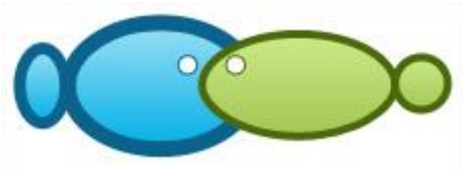

Effect of sodium nitrate, sodium phosphate, and sodium silicate on growth and accumulation of nutritional compounds of microalgae *Nannochloropsis oculata*

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Abstract. This study aimed to determine the optimum levels of sodium nitrate, sodium phosphate and sodium silicate suitable for growing *Nannochloropsis oculata* to achieve its best growth rate and quality. Results showed that the microalgae grown at sodium nitrate level of 112.5 mg L^{-1} achieved the best growth of 314.6 × 10^4 cells mL^{-1}. A 50% increase in sodium phosphate concentration (7.5 mg L^{-1}) compared with that of the control F/2 (5 mg L^{-1}) exhibited the best microalgal growth and a density of 302.4 × 10^4 cells m L^{-1}, while the microalgae in the control (F/2) reached a maximum density of 283.5 × 10^4 cells m L^{-1} at day 10 of culture. Similarly, with a 50% increase in Si content (45 mg/L) in the algal culture medium, the algal density reached 310.4 × 10^4 cells mL^{-1}, which was higher than that of the nutrient medium F/2 (30 mg/L) that reached only a density of 280.5 × 10^4 cells mL^{-1}. Levels of protein, lipid and carbohydrate accumulation in *N. oculata* also differed when increasing or decreasing sodium nitrate, sodium phosphate contents. Increasing the sodium nitrate levels by 50% from that of the control F/2 medium increased the protein (33.67%) and carbohydrate contents (8.54%) but decreased the lipid content (12.45%) compared with that of the nutrients in the F/2 (16.07%) medium. *N. oculata* attained high protein (33.96%) and carbohydrate (8.04%) contents when phosphorus content was increased to 50% (7.5mg L^{-1}) compared to the control medium F/2 (5 mg L^{-1}). However, increasing Si content did not significantly affect the nutritional composition of microalgae when compared to that of the F/2 nutrient medium.
Key Words: carbohydrate, growth, lipid, *Nannochloropsis oculata,* protein.

Introduction. Natural food plays an important role in the success of rearing many aquatic animals. In recent years, although there have been many advanced techniques in the production of artificial food for aquatic larvae, natural foods such as microalgae, rotifers, crustaceans, artemia, worms and the like are still considered an extremely important and irreplaceable food in aquaculture production. Microalgae *Nannochlopsis oculata* is a small-sized algae (2-4 µm) but with moderately high nutritional content in which protein (6–52%), lipid (7–23%) and carbohydrate (5–23%) are the main organic contents (Lavens et al 1996; Brown et al 1997; Binh et al 2021). However, growth and nutrient content of *Nannochlopsis oculata* depends on many ecological factors such as temperature, pH, salinity, light intensity among others, and these depend greatly on the nutrient medium used to culture this microalgae (Cam et al 2016; Tam 2019). Many studies have shown that the F/2 nutrient medium is the ideal nutrient medium for cultivating *N. oculata* biomass (Binh et al 2021). Growth and quality of the microalgae depend greatly on the content of N, P and Si in the nutrient medium used for algae culture. The optimum levels of N, P and Si increase with the algal biomass and the content of nutrients such as protein, lipids and carbohydrates increase several folds (Cam et al 2016; Lien et al 2017). In the light of the above observation, the present study was carried out to determine the optimal content of N, P and Si for the biomass culture of *N. oculata*.

Material and Method

Algal source. *N. oculata* was imported from the Institute of Aquaculture Research North Central (Nghe An) and stored at Department of Plant Cell Technology, Institute of Biotechnology, Hue University, Vietnam. Algae were inoculated in 500 mL bottles of clean treated water and cultured in F/2 medium with 24 h aeration at 30‰ salinity and pH=8. The algae were cultured until the density increased and were again inoculated to create primary seed pots.

Optimal levels of nitrogen, phosphorus and silicate. The studies were carried out at the laboratory of the Faculty of Fisheries, University of Agriculture and Forestry, Hue University. There were 3 separate experiments performed in this study:

1) Determination of the optimal level of sodium nitrate ($NaNO_3$) on growth and proximate composition of the microalgae *N. oculate.* Seven levels of sodium nitrate were prepared, namely, 18.75 mg L^{-1} (T1); 37.5 mg/L (T2); 56.25 mg/L (T3); 93.75 mg/L (T4), 112.5 mg/L (T5); 131.25 mg/L (T6); and F/2 (Control) 75 mg/L. These quantities correspond to the following percentual decrease and increase in sodium nitrate content in the treatments compared to the control level (F/2 nutrient medium): decrease by 75% (T1), 50% (T2), 25% (T3) and increase by 25% (T4); 50% (T5) and 75% (T6).

2) Determination of the optimal sodium phosphate (NaH_2PO_4) content on growth and proximate composition *N. oculate.* Seven levels of NaH_2PO_4 were tested, namely, T1: 1.25 mg/L; T2: 2.5 mg/L; T3: 3.75 mg/L; T4: 6.25 mg/L; T5: 7.5mg/L; T6: 8.75 mg/L; and F/2 as the control treatment: 5 mg/L. These quantities correspond to the following percentual decrease and increase in sodium phosphate in the treatments compared to the control level (F/2 nutrient medium): decrease by 75% (T1), 50% (T2), 25% (T3) and increase by 25% (T4); 50% (T5) and 75% (T6).

3) Determination of optimal level of sodium silicate (Na_2SiO_3) on growth and proximate composition of *N. oculate.* Seven levels of Na_2SiO_3 were tested, namely, T1: 7.5 mg/L; T2: 15 mg/L; T3: 22.5 mg/L; T3: 37.5 mg/L; T4: 45 mg/L; T5: 52.5 mg/L; and F/2 is the control treatment: 30 mg/L. These quantities correspond to the following percentual decrease and increase in sodium silicate in the treatments compared to the control level (F/2 nutrient medium): decrease by 75% (T1), 50% (T2), 25% (T3) and increase by 25% (T4); 50% (T5) and 75% (T6).

The treatments and microalgal innoculants were completely randomized, with 3 replicates for each treatment. Plastic cans of 20 L were used as containers and a total of 21 plastic cans were used for each experiment. The environmental parameters were consistent among treatments: temperature 25°C, salinity 30‰, pH=8, light intensity 3,000 lux, light cycle 24/24 and F/2 algal nutrient medium. All treatments were arranged with a density of 8.5×10^4 cells mL^{-1}.

Environmental parameters and microalgal density. Temperature and pH were measured with a Hanna HI98127 measuring pen; light intensity was measured with an Extech light intensity meter; the cell density was determined by a Sedgewick Rafter counting chamber (capacity of 1 mL, with 1,000 count cells) and a microscope with a ×10 magnification.

Algal samples were taken 1 time day^{-1}, at 8-8:30 am and each time 1 mL of medium was collected. Algal samples were placed in an Ependorf tube and stabilized with a Lugol's Neutral solution (Nga 2007; Cong & Duong 2014).

To determine algal density, a sample was shaken and a Pasteur pipette was used to aspire the sprayed algae into the counting chamber, which was covered. The sample was left to settle for a while and then counted. Cells were counted inside the plot of the counting chamber under the microscope at x10 magnification. Each batch of algal sample was counted 3 times, the formula for calculating the number of *N. occulata* cells was as described by Mai (2009) and Lien et al (2018).

$$\text{Cell density (cells mL}^{-1}) = \frac{Cx1000}{A\,x\,D\,x\,F}$$

Where:
C - countable cells
A: Area of each cell (1 mm^2);
D - height of each plot (1 mm);
F - number of plots to be counted.

Proximate composition of *N. oculata*. The biomass of microalgae *N. oculata* was collected at the end of the growing phase by filtration and centrifugation to determine the protein, lipid, and carbohydrate components in the biomass (Figure 1). All analyzes were performed at the Institute of Biotechnology, Hue University, Vietnam.

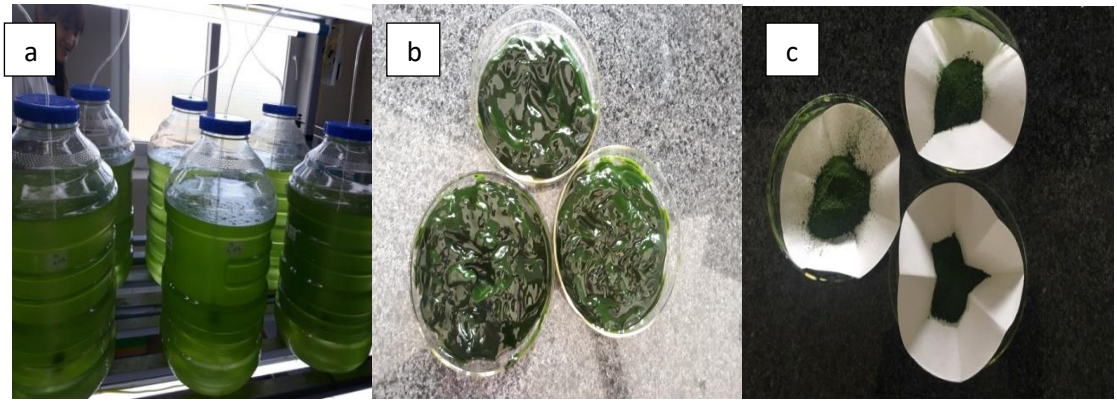

Figure 1. a) Experimental culture of *N. oculata* algae; b) *N. oculata* is collected and concentrated; c) c) *N. oculata* in dried form
Source: Authors' personal archive

Protein content. Protein was extracted according to the method of Barbarino and Louren (Barbarino & Louren 2005). Protein was quantified using the Bradford method by placing 2mL of Bradford's solution into test tubes containing 40µL of sample and optical density at 595 nm was read (Barbarino & Louren 2005).

Lipid content. Lipid content was determined by Soxhlet method with Petroleum ether solvent (Rekha et al., 2012). The lipid content was calculated based on the remaining dry matter weight (Rekha et al., 2012).

$$x = \frac{m1 - m2}{\text{m1}} \times 100\%$$

Where:
x: lipid content (% dry weight) in the sample;
m_1: initial mass of dry sample (g);
m_2: mass of dry sample after extraction (g);

Carbohydrate content. Carbohydrate content was determined using the Dubois method (Dubois, 1956). Glucose was used as a standard at 0.1mg/mL. Optical density (OD) was read at 490 nm. The calibration curve used in the quantification of glucose is shown in Figure 2.

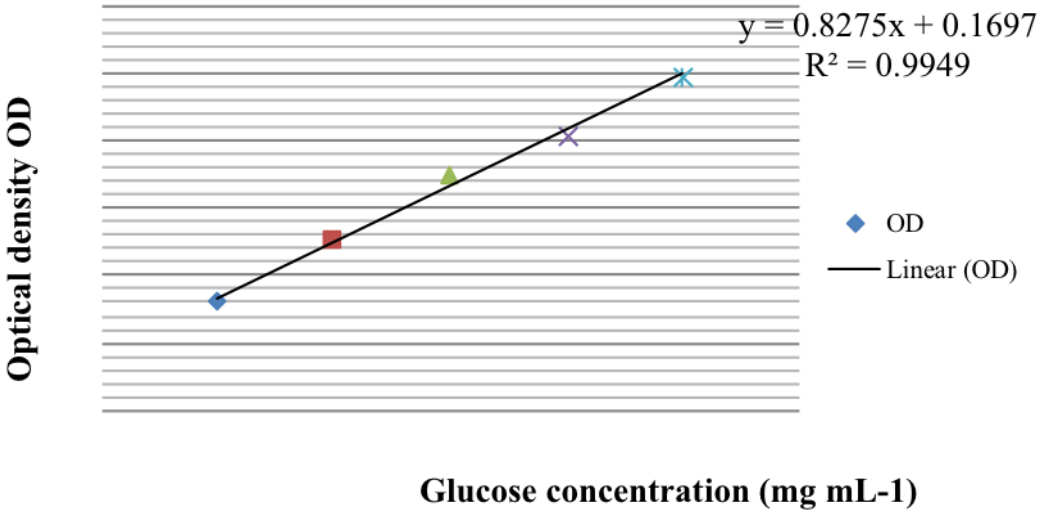

Figure 2. Glucose calibration curve.

The standard curve equation is: y = 0.8275x + 0.1697 with the correlation coefficient R^2=0.9949.

Statistical analyses

Study data were collected and the mean and standard deviation were calculated using the Microsoft Excel 2015. Data were processed with the SPSS 20 software, using the one-way analysis of variance (One-Way ANOVA), and the difference between treatments were tested using Duncan method with 95% confidence interval.

Results

Optimal sodium nitrate concentration. The results of the study on the effect of sodium nitrate content on the growth of microalgae *N. oculata* are shown in Figure 3. Data showed that different sodium nitrate levels exhibited a clear effect on the growth of the algae population of *N. oculata*. In all treatments, the microalgae *N. oculata* grew strongly from day 4 and reached the maximum density at day 9 in treatments with high nitrogen content such as T4, T5 and T6. The density of microalgae cells increased with increasing sodium nitrate content. The highest density was observed at day 9 in T5 (112.5 mg/L), followed T6 in day 10. In contrast, algae in the control treatment F/2, reached a maximum density of 271.8 × 10^4 cells mL^{-1} on the 9th day of culture. However, during the study, we found that adding high sodium nitrate content in T6 (131.2 mg/L) resulted in the rapid growth of the algae during the first few days, especially on the 6th, reaching the highest density of 157.4 × 10^4 cells mL^{-1}; however, immediately after, the algal population died out quickly and the density decreased steeply on the last day of the experiment. T4, T5 and F/2 still maintained high densities on day 12, with T5 maintaining the highest density of 269.8 × 10^4 cells mL^{-1} which was statistically different from the rest of the treatments (p>0.05).

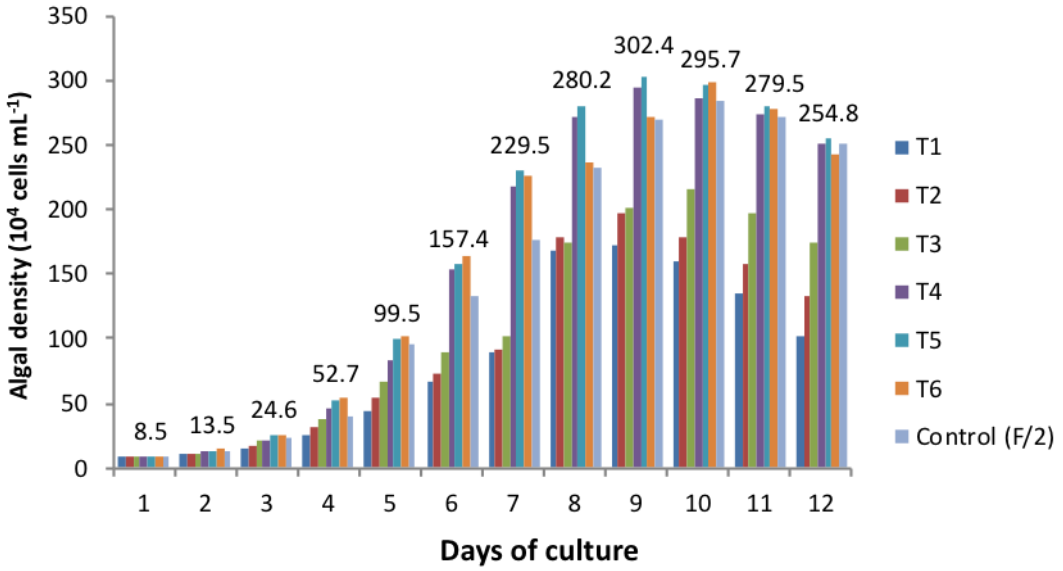

Figure 3. Effect of different nitrogen concentrations on the growth of *N. oculata*.

Figure 3 shows that when sodium nitrate content was reduced, algae density increased slowly and was maintained at a very low level than did the other treatments. In T1 (18.75 mg L^{-1}), the density was only 83.7 × 10^4 cells mL^{-1}, T2 (37.5 mg/L) 149.2 × 10^4 cells mL^{-1} and, T3 (56.25 mg L^{-1}) 176.4 × 10^4 cells mL^{-1} on day 12. Thus, the increase or decrease of sodium nitrate content affected the growth of algae pointing to an optimal level.

Protein, lipid and carbohydrate contents also differed when increasing or decreasing the sodium nitrate content in the algal culture medium. Results are shown in Table 1.

Table 1.

Effect of sodium nitrate concentration on protein, lipid and carbohydrate content in biomass of *N. oculata*

Treatments	*Nutrient content (% dry weight)*		
	Protein	*Lipid*	*Carbohydrate*
T1	24.67 ± 2.81^a	18.63 ± 1.72^c	6.51 ± 1.12^a
T2	24.86 ± 2.15^a	18.58 ± 2.56^c	6.57 ± 0.52^a
T3	26.72 ± 1.64^b	18.42 ± 1.88^c	6.62 ± 1.33^a
F/2 (Control)	30.91 ± 1,86^c	17.50 ± 0.53^b	7.63 ± 0.55^b
T4	31.02 ± 1.88^c	12.67 ± 1.88^a	8.43 ± 1.17^c
T5	33.97 ± 2.61^d	12.45 ± 1.48^a	8.54 ± 1.43^c
T6	31.31 ± 2.63^c	12.26 ± 1.82^a	8.57 ± 1.28^c

*The superscript letters $^{a, b, c, d}$ in the same column between treatments have a statistically significant difference *($p<0.05$).*

Table 1 shows that the protein, lipid and carbohydrate contents in *N. oculata* changed markedly between treatments, in which protein was highest in T5, 33.97%, higher than the control (30.91%). The reduction of sodium nitrate concentration in the algal nutrient medium also affects the quality of algal biomass, the protein content in algae was significantly low which ranged from 24.67% (T1) to 26.72% (T3), much lower than that of the control. A significant difference was observed between these treatments compared to the control ($p<0.05$).

In contrast, lipid content tended to increase with decreasing sodium nitrate content in the algal culture medium. T1 exhibited the highest lipid content (18.63%) when the sodium nitrate was lowest (18.75 mg L^{-1}). Increasing sodium nitrate in the medium reduced the lipid content of the algae specifically in T6 (131.2 mg L^{-1} sodium nitrate) was

only 12.26%. Treatments with higher sodium nitrate content than the control F/2 showed lower lipid accumulation than that of the control. There was a statistically significant differences between these treatments and the control treatment F/2 ($p<0.05$).

Carbohydrate content increased from 8.43 to 8.54 and 8.57 mg/L in treatments T4, T5 and T6, higher than that of the control (7.63 mg L^{-1}) (Table 1). Carbohydrate values of the control and the treatments ($p<0.05$) differed significantly, although values for T4, T5 and T6 were not statistically different ($p>0.05$).

Effects of sodium phosphate. *N. oculata* grew quickly reaching the highest density when P was increased by 50% (7.5 mg/L) in T5 compared with that of the control (5mg L^{-1}). The maximum cell density was 302.4 × 10^4 cells mL^{-1}, while in the control was 283.5 × 10^4 cells mL^{-1}. The microalgae *N. cculata* grew poorly when the sodium phosphate content was lower than that of the F/2 medium. A statistically significant differences was observed between these treatments and the control (Figure 4).

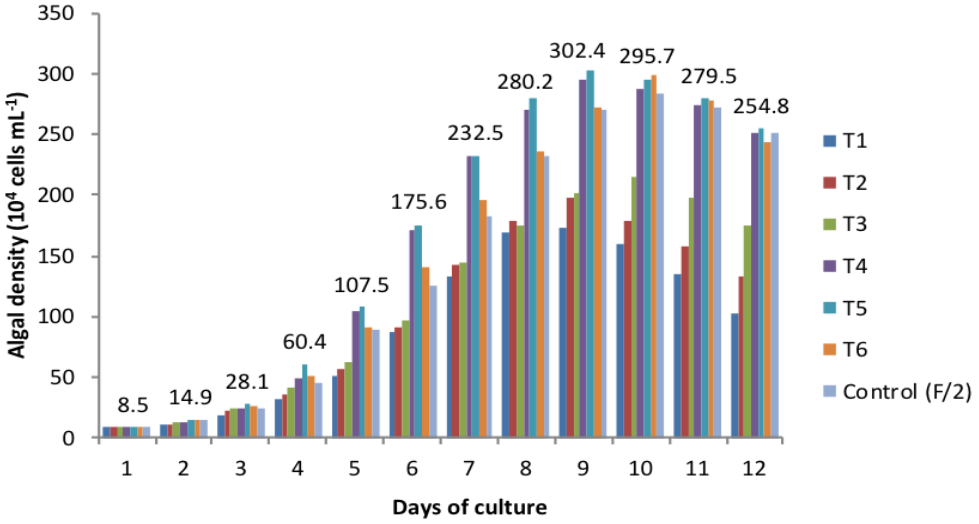

Figure 4. Effect of different concentrations of sodium phosphate on the growth of *N. oculata.*

High total lipid accumulation of the microalgae *N. oculata* was observed at low sodium phosphate concentrations (Table 2). The lipid content gradually increased when sodium phosphate was reduced from 25% (3.75 mg/L) to 75% (1.25 mg/L) than that of the control F/2 (5 mg L^{-1}). In T2 in which 50% reduction in sodium phosphate concentration compared with the control, the lipid content of *N. oculata* exhibited the highest level of 18.88% (T1), higher than the 17.5% in the control.

Table 2.

Effect of sodium phosphate concentrations on protein, lipid and carbohydrate content in biomass of *N. oculata*

Treatments	*Nutrient content (% dry weight)*		
	Protein	*Lipid*	*Carbohydrate*
T1	24.58 ± 3.33^{a}	18.88 ± 1,47^{c}	6.36 ± 1.08^{a}
T2	26.12 ± 2.35^{b}	18.52 ± 0,12^{c}	6,54 ± 1.12^{a}
T3	26.71 ± 2.21^{b}	18.46 ± 0,82^{c}	6.67 ± 1.27^{a}
F/2 (Control)	30.91 ± 1.86^{c}	17.50 ± 0.53^{b}	7.63 ± 0.55^{b}
T4	31.07 ± 2.24^{c}	12.01 ± 0,43^{a}	8.04 ± 1.04^{c}
T5	33.96 ± 2.18^{d}	11,69 ± 0,12^{a}	8.12 ± 1.47^{c}
T6	33.72 ± 2.62^{d}	10,14 ± 0,64^{a}	7.98 ± 1.44^{c}

*The superscript letters $^{a, b, c, d}$ in the same column between treatments have a statistically significant difference *($p<0.05$).*

In contrast, protein and carbohydrate content increased with increasing concentrations of sodium phosphate, the highest value was attained by an increase of 50% sodium phosphate (T5) compared with control treatment (F/2). However, when sodium phosphate was increased to 75% (T6), protein and carbohydrate content did not increase (Table 2).

Effect of sodium silicate concentration. The effect of sodium silicate concentration on algal growth is shown in Figure 5. The concentration of sodium silicate affected the growth of *N. oculata*, in which algal density was low when the concentration of sodium silicate in the medium was low. In contrast, algae grew well in T5 when there was a 50% increase (45 mg L^{-1}) in sodium silicate compared with that of the control (30 mg/L), reaching a maximum of 310.4 × 10^4 cells mL^{-1} compared with the control of 280.5 × 10^4 cells mL^{-1} at day 10. A statistically significant difference was observed when sodium silicate was increased compared with the control ($p<0.05$).

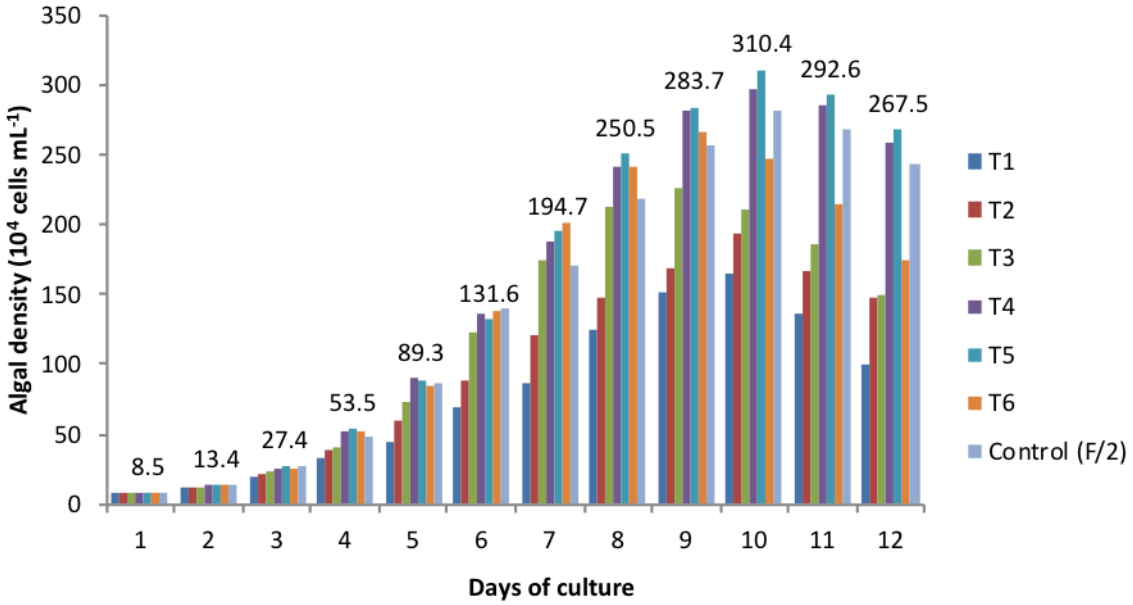

Figure 5. Effect of different concentrations of sodium silicate on the growth of *N. oculata.*

Protein and carbohydrate contents accumulated in *N. oculata* and tended to increase when sodium silicate content was increased compared with the nutrient medium F/2. The highest protein content was 33.22% in T5 followed by T6 (33.06%) and T4 (32.95%). Protein content in all these treatments were higher than that of the control treatment (30.91%). Statistical analysis results also show that there is a statistically significant difference between these treatments compared with the control treatment ($p<0.05$), however when comparing between treatments T4, T5 and T6 did not show a statistically significant difference between these treatments (p.0.05). Accumulated protein content in microalgae tended to decrease when reducing sodium silicate content in algae culture medium (Table 3).

Table 3.
Effect of sodium silicate concentrations on protein, lipid and carbohydrate content in biomass of *N. oculata*

Treatments	*Nutrient content (% dry weight)*		
	Protein	*Lipid*	*Carbohydrate*
T1	24.22 ± 2.14[a]	17.62 ± 1.72[b]	5.76 ± 0.38[a]
T2	26.14 ± 2.31[b]	17.48 ± 2.56[b]	5.34 ± 1.17[a]
T3	26.24 ± 2.42[b]	17.24 ± 1.88[b]	6.46 ± 0.43[b]
F/2 (Control)	30.91 ± 1.86[c]	17.50 ± 0.53[b]	7.63 ± 0.55[c]
T4	32.95 ± 2.24[d]	12.27 ± 1.88[a]	7.83 ± 0,6[c]
T5	33.22 ± 2.18[d]	12.24 ± 1.48[a]	7.74 ± 1,4[c]
T6	33.06 ± 2.62[d]	11.89 ± 1.82[a]	7.71 ± 1,1[c]

*The superscript letters [a, b, c, d] in the same column between treatments have a statistically significant difference *(p<0.05).*

Carbohydrate content in *N. oculata* increased when sodium silicate in medium was increased. However, increasing sodium silicate content did not increase carbohydrate content compared with F/2 medium. No significant differences was observed in these treatments and the control.

Discussion. Nitrogen is an essential component of all structural and functional proteins in algal cells and constitutes more than 7% of the cell's dry weight. Inorganic nitrogen is absorbed by the algae and assimilated into biochemical compounds that provide vital cellular activities in response to changing physiological needs (Ankita et al 2013). Different nitrogen levels have a great influence on the growth of algae in general, which has been mentioned in many studies. According to Pruvost et al (2009), excess or deficiency of nitrogen both reduces growth, metabolism, and nutritional quality of many algae species, including *S. platensis* and *N. oculata* (Pruvost et al 2009). Trang (2013) showed that when the nitrogen level is low (6.18 mg/l), the photosynthesis of algae still takes place but with low intensity, the biomass of algae increases slowly and the equilibrium phase lasts while at 18.63 mg/L, the algae *S. platensis* grew rapidly and achieved the highest biomass (4.90 g/L). In addition to reduced growth, nitrogen deficiency caused algal chlorosis and faster algal dieback times compared with higher nitrogen levels. According to De Pauw et al (1983), the demand for nitrogen is always high, the nitrogen level suitable for the growth of algae *Chaetoceros gracilis* from 12.41 to 17.41 mg/l, algae *C. calcitrans* from 6.69 to 12.69 mg/L and green algae *Tetraselmis sp*. grow well between 17.36 and 22.36 mg/L. Research on Chaetoceros ChTA strain showed that algae grew best in 112.50 mg/L sodium nitrate medium, with maximum biomass of 0.482 g/L (Lien et al 2017). Research by Tam (2019) also shows that different nitrogen concentrations of the microalgae *N. oculata* will grow differently, the microalgae *N. oculata* grew rapidly when the nitrogen concentration was increased compared to the F/2 nutrient medium. At the nitrogen concentration of 99.74 mg/l, microalgae achieved the highest biomass (95.93 mg/L), 9.3% higher than that of the F/2 medium (75 mg/L). Our results are also quite similar to those of Lien et al, (2015) on *Chaetoceros ChTA* and Tam (2019) study on *N. oculata,* microalgae grew best at nitrogen concentration of 112.5 mg/L, the highest algal cell density was 314.6×10^4 cells mL^{-1}, higher than that of the F/2 medium (75mg/L), the microalgae density was 295.3×10^4 cells mL^{-1}.

In addition, nitrogen and phosphorus is an essential nutrient source for the growth and metabolism of algal cells. Restrictions and deficiencies of these important nutrients alter metabolic pathways (Murthy et al 2013). Phosphorus is an integral component of the ATP molecule and is also a structural part of DNA and RNA. In addition, phosphorus is also an important component in phospholipid membranes. The direct effect of phosphorus restriction will be to reduce the synthesis and regeneration of substances in the Calvin-Benson cycle and reduce the rate of light utilization for carbon fixation (Ankita et al 2013). The nutritional composition (nitrogen, phosphorus, silicon and minerals) has

a great impact on the growth ability and biochemical composition of microalgae cells. Macronutrients (carbon, nitrogen, phosphorus) and trace elements play an important role in the growth of microalgae, especially in high density conditions (Tantanasarit et al 2013). Nitrogen and phosphorus deficiencies are the causes of reduced growth rate, biomass, time to maintain maximum density, pigment content, protein, lipids, unsaturated fatty acids, vitamins, carotenoids, phycocyanin and enzymes in many algae species including *C. calcitrans* (Cohen 1999). Dung et al (2016) showed that the growth and density of *C. calcitrans* algae tended to increase with increasing concentrations of nitrogen, silicate and phosphorus. The addition of 1764 µM nitrogen, 159 M silicate and 72.4 µM phosphorus for growth and highest algal density were 18,975 x 10^3 cells/mL, 15,265 x 10^3 cells/mL and 10,358 x 10^3 cells/mL, respectively compared with nutrient medium F/2 (N: 882 µM, P: 36.2 µM and Si: 106 µM). Lien et al (2017) suggested that, compared with sodium nitrate and sodium phosphate, the change in sodium silicate content had less effect on the increase in biomass and total lipid content of *Chaetoceros* ChTA. The maximum biomass yield was 0.383 g/L at the concentration of 5.22 mg/L sodium silicate added to the algae culture medium, while in the F/2 nutrient medium, the biomass of *Chaetoceros* ChTA was 0.381 g/L and there was no statistically significant difference when increasing the sodium silicate content compared to the F/2 medium.

Research on microalgae *N. oculata* of Tam (2019) showed that microalgae *N. oculata* had a phosphorus demand 50% higher than phosphorus in F/2 nutrient medium, at phosphorus concentration 54.3µM (6.25 mg/L), microalgae reached maximum biomass of 214.39 mg/L, while in F/2 microalgae the biomass was only 186.5mg/L, and that the need for sodium silicate was also not high compared to the standard F/2 medium. Our study also found that increasing the content of sodium phosphate and sodium silicate increased the growth of microalgae *N. oculata*.

In terms of biomass quality, lipid content increased under nitrogen deficiency conditions while this was opposite for protein and carbohydrate content. Different nitrogen sources affect the growth of microalgae because nitrogen is required for the formation of protein, which is the main nutrient of algae (Sheehan et al 1998). Research by Trang (2013) showed that different nitrogen levels also greatly affect the protein and lipid content of the algae *S. platensis*. Increasing nitrogen levels in the culture medium increased protein content but decreased lipid accumulation in algal cells. Specifically, at a nitrogen level of 30.88 mg/L, the protein content in algae reached 69.64% and 10.12% lipid. However, at the nitrogen level of 6.18 mg/L, the protein content in the algae reached 52.29% but the lipid content reached 13.48%. Research by Lien (2017) concluded that *Chaetoceros* ChTA strain grew best in nutrient medium containing 112.50 mg/L sodium nitrate, with maximum biomass of 0.482 g/L. However, the lipid content in algae is inversely proportional to the increase of sodium nitrate and sodium phosphate content in the algae culture medium. Total lipid in *Chaetoceros* ChTA algae reached the highest at 25.62% dry weight at low sodium nitrate concentration 18.75mg/L. Similarly, high lipid content was observed at concentrations observed in the medium supplemented with 2.13 mg/L sodium phosphate. Meanwhile, increasing and decreasing sodium silicate did not make a big difference in lipid and protein content compared with F/2 nutrient medium. The carbohydrate content of *Chaetoceros* ChTA algae tends to increase and decrease in proportion to the increase and decrease of the content of sodium nitrate, sodium phosphate and sodium silicate. Phosphorus deficiency also leads to fat accumulation in microalgae cells. Total lipid content in *Scenedesmus sp.* increased from 23% to 53% with a decrease in phosphorus concentration (as phosphate) from 2.0 mg/L reduced to only 0.1 mg/L (Li et al 2010).

Tam's (2019) research on the microalgae *N. oculate*, also showed that increasing the nitrogen and phosphorus content in the algal culture medium increased the protein content but decreased the lipid content in the microalgae. Some authors suggested that the reason was that nitrogen deficiency affected protein synthesis, thereby reducing the amount of soluble protein required for metabolic processes leading to a decrease in cell growth. Nitrogen restriction in the nutrient medium can cause the accumulation of lipids in algae (Lien et al 2017; Tam 2019). Sheehan et al (1998) suggested that the reason for the increase in lipid content was lack of nutrition, the production rate of all cellular

components was lower, but fat production remained high, lead to the accumulation of fat in the cells. The nitrogen-depleted nutrient medium leads to inhibition of cell division, without reducing the immediate rate of lipid production. They also suggested that it was necessary to control the time to harvest algal cells so that the total lipids obtained were highest during the algal culture (Sheehan et al 1998). The results of our study on the microalgae *N. oculata* also found similarities with the above studies. When increasing the content of nutrients N, P, and Si in the algal culture medium (compared with the F/2 nutrient medium) at an appropriate level, the protein and carbohydrate content in these microalgae will increase. However, the lipid content accumulated in microalgae was high when the content of N and P in the algal culture medium was low. The content of nutrients in the microalgae *N. oculata* was recorded as follows: protein (24.14 - 33.97%), lipid (10.14 - 18.23%), carbohydrates (5.34 - 8.57%). These results are similar to those reported in the study of Brown et al (1997) who recorded the nutrient content in the microalgae *N. oculata,* protein from 6 - 52%, lipid 7 - 23% and carbohydrates 5 - 23%. Huang Xu-xiong et al (2004) also showed that protein content in microalgae *N. oculata* ranged from 28.33 to 33.99%, lipid from 6.34 to 22.5% and carbohydrate from 5.21 to 23%.

Conclusions. The different levels of sodium nitrate, sodium phosphate, and sodium silicate in the nutrient medium generated significant effects on growth of *N. oculata*. Specifically, microalgae grown at sodium nitrate level 112.5 mg L^{-1} achieved the best growth of 314.6 × 10^4 cells mL^{-1}. A 50% (7.5 mg/L) increase in sodium phosphate concentration compared with that of the control F/2 (5 mg/L) exhibited the highest cell density of 302.4 × 10^4 cells mL^{-1}, while the control group reached a maximum density of 283.5 × 10^4 cells mL^{-1}. *N. oculata* grew well at 45 mg/L sodium silicate concentration, increasing by 50% compared to the control treatment F/2 (30 mg/L), equivalent density reached a maximum of 310.4 × 10^4 cells mL^{-1} compared with the control treatment 280.5 × 10^4 cells mL^{-1}.

The content of nutrients protein, lipid and carbohydrate also differed when increasing or decreasing the sodium nitrate and sodium phosphate content in the culture medium. Increasing the sodium nitrate levels by 50% compared with the F/2 medium increased the algal protein (33.97%) and carbohydrate contents (8.54%) but decreased the lipid content (12.45%) compared with those of the F/2 medium (17.5%). *N. oculata* achieved high protein (33.96%) and carbohydrate (8.12%) content when sodium phosphate content was increased to 50% compared with the control. However, increasing the sodium silicate content did not significantly affect the nutritional composition of microalgae compared to using F/2 nutrient medium. Depending on the purpose of using microalgae, we can choose the appropriate nutrient environment. The recommended sodium nitrate content based from the results of the present study is 112.5mg/L, sodium phosphate is 7.5mg/L and sodium silicate is 45mg/L used to culture the microalgae *N. oculata.* The results of this study have important practical implications. It helps to select the best nutrient medium for growing *N. oculata*. In particular, the addition of sodium nitrate, sodium phosphate, and sodium silicate content to the F/2 nutrient medium will help this microalgae achieve the best growth and quality.

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