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EFFECT OF LIQUID ORGANIC FERTILIZER OF BAT MANURE (*Chiroptera* sp.) ON THE ABUNDANCE OF Nannochloropsis sp.

Pengaruh Pemberian Pupuk Organik Cair Kotoran Kelelawar (*Chiroptera* sp.) Terhadap Kelimpahan *Nannochloropsis* sp.

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ABSTRACT

Nannochloropsis sp. is a seawater phytoplanton that is often used as a natural food in aquaculture. Nannochloropsis sp. has a high nutritional content, including proteins, lipids, omega-3 fatty acids, and essential vitamins and minerals that are important for fish growth. *Nannochloropsis* sp. culture media using bat manure organic fertilizer can increase the abundance of *Nannochloropsis* sp. and is economical for aquaculture farmers. Bat manure liquid organic fertilizer which has a natural nutrient content rich in nitrogen, phosphorus, and high potassium is used as a nutrient medium in *Nannochloropsis* sp. This study consisted of 4 treatments of 3 replicates of bat manure organic fertilizer doses, treatment A at 0 ml/L (control), treatment B at 80 ml/L, treatment C at 100 ml/L, and treatment D at 120 ml/L. The results obtained from this research are the use of different doses of bat manure fertilizer can increase the abundance of *Nannochloropsis* sp. The best *Nannochloropsis* sp. abundance value in treatment D (120 ml/L) was 24.99 x 10^6 cells/ml.

Keywords: Bat Manure Organic Fertilizer, Nannochloropsis sp. Abundance, Natural Food

ABSTRAK

Nannochloropsis sp. adalah fitoplanton air laut yang sering digunakan sebagai pakan alami dalam budidaya perikanan. *Nannochloropsis* sp. memiliki kandungan nutrisi yang tinggi, antara lain protein, lipid, asam lemak omega-3, serta vitamin dan mineral esensial yang penting untuk pertumbuhan ikan. Media kultur *Nannochloropsis* sp. dengan menggunakan pupuk organik kotoran kelelawar dapat meningkatkan kelimpahan *Nannochloropsis* sp. dan ekonomis bagi pembudidaya perikanan. Pupuk organik cair kotoran kelelawar yang memiliki kandungan nutrisi alami yang kaya akan nitrogen, fosfor, dan kalium yang tinggi digunakan sebagai media nutrisi pada *Nannochloropsis* sp. Penelitian ini terdiri dari 4 perlakuan dengan 3 kali ulangan dosis pupuk organik kotoran kelelawar yaitu perlakuan A dengan dosis 0 ml/L (kontrol), perlakuan B dengan dosis 80 ml/L, perlakuan C dengan dosis 100 ml/L, dan perlakuan D dengan dosis 120 ml/L. Hasil yang diperoleh dari penelitian ini adalah penggunaan dosis pupuk kotoran kelelawar yang berbeda dapat meningkatkan kelimpahan *Nannochloropsis* sp. Nilai kelimpahan *Nannochloropsis* sp. terbaik pada perlakuan D (120 ml/L) yaitu 24,99 x 10^6 sel/ml.

Kata Kunci: Kelimpahan *Nannochloropsis* sp., Pakan Alami, Pupuk Organik Kotoran Kelelawar

INTRODUCTION

Microalgae are microscopic organisms that can be unicellular or multicellular, which obtain nutrients from inorganic substances and produce organic compounds through the process of photosynthesis (Isnansetyo & Kurniastuty, 1995). Microalgae that are unicellular, can live in colonies or as individuals, with diameters ranging from 0.1 to 200µ (Soeprobowati & Hariyati, 2013). Phytoplankton as the main producer in waters has a significant role as a food supplier for marine organisms. In addition to functioning as the main producer, phytoplankton is also an indicator of the level of fertility of waters (Syafriani & Apriadi, 2017). The problem faced by fish farmers is the high nutritional needs in the seeding phase. Lack of nutrition in fish larvae will result in smaller body size and imperfect organ development (Prastiwi, 2016). The feed commonly used in the larval phase is commercial feed which has high feed costs and expensive prices. Therefore, farmers utilize phytoplankton as natural food for fish larvae. To provide natural food, farmers culture *Nannochloropsis* sp. so that the availability of fish food is met.

Nannochloropsis sp. is used as natural food for seawater zooplankton and is used as a maintenance medium for fish larvae. The results of the proximate test show that the nutritional content of *Nannochloropsis* sp. is 17-38% protein, 8-17% carbohydrates, 27% fat, and 96% water. According to Fessenden (1994) *Nannochloropsis* sp. contains various pigments and nutrients, such as protein (52.11%), carbohydrates (16%), fat (27.64%), vitamin C (0.85%), chlorophyll A (0.89%), and has essential amino acids, such as: Glutamic 15.48%, Leucine 15.20%, and Leucine 1.57%. In the culture of *Nannochloropsis* sp. requires the nutritional content needed for the culture of *Nannochloropsis* sp. namely micro and macro nutrients have a very important role in supporting the growth, development, and biomass production of *Nannochloropsis* sp. According to Subarijanti (2005) algae cell tissue is formed by protein, fat, carbohydrates, and nucleoproteins that can operate with the support of enzymes. In the formation of *Nannochloropsis* sp. cell tissue, macro nutrients such as C, H, O, N, P, S, and K are needed. Micro nutrients such as Mg, Fe, Zn, Cl, B, Mo, and Cu are needed for the formation of enzymes. Cultivators usually use chemical fertilizers with high nitrogen, phosphorus, and potassium content as a culture medium for *Nannochloropsis* sp.

Chemical fertilizers are relatively expensive, difficult to obtain, and not environmentally friendly. One alternative that can be used by cultivators is to use liquid organic fertilizer from bat droppings as nutrients in the culture of *Nannochloropsis* sp. Liquid organic fertilizer from bat droppings is one of the fertilizers that has high micro and macro nutrients and meets culture needs. According to research conducted by Suwarno & Idris (2007), bat droppings contain 7-17% nitrogen (N), 8-15% phosphorus (P), and 1.5% to 2.5% potassium (K). There are also nutrients such as 9-13% N, 5-12% P, 1.5-2.5% K, 7.5-11% Ca, and 0.5-1% Mg (Agromedia, 2010). This study aims to determine the best liquid organic fertilizer for bat droppings on the abundance of *Nannochloropsis* sp.

METHODS

Time and Place

This research was conducted from February to March 2024 in the natural feed laboratory of the Batam Marine Aquaculture Center (BPBL).

Tools and Materials

The materials used in this study were *Nannochloropsis* sp., liquid organic fertilizer from bat droppings, sterile seawater. The equipment used in the study included 25-liter plastic jars, water pumps, aerators, water hoses, aeration stones, 50-watt marlin lamps, hemocytometers, water hoses, handcounters, dropper pipes. Water quality measuring instruments in the study were thermometers, pH meters, DO meters, and refractometers.

Research Methods

The method applied in this study was the laboratory experimental method. The experimental design applied was a Completely Randomized Design (CRD) with four treatments and three replications for each treatment, namely:

a. Treatment A: Giving a dose of 0 ml/l of liquid organic fertilizer from bat droppings (Control)
b. Treatment B: Dosage of 80 ml/l liquid organic fertilizer from bat droppings
c. Treatment C: Dosage of 100 ml/l liquid organic fertilizer from bat droppings
d. Treatment D: Dosage of 120 ml/l liquid organic fertilizer from bat droppings

Nannochloropsis sp. Culture Preparation

Culture preparation begins by preparing a culture container, namely a 25 liter jar, 12 containers are washed, dried, and sterilized. After that, at the beginning of the culture, 12.5 liters of water are given and liquid organic fertilizer from bat droppings from Tuban, East Java is added according to the treatment (0, 80, 100, 120 ml/L). Then *Nannochloropsis* sp. inoculant is added from BPBL Batam as much as 4 liters per treatment container. Furthermore, the containers will be placed under sunlight and LED lights and given adjusted aeration. On the 3rd day, the culture will be given additional doses of fertilizer and water. On the 7th day, the *Nannochloropsis* sp. culture is ready to be harvested. Observations were made by taking samples every day at the same time and the number of cells was counted using a Haemacytometer. The Haemacytometer was used by taking a sample of 10 μL using a pipette and dripping it on the surface of the haemacytometer, then covered with a cover glass. The haemacytometer was then placed under a microscope with a magnification of 400x to 1000x and the number of cells was counted based on Figure 1.

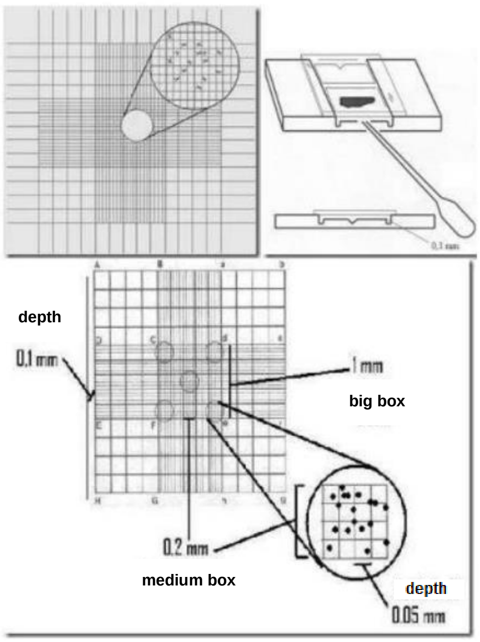

Figure 1. Calculation of the Number of Cells Using a Haemocytometer (Ochtreeani et al., 2014)

In this study, the subculture method was used to extend the life of the culture by periodically transferring some cells from the old culture to new media and enriching *Nannochloropsis* sp. The repeated subculture method can be seen in Figure 2.

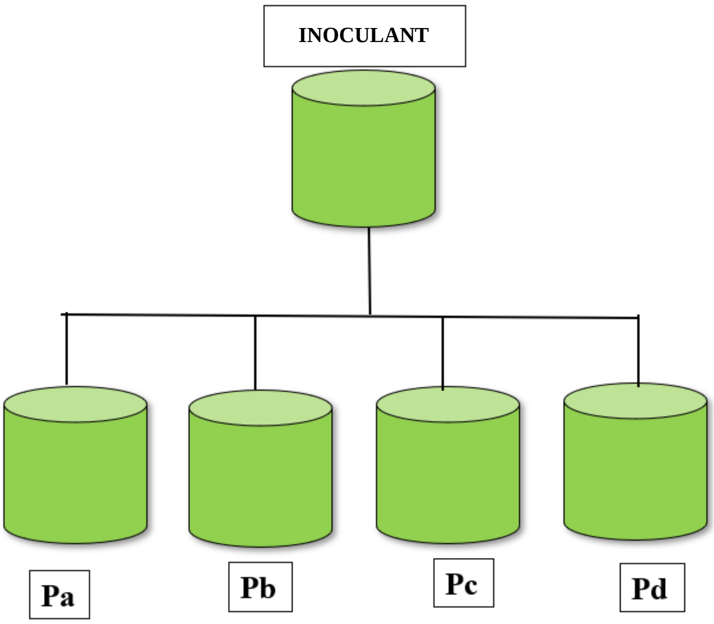

Figure 2. Subculture Method for *Nannochloropsis* sp.

Research Parameters

The parameters in this study are biological, physical, and chemical parameters. Physical chemical parameters are water quality including pH, DO, temperature, salinity, nitrite, and ammonia. While biological parameters are in the form of *Nannocloropsis* sp density. The population density calculation will be carried out using the formula proposed by Erlinda et al. (2015) as follows:

$$K=\frac{n1+n2+n3+n4+n5}{5} \times 25 \times 10^4$$

Description:

K	= Plankton density (cells/ml)
n1+n2+n3+n4+n5	= Number of *Nannochloropsis* sp. in boxes 1 to 5
5	= Number of boxes counted
$25x10^4$	= Surface area of hemocytometer

Calculation of *Nannochloropsis* sp. stock culture to be used for culture using the formula proposed by Djarijah (1995) is as follows:

$$V_1 \times N_1 = V_2 \times N_2$$

Description:

V1	= Seed volume for initial spreading (ml)
N1	= Density of *Nannochloropsis* sp. seed/stock (cells/ml)
V2	= Desired volume of culture media (ml)
N2	= Density of *Nannochloropsis* sp. seed/stock (cells/ml)

The parameters measured during this study to analyze water quality were temperature, pH and DO. Water quality measurements were carried out every day in the morning, afternoon and evening to maintain water quality during the study. The observation methods used are presented in Table 1.

Table 1. Water Quality Parameters

No	Parameter	Unit	Measuring Instrument
1	Temperature	°C	Termometer
2	Dissolved Oxygen	mg/L	DO Meter
3	pH	-	pH Meter
4	Nitrite	mg/L	Spectrophotometry
5	Ammonia	mg/L	Spectrophotometry
6	Salinity	ppm	Refractometer

Data Analysis

The data from the *Nannochloropsis* sp. density test in this study were analyzed statistically through Analysis of Variance (ANOVA) with a 95% confidence level. If there is a significant difference, it is continued with the Duncan's New Multiple Range Test (DNMRT). Water quality consisting of DO, temperature, pH, salinity, nitrite, and ammonia were analyzed using a descriptive approach.

RESULT

Daily Abundance of *Nannochloropsis* sp.

Based on the results of the study, the daily abundance of *Nannochloropsis* sp. during 7 days of observation showed an increase in cell density influenced by the addition of liquid organic fertilizer from bat droppings during maintenance. Differences in doses of organic fertilizer from bat droppings had different effects between treatments (P>0.05) which can be seen in Figure 3.

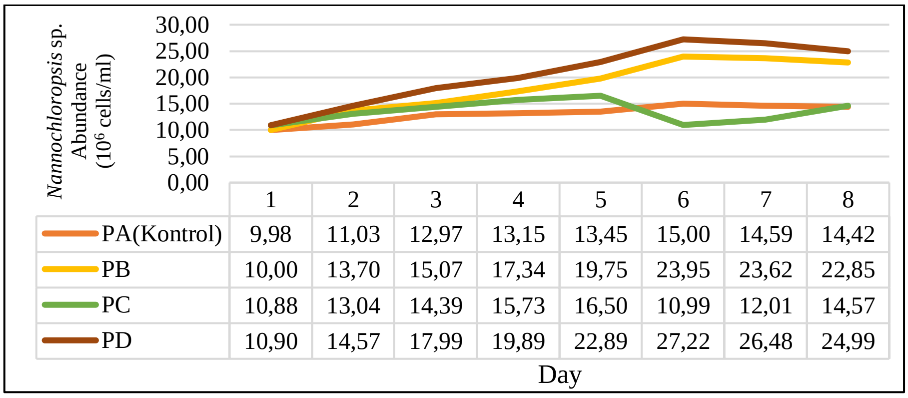

Figure 3. Daily Abundance of *Nannochloropsis* sp.

Table 2. Daily Abundance of *Nannochloropsis* sp.

Treatment	Daily Abundance *Nannochloropsis* sp.
PA: 0 ml/l POC bat droppings (Control)	14.42 ± 0.12[a]
PB: 80 ml/l POC bat droppings	22.85 ± 0.11[b]
PC: 100 ml/l POC bat droppings	14.57 ± 0.11[a]
PD: 120 ml/l POC bat droppings	24.99 ± 0.04[c]

Description: Values followed by the same letter are not significantly different according to Duncan's multiple range test at a 95% confidence level.

Water Quality

Water quality observations are used as supporting parameters that can support the culture of *Nannochloropsis* sp. in research. Water quality observations used as supporting parameters during the study were temperature, dissolved oxygen (DO), acidity (pH), nitrite, ammonia and salinity. The measurement results showed that the water quality still met the quality standards for the maintenance of *Nannochloropsis* sp. (Table 3).

Table 3. Water Quality Measurement Results

Parameter	Measurement Results	Quality Standards
Nitrite (mg/L)	0.08-0.93	0.002-1.00*
Ammonia (mg/L)	0.07-1.18	0.05-2.00**
Temperature (°C)	28 – 29	25 – 30****
DO (mg/l)	6 – 7	>5****
pH	8	6-9*****
Salinity (ppt)	30	28-30***

*Standard according to SNI 19-6964.1-2003
**Standard according to SNI 19-6964.3-2003
***Standard according to SNI 7644: 2010
****Standards according to PP RI No. 22 of 2021

DISCUSSION

Daily Abundance of *Nannochloropsis* sp.

The growth of *Nannochloropsis* sp. can be divided into four phases including the lag phase, exponential phase (logarithmic), stationary phase, and death phase. In the lag phase which lasts from day 0 to day 2, the number of *Nannochloropsis* sp. has not shown an increase. This is due to the adaptation of *Nannochloropsis* sp. to the surrounding environment. At this stage it is very important for the development of microalgae, if environmental conditions are supportive and rich in nutrients, these microalgae are able to adapt well. According to Ru'yatin et al. (2015) various parameters that affect the duration of the adaptation phase include the type and age of microorganism cells, inoculum volume, and the condition of the growth media. Furthermore, there is an exponential phase (logarithmic) which occurs on the 3rd day until the 5th day there is an increase in *Nannochloropsis* sp. cell division. This is due to the ability of *Nannochloropsis* sp. to obtain sufficient nutrients, as well as utilize the available light for photosynthesis which supports its growth. According to Kitaya et al. (2005) environmental factors affect the growth rate of microalgae cells, such as light intensity, radiation, temperature, and nutrient content in the cultivation system. The exponential phase is generally characterized by an increase in the number of cell divisions, even in this phase cell division can reach two times (Sugawara & Nikaido, 2014). The stationary phase occurs on the 5th to 6th day, the growth rate of *Nannochloropsis* sp. is the same as the death rate of *Nannochloropsis* sp. This factor is because *Nannochloropsis* sp. is able to maintain cells by using nutrients available in the environment, so that the number of dead cells is comparable to the number of living cells, in other words, in this phase microalgae grow in stable conditions. Furthermore, the death phase occurs on the 7th day, the number of *Nannochloropsis* sp. cells decreases because the rate of cell death is higher than the rate of cell growth so that the population density decreases. The time needed to approach the death phase depends on the remaining nutrients available for growth and the pH tolerance limit for growth. The death phase is when the death rate of microalgae is higher than that of cells undergoing division (Krisnan et al., 2015). This phase is characterized by a decrease in population density, caused by the death rate exceeding the growth rate (Pelczae et al., 1988).

The high daily abundance rate of *Nannochloropsis* sp. in treatment D is because the dose of organic bat dung fertilizer can meet the needs of nitrogen, phosphorus, potassium, and sunlight to stimulate or stimulate the growth of *Nannochloropsis* sp. According to Nyabuto et al. (2015) nitrogen is an important component in growth, development, and reproduction. All algae that have chlorophyll can grow well in conditions of sufficient nitrogen availability (Burford, 1998). The lowest daily abundance of *Nannochloropsis* sp. in treatment A (fertilizer dose 0 ml/L) because the availability of nutrients in the culture medium is no longer sufficient to grow and reproduce. If the nutrients in the culture medium decrease or run out due to consumption, the availability of nutrients becomes a limiting factor. As a result, the cultured algae will stop growing due to the lack of nutrients in the culture medium which causes nutrient competition which ultimately causes a decrease in the number of cells (Tetelepta, 2011). Furthermore, Hermawan et al. (2017) stated that the availability of non-optimal nutrients in the culture media inhibits the growth of microalgae.

Water Quality

The temperature values for 7 days were obtained during the culture of *Nannochloropsis* sp. namely in treatment A control (0 ml fertilizer dose) of 29.10 ± 0.61°C, treatment B (80 ml fertilizer dose) of 29.53 ± 0.47°C, treatment C (100 ml fertilizer dose) of 29.17 ± 0.45°C, and treatment D (120 ml fertilizer dose) of 28.77 ± 0.21°C. The temperature obtained during the observation was in accordance with the quality standards of PP RI No. 22 of 2021 which explains that the optimal temperature value is 25-30°C. In addition, according to Saputra et al. (2016) the ideal temperature for aquatic microorganisms is 25-30°C. Therefore, microalgae can reproduce well so that they can carry out the photosynthesis process.

Acidity Degree (pH) for 7 days was obtained during the culture of *Nannochloropsis* sp. namely in treatment A (0 ml fertilizer dose) of 8.33 ± 0.15, treatment B (80 ml fertilizer dose) of 8.10 ± 0.17, treatment C (100 ml fertilizer dose) of 8.28 ± 0.25, and treatment D (120 ml fertilizer dose) of 8.25 ± 0.25. The pH obtained during the observation has met the quality standards of PP RI No. 22 of 2021 which explains that the optimal pH value is 6.0-9.0. The use of bat droppings in waters can cause an increase in pH through the release of ammonia and decomposition of organic matter. The more organic matter that is decomposed, the more CO_2 is produced and utilized by *Nannochloropsis* sp. for the photosynthesis process.

Dissolved oxygen (DO) for 7 days was obtained during the culture of *Nannochloropsis* sp. namely in treatment A control (0 ml fertilizer dose) of 6.90 ± 0.20 mg/L, treatment B (80 ml fertilizer dose) of 7.03 ± 0.47 mg/L, treatment C (100 ml fertilizer dose) of 7.10 ± 0.35 mg/L, and treatment D (120 ml fertilizer dose) of 7.07 ± 0.38 mg/L. The DO obtained during the observation has met the quality standards of PP RI No. 22 of 2021 which explains that the optimal DO value is >5 mg / L. A small dissolved oxygen value is very dangerous because it is an indicator of water quality that plays a role in the oxidation process and reduction of organic and inorganic materials.

Ammonia for 7 days was obtained during the culture of *Nannochloropsis* sp. namely in treatment A control (0 ml fertilizer dose) of 0.07 ± 0.02 mg/L, treatment B (80 ml fertilizer dose) of 0.13 ± 0.03 mg/L, treatment C (100 ml fertilizer dose) of 0.59 ± 0.03 mg/L, and treatment D (120 ml fertilizer dose) of 1.18 ± 0.03 mg/L. The average results of observations of ammonia values are already optimal values for *Nannochloropsis* sp. culture because it is in accordance with SNI 19-6964.3-2003, which is in the range of 0.05-2.00 mg/L. The use of bat dung fertilizer in microalgae culture can provide significant benefits in terms of providing nutrients. However, ammonia management is a critical factor that must be considered. According to Mawaddah (2016) ammonia is the main compound that provides nitrogen needed by microalgae for metabolism and new cell formation.

Nitrite for 7 days was obtained during the culture of *Nannochloropsis* sp. namely in treatment A (0 ml fertilizer dose) of 0.08 ± 0.03 mg/L, treatment B (80 ml fertilizer dose) of 0.17 ± 0.02 mg/L, treatment C (100 ml fertilizer dose) of 0.32 ± 0.02 mg/L, and treatment D (120 ml fertilizer dose) of 0.93 ± 0.05 mg/L. The average results of observations of nitrite values are already optimal values for the culture of *Nannochloropsis* sp. because it is in accordance with SNI 19-6964.1-2003, which is 0.002-1.00 mg/L. High nitrite levels can be dangerous and inhibit the growth of *Nannochloropsis* sp. because it can cause cell damage, decreased growth rate, and even death of microalgae, but low levels can help its growth.

Salinity for 7 days was obtained during the culture of *Nannochloropsis* sp. namely in treatment A control (Fertilizer dose 0 ml) of 30.00 ± 0.00 ppt, treatment B (Fertilizer dose 80 ml) of 30.00 ± 0.00 ppt, treatment C (Fertilizer dose 100 ml) of 30.00 ± 0.00 ppt, and treatment D (Fertilizer dose 120 ml) of 30.00 ± 0.00 ppt. The average results of observations of nitrification values are already optimal values for the culture of Nannochloropsis sp. because they are in accordance with SNI 7644: 2010, which is 28-30 ppt. Low salinity values can affect the growth, metabolism, and physiology of *Nannochloropsis* sp.

CONCLUSION

Addition of bat dung fertilizer as a source of nutrition in the growth of *Nannochloropsis* sp. The best abundance of *Nannochloropsis* sp. in P_D treatment with a fertilizer dose of 120 ml/L resulted in a density reaching 24.99 x 10^6 cells/ml.

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