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Nannochloropsis sp phytoplankton culture technique laboratory scale

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Abstract
Nannochloropsis sp. is a phytoplankton often used in marine fish hatchery activities as feed for the mass production of rotifers, and its availability is very much needed for rearing marine fish larvae. This activity aims to study pure culture techniques for *Nannochloropsis* sp on a laboratory scale. The method used is a literature study method and a direct practical method regarding *Nannochloropsis* sp phytoplankton culture techniques laboratory scale and conducted interviews with employees at the BPBL Batam Phytoplankton Live Food Production Unit Laboratory. Based on observations, it was found that the peak growth or optimum cell density of *Nannochloropsis* sp. occurred on the sixth day in a 1000 mL Erlenmeyer, namely 60.95 - 62.10 million cells/mL with an initial density of 10.15 - 10.55 million cells/mL and in a 2000 mL Erlenmeyer, namely 58.75 - 60, 25 million cells/mL with an initial density of 8.25 – 8.45 million cells/mL.

1. INTRODUCTION

The potential for marine fisheries cultivation must be balanced with the availability of quality larvae in terms of quantity, quality, and sustainability. Factors that influence hatchery activities include the provision of sufficient larval food that is available at the same time. The existence of natural food is necessary and cannot be replaced by artificial food (Sururi, 2014). Natural plankton food is essential in providing a source of protein and nutrition for larvae.

Natural food consists of two types of plankton, namely zooplankton and phytoplankton. Zooplankton is a group of heterotrophic plankton organisms that require organic material from other organisms, especially phytoplankton, to live. Meanwhile, phytoplankton is a plankton organism collection that utilizes nutrients, sunlight, and CO_2 to produce its organic material (Zainuddin et al., 2017).

One type of phytoplankton used as natural food in marine culture organism hatchery activities is *Nannochloropsis* sp. Microalgae *Nannochloropsis* sp is a single-celled microalgae that belongs to the Eustigmatophyceae class and is generally cultivated in fish hatcheries as rotifer feed. *Nannochloropsis* sp is important in seeding activities because of its high nutritional content (Sleigh, 1989; Bahua et al., 2015). Considering the role of *Nannochloropsis* sp, which has good nutritional content for rotifers and its availability is very much needed in marine fish hatchery activities, the author is interested in carrying out activities regarding *Nannochloropsis* sp Phytoplankton Culture Techniques. Laboratory Scale at the Batam Marine Aquaculture Center (BPBL), Kepulauan Riau Province.

This activity aims to find out about *Nannochloropsis* sp and study pure culture techniques for the phytoplankton *Nannochloropsis* sp. and determine the growth phase of *Nannochloropsis* sp laboratory scale.

2. RESEARCH METHODS

Time and Place

This activity occurred from January 20 to February 19, 2020, at the Batam Mariculture Fisheries Center on Jl. Raya Barelang Bridge III Setoko Island PO.BOX.60 Sekupang, Batam, Kepulauan Riau Province.

Research Methods

The methods used are direct practice and literature study practice. The direct practice method consists of observing, practicing, and actively participating during culture activities and conducting interviews with laboratory staff at the BPBL Batam live phytoplankton food production unit.

Parameters Measurement

Cell Density

The density of *Nannochloropsis* sp can be calculated using the formula (Guillard, 1973):

$$D = \frac{n1+n2+\cdots+nx}{X} \times 25 \times 104 \text{ cells/mL}$$

Information :

D	= Phytoplankton density (cells/ml)
n	= Number of phytoplankton in the box
X	= Number of boxes observed
25	= Sum of all boxes in a hemocytometer
10^4	= Constant hemocytometer

Data Analysis

The primary and secondary data obtained were collected, grouped, and tabulated in tabular form. Then, the data was analyzed descriptively to provide an overview of cultural techniques and their problems.

3. RESULTS AND DISCUSSION

This was done by calculating the cell growth of *Nannochloropsis* sp. Calculations were carried out using a hemocytometer by taking algae samples in 1000 mL and 2000 mL Erlenmeyer cultures. Meanwhile, cell density was not observed in cultures without aeration because the volume was small, and it was suspected that if samples were taken every day, it would result in contamination. Culture without aeration is still carried out because it is an operational system procedure established by the Batam Marine Aquaculture Center (BPBL). Based on the observations carried out for six days, it was found that the growth density of Nannochloropsis sp. experienced changes every day. According to Astuti (2010), the optimum density achieved on a laboratory scale is 50-60 million cells/mL. The high growth density of *Nannochloropsis* sp is the highest, namely the optimal peak (Figure 1).

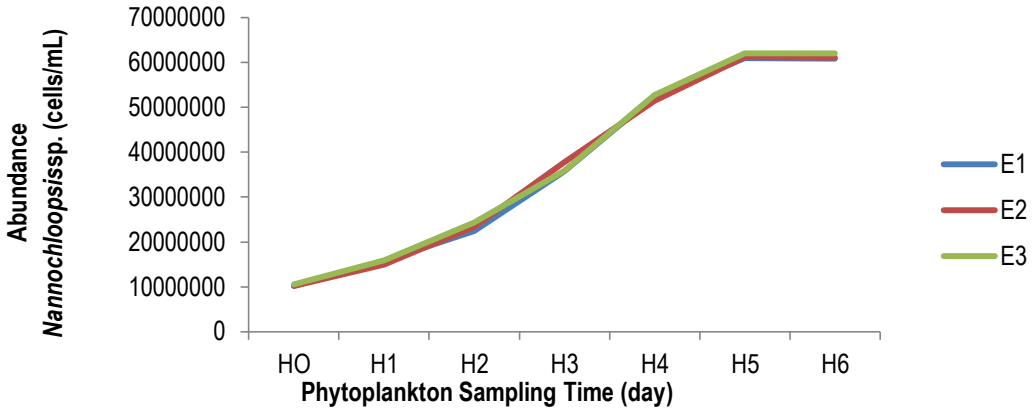

Figure 1. Growth of *Nannochloropsis* sp on a 1000 mL Erlenmeyer

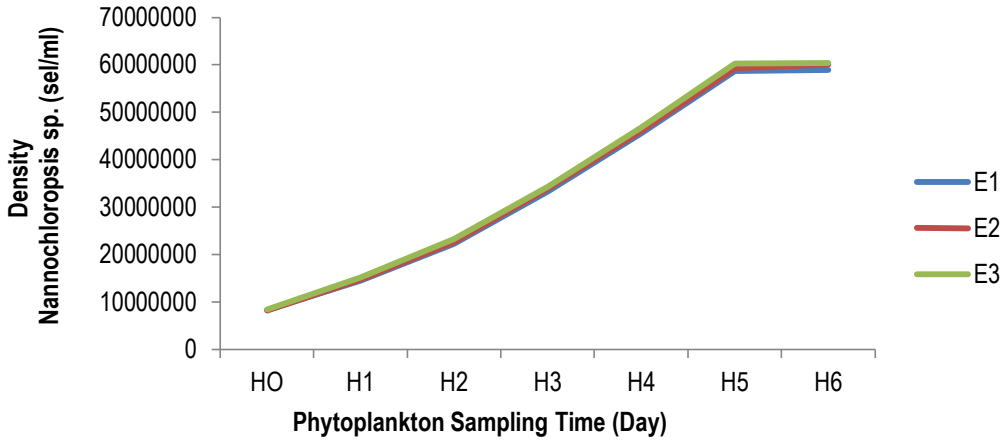

Figure 2. Growth Graph of *Nannochloropsis* sp on a 2000 mL Erlenmeyer
Information: H = Days (Sampling time/culture age); E = Erlenmeyer (Sample)

Based on Figure 1, sampling was carried out on three different Erlenmeyer samples. It was found that the peak growth or optimum cell density of *Nannochloropsis* sp. occurred on H5 (fifth day), namely 60.95−62.10 million cells/mL because on H5, this was the exponential phase of the growth of *Nannochloropsis* sp. and on D6 (sixth day) it has entered a phase of decreasing growth rate so that the cell density of *Nannochloropsis* sp. to 60.85– 62 million cells/mL (Figure 2).

Based on Figure 2, sampling was carried out on three different Erlenmeyer samples. It was found that the peak growth or optimum cell density of *Nannochloropsis* sp occurs on H5 (fifth day), which is 58.75– 60.25 million cells/mL because on H5 this is the exponential phase of the growth of *Nannochloropsis* sp. and on D6 (sixth day) it has entered a phase of decreasing growth rate so that the cell density of *Nannochloropsis* sp. to 58.90– 60.40 million cells/mL.

Greatly influenced by external factors, namely conditions of light intensity, temperature, aeration, and nutrition. In the culture of *Nannochloropsis* sp laboratory scale, seed quality and initial stocking density greatly influence the speed of population growth. The growth of Nannochloropsis sp on the laboratory scale on the sixth day experienced a decrease in growth rate, and the peak phase of growth or optimum density was reached on the fifth day. The time required to reach the highest density varies, depending on several factors: seed quality, stocking density, light intensity, fertilizer, and water quality. The growth curve of *Nannochloropsis* sp. into five phases, namely:

Phase lag. An unreal increase in population characterizes this phase; this phase is referred to as an adaptation phase to the environment, such as increasing levels of enzymes and metabolites involved in cell division and carbon fixation, so they must be synthesized first to continue subsequent cell biochemical activities. Based on the observation results, the lag phase occurs from the first to the second day because the algae adapts to the growth medium. In this phase, the algae cells remain alive but do not reproduce.

Logarithmic phase. This phase is often called exponential because it has experienced division and a fixed growth rate. Growth of *Nannochloropsis* sp. can be maximum depending on the algae species, light intensity, and temperature. Based on observations, the logarithmic phase occurs on the third to the fifth day because cell division has happened during this period. In this phase, the algae are actively reproducing.

The phase of decreasing growth rate. This phase is characterized by a decrease in growth rate compared to the logarithmic/exponential phase because cell growth begins to slow down when nutrients, light, pH, CO_2, or chemical and physical factors begin to limit growth. Based on observations, this phase occurs on the sixth day because there is a decrease in the growth rate on that day, even though the cell density continues to increase.

Stationary phase. This phase is characterized by a balance between the growth and death rates, so the density of *Nannochloropsis* sp. in this phase is relatively constant. The number of cells tends to remain stable, and the growth of new cells is inhibited by dead cells and other limiting factors. Based on observations, the stationary phase is thought to occur on the seventh day.

Death phase. Insufficient nutrients characterize this phase, so population density decreases because the death rate is higher than the growth rate. Based on observations, the death phase is thought to occur on the eighth day.

4. CONCLUSIONS

Based on observations, it was found that the peak growth or optimum cell density of *Nannochloropsis* sp occurred on the sixth day in a 1000 mL erlenmeyer, namely 60.95- 62.10 million cells/mL with an initial density of 10.15 - 10.55 million cells/mL and in a 2000 mL Erlenmeyer, namely 58.75 - 60, 25 million cells/mL with an initial density of 8.25 – 8.45 million cells/mL.

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