The Effect of Long Irradiation on the Growth of the Population
Nannochloropsis sp. Laboratory Scale

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Abstract

Natural feed is a source of nutrients that is very important in the early stages of the development of organisms (larvae or seeds). One type of natural feed that is often used as feed for fish larvae, shrimp, shellfish and also as feed from zooplankton, rotifer and artemia, namely Nannochloropsis sp. synthesis of organic matter in photosynthesis. The minimum duration of irradiation in phytoplankton culture activities itself is 18 hours of light per day. This study aims to analyze the effect of irradiation time on the population growth of Nannochloropsis sp. This study used a completely randomized design (CRD) with treatment; Treatment A (B24:D0), B (B21:D3), C (B18:D6), and D (B15:D9). The results showed that the irradiation duration of 21 hours and 24 hours was not significantly different, but significantly different from the irradiation time of 15 hours and 18 hours in influencing the growth of the population of Nannochloropsis sp. The duration of radiation for 21 hours and 24 hours showed the highest population growth and specific growth 4051.25x10^4 and 4013.75x10^4, while the specific growth was 70.12% and 69.90%.

Keywords: Long the irradiation, Nannochloropsis sp., Photosynthesis

1. Introduction

Natural feed is one type of feed that has a high nutrient content compared to artificial feed. Safitri, et. al. (2013) stated that one type of natural feed that is often used as feed for fish larvae, shrimp, shellfish and also as feed from zooplankton, rotifer and artemia, namely Nannochloropsis sp. species. Nutrient content of Nannochloropsis sp. quite high compared to other microalgae where the protein content of Nannochloropsis sp. reached 52.11%, 16% carbohydrate, 27.64% fat, vitamin C 0.85%, and chlorophyll-a 0.89% (Erlania, 2009). Meanwhile the protein content in Skeletonema costatum was 37.40% and Spirulina platensis was 48.9% (Bangun, 2012). While the fat content in Isochrysis sp. 17.07% and Dunaliella only 6% (Erlania, 2010).
*Nannochloropsis* sp. is an autotroph organism (capable of producing its own food) by absorbing carbon dioxide in the process of photosynthesis and producing oxygen. *Nannochloropsis* sp. can grow and develop by photosynthesis by utilizing sunlight as a source of energy and simple inorganic nutrients such as CO$_2$, dissolved nitrogen and phosphate components. Photosynthesis consists of two reactions, namely dark and bright reactions (photoperiod) (Nurdiana, et. Al., 2017). In bright conditions, the cell will divide asexually, so that the child cells are smaller in size than the parent. Whereas in the dark, cell development occurs to reach normal size. In photoperiod the most important thing is not only the intensity of light but also the duration of irradiation.

The duration of irradiation plays an important role as a supporting factor for the growth of *Nannochloropsis* sp. (Safitri et al., 2013). The duration of irradiation greatly determines the amount of light energy received by phytoplankton when cultured. The amount of light energy received by phytoplankton can affect the growth of phytoplankton populations, where if the light energy received by phytoplankton is more than the ability of phytoplankton to utilize light or too little, it can cause phytoplankton cell reproduction or division (Utami, et al., 2012). The duration of irradiation on phytoplankton culture activities can also affect the biochemical composition of phytoplankton itself and can also affect the synthesis of organic matter in photosynthesis. The minimum duration of irradiation in phytoplankton culture activities itself is 18 hours of light per day (Lavens and Sorgeloos, 1996 in Simatupang, 2014). Based on the description, the study of the effect of the duration of irradiation on the growth of *Nannochloropsis* sp. on a laboratory scale it is important to do it.

2. **Materials and Method**

This research was conducted on September 14, 2018 until September 28, 2018, which was held at the Balai Pengembangan Budidaya Perikanan Pantai (BPBPP) Sekotong, West Lombok Regency. The study used an experimental method, using a Completely Randomized Design (CRD) consisting of 4 treatments of long irradiation on *Nannochloropsis* sp. which was repeated 4 times. The treatment is treated A (24 hours
bright 0 hours dark), B (21 hours bright 3 hours dark), C (18 hours bright 6 hours dark) and D (15 hours bright 9 hours dark).

2.1 Research procedure

2.1.1 Sterilization Tools and materials

Sterilization of the tool in the form of a jar is washed using soap and rinsed thoroughly then the jar is dried, after drying the jar is sprayed with 70% alcohol, and then rinsed using distilled water. Sterilization of aeration hose, aeration and aeration is done by boiling for ±30 minutes. After boiling the aeration hose is dried, then sprayed with 70% alcohol and rinsed with distilled water.

The seawater used previously was deposited for 24 hours and filtered using a physical filter so that the quality of the seawater used is maintained. Sea water that has been left for 24 hours, then sanitized by boiling sea water to boiling at a temperature of 1000C. Seawater that has been through the sanitation process is left for at least 24 hours in an air-conditioned room, then a filtration process using planktonnet is carried out in a maintenance jar with a volume of 0.7 L which is sterile.

2.1.2 Fertilizer preparation

The type of fertilizer used in this study was fertilizer Bowo 36 with a dose of 0.5-1 ml/L. The volume of fertilizer used in this study was 1 mL/L.

2.1.3 Light source preparation and light dark treatment

The light intensity from the lamp to the jar is ±4000 lux. Light received at each jar of Nannochloropsis sp. Culture. comes from 11 watt LED lights. To produce an intensity of ±4000 lux by adjusting the distance from the lamp to the jar and measured using lux meters.

This study uses dark treatment and bright treatment. Each treatment and repetition is given a block made of cardboard wrapped in black plastic and a jar that is used wrapped in aluminum paper. Coating screen using black plastic and coating jars using aluminum paper to produce light intensity 0 lux (Andriyono, 2001). In the light treatment the light intensity used was ±4000 lux. Setting the dark and light time is done using the timer socket tool.

2.1.4 Spread of Nannochloropsis sp. Seeds

Each culture container that has been filled with sea water and given fertilizer, then spreads the seeds of Nannochloropsis sp. with an initial density of 2 x 10^6 cells/mL.
Observation of cell population density was carried out by taking 2 drops of *Nannochloropsis* sp. Seedling samples, and placed in the hemocytometer to be observed further under a light microscope using 10 x 0.25 magnification. Cell density is calculated by the formula: \( N = \text{average cell} \times 25 \times 10^4 \, \text{cells / mL} \), where \( N \) is the cell density (cell / mL) (Andriyono, 2001). Determination of the volume of inoculants during stocking is determined using a dilution formula. Observation of cell population density of *Nannochloropsis* sp. performed every 24 hours for 7 days.

### 2.1.5 Water quality

Water quality parameters as supporting data and supporting the accuracy of observations are measured by several physical and chemical parameters including temperature, salinity and acidity (pH). Temperature measurements were carried out every day at 14.00, while the salinity and pH of the media were measured at the beginning and end of the study.

### 2.2 Test Parameters

The parameters tested in this study were the maximum population density achieved during 7 days of maintenance, specific growth and time of doubling themselves during the exponential phase. Density of *Nannochloropsis* sp. Cell population, determined by microscopic observations on the haemocytometer and calculated using the formula Andriyono (2001):

\[
N = \text{cells average} \times 25 \times 10^4
\]

**Description:**
- \( N \) = Cell density (cell / ml)
- 25 = Number of all large boxes
- \( 10^4 \) = Haemocytometer constant

Specific growth rate (SGR) is determined using the formula Andriyono (2001):

\[
k = \frac{\log\left(\frac{N_t}{N_0}\right) \times 3.32}{T} \times 100\%
\]

**Description:**
- \( k \) = specific growth rate (percent / day)
- \( N_t \) = cell density at the end of observation (cell / ml)
- \( N_0 \) = cell density at the beginning of observation (cell / ml)
- \( T \) = culture time (days)
Self-doubling time is determined using a formula (Mukhlis et al., 2017):

\[
DT = \frac{\log(2) \times \Delta t}{\log(N_t) - \log(No)}
\]  

(3)

Description: 
- \(DT\) = double time (hours),
- \(No\) = initial cell population density (cell / mL)
- \(N_t\) = final cell population density (cell / mL)
- \(\Delta t\) = length of time of observation (hours).

### 2.3 Data analysis

Data from the results of this study were analyzed using the method of analysis of variance (ANOVA) at a real level of 5%. If the treatment gives a significant (significant) effect on the parameters tested, then a further test using Honest Real Difference (Tukey) with a level of 5% is carried out.

### 3. Results

#### 3.1 Population density of *Nannochloropsis* sp.

Based on the results of observations, it can be seen that population density every day has increased which is presented in Table 1.

<table>
<thead>
<tr>
<th>Day</th>
<th>Population density of <em>Nannochloropsis</em> sp. (10^4 cell/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>200±0,00</td>
</tr>
<tr>
<td>1</td>
<td>270±9,13</td>
</tr>
<tr>
<td>2</td>
<td>506,25±16,52</td>
</tr>
<tr>
<td>3</td>
<td>708,75±26,58</td>
</tr>
<tr>
<td>4</td>
<td>1165±14,72</td>
</tr>
<tr>
<td>5</td>
<td>2133,75±19,31</td>
</tr>
<tr>
<td>6</td>
<td>4013,75±41,10</td>
</tr>
</tbody>
</table>

The graph in Figure 1 shows the population growth from the lag phase to the exponential phase on the 6th day. Population growth of *Nannochloropsis* sp. the highest was obtained in treatment B (B21: D3) reaching 4051.25 ± 13.15, followed by treatment A
(B24: D0) reaching 4013.75 ± 41.10, then treatment C (B18: D6) reached 2181.25 ± 8.54, and the lowest in treatment D (B15: D9) reached 1952.5 ± 13.23.

![Growth chart of Nannochloropsis sp.](image)

**Figure 1.** Growth chart of *Nannochloropsis* sp.

Based on ANOVA test conducted on the maximum population density of *Nannochloropsis* sp. showed that irradiation time had a significant effect (P <0.05) on the population growth of *Nannochloropsis* sp. Tukey's further test results gave the results of treatments A and B not significantly different, but significantly different from treatments C and D.

### 3.2 Specific Growth of *Nannochloropsis* sp.

Specific growth rate calculations were carried out to determine the total difference and increase in population of *Nannochloropsis* sp. Specific growth tables are presented in Table 2.

<table>
<thead>
<tr>
<th>Repeat</th>
<th>Specific growth rate of <em>Nannochloropsis</em> sp. (percent / day) treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69.56%</td>
</tr>
<tr>
<td>2</td>
<td>70.11%</td>
</tr>
<tr>
<td>3</td>
<td>69.94%</td>
</tr>
<tr>
<td>4</td>
<td>70.00%</td>
</tr>
<tr>
<td>Average</td>
<td><strong>69.90%±0.0024</strong></td>
</tr>
</tbody>
</table>

Based on ANOVA test conducted on the maximum population density of *Nannochloropsis* sp. showed that the treatment had a significant effect (P <0.05) on the
population growth of *Nannochloropsis* sp. From the results of the Tukey test, the results of treatments A and B were not significantly different, but significantly different from treatments C and D.

### 3.3 Self-Multiplication Time *Nannochloropsis* sp.

Based on the data in Table 3, the maximum doubling time in treatment B (B21:D3) reached an average of $33.18 \pm 0.0357$ hours, in treatment A (B24:D0) $33.28 \pm 0.1144$ hours, in treatment C (B18:D6) reached $41.77 \pm 0.0685$ hours, and treatment D (B15:D9) reached $43.81 \pm 0.1299$ hours.

<table>
<thead>
<tr>
<th>Repeat</th>
<th>Self-Multiplication Time <em>Nannochloropsis</em> sp. (jam)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33,44</td>
</tr>
<tr>
<td>2</td>
<td>33,18</td>
</tr>
<tr>
<td>3</td>
<td>33,26</td>
</tr>
<tr>
<td>4</td>
<td>33,24</td>
</tr>
<tr>
<td>Average</td>
<td>33,28±0,1144</td>
</tr>
</tbody>
</table>

From the results of the statistical analysis it was concluded that the treatment had a significant effect on doubling time ($P < 0.05$). From the results of Tukey's further test, the results of treatments A and B were not significantly different, but significantly different from treatments C and D.

### 3.3 Water Quality

Based on the results of measurements of water quality shows that from each parameter (Temperature, pH and Salinity) is still in the optimal range for culture of *Nannochloropsis* sp. Table of measurement of water quality can be seen in Table 4.

### 4. Discussion

#### 4.1 Population Density

Growth of Nannochlorepsis sp. can be seen from the increase in cell population density. From the observations it can be seen that the growth of *Nannochloropsis* sp. in this study shows a pattern of growth which is divided into the lag phase and the exponential
phase. The lag phase in all treatments is clearly seen in Figure 1 and Table 1. In treatment A (B24:D0) and treatment B (B21:D3) the lag phase lasts for 1 day, namely on day 0 to day 1. The lowest lag phase is found in treatment C (T18: G6) and treatment D (B15:D9) which lasts for 2 days, from day 0 to day 2. This is presumably because the lighting duration for 15 hours and 18 hours per day in treatment D and C is not enough to provide energy for *Nannochloropsis* sp. Cells. to do cell multiplication (reproduce) because. *Nannochloropsis* sp. takes longer to adapt to light (Lavenz and Sorgeloos, 1996). Nurdiana et al. (2017), states that limited light can inhibit the growth activity of *Nannochloropsis* sp. Furthermore Andriyono (2001), states that the lack of light will cause the photosynthesis process to not take place normally so that it disrupts the metabolism. Too long a lag phase will hinder the achievement of peak densities because the energy allocation is more focused on adjusting to the new environment and for maintenance and the possibility of peak population density being achieved is not too high (Andriyono, 2001).

### Table 4. Results of measurements of water quality during the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measurement results</th>
<th>Library Resources</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25.5</td>
<td>25-26</td>
</tr>
<tr>
<td>pH</td>
<td>8.45</td>
<td>8.3-8.6</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>34.5</td>
<td>34-35</td>
</tr>
</tbody>
</table>

The exponential phase is characterized by a rapid increase in the growth rate so that population density increases. The exponential phase of treatment A (B24:D0) and B (B21:D3) takes place on day 1 to day 6 of culture while treatment C (T18: G6) and D (B15:D9) takes place on day -2 to 6th day. From the data of the population density of *Nannochloropsis* sp. Cells. cultured on a laboratory scale presented in Table 1, it is known that the highest population (peak) of culture occurred on the 6th day, namely in treatment B (B21:D3) of 4051.25 10^4 sel / mL and treatment A (B24:D0) of 4013.75 10^4 cells / mL. The high population density in this treatment is due to sufficient lighting time to support the growth of *Nannochloropsis* sp. to carry out photosynthesis. This is in line with Budiman's statement (2009) which states that the duration of irradiation can affect the process of synthesizing organic matter in photosynthesis because only with sufficient energy can the photosynthesis process run smoothly. Lavens and Sorgeloos (1996) suggested that light is
the most important factor for phytoplankton, because light is used as an energy source used by plants in the process of photosynthesis.

4.2 Specific Growth Rate of *Nannochloropsis* sp.

Specific growth rate is a parameter that describes the speed of cell growth in *Nannochloropsis* sp. per unit time. After the adaptation period ends, accelerated growth occurs in the exponential phase, this is reflected in the specific growth constant value (k). Suminto and Hirayama (1996), in their study stated that a greater value of k means that the process of dividing algae cells becomes faster, so that the increase in cells per unit time will be greater than the increase in time itself. Based on BNJ’s further test, good specific growth was shown in treatment A (B24:D0) 70.12% and B (B21:D3) 69.90% (Table 2). However, based on the efficiency of light energy used, treatment B (B21:D3) provides the best specific growth with optimal growth that is equal to 70.12% with a shorter irradiation time than treatment A (B24:D0). Although the maximum specific growth rate occurred at the same time, the growth rate in treatment D (B15:D9) showed a lower value (53.11%) compared to other treatments. The existence of this difference is thought to be due to different lighting times resulting in different cell-specific growth rates. The duration of irradiation in this treatment is below the minimum microalgae culture, which is 15 hours of light and 9 hours dark. According to Lavens and Sorgeloos (1996) states that light irradiation must be appropriate in culturing phytoplankton, if the light is too bright it will inhibit photosynthesis and the minimum duration of artificial lighting must be 18 hours. The duration of irradiation is very decisive in the process of synthesizing organic matter in photosynthesis because only enough energy can run smoothly.

4.3 Relationship between Photosynthesis and The Duration of Irradiation

From the data in Table 1 shows that the duration of irradiation gives significant results (P <0.05) on the growth of *Nannochloropsis* sp. During the photosynthesis process, phytoplankton performs cell division logically. The process of cell division (population growth) can run smoothly if the light energy needed at photosynthesis is sufficient for phytoplankton. Cell division is called the name of mitotic division (Ma’at, 2011). In the process of photosynthesis, light plays a very important role in both light intensity and long irradiation. Novianti (2015) states that the duration of irradiation can affect the synthesis of
organic matter in photosynthesis because only with sufficient energy can the process run smoothly.

*Nannochloropsis* sp. is a photosynthetic organism absorbing light in the form of photons for photosynthesis. The energy of the photon will be used by chlorophyll to break up hydrogen bonds in water, which together with CO$_2$ in photosynthesis will be used to synthesize sugars (Salisbury and Ross, 1995 in Pujiono, 2012). The photosynthesis process according to Bangun (2012) is as follows:

\[
6\text{CO}_2 + 6\text{H}_2\text{O} \xrightarrow{\text{Lights}} \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2
\]

In the (2012), states that, the reactions that occur in the process of photosynthesis are divided into 2 namely bright reactions and dark reactions. Bright reactions depend on the availability of light. Bright reactions are steps to convert light energy into chemical energy. Light absorbed by chlorophyll moves the transport of electrons and hydrogen from water to a receiver (an acceptor) called NADP + which functions as an electron carrier in cellular respiration. Bright reactions use light to reduce NADP + to NADPH by adding a pair of electrons together with the hydrogen nucleus or H +. Bright reactions also produce ATP by giving energy to the addition of phosphate groups which in ADP, this process is called photophosphorylation.

While the dark reaction is the reaction of sugar formation from CO$_2$ that occurs in the stroma. In contrast to light reactions, dark reactions or non-light-dependent reactions can occur during day and night, but during the day the dark reaction rate is certainly lower than the light reaction rate (Inthe, 2012). Dark reactions occur in cyclic series reactions that form sugars from the basic ingredients of CO$_2$ and energy (ATP and NADPH), where the energy used in these dark reactions is obtained from bright reactions (Novianti, 2015). Dark reactions aim to convert compounds containing carbon atoms into sugar molecules.

### 4.4 Time of doubling *Nannochloropsis* sp.

The maximum doubling time is achieved when the specific growth rate also reaches a maximum. Therefore, in this study the maximum doubling time and maximum specific growth rate was reached at the same time, namely on the 6th day of culture, when the growth phase that took place was an exponential phase. In Table 3 shows the comparison of
the maximum doubling time achieved in each treatment. At the time of reaching the maximum value of the 6th day of treatment B (light 21 hours and dark 3 hours) showed a shorter doubling time (33.18 ± 0.0357 hours) compared to treatment A (24 light and 0 hours dark) 33 , 28 ± 0.1144 hours. The slowest doubling time was obtained in treatment C (18 hours light and 6 hours dark) 41.77 ± 0.0685 hours, and treatment D (15 hours light and 9 hours dark) 43.81 ± 0.1299 hours. This is because the photosynthesis process in the treatment does not occur optimally. Tamiya, (1973) in Utami et al., (2012), states that, receiving less light or limited lighting can cause the photosynthesis process is not optimal and the growth of cells is inhibited. Budiardi et al. (2010), states that the optimal photosynthesis process due to lack of light will result in the process of obtaining energy not being optimal, so that the energy for the growth process is less well available.

4.5 Water quality

Water quality is one of the limiting factors for the growth and development of microalgae as one of the organisms that live in water. Fajrin (2014) states that the density of phytoplankton is influenced by several environmental factors, including temperature, light, and pH of water.

The temperature of the culture media during the study ranged from 25oC-26oC. Fachrullah (2011), which states that the optimal temperature for the growth of Nannochloropsis sp. is ranging from 25-30 oC. The results of measuring the pH value of culture media during the study ranged from 8.3 to 8.6. Fajrin (2014), stated that Nannochloropsis sp. can live well in a pH range of 8-9.5. Salinity is one of the limiting factors for the growth and development of phytoplankton. The results of salinity measurements during the study ranged from 35-36 ppt. Rusyani (2012), states that optimal salinity for the growth of Nannochloropsis sp. That is ranging from 25-35 ppt.

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References


