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Density and Growth Rate of *Nannochloropsis oculata* with Different Photoperiods

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ABSTRAK

Nannochloropsis oculata adalah jenis fitoplankton bersel tunggal atau eukariotik yang termasuk dalam kelas Eustigmatophyceae. Mikroalga jenis ini banyak digunakan sebagai pakan alami rotifera dan larva ikan karena memiliki kandungan gizi yang tinggi. Mengingat peran ini, spesies *Nannochloropsis oculata* banyak dibudidayakan dengan cara dikultur. Keberhasilan kultur mikroalga dipengaruhi oleh faktor pertumbuhan seperti intensitas cahaya, CO_2, suhu, dan nutrisi. Namun, dalam prosesnya sering muncul permasalahan mengenai pertumbuhan *Nannochloropsis oculata* yang tidak stabil. Hal ini biasanya disebabkan oleh kurang optimalnya faktor-faktor yang mempengaruhi pertumbuhan, seperti cahaya yang tidak optimal. Berdasarkan permasalahan tersebut maka perlu dilakukan penelitian yang bertujuan untuk mengetahui laju pertumbuhan *Nannochloropsis oculata* dengan perlakuan penyinaran yang berbeda. Metode penelitian yang digunakan adalah metode eksperimental dengan 4 perlakuan yaitu, A (24T : 0G), B (12T : 12G), C (18T : 6 G), dan D (6T : 18G). Hasil penelitian menunjukkan bahwa waktu penyinaran yang berbeda terhadap kepadatan mikroalga *Nannochloropsis oculata* tidak berpengaruh nyata terhadap laju pertumbuhannya. Meskipun hasil uji statistik pemberian fotoperiode tidak mempengaruhi produksi kepadatan sel mikroalga *Nannochloropsis oculata*, tetapi pemberian perlakuan dengan fase gelap menghasilkan pertumbuhan yang lebih baik.

Kata kunci *:* *Nannochloropsis oculata,* mikroalga, photoperiod, kepadatan, laju pertumbuhan

ABSTRACT

Nannochloropsis oculata is a type of single-celled or eukaryotic phytoplankton belonging to the class Eustigmatophyceae. This type of microalgae is widely used as natural food for rotifers and fish larvae because it has a high nutritional content. Given this role, many species of Nannochloropsis oculata are cultivated by culture. The success of microalgae culture is influenced by growth factors such as light intensity, CO_2, temperature, and nutrition. However, in the process problems often arise regarding the unstable growth of Nannochloropsis oculata. This is usually caused by factors that affect growth, such as light that is not optimal. Based on these problems, it is necessary to conduct research that aims to determine the growth rate of Nannochloropsis oculata with different irradiation treatments. The research method used was an experimental method with 4 treatments i.e., A (24L : 0D), B (12L : 12D), C (18L : 6D), and D (6L : 18D). The results showed that different light exposure period on the density of Nannochloropsis oculata microalgae had no significant effect on their growth rate. Even though the results of statistical tests of photoperiod administration did not affect the production of Nannochloropsis oculata microalgae cell density, the treatment with the dark phase resulted in better growth.

Keywords :*Nannochloropsis oculata, microalgae, irradiation time, density, growth rate*

INTRODUCTION

As a maritime country, Indonesia is known as a mega-biodiversity country, with high biodiversity which distributed across islands. Organisms that exist in this region are very diverse, ranging from single-celled organisms such as microalgae to the most complex organisms (Mishbach *et al.*, 2022).

Microalgae as one of the aquatic product commodities, nowadays have become an alternative to be developed because it has great potential to be used as feed or food. Most species of microalgae produce distinctive products such as carotenoids, antioxidants and fatty acids. *Dunaliella salina* and *Nannochloropsis* sp. is an example of microalgae that has the potential to be developed primarily as a source of carotenoids (Darsi, *et al.,* 2012). Because of this content, microalgae become a natural food for fish and their availability in waters can be an indicator of aquatic productivity and has the potential to increase fish production (Bahua, *et al.,* 2015).

*Nannochloropsis oculata*is a type of microalgae that contains lots of nutrients, including; protein (52.11%), carbohydrates (16.00%), fat (27.64%), and chlorophyll pigment (0.89%). The high nutrients contained in *Nannochloropsis oculata* make this type of phytoplankton necessary to help seed growth. By utilizing *Nannochloropsis oculata* as natural food, it is certainly very necessary in sufficiently sustainable and timely quantities. So there is a need for cultivation to develop natural food for the type of *Nannochloropsis oculata*. However, in its development, microalgae cultivation as natural food is still passive and not very developed. Even though microalgae have a size that fits the fish's mouth opening and the nutrients contained can meet the needs of fish growth (Kaparapu, 2018). Therefore, studies are needed to optimize the production of microalgae *Nannochloropsis oculata*, one of which is by knowing the best duration of irradiation as an effort to increase its production to meet the needs of fish feed.

MATERIAL AND METHODS

Experimental Design

This research was conducted from May to July 2022, at the Pescica Marina Laboratory, Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University and used an experimental method with 4 treatments with different duration of light exposures (photoperiod) i.e., A (24L:0D), B (12L:12D), C (18L:6D), and D (6L:18D). the light were connected to an automate timer which had been set and able to switch to the appoint photoperiod treatments. The density and growth of *Nannochloropsis oculate* were collected every four days intervals with total three times during the culture day (i.e., 8 days). The density and growth measurements had two times replication at each observation times.

The culture of Nannochloropsis oculata

The first stage in carrying out this research was the preparation of the culture room, the equipment and materials. The microalgae culture room was covered with dark plastic layer to ensure the light cannot penetrate and disturbed the photoperiod treatments; the temperature was maintained ±18-20 °C. The culture room was provided with two LED light 8 watt (Krisbow™) which connected to automated timer to provide the photoperiod treatments. The microalgae were grown in a 1000 ml of filtered seawater of Duran's bottle which had been sterilized prior the culture. One millilitre of F/2 Guillard media was added to the bottle culture. Approximately 30 ml of microalgae were added to the bottle culture and equipped with glass Pasteur pipette (Normax™)

which connected with aerator to provide oxygen and water motion inside the culture. All the procedures were conducted aseptic and at the end of process, each of the bottle were then sealed by using sterile aluminium foil (Fig. 1). The observations were made for 8 days of cultre.

Calculation of Density and Growth Rate of *Nannochloropsis oculata*

The density data was collected three times during 8 days of culture period. The culture of *Nannochloropsis oculata* was observed on day 0, day 4, and day 8 with two repetitions or duplo and density calculations were carried out for each repetition. The dilution process was carried out prior calculating the density. This dilution was aimed to obtain an artificial sample (cultured microalgae) with the desired concentration. The ratio between seawater and microalgae is 9:1, with 9 mL of seawater and 1 mL of cultured microalgae. The dilution were10 mL test tubes with dilution concentrations of 10^{-1}, 10^{-2}, and 10^{-3}. After the dilution, microalgae density was calculated using a Neubauer Haemacytometer under the trinocular microscope (Olympus™) with 40× magnifications.

Estimation of the cell density of the *Nannochloropsis oculata* microalgae in this study (CSIRO, 2023):

$$N = \frac{n}{4}\ x\ 1\ x\ 10^4$$

N = Density (cells mL^{-1})
n = Number of Cell Density in four chamber of Haemacytometer
V = Volumes total starter of *Nannochloropsis oculata*

Then, to determine the growth rate of microalgae from day 0, day 4, and day 8, it can be calculated using the equation from Janssen *et al.,* (2013):

$$\mu = \frac{\ln Nmax - \ln N_0}{Tmax - T_0}$$

μ = specific growth rate (days),
Nmax = highest cell density (mL $cells^{-1}$)
N_0 = initial cell density (mL $cells^{-1}$)
Tmax = maximum time for microalgae culture (days)
T_0 = initial time of stocking (days).

After that, the percent growth was determined through the formula:

$$\text{growth rate} = \frac{\sum Nmax - \sum N0}{\sum N0}\ x\ 100\%$$

∑Nmax = total cell density of the last day
∑N0 = number of cell densities of the first day

Data Analysis

Data on the density and growth rate of the microalgae *Nannochloropsis oculata* were analyzed by using the T-Test then the data were presented in the form of a graphical display of data tabulation which is

Figure 1. The culture of microalgae *Nannochloropsis oculata*

illustrated descriptively. The percentage of growth was analyzed using charts and graphs. Each graphic display shows the proportion of the frequency of each category series.

RESULTS AND DISCUSSIONS

Density and Growth Rate of *Nannochloropsis oculata*

Observation data on the density and growth rate of *N. oculata* in laboratory-scale culture for 8 days of maintenance illustrated in Table 1.

The result revealed that the density and growth rate of *N. oculata* were not significantly different (*P ≥ 0.05, T-test*) in all treatments. In treatment A (24L : 0D) has an average density value of 6.26 x 10^6 cells mL^{-1}, treatment B (12L : 12D) shows an average density value of 9.18 x 10^6 ± 0.33 cells mL^{-1}, treatment C (18L : 6D) showed that the average cell density was 6.42 x 10^6 cells mL^{-1}. Then, the result of treatment D (6L : 18D) showed an average density of 8.22 x 10^6 ± 0.25 cells mL^{-1}. From the results obtained, it can be seen that the long irradiation or light exposure treatment B has an average density value that is higher than the other treatments, although it was not showed statistically. This is because treatment B has a long irradiation that is in accordance with the needs of *N. oculata* to carry out the photosynthesis process, which was called the presence of optimal dark and light phases (Utami *et al.,* 2012). Whereas treatment A had the lowest average density value because the lighting time given was too excessive without the presence of a dark phase, which could affect the decrease in the number of microalgae cells *N. oculata*.

The results of the average growth rate in this study had a difference that was not that far away as can be seen in Table 1. Treatment A had an average growth rate of 6 ± 4.05 cells day^{-1}, then in treatment B the average growth rate growth was 9 ± 4.58 cells day^{-1}. Furthermore, treatment C had an average growth rate of 9 ± 4.50 cells day^{-1}. Then for treatment D, the average growth rate was 8 ± 4.26 cells day^{-1}. Cell division of *N. oculata* between treatments B and C had the same average growth rate, i.e., every 1 cell of *N. oculata* was able to divide into 9 cells in a day. Treatments B and C had the same results because in the process *Nannochloropsis oculata* received lighting close to optimal light for photosynthesis, namely 14 hours of light and 10 hours of darkness which is the general period for optimum irradiation in microalgae (Isnansetyo, 1995). From these results, it can be seen that the density is directly proportional to the growth rate of microalgae. The high density is also followed by a high growth rate and vice versa. Thus, the different duration of irradiation treatment in this study did not show a strong effect on the growth of *N. oculata*.

Providing different photoperiod to microalgae cultures showed variation on the growth pattern of *N. oculata*. The increase in density from day 0 to day 8 in *N. oculata* indicated that inoculum cells in

Table 1. Density Measurement Results and Growth Rate *N. oculata*

Treatment	Density (cells mL^{-1})	Growth rate (cells day^{-1})
A	6,26 x 10^6 ± 0,25[a]	6 ± 4,05[a]
B	9,18 x 10^6 ± 0,33[a]	9 ± 4,58[a]
C	6,42 x 10^6 ± 0,51[a]	9 ± 4,50[a]
D	8,22 x 10^6 ± 0,25[a]	8 ± 4,26 [a]

Treatment A (24L : 0D), treatment B (12L : 12D), treatment C (18L : 6D) and treatment D (6L : 18D). Numbers with the same superscript are not significantly different (*P* ≥ 0.05, *T-*

microalgae began to utilize nutrients in the growth media so that the cell biosynthetic process was able to reproduce more. In this reproduction also requires energy which of course is obtained from the photoautotrophic process with the help of light. With different irradiation treatments, it can be seen that the duration of light received is related to meeting the needs of microalgae for optimal irradiation duration. The existence of this long irradiation treatment can be manipulated by extending or shortening the irradiation in the microalgae culture process or commonly referred to as the light-dark cycle (Utami et al., 2012). According to Bouterfas et al., (2006), photoperiod affects the process of photosynthesis in microalgae which consists of a dark phase and a light phase. The existence of a dark phase is necessary because in the dark phase two reactions occur, namely photochemical reactions and biochemical reactions. Photochemical reactions are chemical reactions that use light in the process, then biochemical reactions are reactions that make simple compounds into complex compounds. Compounds produced in the light phase are ATP and NADPH which will later be used in the dark phase to synthesize metabolic molecules for microalgae cell growth.

The different photoperiod treatments in this study may possibly affect the photosynthetic process that occurred in the N. oculata culture. The greater the rate of photosynthesis in microalgae, the greater the biomass and cell density produced in microalgae culture (Utami et al., 2012). In its growth, N. oculata is not only influenced by light, but there are other supporting factors such as nutrient content and maintenance of environmental conditions. This will reduce the development of the microalgae population and certainly affect growth. Therefore, the density of N. oculata is not only influenced by the light factor but nutrient and environmental factors also affect the growth of *Nannochloropsis oculata.*

The percentage of growth in treatments A, B, C, and D in Figure 2 showed that the growth pattern of *Nannochloropsis oculata* during 8 days of culture experienced an increase in growth. The percentage of growth on day 0 has no growth rate because this phase has just started the process of protein synthesis and cell metabolism so that the value of the growth rate is 0%. After day 0, the growth rate of *N. oculata* began to enter the exponential phase until day 4, namely 12% for treatment A, 22% for treatment B, 28% for treatment C, and 13.25% for treatment D. For the 8th day is the exponential phase where cells begin to experience division and cause high density. This caused the growth rate to increase, the growth rate for

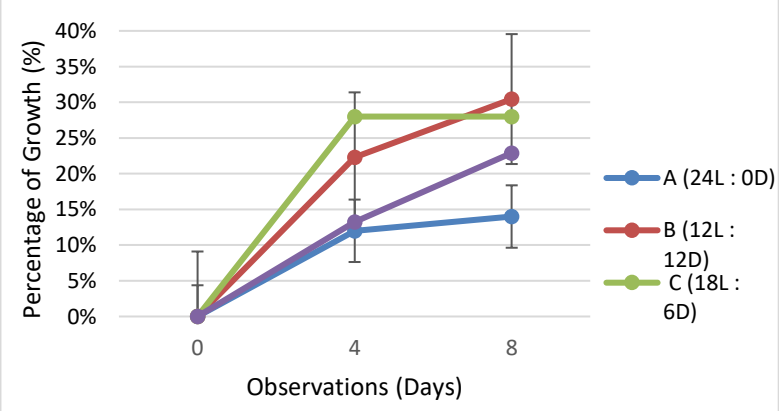

Figure 2. Growth pattern of *Nannochloropsis oculata*

treatment A was 14%, treatment B was 30.45%, treatment C was 28%, and the growth rate for treatment D was 22.9%.

The growth pattern on the culture of the microalgae *N. oculata* is in accordance with the research Arfah *et al.,* (2019), that on day 0 the microalgae *N. oculata* was still in the adaptation phase or adjustment to the culture environment. Then through the first 24 hours, the population density of *N. oculata* cells began to increase, but the growth did not double (exponential phase). Whereas on the 4th day an increase in density has begun to occur which is marked by the increasing number of dividing cells and this phase is called the exponential phase. Then, the peak density was on day 8 where the growth rate increased rapidly but decreased on the following day (Nurhayati *et al.,* 2013). This is in accordance with the results obtained in this study, because there was still an increase on the 8th day. However, the *N. oculata* in treatment C with 18 h exposure of light and 6 h dark experienced a stationary phase after reaching the growth peak at the 4th day with 28%. This could be due to the excessive of light exposure (> 12 h) had impact on the production of excessive energy which may lead to maximum production of microalgae cells. When the density of microalgae is high in the cultured bottle and imbalance with the amount of nutrition inside the media, as consequences, the microalgae will not be able to do more cell development. Based on the research, it revealed that the photoperiod (18L:6D) treatment may become a solution to speed up *N. oculata* production, since it reaches maximum production after the 4th day of culture. After the 8th day, there is a decrease in growth caused by microalgae which have entered the stationary phase or death phase and occurs due to changes in optimum conditions either by light, temperature, or other factors and in this death phase it is also characterized by the number of cells in the culture medium drastically decreased and the color of the water in the culture medium turned brown (Sinaga *et al.,* 2021).

The percentage of growth yield of *N. oculata* in this study is also in line with the statement Fajar *et al.,* (2019) that in the 12 light and 12 dark treatments, the photosynthetic process that occurs in microalgae is quite optimal where 12 hours is the most time for plants to receive light. Therefore, treatment B had better growth percentage results than the other treatments. However, in this case the higher the microalgae growth rate, the process of nutrient utilization is also higher. The process of utilizing nutrients is also related to the uptake of nutrients by microalgae in their growth. Thus, the growth rate is also directly proportional to nutrients. The highest nutrient absorption occurred in treatment B with 12 hours of light and 12 hours of darkness where optimal photosynthetic processes occurred in this treatment causing increased cell metabolism and higher nutrient requirements. Whereas in treatment A, due to the absence of a dark phase and excess quantity of light, the nutrients run out quickly so that it has the lowest density and growth percent. Then for treatment C, the difference in results is not too far from treatment B, because the duration of irradiation in treatment C is still acceptable for photosynthesis. Then for treatment D showed lower results than B and C because the dark phase was longer than the light phase causing nutrient absorption to be less than optimal. These results are in accordance with the statement because the duration of irradiation in treatment C is still acceptable for photosynthesis. Then for treatment D showed lower results than B and C because the dark phase was longer than the light phase causing nutrient absorption to be less than optimal. These

results are in accordance with the statement Soewardi *et al.,* (2005), that the existence of phytoplankton in the waters is very dependent on the available nutrients, especially N, P, and K as well as silicates for diatoms so that changes in the growth phase and specific growth rate in microalgae have a direct relationship with nutrient uptake in the culture media, especially macronutrients i.e., nitrate and phosphate.

Conclusions

In conclusions, the cell density of microalgae *Nannochloropsis oculata* can grow in the four photoperiod treatments. The better growth of microalgae was revealed in treatment B (12L:12D), although it did not significant statistically. Current study suggests that providing proportional of dark and light exposure may have the potential to increase the better growth of *N. oculata*. In addition, manipulating the culture media may also contribute the microalgae growth. Therefore, further investigations are required to convey the ideas in order to have an optimum method to culture *N. oculate* for Laboratory scale practice.

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