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Effect of Different EM4 Dosage in Walne on the Laboratory-scaled *Chlorella* sp. Culture

Pengaruh Dosis EM4 pada Media Walne terhadap Pertumbuhan dan Kelimpahan *Chlorella* sp. Skala Laboratorium

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Abstract

This study aimed to analyze the effects and determine the optimal dosage of EM4 and Walne fertilizer on the abundance, growth rate, and biomass of *Chlorella* sp. cultured under laboratory-scale conditions. A completely randomized design was used, with EM4 applied at three dosages (P2 = 1 mL/L, P3 = 2 mL/L, and P4 = 3 mL/L), each supplemented with 1 mL/L of Walne fertilizer. The control treatment (P1) received only 1 mL/L of Walne fertilizer. All treatments were performed in triplicates. The parameters included abundance (cells/mL), growth rate (cells/mL/day), and biomass (g). All data were analyzed using one-way ANOVA, followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level ($p < 0.05$). The CRD consisted of three replicates per treatment. The results showed no significant differences among treatments ($p < 0.05$), with abundance and daily growth rate observed in P1 and P3 ($p < 0.05$). In conclusion, although EM4 and Walne did not produce significant effects, the exploration of *Chlorella* sp. applications beyond aquaculture should continue to be developed.

Keywords: Chlorella sp., *abundance, EM4, phytoplankton.*

Abstrak

Penelitian ini bertujuan menganalisis pengaruh dan penentuan dosis optimal pemberian EM4 dan Walne terhadap kelimpahan, laju pertumbuhan, dan biomassa *Chlorella* sp. yang dipelihara dalam skala laboratorium. Penelitian ini menggunakan metode rancangan acak lengkap dengan perlakuan EM4 diberikan dalam tiga dosis (P2 = 1 ml/L, P3 = 2 ml/L, dan P4 = 3 ml/L) dan tambahan 1 ml/L pupuk Walne pada setiap perlakuan, sedangkan perlakuan kontrol (P1) hanya diberi 1 ml/L pupuk Walne. Setiap perlakuan diulang sebanyak tiga kali. Parameter yang diamati meliputi kelimpahan (sel/ml), laju pertumbuhan (sel/ml/hari), dan biomassa (g). Seluruh data parameter dianalisis menggunakan analisis sidik ragam satu arah (*one-way ANOVA*) dan dilanjutkan dengan uji jarak berganda Duncan (DMRT) dengan tingkat kepercayaan 95% ($p<0{,}05$). Hasil menunjukkan tidak adanya perbedaan nyata antar perlakuan ($P<0{,}05$), dengan kelimpahan dan laju pertumbuhan harian ditunjukkan pada P1 dan P3 ($p<0{,}05$). Kesimpulannya, meskipun EM4 dan Walne tidak memberikan dampak signifikan, eksplorasi pemanfaatan *Chlorella* sp. di luar sektor akuakultur perlu terus dikembangkan.

Kata Kunci : *Chlorella* sp., kelimpahan, EM4, fitoplankton.

1. Introduction

One of the phytoplankton species frequently cultivated as a natural feed source for fish juvenile and larvae is *Chlorella* sp. (Prayogo and Arifin 2015). In addition to growing rapidly, being suitable for the mouth openings of fish and shrimp larvae, and serving as a regulator of the larval rearing environment's temperature (Ali, 2013), *Chlorella* sp. has a nutrient composition consisting of 51–58% protein, 28–32% oil, 12–17% carbohydrates, 14–22% fat, and 4–5% nucleic acids (Mufidah et al., 2017).

The proliferation process of *Chlorella* sp. involves the use of Walne fertilizer. Walne fertilizer contains nitrogen (N) and phosphorus (P) at concentrations of 0.016 g/L and 0.004 g/L, respectively (Zahro, 2014). These two elements function to accelerate the growth phase of phytoplankton, including *Chlorella* sp. (Meisyara, 2019). However, the use of Walne fertilizer on a large scale (semi-mass and mass) leads to increased costs for *Chlorella* sp. culture. This situation necessitates an alternative through the combination of materials to reduce the use of Walne fertilizer. One potential material that can be combined with Walne fertilizer is EM4 (*Effective Microorganisms-4*).

Based on its nutrient composition, EM4 contains 1.88% organic carbon and 8403.70 ppm potassium salt, which is sufficient to aid the photosynthesis process (Nurlina, 2018). EM4 also consists of fermentative microorganisms, such as lactic acid bacteria (*Lactobacillus* sp.), yeast (*Saccharomyces* sp.), and photosynthetic bacteria (*Rhodopseudomonas* sp. and Actinomycetes) as producers of phytohormones and for maintaining water quality, through the oxidation of organic

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compounds, and the reduction of toxic metabolites (Lestari et al., 2020). The various positive effects of using EM4 are expected to partially replace the role of Walne fertilizer.

This study aims to optimize the abundance, growth rate, and biomass *of Chlorella* sp. as the primary natural feed in larval rearing activities, by varying the doses of EM4 and Walne on a laboratory scale. This study is expected to contribute to the development of environmentally friendly and sustainable aquaculture.

2. Materials and Methods

2.1. Time and Location

This study was conducted in December–January 2024. *Chlorella* sp. samples were commercially obtained from the Cangkringan Center for Aquaculture Technology Development (BPTPB). EM4 was commercially obtained from PT. Songgolangit Persada, Jakarta. The cultivation of *Chlorella* sp. with the addition of EM4 and Walne fertilizer was carried out at the Integrated Laboratory, Tidar University, Magelang, Central Java.

2.2. Research Method

This study employed an experimental design using a completely randomized design (CRD). The treatments consisted of varying doses of EM4 mixed with Walne fertilizer at 1 ml/L of medium. The EM4 doses were as follows: P1 = 0 ml/L EM4, P2 = 1 ml/L, P3 = 2 ml/L, and P4 = 3 ml/L. Each treatment was replicated three times, resulting in 12 experimental units.

2.3 Sterilization of Equipment and Materials

Culture equipment, such as Erlenmeyer flasks (500 ml) and pipettes, was sterilized using an autoclave at 121°C for 15 minutes. Other equipment, such as tubing, was sterilized by soaking in a 1 ml/L chlorine solution for 24 hours. Tap water was filtered and transferred to Erlenmeyer flasks, then covered with aluminum foil. The tap water was subsequently sterilized using an autoclave at 121°C for 15 minutes. The sterilized water was then cooled, treated with a 1 ml/L chlorine solution, and left to stand for 24 hours. The water was then treated with sodium thiosulfate ($Na_2S_2O_3$) at a dose of 1 ml/L and left to stand for 15 minutes (Buwono and Nurhasanah, 2018).

2.4. Chlorella sp. *culture*

Chlorella sp. cultures were grown in Erlenmeyer flasks. Sterilized water mixed with a sodium thiosulfate solution was supplemented with Walne's medium and EM4. The *Chlorella* sp. starter culture was added after the addition of the medium and its treatment. *Chlorella* sp. cultivation lasted approximately one week, or 7 days. Observations of *Chlorella* sp. abundance were conducted daily with an initial density of 100,000 cells/ml, in accordance with Regista et al. (2017). The total culture volume was maintained at 250–300 ml. Illumination was provided using a fluorescent lamp (*Philips TL-D 36W/865*, *Bekasi*) with 24 hours of light (Wardani et al., 2019). During the culture process, culture quality was maintained at a water temperature of 21–23°C, a pH of 7–7.5, and dissolved oxygen (DO) levels of 4.5–5.3 mg/L, in accordance with Mufidah et al. (2017).

2.5. Observation of Abundance and Growth Rate

Observations were conducted daily for 7 days or at least once a day over a 7-day period starting from the first day. Observations were performed using a microscope at magnifications of 10x to 40x, employing a hemocytometer that had been sterilized with 70% alcohol and cleaned with tissue. Then, 1 ml of *Chlorella* sp. phytoplankton was added to the hemocytometer until it was full, and it was covered with *a cover slip* (Isnansetyo and Kurniastuty, 1995).

Counting on the hemocytometer was performed using four squares, each containing sixteen small squares. The sixteen small squares measured 0.05 x 0.05 $^{mm^2}$within four larger squares measuring 1 x 1 $^{mm^2}$(Regista et al., 2017). Growth rate calculations can be performed using data obtained from abundance calculations via the formula (Fadhillah, 2019). The growth rate can be calculated using the formula. According to Hirata et al., 1981, the specific growth rate can be calculated using the following formula:

$$k=3{,}22\,\frac{log\left(\frac{N_t}{N_0}\right)}{T_t\text{-}T_0}$$

Notes:

N_t : Microalgal density at time t

N_0 : Initial microalgal density

3.22 : Constant

T : Observation time

2.6 Biomass

The first step in biomass calculation is to centrifuge the sample. The centrifugation process aims to separate *Chlorella* sp. cells from the water. After centrifugation is complete, the resulting sediment is broken up using a spatula and transferred to an aluminum foil container, then weighed to determine the wet weight using an analytical balance. Next, the samples were dried in an oven at 150°C for 10–20 minutes to obtain the dry weight (Dogaran et al., 2024). After the samples were dry, they were weighed again to obtain the dry weight data for each treatment. The final biomass data are presented in an ANOVA. The initial and final biomass data can be used to determine the weight (Wg).

2.6 Data Analysis

The data obtained in this study were derived from measured parameters, such as abundance (cells/ml), growth rate (cells/ml/day), and biomass (g). All data obtained were analyzed using *one-way* ANOVA with a 95% confidence level. If significant differences were found ($p<0.05$), the data were further analyzed using Duncan's Multiple Range Test (DMRT) to determine the best treatment from the combination of Walne fertilizer and EM4. All data analysis was performed using *SPSS 16.0* software *(IBM Corp., USA*).

3. Results and Discussion

3.1. Chlorella sp. Cell Abundance

The abundance data are presented in growth curves that were analyzed descriptively to characterize the growth phases of *Chlorella* sp., which consist of the adaptation (*lag*) phase, the logarithmic (*log*) phase, the stationary phase, and the death phase (Putra et al., 2015). The growth curves are shown in Figure 1.

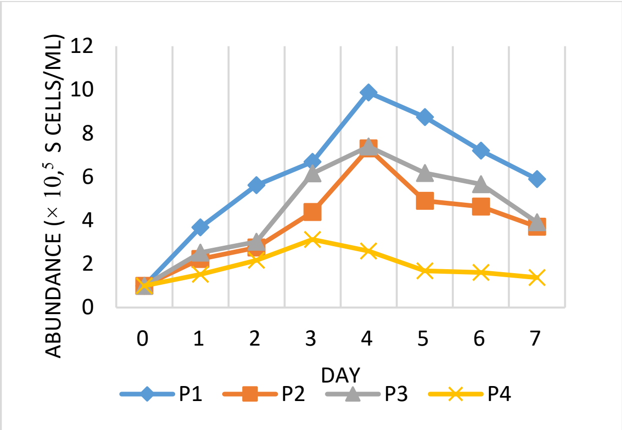

Figure 1. Growth curve of *Chlorella* with different doses of EM4 and 1 ml/L Walne fertilizer. Legend: P1 = Control (0 ml/L EM4 + 1 ml/L Walne), P2 = 1 ml/L EM4 + 1 ml/L Walne, P3 = 2 ml/L EM4 + 1 ml/L Walne, P4 = 3 ml/L EM4 + 1 ml/L Walne.

Based on the *Chlorella* sp. growth graph, a significant increase in the *log* phase was observed in all treatments, specifically on days 1 through 2. However, the first stationary phase was reached in treatment P4 on day 3, while the other treatments reached the stationary phase on day 4. A decline in abundance (death phase) also occurred rapidly in P4 (day 4), while the other treatments showed the death phase on day 5. The acceleration of reaching the stationary phase in P4 is presumed to be due to the antagonistic properties of EM4, which also contains photosynthetic microorganisms, so that the nutrient sources from the Walne fertilizer and EM4 itself were utilized by the EM4 microorganisms and *Chlorella* sp.

The total abundance of *Chlorella* sp. phytoplankton is shown in Table 1. All treatments showed significant differences ($p<0.05$). The highest total abundance was observed in the 0 ml/L EM4 + 1 ml/L Walne fertilizer treatment (P1) at 0.4771 $\times 10^7 \pm$ 0.0636 cells/ml, which was equivalent to P3 (0.3485 $\times 10^7 \pm$ 0.0217 cells/ml; $p > 0.05$).

Table 1.
Total abundance of *Chlorella* sp. under different treatment doses of EM4 and 1 ml/L Walne fertilizer

Treatment	Total Abundance ($\times 10^7$cells/mL)	Notation
P1	0.4771±0.0636	A
P2	0.3485±0.0217	B
P3	0.2991±0.0566	A
P4	0.1409±0.0412	C

Note: Data are presented as mean ± SD. Different symbols indicate significant differences ($p < 0.05$). P1 = Control (0 ml/L EM4 + 1 ml/L Walne), P2 = 1 ml/L EM4 + 1 ml/L Walne, P3 = 2 ml/L EM4 + 1 ml/L Walne, P4 = 3 ml/L EM4 + 1 ml/L Walne.

Based on the analysis of variance, it can be seen that the abundance of *Chlorella* sp. phytoplankton showed significant differences across the EM4 treatment groups (P1, P2, P3, and P4; $p < 0.05$). This study showed a significant effect with the highest EM4 application occurring in P4, followed by P2 and P3. This indicates that EM4 application tends to reduce the total abundance *of Chlorella* sp., as EM4, which also contains photosynthetic microorganisms, generally utilizes the organic matter available in the culture medium, thereby potentially competing with *Chlorella* sp. In accordance with the statement by Andreas and Chilmawati (2024), microorganisms can trigger competition with phytoplankton cell proliferation, thereby disrupting the cell division process and the abundance of *Chlorella* sp. cells.

3.2. *Growth Rate of Chlorella sp.*

The calculation of the growth rate of *Chlorella* sp. was performed to determine the rate of cell increase per unit of time. The growth rate of the phytoplankton *Chlorella* sp. was determined using data from plankton abundance calculations and is shown in Figure 2.

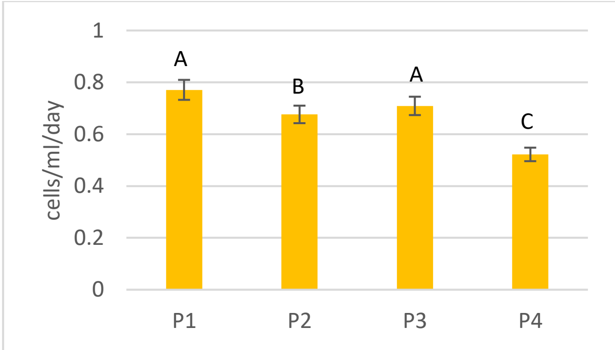

Figure 2. Growth rate of *Chlorella* sp. with different doses of EM4 and 1 ml/L Walne fertilizer. All data are presented as mean ± SD. Different letters above the bars indicate significant differences (DMRT; $p < 0.05$). Legend: P1 = Control (0 ml/L EM4 + 1 ml/L Walne), P2 = 1 ml/L EM4 + 1 ml/L Walne, P3 = 2 ml/L EM4 + 1 ml/L Walne, P4 = 3 ml/L EM4 + 1 ml/L Walne.

The highest growth rates were observed in treatments P1 and P3, at 0.77 ± 0.03 cells/ml/day and 0.71 ± 0.01 cells/ml/day, respectively. Treatment P4 showed the lowest growth rate in the *Chlorella* sp. culture at 0.52 ± 0.06 cells/ml/day. This condition indicates a direct correlation with the total abundance *of Chlorella* sp.
The highest value in treatment P1 indicates that Walne fertilizer contains a complete nutrient profile that can be directly utilized by phytoplankton (Triastuti et al., 2011). However, treatment P3 yielded values equivalent to those of treatment P1, suggesting that the addition of 2 ml/L of EM4 likely provides an optimal supply of phytohormones for the growth of *Chlorella* sp. phytoplankton. However, the highest dose of EM4 (P4) showed a decrease in growth rate, due to the high population of other microorganisms that also utilized the organic matter available in the culture environment, namely from Walne fertilizer and EM4 itself (Nurlina, 2018).

3.3 *Chlorella sp. Biomass*

The biomass of *Chlorella* sp. was obtained from the culture harvest conducted over seven days. The biomass was used to determine the final weight of the culture. The amount of biomass in the *Chlorella* sp. phytoplankton culture is shown in Figure 3.

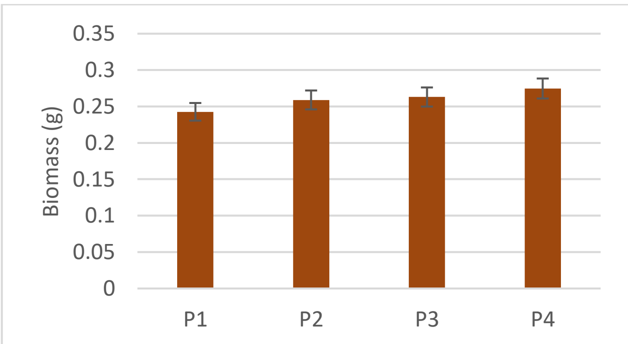

Figure 3. *Chlorella* sp. biomass with different doses of EM4 and 1 ml/L Walne fertilizer. Legend: P1 = Control (0 ml/L EM4 + 1 ml/L Walne), P2 = 1 ml/L EM4 + 1 ml/L Walne, P3 = 2 ml/L EM4 + 1 ml/L Walne, P4 = 3 ml/L EM4 + 1 ml/L Walne.

After seven days of cultivation, biomass samples were collected to determine the weight gain of the *Chlorella* sp. culture during the cultivation period. As shown in Table 1, the lowest value was observed in treatment P1, with a biomass of 0.243 ± 0.010 grams. Next, in treatment P2, the biomass was 0.259 ± 0.011 grams. In treatment P3, the biomass was 0.263 ± 0.008 grams. Treatment four, or P4, had the highest biomass value at 0.275 ± 0.017 grams.

The results of the *one-way* ANOVA regarding final biomass showed no significant difference (p>0.05). Although no significant difference was observed, the biomass *of Chlorella* sp., after being cultured with a combination of EM4 and Walne fertilizer, showed an increasing trend up to treatment P4. This is likely due to the high carbon content, which leads to the accumulation of food reserves within the cells, resulting in a significant mass (Emilia, 2023). It can be inferred that the cells likely undergo enlargement due to the accumulation of food reserves within the vacuoles; however, in high concentrations, nutrient competition with the bacteria present in EM4 occurs, thereby slightly inhibiting cell division. According to Mishbach et al. (2022), microalgal biomass is advantageous for use as a feedstock for biofuels, alternative foods, and pharmaceuticals. Several studies have been conducted to enhance the synthesis of key biomolecules that yield high-quality biomass, such as carbohydrates for bioethanol.

4. Conclusion

1. The addition of EM4 to Walne medium had a significant effect on reducing the abundance and growth rate *of Chlorella* sp., but did not have a significant effect on the final culture biomass. An EM4 dose of 2 ml/L added to 1 ml/L of Walne medium was able to significantly support the proliferation of *Chlorella* sp. cells and was equivalent to the application of 1 ml/L of Walne medium without EM4 (0 ml/L).
2. The highest biomass was obtained in the 3 ml EM4 treatment, but the difference was not statistically significant compared to the other treatments.

References

Andreas, S. Q., dan Chilmawati, D. (2024). Studi pola pertumbuhan dan kualitas sel *Chlorella* sp. yang dihasilkan melalui teknologi pencucian bibit sel. *Journal of Aquaculture Management and Technology, 3*(4): 273-280.

Buwono, N. R., dan Nurhasanah, R. Q. (2018). Studi pertumbuhan populasi *Spirulina* sp. pada skala kultur yang berbeda. *Jurnal Ilmiah Perikanan dan Kelautan*, *10*(1): 26-33.

Isnansetyo, A., dan Kurniastuty, E. (1995). Teknik kultur *phytoplankton* dan *zooplankton*. pakan alami untuk pembenihan organisme laut. *Kanisius.* Yogyakarta.

Lestari, D., dan Suyasa, I. N. G. (2020). Perbedaan kualitas kompos sampah organik menggunakan *Effective Microorganism* 4 (EM4) dan larva *Black soldier fly* di Desa Buduk. *Jurnal Kesehatan Lingkungan (JKL)*, *10*(2): 132-140.

Meisyara, M. A. R. (2019). Potensi mikroalga *Tetraselmis chuii butcher* dalam bioremediasi logam berat timbal (Pb) dari limbah batik. *Skripsi.* Universitas Atma Jaya Yogyakarta. Yogyakarta.

Mishbach, I., Permatasari, N. S., Zainuri, M., Kusumaningrum, H. P., dan Hastuti, E. D. (2022). Potensi mikroalga *Anabaena* sp. sebagai bahan utama bioetanol. *Jurnal Penelitian Biologi, Botani, Zoologi dan Mikrobiologi*. 7(1): 69-76.

Mufidah, A., Agustono, S., dan Nindarwi, D. D. (2017). Teknik kultur *Chlorella* sp. skala laboratorium dan intermediet di Balai Perikanan Budidaya Air Payau (BPBAP) Situbondo Jawa timur. *Journal of Aquaculture and Fish Health*, *7*(2): 50-56.

Nurlina. (2018). Optimasi Penggunaan Probiotik Melalui Perbaikan Kualitas Air Dengan Dosis yang Berbeda terhadap Tingkat Kepadatan *Phytoplankton*. *Skripsi*. Universitas Muhamadiyah Makassar. Makassar.

Prayogo, I., dan Arifin, M. (2015). Teknik kultur pakan alami *Chlorella* sp. dan *Rotifera* sp. skala massal dan manajemen pemberian pakan alami pada larva kerapu cantang. *Jurnal Ilmu Perikanan*, *6*(2): 125-134.

Regista, R., Ambeng, A., Litaay, M., dan Umar, M. R. (2017). Pengaruh pemberian vermikompos cair *Lumbricus rubellus hoffmeister* pada pertumbuhan *Chlorella* sp. *Jurnal Biologi Makassar*, 2(1): 1-8.

Triastuti, R. J., Mubarak, A. S., dan Prabandari, L. (2011). Pengaruh penambahan pupuk bintil akar kacang tanah sebagai sumber nitrogen dan fosfor terhadap populasi Chlorella sp. Jurnal Ilmiah Perikanan dan Kelautan. 3(2).

Wardani, N. K., Supriyantini, E., dan Santosa, G. W. (2022). Pengaruh konsentrasi pupuk walne terhadap laju pertumbuhan dan kandungan Klorofil-A *Tetraselmis Chuii*. *Journal Of Marine Research*, *11*(1): 77-85.

Zahro, A. K. (2014). Uji toksisitas Minyak dan Asam Lemak Mikroalga *Chlorella* sp. terhadap Larva Udang *Artemia salina*. *Skripsi.* Malang.