Tomini Journal of Aquatic Science, Vol 4(1): 10–17, November 2025
Received: 2025-07-07; Accepted: 2025-10-28

e-ISSN: 2722-3787
Tomini Journal of Aquatic Science
Homepage: http://ejurnal.ung.ac.id/index.php/tjas

The Effect of Liquid Organic Fertilizer Application on the Growth of Microalgae *Chlorella* sp.

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ARTICLE INFO

Keywords:
Chlorella sp., cell density, growth rate, liquid organic fertilizer, microalgae growth.

How to cite:
Wijayanti, L. A. S., Utama, M. I. C., Afifah, A. N., Septiani, A. R., Pratidnia, M. W. G., Yuningsih, Y., Umbekna, S. (2025). The Effect of Liquid Organic Fertilizer Application on the Growth of Microalgae Chlorella sp.. Tomini Journal of Aquatic Science, 4(1): 10–17

ABSTRACT

Microalgae such as *Chlorella* sp. are valuable for their potential in sustainable food, feed, and bioenergy production, but their cultivation often relies on costly synthetic nutrients. Exploring alternative nutrient sources, such as liquid organic fertilizers, may offer a more economical and environmentally friendly approach to biomass production. This study aims to evaluate the effect of liquid organic fertilizer application on the growth of *Chlorella* sp. in a closed culture system. Seven treatments were used: one control without fertilizer (K) and six treatments with increasing fertilizer doses (P1–P6). The observed parameters included initial and final cell density, as well as daily growth rate during a seven-day incubation period. The results showed that fertilizer application had a significant effect on the growth enhancement of *Chlorella* sp. The highest final cell density was recorded in treatment P6, exceeding 6.3×10^7 cells/ml, while a population decline was observed in the control group. The daily growth rate also increased with higher fertilizer doses, with the highest value ($\mu = 0.36$) observed in P6. Linear regression analysis showed a strong relationship between fertilizer amount and growth rate ($r = 0.8493$; $p = 0.0156$; $R^2 = 0.7213$), indicating that growth variation could be significantly explained by the amount of fertilizer applied. The conclusion of this study is that liquid organic fertilizer has great potential as an efficient and environmentally friendly alternative for microalgae culture media. Proper dosage formulation can support optimal microalgal biomass production. Further research is needed to identify the dominant nutrient content in the fertilizer and to assess the biomass quality for potential applications in food, feed, or bioenergy sectors.

INTRODUCTION

Microalgae are microscopic photosynthetic organisms that play an important role in aquatic ecosystems and various biotechnological applications, including as sources for aquaculture feed, biofertilizer, bioenergy, as well as raw materials for pharmaceuticals and cosmetics. One of the most widely cultivated microalgae is *Chlorella* sp., due to its rapid growth rate, high nutritional content, and ability to adapt to various environmental conditions (Andriani et al. 2023; Widiyanto et al. 2014). The success of microalgae cultivation largely depends on the culture medium used (Mufidah et al. 2017; Skifa et al. 2025), particularly the availability of essential nutrients required for its growth.

So far, synthetic media such as Guillard's (f/2) medium are often used as a standard in *Chlorella* sp. cultures. However, the high production cost and dependence on inorganic fertilizers are major obstacles, especially for large-scale or community-level applications. As attention to sustainability issues increases, various studies have begun to explore the use of organic fertilizers as a more eco-friendly and economical alternative. One promising innovation is the use of organic waste, such as skipjack tuna waste, which is processed into liquid fertilizer. Research has shown that liquid organic fertilizer from fish waste can enhance the growth and chlorophyll-a content of *Chlorella* sp., with performance comparable to Guillard's medium (Sujarta et al. 2025). This indicates that fishery waste can be efficiently utilized within a circular economy framework.

In addition to fishery waste, various other organic sources have also been studied, such as the aquatic plant *Azolla* sp., known to contain nitrogen, phosphorus, and bioactive compounds that support microalgal metabolism. The use of liquid organic fertilizer derived from *Azolla* at a concentration of 12 mL L^{-1} has been reported to significantly increase cell density and growth rate of *Chlorella* sp. (Taradifa et al. 2022). These successful outcomes have encouraged further research into other natural materials, such as *Lemna* sp., white sesbania (*Sesbania grandiflora*) leaves, and water hyacinth, which also show potential as raw materials for liquid organic fertilizers (Andriani et al. 2023). The main advantages of this approach include easy access to raw materials, simple processing methods, and the potential for application at the community level.

Although several organic fertilizers have shown positive results, some studies still indicate that inorganic fertilizers offer greater stability and effectiveness, as seen in comparative studies using soil extracts (Taradifa et al. 2022; Sahu et al. 2024). However, in the context of long-term sustainability, the use of organic fertilizers from natural materials and waste remains highly relevant for the development of low-cost and sustainable microalgae cultivation systems. Based on this background, this study aims to examine the effect of liquid organic fertilizer application on the growth of *Chlorella* sp., specifically by assessing changes in cell density and chlorophyll-a content as key indicators. The results of this study are expected to enrich information on alternative culture media formulations and support the development of more efficient, economical, and environmentally friendly microalgae cultivation systems. An introduction is about 400-600 words, covering the aims of the research and providing an adequate background. Indicate the aims of research in the last paragraph.

MATERIAL AND METHODS

Study site. This research was conducted in 2025 at the plankton laboratory, Padjadjaran University, and observations were carried out for one week.

Study design. This study used a Completely Randomized Design (CRD) with 7 concentration treatments, each replicated 3 times. The concentrations for the 7 treatments were as follows:

- P0: Control (no fertilizer)
- P1: 2 mL/L liquid organic fertilizer

- P2: 2.5 mL/L liquid organic fertilizer
- P3: 3 mL/L liquid organic fertilizer
- P4: 3.5 mL/L liquid organic fertilizer
- P5: 4 mL/L liquid organic fertilizer
- P6: 4.5 mL/L liquid organic fertilizer

Data collection. The data collected during the study consisted of the growth of *Chlorella* sp. from the beginning to the end of the culture period. Culture samples were taken using a sterile pipette and placed on the counting area of a hemocytometer. The hemocytometer was then placed under a microscope for observation. *Chlorella* sp. cells located within the counting grid were carefully observed and counted. Counting was performed in multiple grids to obtain more representative results. Based on the cell count data obtained from the hemocytometer, calculations of initial density, stocking density, and final density were calculated using the following formula:

$$\text{Cell Density (cells/mL)} = \frac{\text{Total Cells Counted}}{\text{Number of Counting Squares}} \times \frac{1}{\text{Volume per Square (mL)}} \times \text{Dilution Factor}$$

Data Analysis. To determine the relationship between the applied fertilizer dose and the growth of *Chlorella*, statistical methods such as correlation and simple linear regression analysis were used. The correlation analysis aimed to assess the strength of the relationship between the fertilizer dose (independent variable, X) and *Chlorella* growth (dependent variable, Y), which was measured in terms of cell density (cells/ml). Meanwhile, the regression analysis was used to predict and explain changes in variable Y (*Chlorella* growth) based on variations in X (fertilizer dose). Thus, regression analysis not only evaluates the relationship but also indicates the direction of influence and the extent to which the independent variable contributes to the dependent variable. The analysis results were visualized in the form of tables and graphs. All analyses and visualizations were performed using the Python programming language on the Google Collaboratory scientific computing platform.

Pearson correlation test was employed because both variables are quantitative and assumed to be normally distributed. The Pearson correlation coefficient, r, ranges from –1 to 1, and is interpreted as follows:

$$r = \frac{n\sum(XY) - \sum X \sum Y}{\sqrt{[n\sum X^2 - (\sum X)^2][n\sum Y^2 - (\sum Y)^2]}}$$

- $r > 0$ indicates a positive relationship (the higher the fertilizer dose, the greater the *Chlorella* growth).
- $r < 0$ indicates a negative relationship.
- $r = 0$ indicates no linear relationship.

Simple linear regression analysis was conducted to determine the mathematical model describing the relationship between fertilizer dose (X) and *Chlorella* growth (Y), with the equation:

$$Y = a + bX$$

Where:

- Y = predicted value of *Chlorella* growth
- X = fertilizer dose
- a = intercept (Y value when X = 0)
- b = regression coefficient (indicating the magnitude of change in Y for each unit change in X)

RESULTS

The results of the study showed that the application of liquid organic fertilizer had a significant effect on the growth of *Chlorella* sp (Figure 1). The initial cell density across all treatments was relatively uniform, approximately 5 × 10^6 cells/ml. However, after seven days of incubation, significant variations in final cell density were observed among the treatments. In the control treatment (K), cell density drastically decreased, indicating the absence of growth without fertilizer addition. Treatments P1 and P2 resulted in relatively low increases in cell density, while treatments P3 to P6 showed much higher increases, peaking at P6 with more than 6.3 × 10^7 cells/ml. This pattern indicates that the higher the fertilizer concentration applied, the greater the biomass growth of *Chlorella* sp.

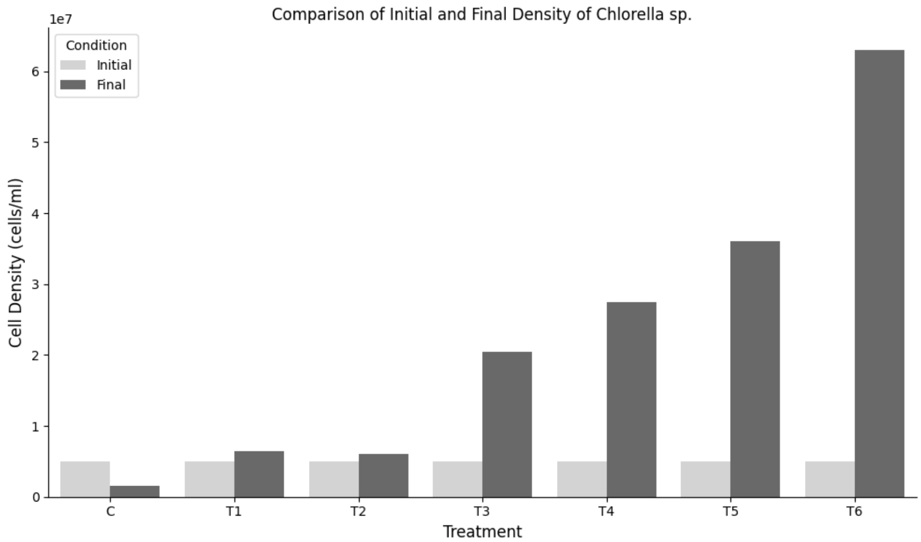

Figure 1. Comparison graph of cell density at the initial and final culture stages.

The positive relationship between fertilizer amount and biomass growth was also evident in its correlation with growth rate (Figure 2). The daily growth rate of *Chlorella* sp. showed noticeable differences among treatments. In the control treatment (K), the growth rate was negative ($\mu = -0.19$), indicating a population decline during the incubation period. In contrast, treatments P1 and P2 showed positive but still low growth rates ($\mu = 0.04$ and 0.03), suggesting that the fertilizer concentrations in these treatments were not yet optimal to stimulate significant growth. A marked increase in growth began at treatment P3 ($\mu = 0.20$) and continued in subsequent treatments, reaching the highest growth rate at P6 ($\mu = 0.36$). This pattern demonstrates a positive relationship between the amount of fertilizer applied and the growth rate of *Chlorella* sp., indicating that nutrient availability from the fertilizer plays a crucial role in accelerating the growth of the microalgae.

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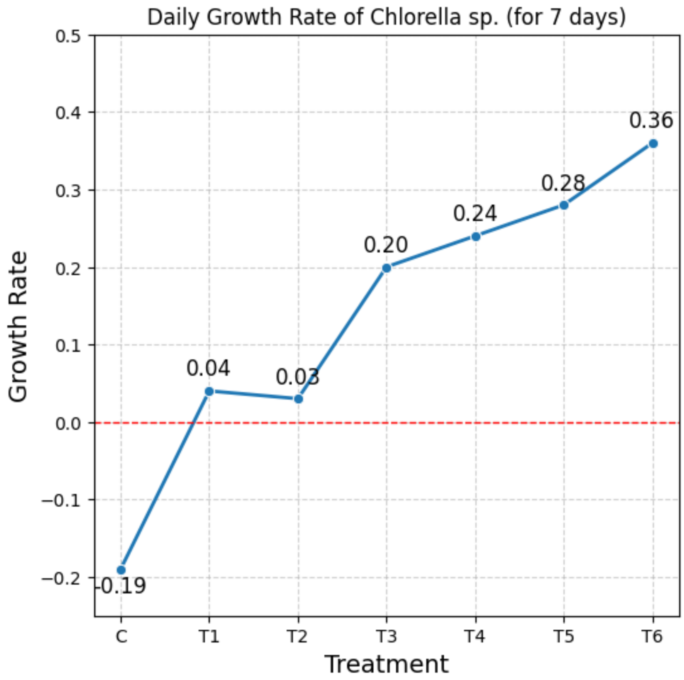

Figure 2. Graph of daily growth rate for each treatment.

Linear regression analysis between fertilizer amount and daily growth rate of *Chlorella* sp. showed a statistically significant relationship (Figure 3). The resulting regression equation was: $y = 1.75 \times 10^6x - 2.30 \times 10^6$, with a correlation coefficient $r = 0.8493$, indicating a strong positive relationship between fertilizer amount and daily growth rate. The coefficient of determination $R^2 = 0.7213$ means that 72.13% of the variation in daily growth rate can be explained by the variation in fertilizer amount. A p-value of 0.0156 (< 0.05) indicates that this relationship is statistically significant. These results further support previous findings that increasing fertilizer concentration directly contributes to the acceleration of *Chlorella* sp. growth.

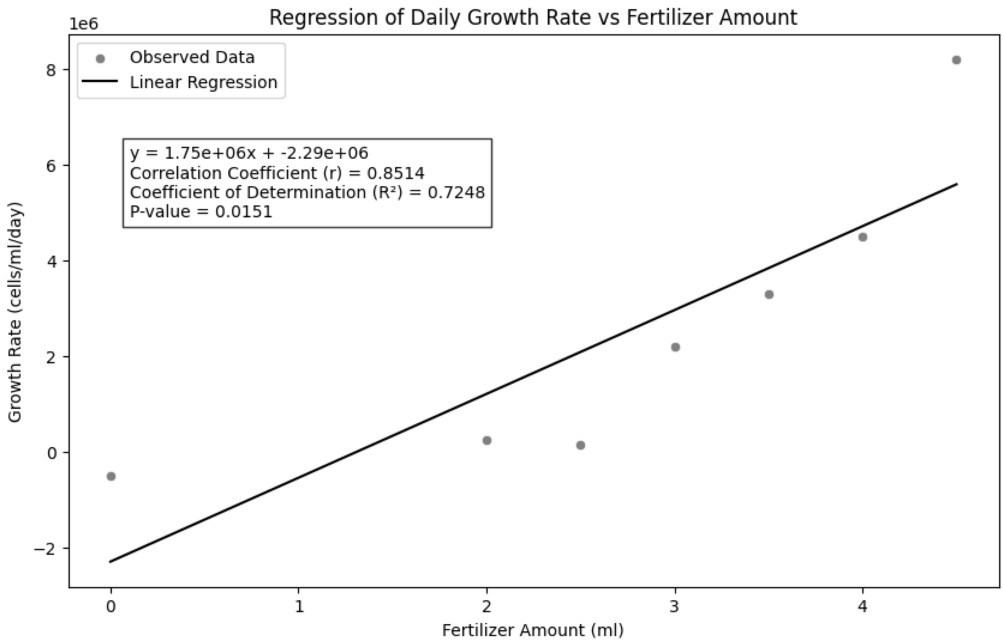

Figure 3. Graph of daily growth rate for each treatment.

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DISCUSSION

This study demonstrates that the application of liquid organic fertilizer has a positive effect on the growth of *Chlorella* sp., as indicated by the increased cell density observed across treatments. Final cell density increased significantly with higher fertilizer doses, while in the control treatment without fertilizer, a decrease in density was observed. These results are consistent with findings by Sujarta et al. (2025), who reported that liquid fertilizer made from skipjack tuna waste significantly enhanced *Chlorella* sp. growth. The presence of nitrogen, phosphorus, and other organic compounds in organic fertilizers plays a crucial role in supporting the anabolic processes of microalgal cells (Simarmata et al. 2012). Thus, external nutrient supplementation is essential in closed culture systems to maintain population productivity (Zainuddin & Nofianti, 2022).

In addition to increased density, the pattern of daily growth rate of *Chlorella* sp. also showed a strong correlation with fertilizer concentration. The negative growth rate in the control treatment indicates that the absence of nutrients leads to cell death. In contrast, the gradual increase from P1 to P6 shows that *Chlorella* sp. can physiologically respond to improved nutrient availability. This phenomenon aligns with the nutrient limitation theory described by Richmond & Hu (2013), which states that microalgal growth is highly dependent on the balance of nutrients in the medium. Treatment P6, which showed the highest growth rate, likely approached optimal conditions for metabolism and cell division. This is supported by Afifah et al. (2021), who found that the addition of 0.3 mL of liquid NPK fertilizer produced the best results for *Chlorella* sp. cultivation.

However, it is important to note that although the growth rate increased with fertilizer dose, it remains unclear whether even higher concentrations would continue to produce positive effects. Research by Afifah (2021) showed that beyond certain concentrations, excess nutrients may lead to saturation or even toxicity, which could inhibit microalgal growth. Therefore, determining the optimal dose is a critical step in developing waste-based liquid fertilizer formulations. In this context, the study’s findings provide preliminary evidence that the fertilizer concentration in treatment P6 remains within an acceptable range and supports optimal *Chlorella* sp. growth.

Linear regression analysis further supports previous observational findings. The relationship between fertilizer amount and daily growth rate showed a strong and statistically significant correlation ($r = 0.8493$; $p = 0.0156$), with a coefficient of determination of 72.13%. This indicates that the majority of the variation in growth rate can be explained by the amount of fertilizer applied. These findings are consistent with reports by Markou & Vandamme (2014) and Yaakob et al. (2021), which emphasize that nutrient availability—particularly nitrogen—directly affects the growth rate and biomass efficiency of microalgae. Therefore, this regression model can serve as a foundation for formulating appropriate fertilization strategies to improve the efficiency of microalgae cultivation.

Overall, the results of this study reinforce the potential of using liquid organic fertilizer as an efficient and environmentally friendly alternative microalgae culture medium. In addition to supporting competitive growth compared to synthetic media, the use of organic waste as raw material contributes to waste management and the circular economy. Further studies are needed to identify the dominant nutrient components in the fertilizer and their effects on biomass quality parameters, such as protein and lipid content. In addition, sustainability trials on a larger production scale are necessary to ensure the long-term effectiveness and stability of the cultivation system.

CONCLUSION

This study demonstrates that liquid organic fertilizer has a significant effect on the growth of *Chlorella* sp. The application of fertilizer with gradually increasing concentrations resulted in higher final cell densities and daily growth rates of the microalgae. The treatment with the

highest concentration (P6) yielded optimal growth, showing the highest growth rate and cell density, far exceeding those of the other treatments. The positive and statistically significant relationship between fertilizer amount and daily growth rate was further supported by linear regression analysis, indicating that nutrient availability in the fertilizer is a key factor in supporting the metabolism and cell division of *Chlorella* sp.
Therefore, liquid organic fertilizer has great potential as an environmentally friendly and cost-effective alternative culture medium for microalgae cultivation. Further research is needed to precisely determine the optimal dosage, evaluate the quality of the cultivated biomass, and test its effectiveness on a mass production scale to support applications in food, feed, and bioenergy sectors.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the Fisheries Department of Pangandaran Regency for their support and cooperation during the research process. Appreciation is also extended to the laboratory team and all individuals who contributed to the sampling, laboratory analyses, and the preparation of this scientific report.

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