Original article DOI: 10.19027/jai.24.1.92-101

Utilization of chicken manure enriched with *Lemna* liquid organic fertilizer *Lemna minor* on cell density of *Chlorella* sp.

Pemanfaatan kotoran ayam yang diperkaya pupuk organik cair *Lemna Lemna minor* terhadap kepadatan sel *Chlorella* sp.

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(Received December 26, 2023; Revised February 16, 2024; Accepted October 1, 2024)

ABSTRACT

One of the organic fertilizers that has the potential to be used to culture *Chlorella* sp. is chicken manure. However, to increase the content of nutrients in chicken manure, it must be enriched with liquid organic fertilizer (POC) in different doses. The purpose of this study was to determine the optimal dose of chicken manure enrichment using *Lemna* POC on the cell density of *Chlorella* sp. This study used a complete randomized design (CRD) with five treatments of three replicates, namely P0 giving 3 g/L chicken manure (control), P1 = 3 g/L chicken manure enriched with 4% *Lemna* POC, P2 = 3 g/L chicken manure enriched with 5% *Lemna* POC, P3 = 3 g/L chicken manure enriched with 6% *Lemna* POC, and P4 = 3 g/L chicken manure enriched with 7% *Lemna* POC. The results obtained the highest cell density of *Chlorella* sp. in the P3 treatment of $516.67 \pm 6.29 \times 10^4$ cells/mL, a specific growth rate of 0.142 cells/mL/day, and a biomass weight of 0.39 g/L. From the results of the study, it can be concluded that the optimal dose of chicken manure enrichment to increase the cell density of *Chlorella* sp. is 6% *Lemna* POC in P3 treatment.

Keywords: cell density, chicken manure, Chlorella sp., Lemna POC

ABSTRAK

Salah satu pupuk organik yang memiliki potensi dimanfaatkan untuk kultur *Chlorella* sp. adalah kotoran ayam. Namun untuk meningkatkan kandungan unsur hara pada kotoran ayam maka harus diperkaya dengan pupuk organik cair (POC) *Lemna* dengan dosis berbeda. Tujuan dari penelitian ini untuk mengetahui dosis pengayaan kotoran ayam yang optimal menggunakan POC *Lemna* terhadap kepadatan sel *Chlorella* sp. Penelitian ini menggunakan rancangan acak lengkap (RAL) dengan lima perlakuan tiga ulangan, yaitu P0 pemberian 3 g/L kotoran ayam (kontrol), P1 = 3 g/L kotoran ayam diperkaya 4% POC *Lemna*, P2 = 3 g/L kotoran ayam diperkaya 5% POC *Lemna*, P3 = 3 g/L kotoran ayam diperkaya 6% POC *Lemna*, dan P4 = 3 g/L kotoran ayam diperkaya 7% POC *Lemna*. Hasil penelitian diperoleh kepadatan sel *Chlorella* sp. tertinggi pada perlakuan P3 sebanyak 516,67 ± 6,29 ×10⁴ sel/mL, laju pertumbuhan spesifik sebesar 0,142 sel/mL/hari dan berat biomassa sebesar 0,39 g/L. Dari hasil penelitian dapat disimpulkan bahwa dosis pengayaan kotoran ayam yang optimal untuk meningkatkan kepadatan sel *Chlorella* sp. adalah 6% POC *Lemna* pada perlakuan P3.

Kata kunci: Chlorella sp., kepadatan sel, kotoran ayam, POC Lemna

INTRODUCTION

Chlorella sp. is a single-celled microorganism from the green algae group, classified under the phylum Chlorophyta, known for its high nutritional value (Rosyadi et al., 2022). Chlorella sp. contains 51-58% protein, 28-32% fat, 4-5% nucleic acid (Mufidah et al., 2017); 16.7% carbohydrates, 2.8% chlorophyll, vitamins A, B1, B2, B6, B12, C, E, K (Hafizhah et al., 2012); and 32.74% antioxidants (Iriani et al., 2017a), making it useful as natural feed (Hadi & Rosyadi, 2022; Roza et al., 2022), a supplement and health food (Iriani et al., 2017b; Novianti et al., 2019), a biofuel source (Ammar et al., 2022), a flavor preservative (Indriana et al., 2020), and a cosmetic ingredient (Putri et al., 2019; Silva et al., 2019; Iriani et al., 2023).

Chlorella sp. can be obtained through culturing. One of the crucial factors influencing the growth and biomass of *Chlorella* sp. is the availability of nutrients in the culture medium (Hadi & Rosyadi, 2022). Currently, microalgae culture predominantly relies on synthetic culture media, which are relatively expensive and have limited supply (Goa *et al.*, 2019). Using organic fertilizers has advantages, such as easy availability and the presence of NPK and other nutrients essential for plant growth (Indriana *et al.*, 2020).

Chicken manure has significant potential as fertilizer because not only improves the physical, chemical, and biological properties of the soil but also contains higher levels of nitrogen, phosphorus, and potassium compared to other livestock manures. Chicken manure contains 2.44% nitrogen (N), 0.67% phosphorus (P), 1.24% potassium (K), and 16.10% organic carbon (Sari *et al.*, 2016). To enhance and increase the nutrient content of chicken manure, it can be enriched with other organic fertilizers, such as *Lemna*. *Lemna* is a small, floating aquatic weed.

Besides being used as fish feed, *Lemna* can also be processed into liquid organic fertilizer. Currently, *Lemna* is underutilized, often left to grow freely, which can harm the environment by reducing water quality if its population becomes excessive. *Lemna* contains 0.8–7.8% nitrogen (N) and 0.03–2.8% phosphorus (P) based on its total dry weight, along with other nutrients (Iskandar *et al.*, 2020), making it a potential nutrient source for microalgae. Therefore, this study aimed to investigate the effect of chicken manure enriched with *Lemna*-based liquid organic fertilizer (POC)

at different doses on the cell density of *Chlorella* sp.

MATERIALS AND METHODS

Preparation of chicken manure fertilizer

The chicken manure used was obtained from the poultry farm of PT. Rosna, located in Baru Village, Siak Hulu Sub-district, Kampar Regency, Riau Province. Before use, the chicken manure was dried for three days. Once dried, the manure was ground into a fine powder. The manure was then weighed according to the dosage for each treatment and dissolved in 500 mL of water. The solution was left to stand for one week, after which it was filtered using a clean cloth and then filtered again using a plankton net.

Preparation of Lemna liquid organic fertilizer

The Lemna (Lemna minor) used in this study was cultivated at the Fish Hatchery Center of the Faculty of Agriculture, Universitas Islam Riau, Pekanbaru. The preparation of Lemna liquid organic fertilizer (POC) began by weighing 300 g of Lemna, which was then blended into a fine mixture. The blended Lemna was dissolved in 1,500 mL of distilled water (akuades) and supplemented with 30 mL of EM4 (effective microorganism 4) and 30 g of brown sugar.

After thorough mixing, the solution was fermented anaerobically for seven days. Following fermentation, the *Lemna* fertilizer was filtered using a fine cloth to obtain the liquid organic fertilizer (Astrid *et al.*, 2013; Indriana *et al.*, 2020). The liquid organic fertilizer was then ready for use. The EM4 used in this process was specifically for plants and contained fermentative microorganisms (*Lactobacillus* sp. and *Saccharomyces* sp.) (Hadi & Rosyadi, 2022). This material was obtained commercially.

Preparation of containers and culture of *Chlorella* sp.

The *Chlorella* sp. culture in this study used inoculum sourced from the Laboratory of Microalgae and Fish Nutrition, Faculty of Agriculture, Universitas Islam Riau. The culturing process began with the preparation of 15 containers, each with a 20 L capacity, equipped with aeration systems. The culture volume for each container was set to 16 L, and lighting was provided using 40 watt TL lamps with a light intensity of 2,500 lux, measured using

a lux meter (Smart Sensor) (Hadi & Rosyadi, 2022). Fertilization was carried out by first adding chicken manure fertilizer, followed by the addition of *Lemna* liquid organic fertilizer (POC) at the respective treatment doses. The *Chlorella* sp. inoculum was introduced at a rate of 30 mL/L, with an initial density of 17.48×10⁴ cells/mL. Cell growth was monitored every two days over a 14-day cultivation period.

Experimental design

This study employed an experimental method with a completely randomized design (CRD) consisting of five treatments and three replications. The treatments were as follows P0 as 3 g/L of chicken manure (control), P1 as 3 g/L of chicken manure enriched with 4% *Lemna* liquid organic fertilizer (POC), P2 as 3 g/L of chicken manure enriched with 5% *Lemna* POC, P3 as 3 g/L of chicken manure enriched with 6% *Lemna* POC, P4 as 3 g/L of chicken manure enriched with 7% *Lemna* POC.

Measured parameters

The cell density of *Chlorella* sp. was calculated using the formula provided by Mukhlis *et al*. (2017), as follows $N = n \times 10^4$ (cells/mL), N is the total cell count result (cells/mL) and n is the total number (cells/mL) in each sample. The specific growth rate (SGR) is calculated using the formula proposed by Kumar *et al*. (2014):

$$\mu = \frac{\ln N2 - \ln N1}{(t2 - t1)}$$
 (cells/mL/day)

Note:

 μ = Specific growth rate (cells/mL/day)

t = day

N1 = The cell counts at time 1 (t1) during the exponential phase

N2 = The cell counts at time 2 (t2) during the exponential phase.

The biomass weight of *Chlorella* sp. is calculated using the formula from Ogbonna & Ogbonna (2018):

$$G = Bx - Bo(g/L)$$

Note:

G = Biomass weight (g/L) Bx = Final weight (g/L) Bo = Initial weight (g/L)

Data analysis

The data in this study are presented in the form of tables and graphs. The effect of chicken manure enriched with *Lemna* liquid organic fertilizer on the cell density of *Chlorella* sp. was tested using ANOVA with SPSS 25 software. If the analysis of the treatments shows a significant or highly significant difference, a Tukey test will be conducted to determine the differences between treatments (Hadi & Rosyadi, 2022).

RESULTS AND DISCUSSION

Result

The density of Chlorella sp. cells

The cell density of *Chlorella* sp. on the peak day after being cultured at the laboratory scale for 14 days can be seen in Table 1, while the growth pattern of the cells is presented in Figure 1. It can be seen at Table 1 that the highest cell density of *Chlorella* sp. during the observation was found in treatment P3, with $516.67 \pm 6.29 \times 10^4$ cells/mL, followed by treatments P4, P2, and P1 with $472.50 \pm 15.61 \times 10^4$ cells/mL, $450.83 \pm 24.02 \times 10^4$ cells/mL, and $390.83 \pm 6.29 \times 10^4$ cells/mL, respectively, with the peak day occurring on

Table 1. Cell density of *Chlorella* sp. on the peak growth day.

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Treatments	Peak day	The average of cell density (×10 ⁴ cells/mL)
P0: 3 g/L of chicken manure without enrichment	7	350.00 ± 15.00^{a}
P1: 3 g/L of chicken manure (control) enriched with 4% <i>Lemna</i> POC	9	$390.83 \pm 6.29^{\text{b}}$
P2: 3 g/L of chicken manure (control) enriched with 5% <i>Lemna</i> POC	9	$450.83 \pm 24.02^{\circ}$
P3: 3 g/L of chicken manure (control) enriched with 6% <i>Lemna</i> POC	9	516.67 ± 6.29^{d}
P4: 3 g/L of chicken manure (control) enriched with 7% <i>Lemna</i> POC	9	472.50 ± 15.61°

Note: Different superscript letters indicate a significant difference (p<0.05).

day 9 for each treatment. However, in treatment P0, the peak occurred earlier (on day 7) with a cell density of $350.00 \pm 15.00 \times 10^4$ cells/mL. Based on statistical analysis, the addition of chicken manure enriched with *Lemna* POC had a significant effect on the cell density of *Chlorella* sp. (p<0.05). This indicates that the addition of chicken manure enriched with *Lemna* POC can be utilized as an organic material source for the growth and cell division of *Chlorella* sp.

The cell growth of *Chlorella* sp. at the beginning of the culture did not show an adaptation phase (lag phase) to the new culture medium. From Figure 1, it can be observed that *Chlorella* sp. was able to utilize the nutrients provided and immediately entered the exponential phase. The stationary phase for each treatment appeared for only one day. Treatment P0 reached the stationary phase on day 8, while treatments P1, P2, P3, and P4 reached it on day 10. During the stationary phase, a balance was achieved between the cell density of *Chlorella* sp. and the nutrients in the culture medium, so when the nutrient levels decreased, *Chlorella* sp. entered the death phase.

Specific growth rate (SGR) is one of the factors that can determine the amount of *Chlorella* sp. production during cultivation. The faster the growth rate, the earlier the peak cell density of *Chlorella* sp. will occur. Figure 2 shows that the application of 3 g/L chicken manure (without enrichment) (treatment P0) experienced a faster specific growth rate compared to the other treatments. This is because chicken manure without enrichment with *Lemna* POC can be easily absorbed and utilized by *Chlorella* sp. for growth and development. The slower specific growth rate in treatments P1, P2, P3, and P4 (chicken manure

enriched with *Lemna* POC) is due to the fact that, when enriched, the culture medium for *Chlorella* sp. appeared darker, making it more difficult for *Chlorella* sp. to perform photosynthesis. Although the addition of chicken manure enriched with *Lemna* POC showed a delayed specific growth rate, it was able to meet the nutrient needs for *Chlorella* sp. and resulted in a higher cell density compared to treatment P0.

The high cell density of *Chlorella* sp. is always related to the amount of biomass produced. The higher the cell density of *Chlorella* sp., the higher the biomass weight, and conversely, if the cell density is low, the biomass weight will also be lower. The highest biomass weight was obtained in treatment P3, with 0.39 g/L, followed by treatments P4, P2, and P1 with 0.38 g/L, 0.36 g/L, and 0.34 g/L, respectively. The lowest biomass weight was obtained in treatment P0, with 0.30 g/L. For more details, please refer to Figure 3.

Utilization of nutrients for growth

Nutrients are essential for the growth and development of *Chlorella* sp.. The availability of macronutrients such as nitrate, phosphate, and ammonia are factors that can influence the cell density of *Chlorella* sp.. In Figure 4a, it can be seen that the nitrate levels in each treatment began to be utilized by *Chlorella* sp. from day 7 to day 14. Meanwhile, the phosphate levels (Figure 4b) in each treatment were utilized by *Chlorella* sp. until the end of the study. Additionally, the ammonia levels (Figure 4c) in the culture medium were also utilized by *Chlorella* sp. until day 7 and increased until day 14. The increase in ammonia levels was caused by *Chlorella* sp. in each treatment entering the death phase.

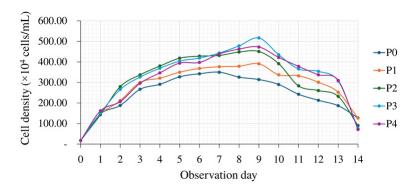


Figure 1. Growth pattern of *Chlorella* sp. cells cultured using chicken manure and enriched with *Lemna* POC (P0 = 3 g/L of chicken manure (without enrichment), P1 = 3 g/L of chicken manure enriched with 4% *Lemna* POC, P2 = 3 g/L of chicken manure enriched with 5% *Lemna* POC, P3 = 3 g/L of chicken manure enriched with 6% *Lemna* POC, and P4 = 3 g/L of chicken manure enriched with 7% *Lemna* POC).

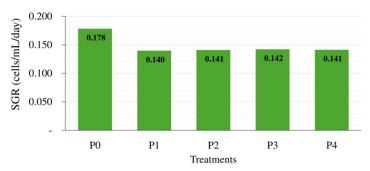


Figure 2. The difference in the specific growth rate of *Chlorella* sp. cultured using chicken manure and enriched with *Lemna* POC (P0 = 3 g/L of chicken manure (without enrichment), P1 = 3 g/L of chicken manure enriched with 4% *Lemna* POC, P2 = 3 g/L of chicken manure enriched with 5% *Lemna* POC, P3 = 3 g/L of chicken manure enriched with 6% *Lemna* POC, and P4 = 3 g/L of chicken manure enriched with 7% *Lemna* POC).

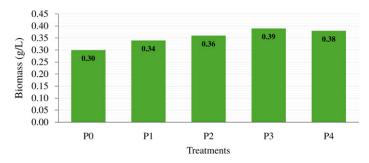


Figure 3. The difference in biomass weight of *Chlorella* sp. cultured using chicken manure and enriched with *Lemna* POC (P0 = 3 g/L of chicken manure (without enrichment), P1 = 3 g/L of chicken manure enriched with 4% *Lemna* POC, P2 = 3 g/L of chicken manure enriched with 5% *Lemna* POC, P3 = 3 g/L of chicken manure enriched with 6% *Lemna* POC, and P4 = 3 g/L of chicken manure enriched with 7% *Lemna* POC).

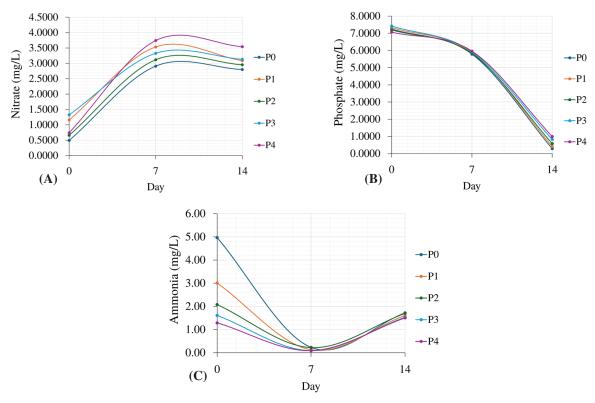


Figure 4. Pattern of utilization (a) nitrate levels (b) phosphate levels (c) ammonia levels in chicken manure enriched with *Lemna* POC (P0 = 3 g/L of chicken manure (without enrichment), P1 = 3 g/L of chicken manure enriched with 4% *Lemna* POC, P2 = 3 g/L of chicken manure enriched with 5% *Lemna* POC, P3 = 3 g/L of chicken manure enriched with 6% *Lemna* POC, and P4 = 3 g/L of chicken manure enriched with 7% *Lemna* POC).

Relationship between cell density and water quality parameters

The cell density of Chlorella sp. is related to water quality parameters such as temperature, dissolved oxygen, and pH. In Figure 5a, the temperature fluctuations during the culture period were not significantly different, ranging from 27–29°C. These temperature fluctuations can affect the specific growth rate, survival, cell composition, and productivity of Chlorella sp. Physicochemically, temperature can influence the mass of CO2 and O2 (Serra-Maia et al., 2016). In addition to temperature, the cell density of Chlorella sp. is also related to the dissolved oxygen content and the pH of the culture medium. As seen in Figures 5b and 5c, on day 7, the dissolved oxygen content and pH of the culture medium increased. The increase in dissolved oxygen content and pH was due to the rise in cell density of Chlorella sp. on day 7. However, the dissolved oxygen content and pH on day 14 decreased in each treatment, which is caused by Chlorella sp. entering the death phase.

Discussion

Cell density of *Chlorella* sp.

The growth of *Chlorella* sp. cells did not experience a lag phase at the beginning of the culture but directly entered the exponential phase. This was because the culture stock was already in the exponential phase, so the inoculated cells could adapt well to the new culture medium. The absence of a lag phase at the beginning of the culture could be due to the efficient absorption of nutrients available in the culture medium. Istirokhatun *et al.* (2017) stated that the lag phase will be shorter or even absent if the inoculated cells come from a culture in the exponential phase.

As seen in Figure 1, the growth pattern of *Chlorella* sp. was almost identical across treatments and measurement days. Treatment P3 exhibited the highest cell density compared to other treatments. This is because *Chlorella* sp. requires nutrients in optimal amounts for its growth, and chicken manure enriched with 6% *Lemna* POC was found to be the optimal enrichment dose for *Chlorella* sp. Ambarwati *et*

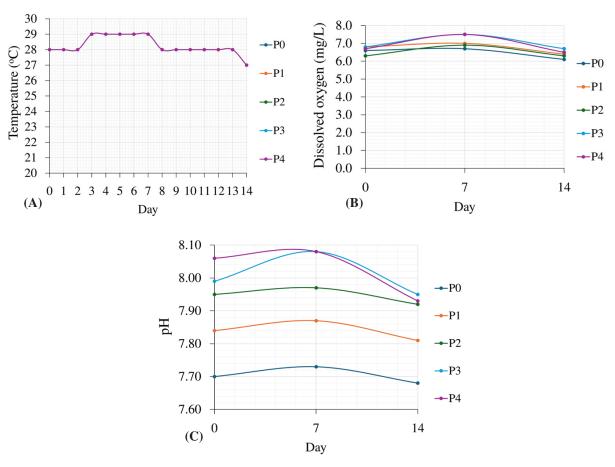


Figure 5. The relationship between cell density and (a) temperature (b) dissolved oxygen (c) pH in the culture medium (P0 = the application of 3 g/L chicken manure (without enrichment), P1 = 3 g/L chicken manure enriched with 4% *Lemna* POC, P2 = 3 g/L chicken manure enriched with 5% *Lemna* POC, P3 = 3 g/L chicken manure enriched with 5% *Lemna* POC, and P4 = 3 g/L chicken manure enriched with 5% *Lemna* POC).

al. (2018) mentioned that when nutrient levels in the culture medium are optimal, *Chlorella* sp. will enter the exponential phase and produce a more intense culture color as cell density increases. The stationary phase in all treatments lasted for only one day.

This phase occurred due to the balance between the nutrient levels in the culture medium and the cell density of Chlorella sp. Thus, if the nutrient availability decreases, Chlorella sp. will enter the death phase (Hadi & Rosyadi, 2022; Mukti et al., 2024). Furthermore, cell death is also caused by changes in water quality, which become unfavorable, environmental conditions that are no longer beneficial, and the culture's extended duration (Meritasari et al., 2012; Rosahdi et al., 2015; Rosyadi et al., 2023). Table 1 shows that 3 g/L of chicken manure enriched with 6% Lemna POC produced the highest cell density of *Chlorella* sp., which was 516.67 ± 6.29 ×10⁴ cells/mL. This result is significantly higher compared to the study by Febtisuharsi (2016), which used a dose of 20 g/L of chicken manure and achieved a cell density of 78.83 ×104 cells/

The comparison between these two studies indicates that enriching chicken manure with Lemna POC can supplement the deficiencies in the culture medium's nutrients, leading to higher Chlorella sp. cell density. The high cell density of Chlorella sp. in this study is related to the specific growth rate (SGR) and biomass weight produced each day. As the specific growth rate increases, the cell density of Chlorella sp. will also increase, causing the nutrients in the culture medium to be depleted more quickly, and the peak cell density will be reached sooner. Hadi & Rosyadi (2022) reported that the SGR of Chlorella sp. could be influenced by the concentration of fertilizer in the culture medium. Higher fertilizer concentrations make the culture medium darker, which disrupts the photosynthesis process, thus decreasing the daily growth rate. Nurfadillah et al. (2012) further noted that the darkened color of the culture medium can block light penetration, interfering with phytoplankton's ability to photosynthesize.

Utilization of nutrients for cell growth

Nutrients are essential parameters that support cell growth and biomass production in *Chlorella* sp.. The nitrogen content in nitrate significantly affects cell growth and biomass productivity in *Chlorella* sp. because it is needed for protein and chlorophyll formation (Juneja *et al.*, 2013; Nyabuto

et al., 2015; Ulya, 2018). As seen in Figure 4a, the nitrate concentration in each treatment increased until day 7, after which it began to be utilized. This increase is due to the slow decomposition process of the fermented fertilizer, which results in a slow formation of nitrate (Rosyadi et al., 2022). After that, the utilization of nitrate in the culture medium by *Chlorella* sp. is observed as the nitrate concentration decreases until the end of the study (Hadi & Rosyadi, 2022).

In addition to nitrate, the phosphate concentration in the culture medium is crucial for energy transfer from outside the cell to within the organism. Phosphate is one of the main elements required for the growth of microalgae and increasing microalgal biomass (Natalia *et al.*, 2019; Mutia *et al.*, 2021). Moreover, *Chlorella* sp. uses phosphate for cell division, metabolism, and chlorophyll formation (Hadi & Rosyadi, 2022). Figure 4b shows that the phosphate concentration was utilized by *Chlorella* sp. from the beginning to the end of the study. According to Nurdiana *et al.* (2021), phosphate utilization can be seen from the decrease in phosphate concentration in the culture medium.

Another nutrient element that plays a role in *Chlorella* sp. cell growth is ammonia concentration. Afifah *et al.* (2021) explain that ammonia is a macro-nutrient, a nutrient required in large amounts to support microalgal growth. According to Omairah *et al.* (2019), ammonia contains a high nitrogen content, making it a valuable nutrient source for phytoplankton.

Relationship between cell density and water quality parameters

The growth of *Chlorella* sp. can remain stable if the physical and chemical parameters of the culture medium are optimal. Temperature is one of the physical parameters that can affect cell growth (Kumar *et al.*, 2014), photosynthesis, metabolism (Ernawati *et al.*, 2023), and directly influence the production of *Chlorella* sp. (Jie *et al.*, 2021). The temperature range during the study was optimal for *Chlorella* sp., as stated by Boroh *et al.* (2019), where temperatures between 25 to 32°C are considered ideal for *Chlorella* sp. culture.

Another factor related to *Chlorella* sp. cell growth is the dissolved oxygen (DO) concentration. Based on Figure 5b, the DO range during the study was relatively consistent, between 6.1–7.5 mg/L. The DO values for each treatment showed an increase on day 7, which was

due to the increased cell density of *Chlorella* sp., leading to an enhancement in photosynthesis by the cell's metabolism. This increase in DO was more influenced by a substantial supply from photosynthesis and aeration.

However, on day 14, the DO values in the culture medium decreased due to incomplete photosynthesis caused by the condition of the culture medium. *Chlorella* sp. is active in photosynthesis, converting CO2 into oxygen, thus making oxygen productivity higher. The dynamics of dissolved oxygen in aquatic ecosystems depend on the balance between oxygen production and consumption. As stated by Puspitaningrum *et al.* (2012) and Panggabean & Prastowo (2017), oxygen is produced through photosynthesis by autotrophic communities, while oxygen is consumed by all organisms through respiration and organic matter decomposition.

The pH level is a parameter that indicates the acidity of a solution (Azmi *et al.*, 2016). The pH in the culture medium can affect the metabolism and growth of *Chlorella* sp., such as altering the balance of inorganic carbon, nutrient availability, and affecting cell physiology. During the study, the pH ranged from 7.68 to 8.08. This range still supports the growth of *Chlorella* sp., as Maharsyah *et al.* (2013) stated that the optimal pH for *Chlorella* sp. growth is between 4.5 and 9.3. According to Wulandari *et al.* (2019), changes in pH in the culture medium generally affect the biomass, lipids, and primary metabolite content of *Chlorella* sp.

CONCLUSION

According to these findings of study, it can be concluded that the optimal dose of *Lemna* POC for enriching chicken manure to increase the cell density of *Chlorella* sp. is 6%, where the cell density, specific growth rate, and biomass weight obtained were 516.67 ×10⁴ cells/mL, 0.142 cells/mL/day, and 0.39 g/L, respectively.

ACKNOWLEDGMENT

The authors would like to express their gratitude to the Directorate of Research and Community Service (DPPM) of Universitas Islam Riau for providing research funding with contract number: 641/KONTRAK/P-PT/DPPM-UIR/06-2023. Special thanks are also extended to Mauli Warman, a student of the Aquaculture Program, Faculty of Agriculture, Universitas

Islam Riau, for assisting in the successful completion of this research.

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