexane is continuously applied until hexane has no change in colour. The lipid is separated from hexane by evaporating it using vacuum evaporator series.

Analysis of lipid percentage is applied by measuring a dry mass of biomass and a lipid mass. A percentage of lipid is determined by dividing a lipid mass and a dry mass of biomass. Percentage of lipid obtained in every variation is compared then the maximum lipid contained in *Nannochloropsis oculata* is obtained.

3. Results and Discussion

According to the obtained data of each cultivation, the results using *Nannochloropsis oculata* with CO_2 concentration of 2%, light intensity of 4000 lux, nutrition (NaH_2PO_4) concentration of 5, 10 and 15 ppm, and salinity of 33, 34, 35 and 36 ppt can be seen in Table 1.

Table 1. Data of optimum salinity dan nutrition (NaH_2PO_4) determination to gain maximum lipid in *Nannochloropsis oculata*

Salinity (ppt)	NaH_2PO_4 (ppm)	Dry Mass (mg)	Lipid Mass (mg)	Percentage of Lipid (%)
33 ppt	5	4326,1	549,0	12,69
	10	2760,1	113,7	4,12
	15	1333,5	65,84	4,94
34 ppt	5	3785,9	846,53	22,36
	10	2220,6	399,40	17,99
	15	3151,3	379,05	12,03
35 ppt	5	2077,9	782,90	37,68
	10	3842,9	854,23	22,23
	15	2555,9	464,92	18,19
36 ppt	5	1523,4	95,52	6,27
	10	1082,7	62,15	5,74
	15	1741,2	61,12	3,51

$$\text{Percentage of Lipid} = \frac{\text{Lipid Mass}}{\text{Dry Mass of Microalgae}} \times 100\%$$

Figure 2 shows lipid content in *Nannochloropsis oculata* with salinity of 33, 34, 35 36 ppt, and nutrition (NaH_2PO_4) of 5, 10 dan 15 ppm.

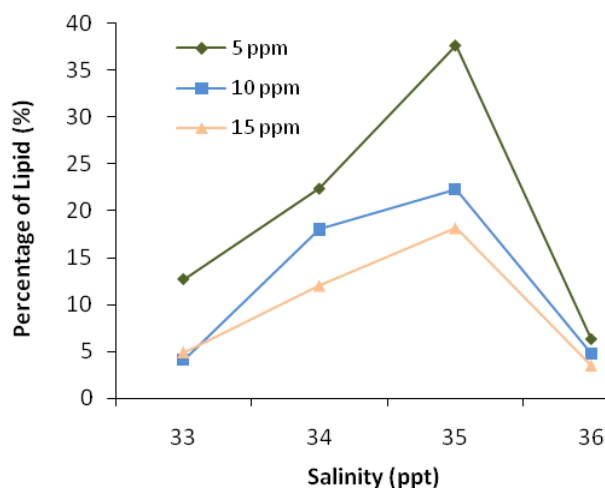


Figure 2. The profile of salinity to gain the maximum lipid in *Nannochloropsis oculata*

The higher the salinity in the culture of *Nannochloropsis oculata*, the higher the percentage of lipid until it reached 35 ppt. At the salinity of 36 ppt, the percentage of lipid decreased for each NaH_2PO_4 concentration. It's caused by the hypersaline condition in culture and *Nannochloropsis oculata* couldn't adapt it well. This condition then affect the lipid contained in *Nannochloropsis oculata*.

This result is compared to a research carried out by [10]. This research has a purpose of knowing the influence of salt concentration added to *Dunaliella tertiolecta* culture to lipid contained in microalgae. An increase of NaCl concentration from 0,5 M until 1 M results a different lipid content, 60% for the addition of 0,5 M NaCl and 67% for the addition of 1 M NaCl. The researcher reported that the high intra cell lipid accumulation was suspected as a respond of cell to keep growing over the high salinity in culture. This phenomena run simultaneously with the production of glycerol in some microalgae as the form of cell adaptation to an extreme environment, especially a hypersaline culture. Intracellular glycerol concentration is directly related to the external concentration of salt. Another research [11] was carried out with the purpose of knowing the influence of salinity to the growth and lipid contained in microalgae. The researcher reported that the inhibited malate enzyme formation probably can result a very low lipid productivity. The genetic ability in microalgae to produce a malate enzyme is needed for lipid accumulation. Furthermore the researcher reported that *Chlamydomonas sp.* produce a moderate lipid content at a low salinity, but a low lipid content is produced at a high salinity. Nevertheless *Tetraselmis sp.* and *Dunaliella tertiolecta* produce a higher lipid content at a high salinity. By seeing the results, the researcher couldn't state that salinity can influence malate enzyme activity or not. Therefore it's necessary to carry out a research with a purpose of knowing that salinity can inhibit the enzyme activity or not. In research [12], it was reported that the formation of β -ketoacyl-conzyme A (CoA) is increased by adding 0,5 M NaCl to 3,5 M NaCl. β -ketoacyl-conzyme A (CoA) is a coenzyme A which has a function to accelerate the first step and rate-limiting step in a chain elongation of fatty acid. To adapt to the hypersaline environment, *Dunaliella salina* need to modify the composition of a fatty acid. Analysis of lipid showed that microsome (not a plasma membrane or thylakoid) in the microalgae at the addition of 3,5 M NaCl contained a higher ratio of 18C to 16C than when 0,5 M NaCl was added to the culture. The higher the ratio of 18C to 16C, the higher the lipid content in microalgae. Figure 2 shows that there is a significant decrease of a lipid content at the salinity of 36 ppt. the cause of the significant lipid decrease still can't be defined.

By comparing the results with another researcher reports, it can be concluded that the lipid content in microalgae will be different for some kinds of microalgae because each kind of it has a different respond to a salinity. It's still can not be concluded that a high salinity can inhibit the activity of malate enzyme which able to influence the lipid accumulation in microalgae.

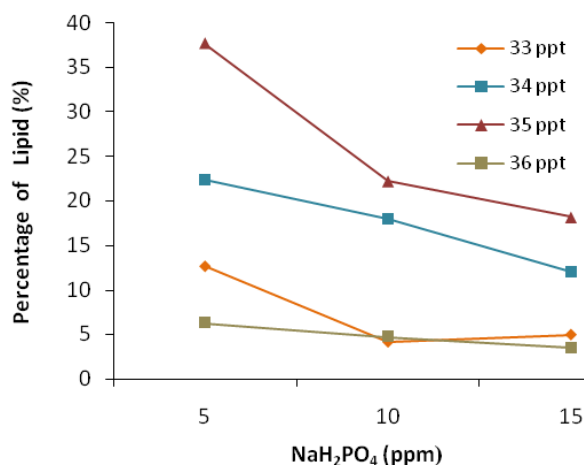


Figure 3. The profile of nutrition (NaH_2PO_4) to gain the maximum lipid in *Nannochloropsis oculata*

Figure 3 shows how NaH_2PO_4 influence the lipid in *Nannochloropsis oculata*. It shows that the higher NaH_2PO_4 added to the culture, the lower the percentage of lipid. A decrease in the percentage of lipid was happened because when lipid in an excess condition, the path of the lipid metabolism will be reversible (at the path of glycolysis) and form glucose-6-phosphate. It was supported by the addition of phosphor in a big amount in the culture so the excess lipid was gained. In glycolysis, phosphor is used to form *adenosine triphosphate* (ATP) then this product is used in every step in glycolysis. If phosphor existed to the culture was enough, an excess lipid will not be gained and the maximum lipid can be reached [13]. The result then compared to a research carried out by [14]. In this research, a different phosphate concentration was added to the culture of *Dunaliella salina* (30 ppm, 20 ppm, 10 ppm and 5 ppm). The highest lipid content gained is 13% when the addition of 10 ppm phosphate. By comparing the results, it can be concluded that if the phosphor added to culture is lower in the concentration, the percentage of lipid will be higher.

The lipid percentage contained in *Nannochloropsis oculata* is 38%-60%. Nevertheless the maximum lipid percentage in *Nannochloropsis oculata* gained in this research is 38,67%. It can be caused by the method used when preparing the dry microalgae to the extraction step.

The reason why a low percentage of lipid is gained has been showed by the former researches. One of the researches is a research carried out by [15]. The results show that lipid contained in microalgae can be influenced by a drying temperature. In this research, the drying temperature used are 0°C, 60°C, 80°C and 100°C. The maximum lipid was gained at 0°C, 52.5% and the lowest one was gained at 80°C, 48.75%, so that it can be stated that the highest lipid content is gained at a low drying temperature.

The drying temperature used is 80°C and by comparing the results, it can be concluded that a low lipid percentage can be caused by a drying temperature.

Furthermore a cell disruption method used can influence a microalgal lipid content. Cell disruption is a method which used to break the cell wall. In a research carried out by [16], cell disruption method of autoclaving, bead-beating, microwaves and sonication was applied to *Botryococcus sp.*, *Chlorella vulgaris* and *Scenedesmus sp.* The result shows that by using microwaves method, the highest lipid percentage was gained after the microalgae was extracted. Meanwhile the lowest lipid percentage was gained by using sonication method.

The method used for the cell disruption in this research is sonication method and it can be concluded that a cell disruption method can cause a low lipid percentage.

A selection of solvent of extraction can influence the lipid content we gain. A research carried out by [17] has a purpose of determining the best solvent in extraction. The solvent used are chloroform-methanol with volume ratio of 2:1, isopropyl alcohol-hexane with a volume ratio of 2:3 and hexane. The result shows that chloroform-methanol can extract lipid a lot more than the other solvents and isopropyl alcohol-hexane can extract lipid a lot

more than hexane. Nevertheless there's a literature that for a safety reason it's better to use isopropyl alcohol-hexane than chloroform (carcinogenic).

The solvent used in this research is isopropyl alcohol-hexane. It can be concluded that a solvent selection can result a low lipid percentage.

4. Conclusions

Based on the results, the conclusion are as follows:

- a. The maximum lipid in *Nannochloropsis oculata* obtained at the salinity of 35 ppt and the addition of 5 ppm NaH_2PO_4 is 37.68%
- b. At the salinity of 36 ppt, *Nannochloropsis oculata* is not able to adapt so that the low lipid content is obtained
- c. At the addition of 15 ppm NaH_2PO_4 , the microalgal lipid content is the lowest in this research

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