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Evaluation of Shrimp Head-Based Liquid Organic Fertilizer as a Sustainable Alternative Nutrient Source for *Nannochloropsis* sp. Culture

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Abstract

Laboratory-scale *Nannochloropsis* sp. cultures typically rely on expensive commercial nutrients for aquaculture feed production. Shrimp head waste contains important nutrients that can be used to produce liquid organic fertilizers. This study aimed to evaluate the effect of using commercial fertilizer combined with liquid organic shrimp head fertilizer (LOF) on the growth of *Nannochloropsis* sp. on a controlled scale. The experimental design included four treatments (P0:100% commercial fertilizer), P1 (50% commercial + 50% LOF), P2 (75% commercial + 25% LOF), and P3 (100% LOF). Cell growth data were subjected to ANOVA and Duncan's tests. The use of LOF significantly influenced the growth of *Nannochloropsis* sp. on day 7. Peak growth occurred on day 7. P0 treatment (100% commercial fertilizer) showed the highest growth (15.7×10^4 cells/ml), although it was not statistically different from P1 (50% commercial + 50% LOF) and P2 (75% commercial + 25% LOF). The highest Specific Growth Rate was observed in the P0 treatment (0.22). This suggests that while shrimp head LOF alone may not be sufficient, its combination with commercial fertilizers holds promise for the sustainable cultivation of *Nannochloropsis* sp.

Keywords: Aquaculture, LOF, *nannochloropsis*, nutrient, microalgae

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Introduction

The aquaculture industry is experiencing significant growth and offers a sustainable solution to meet the increasing global demand for seafood. It has diversified to include various species and is driven by technological advancements and a focus on sustainability (Bagotia et al., 2024). One potential resource for aquaculture is microalgae. Microalgae have long been considered alternative unconventional protein sources and food supplements for animal and human nutrition, but their commercial large-scale production started only a few decades ago (Zanella & Vianello, 2020). Reliable microalgae production is essential in aquaculture, as microalgae form the beginning of any food chain in aquaculture and play an important role as live feed for the direct consumption of mollusks at all life stages. This is an essential part of successful production. *Nannochloropsis* sp. is a marine eustigmatophyte with high nutritional value, particularly high eicosapentaenoic acid content, making it an interesting feed for aquaculture. *Nannochloropsis* sp. are microalgae that play an important role in aquaculture by providing live food for larvae, shrimp, mollusks, crustaceans, and zooplankton. Microalgae must meet several criteria, including ease of growth, non-toxicity, and highly nutritious (Hemaiswarya et al., 2011). *Nannochloropsis* sp. has a high carbohydrate nutrient content of 16.00%, 52.11% protein, and fat 27.64% (Ariany et al., 2021), which is composed of eicosapentaenoic acid (EPA, 215 g/kg total fat) and docosahexaenoic acid (DHA, 32 g/kg total fat) (Hulatt et al., 2017). When suplemented in fish feed, these fatty acids enhance lipid metabolism, enrich fatty acid profiles, and boost antioxidant capacity and hematological characteristic (Jin et al., 2017). Additionally, *N. oculata* in fish diets stimulates the production of functional growth hormone. Adding *Nannochloropsis oculata* to the diet of Nile tilapia is beneficial. It acts as a good source of

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protein and fat, improving the fish's growth and healthy fatty acid profile. This high-quality microalga also reduces the need for fish oil in feed. Furthermore, including *N. oculata* positively impacts genes related to antioxidants, immune response, fat metabolism, and cell health. Ultimately, using *N. oculata* in aquafeed can lead to healthier fish and more sustainable aquaculture practices (Zahran et al., 2023).

Cultural activities are an effort to develop and meet the needs of *Nannochloropsis* sp., so that the supply of *Nannochloropsis* sp. does not depend only on nature. One of the goals of algal culture is to produce the largest number of cells with the optimal nutritional content. The fulfillment of the nutrient requirements of *Nannochloropsis* sp. is highly dependent on its availability in the culture medium. The growth of *Nannochloropsis* sp. is strongly influenced by the high levels of nutrients in the culture media, which are required for growth and survival. Fertilizers as a source of nutrients provided in *Nannochloropsis* sp. culture generally use inorganic fertilizers (urea, TSP, and ZA). Excessive use of inorganic fertilizers produces waste residues, which can pollute and endanger aquatic organisms. Therefore, it is necessary to alternatively use organic fertilizers that contain nutrients suitable for the growth of *Nannochloropsis* sp., are not harmful to aquatic ecosystems at a relatively low cost, and are easy to obtain.

Research has been conducted on the use of various nutrients to increase the growth of *Nannochloropsis* sp.. *Nanochloropsis* grown on media with *Azolla mycropilla* liquid fertilizer was able to provide growth that was no different from that obtained using commercial Walne fertilizer. The highest population density was in the walne treatment (control) on day 6 with a value of 483.5 × 10^5 (cells/mL) and treatment with 4 mL *A. microphylla* fertilizer on day 5 with a value of 437.3 x 10^5 (cells/mL) (Padli et al., 2024). Water-extracted shrub *Clerodendrum minahassae* L. leaf application gave the highest growth of *Nannochloropsis* at 4% shrub extract concentration was $82.3x10^7 \pm 4.7x10^7$ cells in day-4, and 6% shrub extract concentration yielded the highest density in day-3, $70.7x10^7 \pm 19.0x10^7$cells (Dangeubun et al., 2020). Another study showed that a 10% duckweed LOF concentration could increase the growth of *Nannochloropsis* sp. cell population (Ariany et al., 2021). Nitrate, temperature, and pH have a positive effect on biomass production, and protein concentration is significantly influenced by the interactions of pH and nitrate, and carbohydrate and lipid concentrations are influenced by the interactive effects of pH, temperature, and nitrate (Kumaran et al., 2021).

Therefore, alternative fertilizers derived from natural ingredients that are available at affordable costs are required. One of the largest sources of waste is shrimp heads. Shrimp heads are industrial waste produced from fresh shrimp for export purposes. Shrimp head flour contains 84.75% dry matter; crude protein 21.76% and crude fiber 23.32% (Perkasa & Sudjarwo, 2019). Many shrimp head wastes are processed into liquid organic fertilizers. The characteristics of liquid organic fertilizer from shrimp heads are dark brown, very odorous, and a temperature of 33 °C; the chemical properties are N-total: 0.24%, P2O5:0.175%, and K2O: 0.175%, where the total value is N-Total + P2O5 + K2O = 0.59%, C-Organic = 3.585%, N-organic = 0.13%, and pH = 5.36. The potential of shrimp heads as a source of nutrition in the form of liquid fertilizer is very possible to be developed with the right processing techniques. Previous study reported that iquid fertilizer derived from shrimp head waste provides nutrients such as N = 4.475%, P = 0.048%, K = 0.0216%, C = 1.790%, Fe: 99.02 ppm, Mg = 0.0112 ppm, and has a pH of 6.24 (Rahmadiarto M. F., Ridwan, 2021).

The use of shrimp head meal as a fertilizer has been extensively researched, but its application is still limited to horticultural crops. The use of shrimp head waste as liquid fertilizer for plant cultivation is an example of a blue and green economy integration model (Adiwena et al., 2025). No studies have reported the effects of liquid shrimp head fertilizer on microalgae cultures. This research will examine the effect of adding liquid shrimp head fertilizer to the media on the growth of *Nannochloropsis* on a laboratory scale.

Research Method

Material

Nannochloropsis sp. with a density of 3 × 10^4 cells/ml was obtained from the culture

collection of microalgae at Koperasi Nelayan Kaltara. Commercial nutrition using KW21 composed of nitrogen (49 g/l), phosphoric acid (4 g/l), boron, manganese, cobalt, zinc, EDTA Amino acid complexes, and vitamin complexes (B1, B12, biotin, etc.). The medium pH was 7.00, and cultures were incubated at 25 ± 2ºC, 24-hour LED lighting, with aeration.

Preparation Of Liquid Organic Fertilizer (LOF) Shrimp Heads

Shrimp heads were thoroughly washed with clean water, oven-dried, and ground into a fine powder. A total of 500 g of shrimp head meal was mixed with 5 L of water containing 0.5% EM4 (Effective Microorganisms) and 250 g of brown sugar. The mixture was stirred thoroughly and allowed to ferment for 30 days under anaerobic conditions. During fermentation, the cultures were manually shaken three times daily to prevent solid particles from adhering to the container walls. After fermentation, the liquid organic fertilizer (LOF) was filtered and analyzed for nutrient contents, including total nitrogen (N), phosphorus (P), potassium (K), organic carbon (C-organic), and pH, at the Laboratorium Ilmu Tanah, Faculty of Agriculture, Universitas Borneo Tarakan (UBT).

Experimental Design

This experimental study used a randomized block design consisting of four treatments and three replicates. The treatment design consisted of control treatment (P0), 100% commercial fertilizer; Treatment 1 (P1), 50% commercial fertilizer + 50% liquid organic fertilizer (LOF); Treatment 2 (P2), 75% commercial fertilizer + 25% liquid organic fertilizer; and Treatment 3 (P3), 100% liquid organic fertilizer.The design and layout of the culture medium are shown in figure 1.

Culture of Nannochloropsis sp.

The inoculum microalgae sample was inoculated in medium with an initial density of 3 x 10^4 cell mL^{-1}, Cells were cultured for 14 days using mini aquarium. Cell density was calculated under a microscope every day using haemocytometer. Water quality measurements of *Nannochloropsis* sp., namely temperature, pH, and salinity, were performed every two days. Nitrate and phosphate measurements were performed at the beginning and end of the study (days 7 and 14) using a spectrophotometer.

Cell density was calculated using a Neubauer hemocytometer as follows:

$$\text{Cell Density: } \frac{Number\ of\ Cell}{Number\ of\ Square}\ x\ 10^4 \quad (1)$$

Spesific Growth Rate (SGR)

The specific growth rate value is calculated using the formula:

$$SGR : \frac{(\ln wt - \ln w0)}{t1 - t0}\ x\ 100\% \quad (2)$$

Wo : initial growth

Wt : peak growth in the exponential phase

T : culture time

Data Analysis

The data obtained, such as cell density at day 7, were analyzed statistically using analysis of variance (ANOVA) with a significance level of 95% using the SPSS 23 software. If there were real differences, they were further tested using the Duncan Multiple Range Test (DMRT).

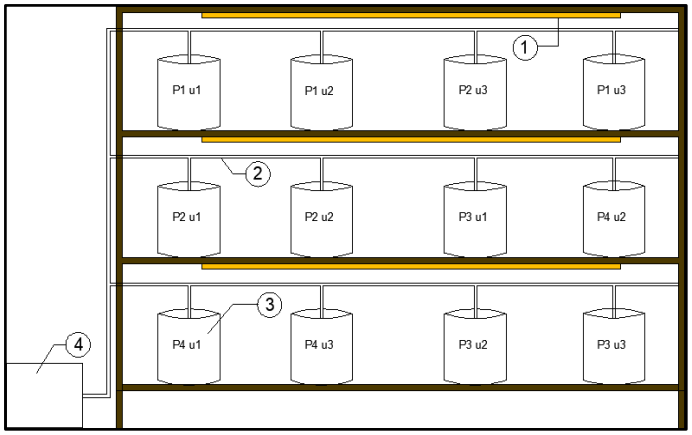

Figure 1. Research Design in Laboratorium
P1, P2, P3, P4 (Treatment); u1, u2, u3, and u4 (repetitions), 1. Lamp; 2. Aeration Pipes, 3. Culture Media, and 4. Aerators.

Result and Discussion

Nutrient Content Of Organic Liquid Fertilizer

The nutritional content of shrimp head waste liquid fertilizer measured in this study is $N+P_2O_5+K_2O$, C-Organic, and pH shown in Table 1.

The results of measuring the nutrient content of liquid organic fertilizer on the chemical properties above with a total value of $N+P_2O_5+K_2O$, C-Organic do not meet the requirements of Fertilizer criteria No.261/KPTS/SR.310/M/4/2019, while the pH value is 6.77 can fulfill the requirements of Fertilizer Standard No.261/KPTS/SR.310/M/4/2019. Previous research reports show that liquid organic fertilizer from shrimp heads contains 0.24%, P_2O_5:0.17%, K_2O: 0.17% where the total value of N-Total + P_2O_5 + K_2O is 0.59%, C-Organic: 3.58%, N -organic: 0.13%, and pH: 5.36 Compared to which results of those obtained in this study lower P_2O_5 and K_2O values were obtained. However, the C-Organic value was higher (7.28 %). C-Organic content is an important factor determining the mineral quality of a material. The higher the total C-organic content, the better the mineral quality. Organic materials play an important role in improving the physical properties of growing media, increasing the biological activity of the media, and increasing the nutrient availability for plants. The length of the fermentation time affected the nutritional content of the liquid organic fertilizer. The length of fermentation has an influence on crude protein content; the longer the fermentation, the more the crude protein content increases up to a certain time. Several factors influence the fermentation process, including the ingredients used, microbes added, and incubation time (Guillard & Sieracki, 2005). Raden et al., (2017) observed a decline in Total-N content after 20 days of soaking, reaching 0.17% by 40 days. A similar pattern was noted for P_2O_5, where a 10-day soaking period resulted in significantly higher P_2O_5 levels compared to soaking times of 20, 30, and 40 days.

The Cell Density of *Nannochloropsis* sp.

The cell population density of *Nannochloropsis* sp. was observed and counted daily for 14 days. The average growth rate is listed in Table 2. As shown in Table 2, the peak cell growth occurred on day 7, and statistical analysis was performed to determine the effect between treatments. The results showed that the treatments had significantly different effects on the growth of *Nannochloropsis* sp., with a sig value of 0.001 ($P<0.05$).

Based on observations over 14 days, the growth of *Nannochloropsis* sp. cells showed significant variation among the treatments. Treatments P0 (100% commercial fertilizer) and P1 (50% commercial fertilizer + 50% LOF) showed relatively high and stable growth rates until day 7, with peak densities of 15.7×10^4 cells mL^{-1} and 15.3×10^4 cells mL^{-1}, respectively.

Treatment P2 (75% commercial fertilizer + 25% LOF) also showed good growth with a peak of 13.7×10^4 cells mL^{-1}, whereas P3 (100% LOF) produced the lowest growth with a maximum density of only 9.7×10^4 cells mL^{-1}. After reaching the peak phase (day 7), all treatments experienced a decrease in cell number, indicating that the culture entered the stationary phase and then the death phase due to nutrient depletion and metabolite accumulation. These results indicate that a combination of commercial fertilizer and LOF in balanced proportions (P1 and P2) can increase the growth of *Nannochloropsis* compared to their single use.

Available nutrients and N:P balance commercial fertilizers are specifically formulated for microalgal cultures, providing the appropriate ratio of N, P, and trace elements for *Nannochloropsis*. Shrimp head waste/LOF contains nutrients (N, P, K, organics, protein, calcium/chitin), but the N:P ratio is often not ideal for microalgae, and if used undiluted, it can limit their growth (Nisa et al., 2021). This explains why P3 (100% LOF) performed poorly.

Cell Growth Phase

Figure 2 shows that there was an increase in the growth of *Nannochloropsis* sp. in each treatment from day to 2-7, and after day 7, the growth of *Nannochloropsis* sp. In all treatments, it decreased. The lag phase of *N. oculata* culture occurs on the first and second days (Widihastuti et al., 2022). The peak of growth occurred on the day-7 and after that , cell growth entered the stationary phase and continued to experience a decline in growth until the 14th day.

Table 1. Nutrient content of organic liquid fertilizer

Nutrient	Content	Unit	The SNI of Liquid Organic Fertilizer No. 261/KPTS/SR.310/M/4/2019
N-Total	0,26	%	N+P_2O_5+K_2O=2-6
P_2O_5	0,015	%	
K_2O	0,007	%	
pH	6,77		4-5
C-Organic	7,28	%	Min 10

Table 2. Cell density of *Nannochloropsis* sp.

Day Culture	Cell averages × 10^4 Cell ml^{-1}			
	P0	P1	P2	P3
1	4.3±0.58	5.7±0.57	6.0±2.00	4.3±0.57
2	6.3±0,58	7.0±0.57	7.0±2.00	4.7±0.57
3	7.3±1,53	7.7±1,00	8.7±1.00	5.3±0.57
4	8.7±1,15	8.7±1,00	9.0±1.00	6.0±0.07
5	9.3±1,53	10.0±1,00	10.0±1.05	6.7±0.57
6	12.3±0,58	11.0±0,01	12.0±1.05	7.3±0.57
7	15.7±0,58a	15.3±1,00a	13.7±1,15a	9.7b±1.00b
8	13.3±1,15	13.0±1,00	13.0±1,15	8.0±0.57
9	13.0±1,00	12.3±1,00	12.3±1,10	7.0±1.00
10	10.3±1,15	10.3±0.57	11.0±1,00	6.7±1.00
11	9.0±1,00	9.3±0.57	9.7±1,15	5.3±1.15
12	8.0±1,00	8.3±0.57	9.0±1,10	4.0±1.05
13	7.0±1,70	7.3±0.57	7.7±1,00	3.3±1.00
14	6.0±1,00	6.7±0.57	6.3±1,00	2.3±1.00

The average value that has the same lowercase letter does not show a real difference at the α level = 5%

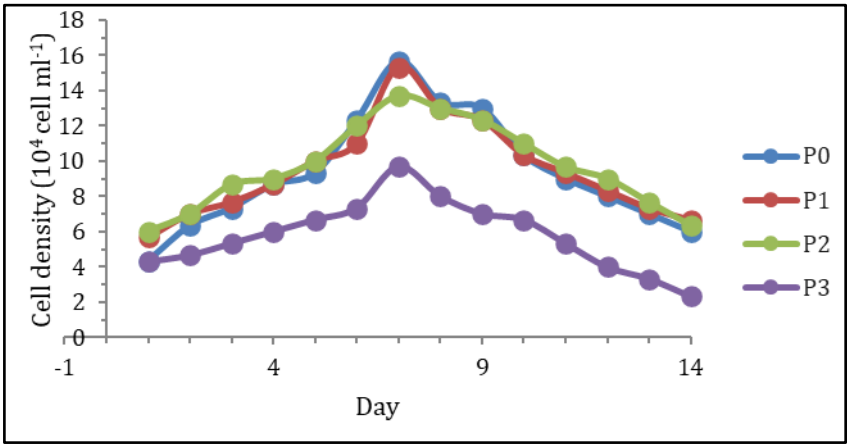

Figure 2. Growth of Cell Phase *Nannochloropsis* sp. in 14 days

Growth observations were carried out by counting the population of *Nannochloropsis* sp. to determine the peak population density, that is, when the population of *Nannochloropsis* sp. is at its highest point during its growth period. The final population density was determined at the time of death. The population growth of *Nannochloropsis* sp. is influenced by nutrients, with nitrogen (N) and phosphorus (P) playing major roles. N element in culture media functions in the form of proteins, fats, and chlorophyll (Fery et al., 2020). P is one of the main macronutrients in cell metabolic processes, as it forms various structural and functional components that cells need for the growth and reproduction of microalgae.

Increase in cell density in the P0 treatment was considered good. This may be because the nutrients in the P0 treatment were absorbed well and were at appropriate concentrations to support the growth of *Nannochloropsis* sp. cells. compared with the other treatments. The treatment of 100% LOF (P3) was less optimized in *Nannochloropsis* sp. cells. This may be because the nutrient content in the culture medium was insufficient to meet the needs of the cells. Previous studies have reported that the use of nutrients in microalgae culture media has a maximum limit that can be absorbed by cells; if it exceeds this maximum limit, there will be an inhibition of the biosynthesis process, especially protein biosynthesis (Arfah et al., 2019). Likewise, if the nutrient content is low, cell growth is hampered because the available nutrients are insufficient to meet the needs to support cell growth. Treatment P3 had the lowest cell density growth among the other treatments because the content of liquid organic fertilizer from shrimp head waste did not meet the fertilizer standards (SNI) required for the cell growth of *Nannochloropsis* sp.

Population growth curves for *Nannochloropsis* sp. Figure 2. shows the differences in the growth for each treatment. Treatments P0, P1, and P2 had an adaptation phase starting from day 1 to day 2, whereas treatment P3 experienced an adaptation phase starting from day 1 to day 3. The different adaptation processes in each treatment occurred because of differences in nutritional provision in the culture media; in this phase, the *Nannochloropsis* sp. cells. experiencing adjustments to new media. The adaptation phase is characterized by a slight increase in cell density (Suantika & Hendrawandi, 2009).

The exponential phase in the P0, P1, and P2 treatments began on days 3–7, whereas in treatment P3, the exponential phase occurred on days 4–7. Each treatment experienced a different exponential phase, which is thought to be due to differences in the time interval in the adaptation phase, where treatments with a high concentration of commercial fertilizers have higher nutrient content, which results in microalgae cells taking longer to adapt to the culture medium. which are given. The exponential phase is characterized by cell division, and the growth rate continues to increase (Leksono et al., 2017). The exponential phase occurred until the cell population reached its maximum peak (Gonzalez & Aranda, 2023).

The stationary phase occurs after going through the exponential phase and is characterized by growth that begins to decrease compared with the exponential phase. The stationary phase only occurred at P0, namely on days 8 and 9, showing the same number of cells, whereas in other treatments on days 8 to 14, there was a decrease in the number of cells. When the culture is in the stationary phase, the composition of the microalgae changes significantly because of the limited nitrate content in the culture medium, which results in the carbohydrate content increasing to twice the protein content (Leksono et al., 2017). The total carbohydrate content increased with age of the microalgae culture. In the stationary phase, the rate of reproduction or cell division is the same as the rate of death, in the sense that the addition and reduction of microalgae is relatively the same, as the microalgae density tends to remain constant.

The peak of cell growth occurred on the 7th day and the 8th day had decreased until the 14th day. Therefore, the cell growth pattern (Fig.2) did not show a stationary phase, but on the 8th day it decreased continuously (death phase). The cell death phase (decline phase) is the last stage in the growth curve of microorganisms, including microalgae, following the stationary phase. This indicated that all treatments gradually entered the cell death phase during this period. Previous studies also reported that On day seventh, *N. Oculata* cultures enter the stationary phase until death (Widihastuti et al., 2022). Several factors can lead to reduced algal growth, including insufficient nutrient levels, self-shading (where a dense population limits light penetration), and alterations in the culture environment, such as elevated pH, accumulation of metabolic byproducts, or presence of autoinhibitory compounds secreted by certain species (Widihastuti et al., 2022). The cell death phase is characterized by a cell population whose growth pattern tends to decrease constantly (Arfah et al., 2019). In this phase, microalgae compete for space in the culture media, and there is a significant decrease in nutrients in the culture owing to a lack of nutrients (Yatin, 2015).

Spesific Growth Rate (SGR)

In this experiment, *Nannochloropsis* showed an average Specific Growth Rate (μ) value for days 1–7 of: P0 (100% commercial nutrient) = 0.22 d^{-1}; P1 (50% commercial nutrient + 50% LOF) = 0.17 d^{-1}; P2 (75% commercial nutrient + LOF) = 0.14 d^{-1}; P3 (100% LOF) = 0.14 d^{-1} (Fig. 2). In general, P0 provided the highest growth rate, whereas the treatment containing liquid shrimp head fertilizer (LOF) had a lower growth rate. The higher the LOF percentage, the lower the SGR value.

P1 (50% commercial + 50% LOF) showed a better intermediate SGR than P2/P3, suggesting that supplementing with some commercial nutrients can compensate for the delayed release of nutrients from LOF. This blending strategy is often recommended in agricultural and aquaculture practices to balance cost efficiency and rapid nutrient availability (Brito-Lopez et al., 2025).

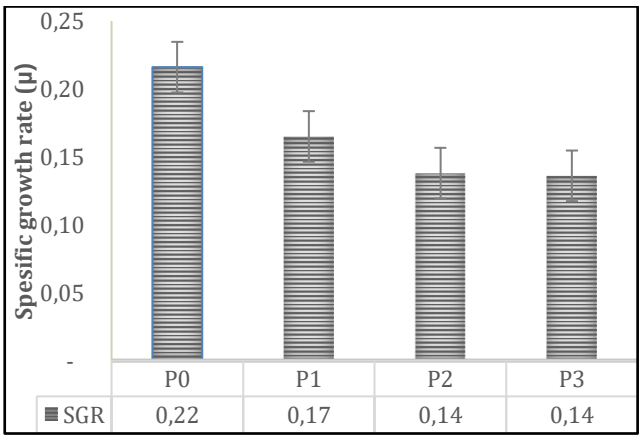

Figure 2. *Specific growth rate* (SGR)

Commercial media are generally formulated to provide nitrogen and phosphorus in rapidly assimilated inorganic forms (e.g., NO_3^-, NH_4^+, PO_4^{3-}). This rapid and proportional nutrient availability supports a rapid cell division phase, thus increasing μ. The P0 > P1–P3 result is consistent with the documentation that the μ of *Nannochloropsis* increases when readily available N/P sources are provided (Barten et al., 2022). Shrimp heads contain proteins, lipids, and chitin, which must be hydrolyzed by microbial enzymes (proteases and chitinases) before being converted into soluble inorganic forms usable by microalgae. This mineralization process occurs gradually; therefore, the availability of inorganic nutrients in media containing LOF is generally lower in the early phase of culture, which explains the decreased SGR in both 100% full LOF treatments and treatments combined with commercial fertilizers. Studies using biological waste as organic fertilizers have confirmed that nutrient release from organic substrates is slower than that from inorganic fertilizers (Anwar et al., 2024).

The decomposition of proteinaceous material in shrimp heads (and microbial activity) can increase ammonia/ammonium (NH_4^+/NH_3) concentrations. At a certain threshold, NH_3 is toxic to many microalgae and can suppress growth or alter physiology. Therefore, the use of LOF without controlling pH/ammonia solubility can negatively impact Nannochloropsis growth rates. Studies on the interaction of NH_4^+/NH_3 with algal growth support the need to pay attention to these parameters when using organic/waste nutrient sources (Lin et al., 2017).

Water Quality

Water quality is an important component in the culture process of *Nannochloropsis* sp. Temperature, pH, and salinity measurements were carried out every two days, while nitrate and phosphate measurements were carried out on days 7th and 14th days (table 3).

Based on the results of this study, the water quality of the culture media with the addition of fertilizer from each treatment was still relatively good because the microalgae cells could still reproduce and grow. However, the addition of LOF from shrimp head waste affected the acidity (pH) of the culture medium. If the concentration added to the medium is higher, a lower pH is obtained in the culture medium. The low pH level in LOF from shrimp head waste is caused by the fermentation process used to make the fertilizer. CO_2 gas, which is acidic (H_2CO_3), is produced.

Temperature measurements during the culture period ranged from to 24-28 ºC. Temperature greatly influences the life and growth of the aquatic biota. Temperature can affect the enzyme activity within microalgae cells, which in turn affects the metabolic processes and growth of microalgae cells (Suparmaniam et al., 2022). While a optimal temperature encourages microalgae to grow well, too much heat can be harmful. It may cause their proteins to break down, which then shuts down enzymes, throws their metabolism into disarray, and can even lead to the death of the algal cells (Manhaeghe et al., 2019). The temperature in this study met the maximum limit at which *Nannochloropsis* sp. could grow well in the range of 25-30 ºC. The pH can influence the metabolism and growth of microalgae cultures, including changing the balance of inorganic carbon, changing nutrient availability, and affecting cell physiology. The pH value obtained during the culture period ranges from 7.11–7.44. *Nannochloropsis* sp. can grow well in the pH range of 7-8 (Sahira et al., 2017). Changing salinity can affect the growth of microalgae; some microalgae can grow in high salinity ranges, but microalgae can also grow at low salinity. Water in culture media that experiences high salinity can be added to fresh water to neutralize salinity. The salinity parameter measured during the culture period was 35-38 ppt. Salinity affects organisms in maintaining osmotic pressure within their environment (Farias et al., 2024).

Nitrate and phosphate are the primary nutrients that are essential for the growth of microalgae (Kartina et al., 2025). The nitrate phosphate content in the culture media is explained in the following table 4.

Table 3. water quality of culture media

No.	Parameters	Unit	Range Value
1.	Temperature	ºC	24-28
2.	pH	-	6.5-7,44
3.	Salinity	mg/L	34-38

Table 4. Nitrate and Phosphate content in medium culture

	Day	P0	P1	P2	P3
Nitrate	7	1,98	0,48	0,74	0,34
(mg/L)	14	2,07	0,71	2,00	0,27
Phosphate	7	0,22	0,28	0,40	0,26
(mg/L)	14	0,31	0,29	0,28	0,19

P0 (100% commercial fertilizer) showed the highest nitrate value (1.98–2.07 mg/L), indicating that commercial fertilizer (KW21) is a stable and easily absorbed inorganic nitrogen source. P1 and P3 showed a significant decrease in nitrate on the 7th day, indicating that nitrogen from organic shrimp head fertilizer is a slow-release fertilizer because it is still in the form of protein and amino acids that require microbial decomposition before they can be utilized. P2 (75% commercial + 25% LOF) approached the P0 level on the 14th day (2.00 mg/L), indicating that a small combination of organic and commercial fertilizers can provide continuous nitrogen, increasing efficiency without nutrient deficiencies.

Nitrate (NO_3-) is the main nitrogen compound absorbed by various microalgae during growth. Microalgae use nitrate for protein synthesis and cell tissue formation (Matthews, 2014). In addition to nitrate, phosphorus is an important macronutrient for microalgal growth. The phosphorus content in the nutrient medium is very important because it plays a role in the transfer of energy from adenosine diphosphate (ADP) to adenosine triphosphate (ATP), which occurs in the mitochondria (Bergman et al., 1999). Nitrogen can affect phosphorus use; therefore, adding both nutrients can increase the rate of algal growth (Fried et al., 2003). The nitrate value obtained during culture on the 7th day ranged from 0.34 to 1.978 mg/l, whereas on the 14th day, the nitrate value obtained ranged from 0.27 to 2.07 mg/l. In this study, the nitrate value on the 14th day increased, especially in P0 treatment and P2 treatment the optimum value of nitrate for algae growth in waters is 0.9-3.5 mg^{-1} (Nuraini, 2006).

The phosphate value obtained during culture on day 7 ranged from 0.224 – 0.399 mg/l. Phosphate levels in water are generally low, ranging from 0.05 to 0.20 mg/l, and phosphate mobility is very low. High phosphate levels in natural waters can cause the excessive growth of aquatic plants and algae (Subarijanti, 2006). The low phosphate value in the P3 treatment is thought to be because shrimp head LOF has a low phosphate content (Table 4.1), which affects the low cell density of *Nannochloropsis* sp. The phosphate content of water is also a characteristic of its fertility (Arizuna et al., 2014).

Conclusion

Based on the research results, it can be concluded that the use of 100% commercial fertilizer KW21 provides the highest cell density and specific growth rate *Nannochloropsis* sp. compared to the use of 100% organic fertilizer LOF. However, the combination of 50% commercial fertilizer + 50% LOF yielded cell density and specific growth rate values that were not significantly different from the 100% commercial fertilizer treatment.

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