

THE DETERMINATION OF SALINITY AND NUTRITION (NaH₂PO₄) PROFILE IN *Nannochloropsis oculata* CULTIVATION TO GAIN MAXIMUM LIPID

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Abstract

The determination of salinity and nutrition (NaH₂PO₄) profile in Nannochloropsis oculata cultivation to gain maximum lipid was investigated. The purpose of this research was to determine the optimum salinity and nutrition (NaH₂PO₄) to gain maximum lipid in Nannochloropsis oculata. Phosphor can be obtained by adding NaH₂PO₄ and a salinity enhancement can be applied by adding NaCl to the culture. This research was run by matching each variable of salinity (33, 34, 35, and 36 ppt) to each variable of NaH₂PO₄ (5, 10 and 15 ppm) randomly. The results show that the percentage of maximum lipid in Nannochloropsis oculata was obtained at 35 ppt and 5 ppm NaH₂PO₄ (37.68 %). Nevertheless the maximum lipid percentage is low and it can be caused by some factors, such as the drying temperature for preparing the dry microalgae to extract, the cell disruption method and the extraction solvent used. By seeing the results, salinity can affect the total lipid in Nannochloropsis oculata. The greater the salinity in culture the greater the lipid content in Nannochloropsis oculata. Otherwise a different response was obtained by adding NaH₂PO₄. If NaH₂PO₄ concentration is greater, the lipid contained Nannochloropsis oculata will be lower

Keywords: maximum lipid; nannochloropsis oculata; nutrition (NaH₂PO₄); salinity

Abstrak

PENENTUAN PROFIL SALINITAS DAN NUTRISI (NaH₂PO₄) PADA BUDIDAYA *Nannochloropsis oculata* UNTUK MEMAKSIMALKAN KADAR LEMAK. Penentuan profil salinitas dan nutrisi (NaH₂PO₄) pada pembiakan *Nannochloropsis oculata* untuk memperoleh lipid maksimum diteliti. Tujuan penelitian ini adalah untuk menentukan salinitas dan nutrisi (NaH₂PO₄) optimum untuk memperoleh lipid maksimum pada *Nannochloropsis oculata*. Fosfor dapat diperoleh dengan menambahkan NaH₂PO₄ pada kultur dan peningkatan salinitas dapat dilakukan dengan menambahkan NaCl pada kultur. Penelitian ini dijalankan dengan mencocokkan setiap variabel salinitas (33, 34, 35, dan 36 ppt) dengan setiap variabel NaH₂PO₄ (5, 10 dan 15 ppm) secara acak. Hasil menunjukkan bahwa persentase lipid maksimum pada *Nannochloropsis oculata* diperoleh pada salinitas 35 ppt dan 5 ppm NaH₂PO₄ (37,68 %). Walaupun demikian, persentase lipid maksimum masih tergolong rendah dan hal ini dapat disebabkan oleh beberapa faktor, seperti temperatur pengeringan yang diterapkan, metode cell disruption dan pelarut ekstraksi yang digunakan. Dengan mempelajari hasil yang telah didapat, salinitas dapat mempengaruhi lipid total pada *Nannochloropsis oculata*. Semakin tinggi salinitas pada biakan, semakin tinggi kandungan lipid pada *Nannochloropsis oculata*. Sebaliknya didapatkan respon yang berbeda saat menambahkan NaH₂PO₄. Apabila konsentrasi NaH₂PO₄ semakin tinggi, maka lipid yang terkandung pada *Nannochloropsis oculata* akan menjadi lebih rendah.

Kata kunci: lipid maksimum; nannochloropsis oculata; nutrisi (NaH₂PO₄); salinitas

INTRODUCTION

Microalgae is a kind of microorganism which capable to utilize light to convert CO₂ into biomass through photosynthesis reaction. Microalgae has a big

potential to be converted to some products for human, such as food materials, feed for animals, medicines, and energy (Chisti, 2007).

The concept of material selection in producing biodiesel is intended to the quality of the raw material. Microalgae is observed as one of the raw 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 lipid content more than 50%. A high lipid content identify how high the fatty acid in microalgae. The higher 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 a kind of microalgae and a growth condition. Lipid content is ranged between 2-60% of dry weight (Wijffles, 2006). Some of them which able to be converted to algal oil are *Chlorella* (32%), *Dunaliella* (23%), *Isochrysis galbana* (35%), dan *Nannochloropsis oculata* (68%) (Guschina and Harwood, 2006).

Lipid can be utilized as a material for producing a liquid fuel. Triglyceride and fatty acid which are lipid component can be converted to methyl ester. Methyl ester produced has some advantages if it is compared to fossil energy. It is 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 (Wang, 2008).

Nannochloropsis oculata has a high lipid content (31-68%) (Chisti, 2007) and it has been cultivated in Lampung province. It is often utilized as feed to *Brachionus plicatilis*, a zooplankton cultivated for feeding fish larvae (Ismi and Sutarmat, 1996). *Nannochloropsis oculata* is classified as *Chrysophyta* which 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. The optimum salinity for *Nannochloropsis oculata* growth is 33-35 ppt (Wijaya, 2006).

Many studies 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 (Borowitzka *et al.*, 1990). Furthermore a lipid content in microalgae can be influenced by some nutrients (phosphor, nitrogen, sulphur, iron, ammonia, etc.) (Wang, 2008) 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. Budiman (2009) carried out a research with a purpose of determining the optimum light intensity on *Nannochloropsis oculata* growth and lipid rate. Three different light intensities (2000, 3000, 4000 lux) were applied to *Nannochloropsis oculata*. At

4000 lux, there's a significant influence on the growth of *Nannochloropsis oculata* with cell density of 38.33×10^6 cell/ml. Afterward the culture was centrifuged and a lyophilization was applied to the obtained biomass. The biomass was extracted by using chloroform-methanol (2:1 (v/v)). The maximum lipid percentage obtained is 38.02% at 4000 lux. Yi *et al.* (2009) carried out a research with a purpose to study the growth and lipid productivity in *Nannochloropsis oculata*. The variables applied are 2%, 5%, 10% and 15% of CO_2 aeration. In this research, *Nannochloropsis oculata* was cultivated at a photobioreactor (height = 30 cm and diameter = 7 cm) with aeration of 200 mL/min. The maximum cell density of *Nannochloropsis oculata* was obtained at 2% CO_2 (0.48 ± 0.029) cell/mL and lipid percentage of 29.7 ± 2 . Wijaya (2006) investigated the effect of different concentration of urea to the *Nannochloropsis oculata* growth. The variables applied are 0 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm urea. Each run is repeated three times. The result shows that a different concentration of urea can give a different cell density. The addition of 40 ppm urea can result the maximum cell density (16.27×10^6 cell/mL) at the sixth day and the maximum protein content (95.89 $\mu\text{g/ml}$ media). Converti *et al.* (2009) carried out a research with a purpose of studying the effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Two variables applied to *Nannochloropsis oculata* are temperature (15, 20, 25°C) and NaNO_3 concentration (0.075, 0.150, 0.300 g L^{-1}). The results show that there's an increase in the lipid component passing from 25°C (13%) to suboptimal temperature condition (7.9% at 20°C and 14.9% at 15°C). Nevertheless the growth rate was significantly affected by changes in temperature, thus leading a lipid productivity. The reduction of nitrate concentration in the growth medium increase the lipid fraction. The maximum lipid content (7.88 ± 0.21) was obtained at addition of 0.075 g L^{-1} NaNO_3 .

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 (Budiman, 2009), CO_2 concentration of 2% (Yi *et al.*, 2009), 40 ppm urea (Wijaya, 2006), temperature of 25°C (Converti *et al.*, 2009). As mentioned above, there are many results with a purpose of gaining maximum lipid. 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*. By knowing the optimum variables, it is expected that it will can used

as a literature to produce biodiesel in a great quantity respectively.

MATERIALS AND METHOD

Materials

The materials used were *Nannochloropsis oculata*, an ozonated brine (taken from marine and has been treated), NaH_2PO_4 , urea ($\text{NH}_2\text{COONH}_2$), NaCl , CO_2 , air, isopropyl alcohol and hexane.

Instruments

The instruments used in this research were photobioreactor (diameter of 10 cm and height of 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 scales, 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.

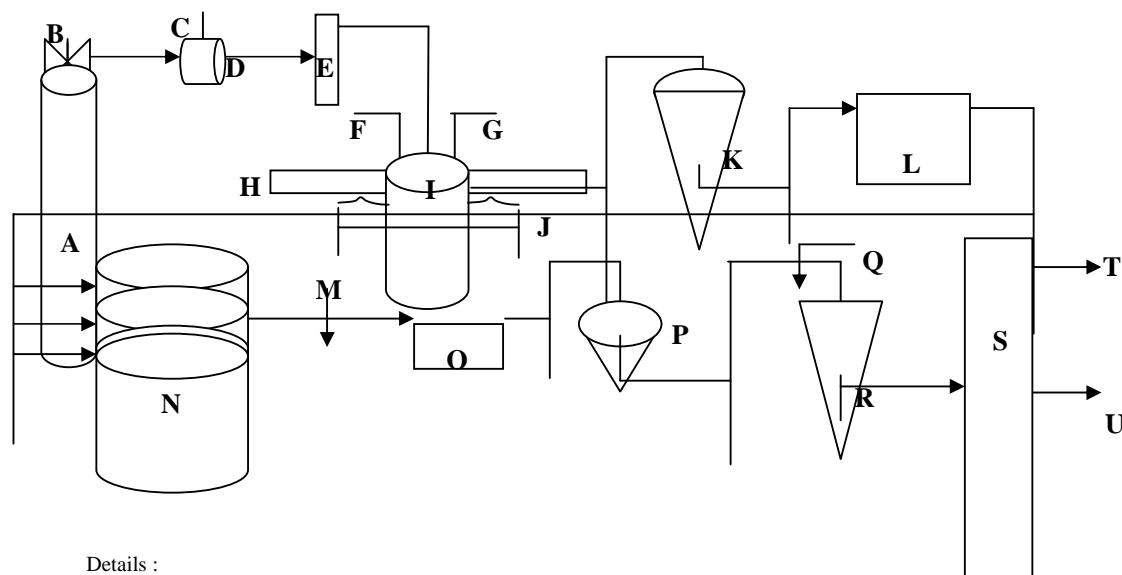
Variables

The variables observed in this research is the salinity (33-36 ppt) and nutrition (5-15 ppm NaH_2PO_4). The both variables are matched randomly. Therefore 12 runs (33 ppt, 5 ppm NaH_2PO_4 ; 33 ppt, 10 ppm NaH_2PO_4 ; 33 ppt, 15 ppm NaH_2PO_4 ; 34 ppt, 5 ppm NaH_2PO_4 ; 34 ppt, 10 ppm NaH_2PO_4 ; 34 ppt,

15 ppm NaH_2PO_4 ; 35 ppt, 5 ppm NaH_2PO_4 ; 35 ppt, 10 ppm NaH_2PO_4 ; 35 ppt, 15 ppm NaH_2PO_4 ; 36 ppt, 5 ppm NaH_2PO_4 ; 36 ppt, 10 ppm NaH_2PO_4 ; 36 ppt, 15 ppm NaH_2PO_4) are applied.

Experiments

Before starting to culture, the cell density of *Nannochloropsis oculata* should be measured and the instruments should be set. Air and CO_2 were aerated by using an aerator and turning the CO_2 gas tank valve on. A CO_2 concentration of 2% at 4 L/min aeration was set by using a CO_2 regulator and a flowmeter. Afterward it was flowed passing the mixing tube and it was separated to 4 lines which connected to 4 photobioreactors. The cultivation would be applied at temperature of 25°C and a heater was used to make the temperature of cultivation constant. A lamp was put behind the photobioreactors and the light intensity of 4000 lux was set by using a luxmeter. The culture was prepared by applying a ratio of 1:4 (ratio of *Nannochloropsis oculata* culture volume to ozonated brine volume). The less the culture volume, the less the microalgal lipid obtained and it will cause a difficulty in lipid weighing. Therefore it should be applied at a sufficient volume and 2 L of culture is applied. After adding 40 ppm of urea, NaCl and NaH_2PO_4 (based on each run), and 1600 mL of ozonated brine to a photobioreactor, 400 mL of *Nannochloropsis oculata* culture was added and the cultivation was ready to start.



Details :

- | | | |
|------------------------------|--------------------------|-----------------------------|
| A. CO_2 Gas Tank | H. Fluorescent Lamp | O. Ultrasound Sonifier |
| B. CO_2 Regulator | I. Photobioreactor | P. Filter |
| C. Air Pump | J. Heater | Q. Hexane |
| D. Mixing Tube | K. Centrifuge Instrument | R. Separating Funnel |
| E. Flowmeter | L. Freezer | S. Rotary Vacuum Evaporator |
| F. NaCl | M. Isopropyl alcohol | T. Hexane |
| G. NaH_2PO_4 | N. Freeze Dryer | U. Lipid |

Figure 1. Scheme of instrument series

The cultivation was carried out until the cell density of *Nannochloropsis oculata* reach an exponential phase then the cultivation time was obtained. The cell density count was applied every 2 hours by using a microscope and a haemocytometer. Afterward the determination of cultivation time was applied for other 11 runs. By obtaining the cell density at exponential phase and cultivation time needed for *Nannochloropsis oculata* to grow and reach the exponential phase, the growth rate for each run can be found by using the formula as shown in equation (1).

$$\mu = \frac{\ln(N_t/N_0)}{t - t_0} \quad (1)$$

Where, μ is growth rate (day^{-1}); N_t is number of the cells at the end of the cultivation time (start of the exponential phase); N_0 is the number of cells at the start of culturing; t is the cultivation time; t_0 is time of zero.

The cultivations for each run was repeated by applying the obtained cultivation time. After the culture was centrifuged, it was freezed by using a freezer then the water was separated by using a freeze dryer at 80°C until dry mass was obtained. Dry biomass was obtained and the weight was measured by using a digital balance. The dry biomass was dissolved in 20 ml of isopropanol and it was put in a sonication device (ultrasound sonifier) to disrupt the cells in 30 minutes. The mixture of dry biomass and isopropanol was separated by screening it using a filter. The result was mixed with 20 ml of hexane in a separating funnel and then the hexane was separated from isopropanol. The addition of hexane was continuously applied until hexane has no change in colour. The lipid was separated from hexane by evaporating it using vacuum evaporator series at 40°C . Afterward the weight of lipid obtained was measured by using a digital balance.

Analysis

Analysis of lipid percentage was applied first by measuring dry mass and lipid mass by using a digital scales. Dry mass was measured before it was dissolved with isopropyl alcohol and lipid mass was measured after the extraction product was evaporated by using vacuum evaporator series. Lipid content was determined by using the formula as shown in equation (2). Afterward the lipid content for 12 runs was compared then the maximum lipid contained in *Nannochloropsis oculata* could be determined.

$$\% \text{ Lipid} = \frac{\text{Lipid Mass}}{\text{Dry Mass of Microalgae}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

The cultivation time obtained for each run by setting the growth to the exponential phase is shown in Table 1. The cultivation time for each run is different and it can be caused by the different response of *Nannochloropsis oculata* to adapt the culture condition. There's no significant change in cultivation

time for 33 and 34 ppt. The maximum growth rate at 33 and 34 ppt is 0.3611/day and 0.4912/day respectively. At 35 ppt and addition of 5 ppm NaH_2PO_4 , it is obtained that the culture was cultivated in 3 days 22 hours to grow and reach the exponential phase. *Nannochloropsis oculata* gave a different response at the other additions of NaH_2PO_4 . It can be seen in the cultivation time obtained, 3 days and 15 hours at the addition of 10 ppm NaH_2PO_4 , and 3 days and 9 hours at the addition of 15 ppm NaH_2PO_4 . If these are compared to run of 33 and 34 ppt, the culture needed a longer time to be cultivated. The maximum growth rate for 35 ppt is 0.3116/day. At 36 ppt and addition of 5 ppm NaH_2PO_4 , The culture needed 2 days 5 hours to grow and reach the exponential phase. A shorter cultivation time was obtained at the other additions of NaH_2PO_4 , 1 day 22 hours at the addition of 10 ppm NaH_2PO_4 , and 2 days and 2 hours at the addition of 15 ppm NaH_2PO_4 . The maximum growth rate for 36 ppt is 0.3155 /day.

A study applied by Jayappriyan *et al.* (2010) examined the effect of different conditions to a culture of *Dunaliella salina*. Different concentrations of NaCl was added to the culture (0.5-5 M) and it was found that the maximum cell density was obtained at the addition of 1.0 M NaCl, 6.075 \log_{10} cell /mL. At the addition of 1.5-5.0 M NaCl, the cell density of *Dunaliella salina* was getting decreased. By comparing the study applied by Jayappriyan to the results as shown in Table 1, it can be stated that *Nannochloropsis oculata* responded as same as *Dunaliella salina* did. Although *Nannochloropsis oculata* and *Dunaliella salina* are classified as a marine microalgae, these two microalgae can't adapt as well as when the culture is in a lower salinity.

As shown in Table 1, there is a significant difference at the growth rate for each addition of NaH_2PO_4 . Jayappriyan *et al.* (2010) studied the effect of different concentrations of NaH_2PO_4 (0.10-1.0 mM) added to the culture of *Dunaliella salina*. It was found that high concentrations of phosphate added to the culture would inhibit the growth of *Dunaliella salina*. The higher the concentration of phosphate, the more the growth will be inhibited. If the result as shown in Table 1 is compared to the study applied by Jayappriyan *et al.* (2010), high concentration of NaH_2PO_4 will inhibit the growth of *Nannochloropsis oculata*. From this study, it can be stated that *Nannochloropsis oculata* growth will be inhibited if the addition more than 10 ppm NaH_2PO_4 is applied.

The difference in the cultivation time obtained for each run can be seen at the growth rate precisely. If the growth rate for each run is compared, the maximum growth rate was obtained at 33 ppt and the addition of 10 ppm NaH_2PO_4 , 80.7125.10⁴ cell. mL⁻¹.day⁻¹. It can be stated that *Nannochloropsis oculata* gave the best response at this run. *Nannochloropsis oculata* responded better at the salinity of 33 ppt and addition of 10 ppm NaH_2PO_4 .

Table 1. Data of *Nannochloropsis oculata* cell density and cultivation time at the exponential phase for each run (the cell density is multiplied by 25×10^4 cell/mL)

Salinity (ppt)	NaH ₂ PO ₄ (ppm)	Cell Density					Cultivation Time	Growth Rate (/day)
		Starter	Day 1	Day 2	Day 3	Day 4		
33	5	2.33	3.67	6.33	4.67	-	2 days and 11 hours	0.2828
	10	3.67	6.33	8.00	7.67	-	2 days and 9 hours	0.3104
	15	1.33	3.00	4.67	3.33	-	2 days and 13 hours	0.3611
34	5	3.67	4.00	4.67	5.00	4.67	3 days and 10 hours	0.0705
	10	2.33	7.00	8.00	7.33	-	2 days and 8 hours	0.4912
	15	1.33	2.67	3.67	7.00	6.67	3 days and 8 hours	0.4837
35	5	2.33	4.33	5.00	5.67	5.33	3 days and 22 hours	0.2112
	10	3.67	5.33	6.67	8.33	7.67	3 days and 15 hours	0.2033
	15	2.33	6.33	7.67	8.33	6.67	3 days and 9 hours	0.3116
36	5	1.33	2.33	3.67	2.67	-	2 days and 5 hours	0.3155
	10	3.67	4.00	4.67	4.33	-	2 days and 2 hours	0.0793
	15	2.33	3.67	3.33	-	-	1 day and 22 hours	0.1863

Table 2. Data of optimum salinity and nutrition (NaH₂PO₄) determination to gain maximum lipid in *Nannochloropsis oculata*

Salinity (ppt)	NaH ₂ PO ₄ (ppm)	Dry Mass (mg)	Lipid Mass (mg)	Lipid Content (%)
33	5	4326.1	549.0	12.69
	10	2760.1	113.7	4.12
	15	1333.5	65.84	4.94
34	5	3785.9	846.53	22.36
	10	2220.6	399.40	17.99
	15	3151.3	379.05	12.03
35	5	2077.9	782.90	37.68
	10	3842.9	854.23	22.23
	15	2555.9	464.92	18.19
36	5	1523.4	95.52	6.27
	10	1082.7	62.15	5.74
	15	1741.2	61.12	3.51

Although the culture gave the best result in growth at 33 ppt and addition of 10 ppm NaH₂PO₄, it can't be determined that by applying this run, the maximum lipid in *Nannochloropsis oculata* will be obtained. According to the obtained data for each run, the results in cultivating *Nannochloropsis oculata* by applying CO₂ concentration of 2%, light intensity of 4000 lux, nutrition (NaH₂PO₄) concentration of 5, 10 and 15 ppm, and salinity of 33, 34, 35 and 36 ppt is shown in Table 2.

Figure 2 shows lipid content in *Nannochloropsis oculata* with salinity of 33-36 ppt, and nutrition (NaH₂PO₄) of 5-15 ppm. It's getting increase from 33 to 35 ppt and there is a significant decrease from 35 to 36 ppt. 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 NaH₂PO₄ concentration. The factor why there is an increase and significant decrease will be discussed

by comparing the data obtained with the results of the former research.

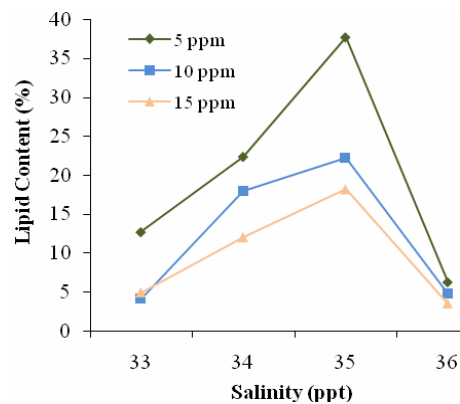


Figure 2. The profile of salinity to gain the maximum lipid in *Nannochloropsis oculata* (5-15 ppm NaH₂PO₄)

The lipid productivity of *Nannochloropsis oculata* is the form of cell adaptation collaterally with the production of glycerol. Takagi (2006) carried out a research with 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. By seeing the data obtained, there's an increase in lipid component along with a salinity increase from 33 ppt (12.69%) to the higher salinity (22.36% at 34 ppt and 37.68% at 35 ppt) at the addition of 5 ppm NaH_2PO_4 .

Not only triggered by the form of cell adaptation which led to the production of glycerol, but the formation of coenzyme A can be a cause why the increase of lipid percentage was obtained. Azachi *et al.* (2002) 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 is able to accelerate the first step and rate-limiting step in a chain elongation of fatty acid. To adapt to the hypersaline environment, *Dunaliella salina* needs 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.

By comparing the data obtained with what Azachi *et al.* (2002) reported, it can be concluded that a high salinity definitely can inhibit the activity of malate enzyme which able to influence the lipid accumulation in *Nannochloropsis oculata*. There's an increase of 18C to 16C ratio along with the increase of salinity from 33 to 35 ppt. If there is a higher ratio of 18C to 16C, it will lead to higher lipid content and it can be seen from the results obtained. There is an increase from 33 ppt (12.69%) to 34 ppt (22.36%) and 35 ppt (37.68%) at the addition of 5 ppm NaH_2PO_4 .

Nevertheless there's a significant decrease of lipid content from salinity of 35 ppt (37.68%) to 36 ppt (6.27%). It can be caused by the incapability of *Nannochloropsis oculata* to adapt as well as at the salinity of 33-35 ppt. Not only the incapability of *Nannochloropsis oculata* to adapt well at 35 ppt, but the formation of malate enzyme probably can be a cause anyway. Sonnekus (2010) carried out a research in a 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 greater lipid content at a high salinity. Furthermore a significant decrease of 18C to 16C ratio can be a factor why a low lipid productivity was obtained (Azachi *et al.*, 2002).

Figure 3 shows how NaH_2PO_4 influence the lipid in *Nannochloropsis oculata*. It shows that the greater NaH_2PO_4 in concentration added to the culture, the lower the percentage of lipid. There is a decrease of lipid content from 5 ppm NaH_2PO_4 to 15 ppm NaH_2PO_4 . The maximum lipid content was obtained at 5 ppm NaH_2PO_4 (12.69% at 33 ppt, 22.36% at 34 ppt, 37.68% at 35 ppt, 6.27% at 36 ppt) minimum lipid content was obtained the addition of 15 ppm NaH_2PO_4 (4.94% at 33 ppt, 12.03% at 34 ppt, 18.19% at 35 ppt, 3.51% at 36 ppt).

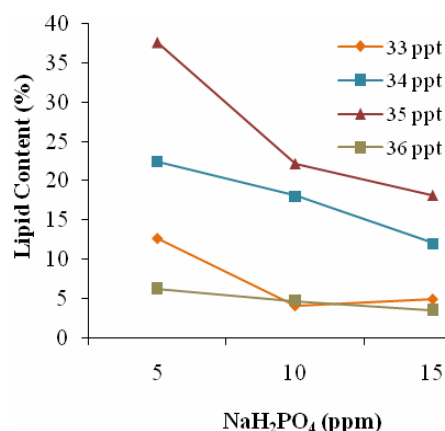


Figure 3. The profile of nutrition (NaH_2PO_4) to gain the maximum lipid in *Nannochloropsis oculata* (33-36 ppt)

A decrease in the lipid content 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. Gunstone *et al.* (2007) stated that this reversibility 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 in the culture was sufficient, an excess lipid will not be gained and the maximum lipid can be reached.

The data obtained is compared to other research carried out by Sunarko (2010). 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 maximum lipid content gained is

13% when the addition of 10 ppm phosphate. By comparing the results, it can be stated that if the phosphor added to culture is lower in the concentration, the percentage of lipid will be greater.

The lipid content in *Nannochloropsis oculata* is 38%-61%. 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 research (Widjaja, 2009). The results show that lipid contained in microalgae can be influenced by a drying temperature. In this research, the drying temperatures (0°C, 60°C, 80°C and 100°C) are applied in the drying of *Chlorella vulgaris* culture. 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 a higher lipid content will be gained at a lower drying temperature.

Furthermore a cell disruption method used can influence microalgal lipid content. Cell disruption is a method which used to break the cell wall. Lee (2009) carried out a research by applying cell disruption methods of autoclaving, bead-beating, microwaves and sonication to *Botryococcus sp.*, *Chlorella vulgaris* and *Scenedesmus sp.* The result shows that by using microwaves method, the maximum lipid percentage was gained after the microalgae was extracted. Meanwhile the minimum lipid percentage was gained by using sonication method.

A selection of solvent of extraction can influence the lipid content. Walter (2009) carry out a research in 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 explained that for a safety reason it's better to use isopropyl alcohol-hexane than chloroform (carcinogenic).

CONCLUSIONS

The maximum lipid in *Nannochloropsis oculata* is obtained at the salinity of 35 ppt and the addition of 5 ppm NaH_2PO_4 is 37.68%. At the salinity of 36 ppt, *Nannochloropsis oculata* is not able to adapt so that the low lipid content is obtained. Nevertheless by comparing this result to the other research, it 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. At the addition of 15 ppm NaH_2PO_4 , the minimum microalgal lipid content is obtained. This result is supported by other results and it can be concluded that the lower the phosphor concentration, the greater the percentage of lipid. It is affected by the addition of phosphor in a big amount to the culture so the excess lipid is gained. If phosphor

existed in the culture is sufficient, an excess lipid will not be gained and the maximum lipid can be reached. By comparing the results to others, it can be stated that a different preparation before extracting dry microalgae can be a cause why the maximum lipid content obtained is low. Sonication which is one of cell disruption methods was applied in this research. In a former research, a disruption method of microwaves definitely will contribute greater microalgal lipid content. In the results of the former research, some cell disruption methods was applied to three kinds of microalgae and microwaves method contributed the maximum lipid. Thereby it makes a sense that the cell disruption method will contribute a maximum lipid in *Nannochloropsis oculata*.

By evaluating the results and making some comparisons to the former research, it is suggested that the maximum lipid contained in *Nannochloropsis oculata* will be greater than lipid obtained in this study by changing the cell disruption method to microwaves, applying drying temperature of 80°C and using chloroform-methanol as the extraction solvent. By implementing the suggestions it is expected that the maximum lipid in *Nannochloropsis oculata* will be greater than the maximum lipid obtained in this study.

The aim of this study is to determine the optimum salinity and nutrition (NaH_2PO_4) to gain maximum lipid in *Nannochloropsis oculata*. By seeing the results, it can be concluded that the maximum lipid in *Nannochloropsis oculata* was obtained at salinity of 35 ppt and nutrition (NaH_2PO_4) of 5 ppm. This result was obtained after applying drying temperature of 80°C, sonication as a cell disruption method and isopropyl alcohol-hexane as an extraction solvent.

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