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Utilization of Cow's Rumen Bokashi Enriched with Chicken Manure on *Chlorella* sp. Cell Density

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Abstract

One of the organic fertilizers that has the potential to cultivate *Chlorella* sp. is the cow's rumen. To increase the nutrient content in the cow's rumen, the cow's rumen must be made into bokashi and enriched with chicken manure. This study aimed to determine the use of cow's rumen bokashi enriched with chicken manure at different doses on *Chlorella* sp cell density. The research design used was a Completely Randomized Design (CRD) with 5 treatments and 3 replications, namely P0 (2 g/L cow's rumen bokashi), P1 = 2 g/L cow's rumen bokashi enriched with 2.5 g/L chicken manure, P2 = 2 g/L cow's rumen bokashi enriched with 3.0 g/L chicken manure, P3 = 2 g/L cow's rumen bokashi enriched with 3.5 g/L chicken manure, and P4 = 2 g/L cow's rumen bokashi enriched with 4.0 g/L chicken manure. The results showed that the highest cell density of *Chlorella* sp. occurred in treatment P3 (i.e. 456.1 ± 15.1 × 10^4 cells/mL) and the highest specific growth rate occurred in treatment P0 (i.e. 0.20 ± 0.0/day). Nutrient levels in the culture medium are optimal for *Chlorella* sp., with nitrate ranging from 0.1375 to 0.2833 mg/L and phosphate ranging from 2.4889 to 2.8650 mg/L. From the results of this study, it can be concluded that the use of cow's rumen bokashi enriched with chicken manure had a very significant effect on cell density and specific growth rate of *Chlorella* sp. ($P<0.01$).

INTRODUCTION

Seeding is the first step in the development of aquaculture because this business is related to the availability of production factors. Natural feed is good feed for fish rearing activities because this type of feed has a higher nutritional content compared to artificial feed (Mufidah *et al*., 2017; Rihi, 2019). One natural food that can provide fish growth is *Chlorella* sp. (Supryady *et al*., 2022).

Enyidi (2017) found that *C. vulgaris* is a viable alternative to replace fish meal in catfish (*Clarias gariepinus*) feed rations. Huang *et al*. (2023) found that feeding tilapia with *Chlorella* sp. can improve the immune response and composition of the gut microbiota. Yulita (2014; 2015) also found

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that *C. vulgaris* which was cultured using liquid waste from the rubber industry had the nutrients needed by fish larvae for growth, namely unsaturated fat 0.44 mg/kg, protein 2.3%, fatty oil 141 mg/L, chlorophyll a 2.7094 mg/L, chlorophyll b 0.8424 mg/L, vitamin A 825 IU/100 g, vitamin B1 10.1 mg/kg, vitamin E 13.1 mg/100 g, beta-carotene 48.8 mg/kg, and calcium 187 mg/100 g. Currently, cultivating *Chlorella* sp. can be gained by culturing them using organic fertilizer. One potential organic fertilizer is cow's rumen bokashi. This waste has the potential to be used as fertilizer because it contains the nutrients needed by *Chlorella* sp.

Rahman (2022) has researched the effect of giving cow's rumen bokashi at different doses. The results of his research showed that the highest cell density of *Chlorella* sp. occurred at a dose of 2.0 g/L, namely 336.1 × 10^4 cells/mL. These results are still relatively low when compared with the research results of Roza *et al*. (2022) who used liquid organic fertilizer (LOF) vegetable waste including spinach (*Amaranthus* spp), cabbage (*Brassica oleracea var. capitata*), katuk (*Sauropus androgynus*), and mustard greens (*Brassica juncea* L) which obtained a density of 1,118.3 ×10^4 cells/mL.

To increase the production of *Chlorella* sp. cultured with cow's rumen bokashi, the nutrients contained in the bokashi must be increased because the nutritional content is still low, so the cow's rumen bokashi needs to be enriched with other organic materials. One of them is chicken manure.

According to Utomo *et al*. (2005), chicken manure can be used to replace chemicals that can meet the availability of macronutrients. Febtisuharsi (2016) research using livestock manure (cow, goat, and chicken manure) resulted in the highest cell density of *Chlorella* sp. by using 20 g/L chicken manure, namely 78.83 × 10^4 cells/mL. Therefore, this research was conducted to determine the use of cow's rumen bokashi enriched with chicken manure on the cell density of *Chlorella* sp.

METHODOLOGY

Ethical Approval

In this study, it is noteworthy that no animals were employed. Ethical approval for this research aligns with established guidelines for humane and responsible scientific inquiry, ensuring the welfare and ethical treatment of living organisms are upheld under applicable standards.

Place and Time

This research was carried out in June-July 2022 at the Microalgae and Fish Nutrition Laboratory, Faculty of Agriculture, Universitas Islam Riau, Pekanbaru. Analysis of nitrate and phosphate nutrients was carried out at the Marine Chemistry Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

Research Materials

The materials used in this research were microalgae *Chlorella* sp. with a density of 30.5 ×10^4 cells/mL, cow rumen, chicken manure, and fresh water. Cow's rumen was obtained from the Slaughterhouse on Cipta Karya Street which is managed by the Pekanbaru City Agriculture and Livestock Service. Chicken droppings taken from the Rio Aje Rooster chicken farm located on Melati III Street, East Sidomulyo, Marpoyan Damai District, Pekanbaru City. The fermentation material used is EM_4 for plants, which is obtained commercially. This material contains fermentor microorganisms such as *Lactobacillus* sp. and *Saccharomyces* sp.

The equipment used was a 20 L capacity gallon, fluorescent lamp (Philips TLD 36 W, China), aerator (Atman HP 8,000, China), aeration stone, aeration hose, microscope (Olympus CX23 LEDRFS1, China), haemacytometer (Neubauer improved, Germany), spectrophotometer (Spectronic 20D+, USA), DO meter (Lutron PDO-519, Taiwan), ammonia MR (HI715, Romania), pH tester (H198108, Romania), 1000 mL measuring cup, micropipette, plankton net, analytical balance, and thermometer.

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Research Design

This research used a Completely Randomized Design (CRD) with 5 treatments and 3 replications. Determination of the dose of cow's rumen bokashi used referred to research conducted by Rahman (2022) which found that the best dose was 2 g/L.

P0: control (2 g/L cow's rumen bokashi); P1: 2 g/L bokashi cow rumen enriched with 2.5 g/L chicken manure; P2: 2 g/L bokashi cow rumen enriched with 3.0 g/L chicken manure; P3: 2 g/L bokashi cow rumen enriched with 3.5 g/L chicken manure; and P4: 2 g/L bokashi cow rumen enriched with 4.0 g/L chicken manure.

Work Procedure

The Making of Cow's Rumen Bokashi

Cow's rumen obtained from the Slaughterhouse was filtered using a cloth filter before use. This filtering was done to separate the liquid from the dregs. Next, the dregs were dried in the sun to reduce the water content contained in the rumen. After that, the dry rumen was mixed evenly with the EM_4 solution until the rumen was moist. The EM_4 solution consisted of 10 L of water plus 150 g of brown sugar and 350 mL of EM_4. The rumen bokashi was fermented for 7 days until the smell and color of the cow's rumen bokashi changed. The temperature of the cow's rumen bokashi was checked every day so that it was always in the temperature range of 40-50 °C, if the temperature exceeded 50 °C the rumen was inverted so that the temperature decreased. When the cow's rumen bokashi was ready to be used, 500 mL of water from the culture medium was mixed with the rumen bokashi and then allowed to sit for a while until the color of the water turned brown. After that, the water was filtered with sterile and clean cloth material (Rahman, 2022).

Chicken Manure Preparation

Preparation of chicken manure was done by drying the chicken manure in the sun for 3 days. After the chicken manure was dry, grinding was conducted so that the chicken manure formed fine granules. Next, chicken manure was weighed according to the dose for each treatment and dissolved in 500 mL of water from the culture medium. Next, the solution was waited for up to one week. After that, the solution was filtered using a clean cloth. In the next stage, the solution was filtered again using a plankton net (Utomo *et al*., 2005).

Preparation of Containers and Culture of *Chlorella* sp.

The culture was carried out by preparing a container in the form of a 20 L capacity gallon filled with fresh water and equipped with an aeration installation. Lighting used neon lights. Next, cow's rumen bokashi water and chicken manure were added to the container containing fresh water according to the specified dosage. Next, the test container was added with an initial density of 30.5×10^4 cells/mL of *Chlorella* sp. Calculation of *Chlorella* sp. cell density was carried out every 2 days with 3 repetitions for each sample. Nitrate and phosphate contents were analyzed 3 times during the study. Water quality parameters such as temperature, acidity, and dissolved oxygen were measured every two days. *Chlorella* sp culture was carried out for 16 days.

The total cell density of *Chlorella* sp. was measured using a Neubauer-type haemacytometer and then calculated using the formula according to Mukhlis *et al*. (2017):

$N = n \times 10^4$ (cells/mL)

Where:

N = total cells calculated (cells/mL)
n = total number of cells/mL in each sample

Specific growth rate (μ) was calculated using the formula according to Wood *et al*. (2005):

$\mu = Ln\ (N2/N1)/t2\text{-}t1$

μ = specific growth rate (/day)
N2 = population density at time t
N1 = cell population density at time 0,
t1 = initial time
t2 = observation time (days)

Analysis of Nitrate and Phosphate Levels

Nitrate content analysis was carried

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out by taking 10 mL of a filtered sample and then adding 4 drops of 0.01 m EDTA solution to the sample. The EDTA solution was flown through the Cd-Cu reduction pool. Next, 10 drops of sulfanilamide solution were added to the sample and left for 1-2 minutes. Next, 10 drops of N-Naptyl solution were added then shaken and left for 5-8 minutes. The absorbance of each sample was then measured with a spectrophotometer at a wavelength of 543 nm (Hadi, 2022).

Phosphate checking was conducted by taking 12.5 mL of filtered samples. A total of 10 drops of ammonium molybdate solution were added to the sample. Next, 5 drops of $SnCl_2$ $2H_2O$ solution were added, shaken until homogeneous, and waited for approximately 2 minutes. The absorbance was then measured with a spectrophotometer at a wavelength of 690 nm (Hadi, 2022).

Data Analysis

Research data was analyzed statistically using ANOVA (Analysis of Variance) with SPSS 25 software to determine the effect of the treatment given. If the results of the treatment analysis showed the effects were significantly different or very significantly different, then the Tukey test was carried out to determine the differences between treatments (Hadi and Rosyadi, 2022). The results of the ANOVA analysis were presented narratively with graphs, while water quality was analyzed descriptively.

RESULTS AND DISCUSSIONS

Cell Density of *Chlorella* sp.

Cell density can describe the number of developing *Chlorella* sp. cells per day. *Chlorella* sp. cell density in each treatment cultured for 16 days is presented in Figure 1.

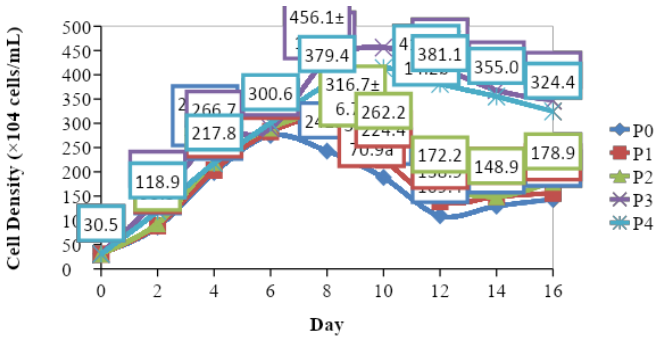

Figure 1. *Chlorella* sp. cell density was cultured using cow rumen bokashi enriched with chicken manure in each treatment.

Description: P0 (control 2 g/L cow's rumen bokashi), P1 (2 g/L cow's rumen bokashi enriched with 2.5 g/L chicken manure), P2 (2 g/L cow's rumen bokashi enriched with 3.0 g /L chicken manure), P3 (2 g/L cow's rumen bokashi enriched with 3.5 g/L chicken manure), P4 (2 g/L cow's rumen bokashi enriched with 4.0 g/L chicken manure). The values shown are the average ± standard deviation, different superscript letters indicate very significantly different ($P<0.01$).

Figure 1 shows that the peak cell density of *Chlorella* sp. was different for all treatments. The results of statistical tests showed that using cow's rumen bokashi enriched with chicken manure significantly affected the cell density of *Chlorella* sp. ($P<0.01$). Tukey test results showed that treatments P3 and P4 significantly differed from treatments P0, P1, and P2 regarding *Chlorella* sp. cell density. *Chlorella* sp. cell density in this study was higher when compared with the results of Rahman (2022) study which only obtained a cell density of 336.1×10^4 cells/mL when using cow's rumen bokashi. Meanwhile, the results of research by Febtisuharsi (2016) which only used chicken manure produced a density of 78.83×10^4 cells/mL indicating that cow's rumen bokashi enriched with chicken

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manure can increase the cell density of *Chlorella* sp. According to Lussy *et al.* (2017), this is because chicken manure contains 1.62 mg/L of nitrogen (N) and 54 mg/L of phosphorus (P).

This research resulted in the highest cell density of *Chlorella* sp., namely 456.1 ± 15.1 × 10^4 cells/mL, in cow rumen bokashi enriched with 3.5 g/L chicken manure (P3). The high cell density was due to the enrichment of cow's rumen bokashi with 3.5 g/L chicken manure which was optimal compared to other treatments, so that the media could stimulate the growth of *Chlorella* sp. cells. According to Nur *et al*. (2023), the cell density of *Chlorella* sp. will be more abundant if the dose of fertilizer given is optimal. Doses given too little or more than needed can inhibit growth. Roza *et al*. (2022) explained that nutrients are an important parameter to support the growth of *Chlorella* sp cells and can be a limiting factor in cell development.

Figure 1 shows that all treatments did not experience a lag phase and immediately entered the exponential phase. Treatment P0 entered the exponential phase until day 6; treatments P1 and P2 entered the exponential phase on day 8; and treatments P3 and P4 entered the exponential phase on day 10. The exponential phase occurred because *Chlorella* sp. could absorb nutrients from the cow's rumen which was processed into bokashi and enriched with chicken manure well, so that the cell density of *Chlorella* sp. continued to improve. According to Fadila *et al*. (2021), the exponential phase occurs in all treatments because the microalgae have been able to adapt to the culture media. Umainana *et al*. (2019) said that the growth of *Chlorella* sp. cells at the exponential phase is characterized by an increase in the number of cells starting from the first day until the peak day. Fadilla (2010) added that the growth rate in the exponential phase can reach a maximum because in this phase the cells consume nutrients well.

The stationary phase in all treatments only occurred for one day, namely treatment P0 on day 8, P1 and P2 on day 10, while P3 and P4 on day 12 where the highest cell density was in treatment P3, namely 415.6 × 10^4 cells/mL, followed by P4 (381.1 × 10^4 cells/mL), P2 (262.2 × 10^4 cells/mL), P0 (242.8 × 10^4 cells/mL), and P1 (224.4 × 10^4 cells/mL). It turned out that enrichment of cow's rumen bokashi with a higher dose of chicken manure was not always accompanied by a high cell density. This happened because *Chlorella* sp. required nutrients in optimal amounts to support cell growth. As stated by Nur *et al*. (2023) *Chlorella* sp. requires nutrients in optimal amounts to grow and develop well.

Furthermore, the final phase was marked by a decrease in the cell density of *Chlorella* sp. drastically at the death phase. This phase started on day 10 in treatment P0, day 12 in treatments P1 and P2, then day 14 in treatments P3 and P4, and ended on day 16. The decrease in cell density occurred because there was no addition of new nutrients from outside the culture media (Utomo *et al*., 2020). The death phase was marked by a decrease in *Chlorella* sp. cell density drastically (Taradifa *et al*., 2022).

Chlorella sp. cell density in treatments P0, P1, and P2 began to rise again on the 14th and 16th days. This occurred because *Chlorella* sp. and dead bacteria underwent a decomposition process and were then used again by *Chlorella* sp. as a source of nutrients for growth. This phenomenon follows the statement of Nurlaili *et al*. (2015) that dead bacteria and algae will be decomposed by aerobic microbes into nutrients that can be used again as nutrients in culture media.

Chlorella sp. cell density in this study was not optimal and was still relatively low when compared with the research results of Taradifa *et al*. (2022) who utilized liquid organic fertilizer (LOF) Azolla to obtain a cell density of 904.3 ×

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10^4 cells/mL at a dose of 12 mL/L. Similar conditions were also found in the research results of Roza *et al*. (2022) showing that POC of vegetable waste (spinach, cabbage, katuk, and mustard greens) produced a higher cell density, namely 1,118.3 × 10^4 cells/mL in mustard vegetable waste. The differences in cell density levels in several studies were caused by differences in fertilizer and nutrient content used so the ability of *Chlorella* sp. to absorb the nutrients in each fertilizer was different.

Specific Growth Rate of *Chlorella* sp.

Specific growth rate (SGR) is a parameter that can describe the growth of *Chlorella* sp. cells per unit time. The average specific growth rate values for each treatment are presented in Figure 2.

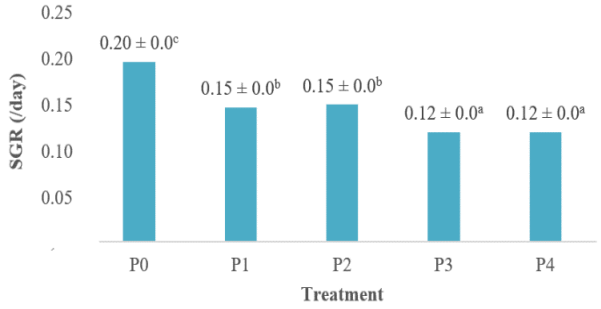

Figure 2. The specific growth rate of *Chlorella* sp. cultured using cow's rumen bokashi enriched with chicken manure in each treatment.

Description: P0 (control 2 g/L cow's rumen bokashi), P1 (2 g/L cow's rumen bokashi enriched with 2.5 g/L chicken manure), P2 (2 g/L cow's rumen bokashi enriched with 3.0 g /L chicken manure), P3 (2 g/L cow's rumen bokashi enriched with 3.5 g/L chicken manure), P4 (2 g/L cow's rumen bokashi enriched with 4.0 g/L chicken manure). The values shown are the average ± standard deviation, different superscript letters indicate very significantly different ($P<0.01$).

Based on Figure 2, it can be seen that the use of cow's rumen bokashi enriched with chicken manure had a very significant effect on the SGR value of *Chlorella* sp. ($P<0.01$). The differences in SGR values in all treatments were caused by environmental differences, namely the level of turbidity in the turbidity media. The higher the dose of chicken manure added to the cow's rumen bokashi, the higher the nutrients and level of turbidity in the culture media, so the phosphate levels were less utilized by *Chlorella* sp. This can be seen from the utilization of phosphate levels in media enriched with chicken manure ranging from 2-10%, while the utilization without enrichment (P0) was 19%.

The SGR values in treatments P1, P2, P3, and P4 were lower than the SGR values in treatment P0. This happened because without enriching chicken manure in the cow's rumen bokashi, *Chlorella* sp. could quickly absorb nutrients from the cow's rumen bokashi well and experience an exponential phase quickly. Meanwhile, the SGR values of *Chlorella* sp. in treatments P1, P2, P3, and P4 were lower than in treatment P0. This occurred because cow's rumen bokashi enriched with chicken manure had different doses, so the level of turbidity in the culture media was also different. According to Nurfadillah *et al*. (2012), the cause of the slow growth rate is turbidity. Turbidity can prevent light penetration which can interfere with phytoplankton carrying out photosynthesis.

The higher the dose of chicken manure given to the cow's bokashi rumen, the higher the level of turbidity in the culture medium, making it difficult for light to enter. Utilization of nutrients for the growth of *Chlorella* sp. at a slow rate

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could be caused by several factors, namely the photosynthesis process, the availability of sufficient nutrients, and turbidity.

Quality of Cultural Media

Nutrients in the culture media such as nitrate are one of the important factors that can influence the cell density of *Chlorella* sp. Nitrate levels in the culture media are presented in Figure 3.

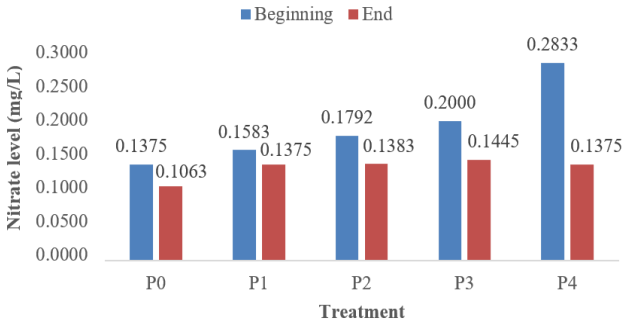

Figure 3. Results of nitrate measurements in cow's rumen bokashi culture media enriched with chicken manure in each treatment.

Description: P0 (control 2 g/L cow's rumen bokashi), P1 (2 g/L cow's rumen bokashi enriched with 2.5 g/L chicken manure), P2 (2 g/L cow's rumen bokashi enriched with 3.0 g /L chicken manure), P3 (2 g/L cow's rumen bokashi enriched with 3.5 g/L chicken manure), P4 (2 g/L cow's rumen bokashi enriched with 4.0 g/L chicken manure).

Figure 3 shows that the nitrate content at the start of the study increased with increasing addition of chicken manure to the cow's bokashi rumen, namely in the range of 0.1375-0.2833 mg/L. This range of nitrate content still supported the growth of microalgae cells. According to Aprilliyanti *et al.* (2016), optimal growth of phytoplankton requires nitrate ranging from 0.9-3.5 mg/L. Patahiruddin (2010) stated that a nitrate content in the range of 0.9-3.5 mg/L supports the growth of *Chlorella* sp. cells. If the nitrate level is <0.1 mg/L or > 45 mg/L, then nitrate can be a limiting factor for fertility. As stated by Dianita *et al.* (2020) nutrients that are too high can be toxic in the culture media, so they can inhibit growth and have low nutrient utilization efficiency.

The end of the culture showed that the utilization of nitrate levels in each treatment was different. The highest utilization of nitrate levels occurred in treatment P4, namely 51%, followed by treatments P3 (28%), P2 (23%), P0 (23%), and P1 (13%). The higher the utilization of nitrate in the culture media, the higher the cell density of *Chlorella* sp. (Nyabuto *et al.*, 2015). Nitrate was utilized by *Chlorella* sp. for cell growth and development. According to Roza *et al.* (2022) and Juneja *et al.* (2013), the use of nitrate is characterized by a decrease in the nitrate content in the culture media because it is used by *Chlorella* sp. to grow. Nyabuto *et al.* (2015) said that the less nitrate in the water, the higher the density of microalgae cells. Dahril *et al.* (2020) reported that nitrate is one of the nutrients that affect the cell density of *Chlorella* sp.

The high utilization of nitrate in the P4 treatment (51%) was not always accompanied by a higher cell density. This occurred due to the development of *Chlorella* sp. cells. Not only did it need nitrate but it also needed phosphate for cell division. Treatment P4 showed low phosphate utilization, namely only 2% compared to other treatments (Figure 4). This caused a decrease in *Chlorella* sp. cell density in treatment P4.

The results of this research show that the higher the enrichment of the cow's rumen with chicken manure, the higher the nitrate absorption capacity by

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Chlorella sp. This phenomenon occurred due to the processing of fertilizer into bokashi so that nutrients in the form of nitrate could be utilized properly by *Chlorella* sp. Apart from nitrate, another macronutrient important for the cell density of *Chlorella* sp. is phosphate. Utilization of phosphate content in culture media during the research is presented in Figure 4.

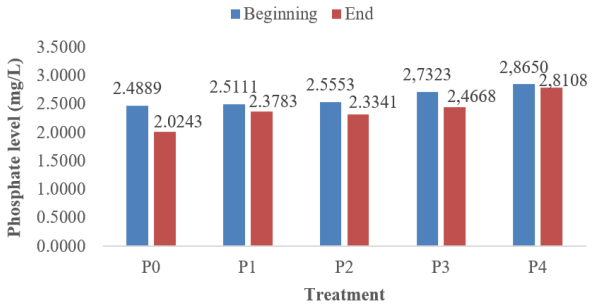

Figure 4. Results of phosphate measurements in cow rumen bokashi culture media enriched with chicken manure in each treatment.

Description: P0 (control 2 g/L cow's rumen bokashi), P1 (2 g/L cow's rumen bokashi enriched with 2.5 g/L chicken manure), P2 (2 g/L cow's rumen bokashi enriched with 3.0 g /L chicken manure), P3 (2 g/L cow's rumen bokashi enriched with 3.5 g/L chicken manure), P4 (2 g/L cow's rumen bokashi enriched with 4.0 g/L chicken manure).

Based on Figure 4, the initial phosphate content of the culture ranged from 2.4889-2.8650 mg/L. This range was still optimal for the development of *Chlorella* sp. cells as stated by Boroh *et al*. (2019) that the optimum phosphate range is 0.27-5.51 mg/L. Treatment P0 had a percentage value of phosphate absorption effectiveness of 19%, which was a higher value than treatments P1 (5%), P2 (9%), P3 (10%) and P4 (2%). The high percentage value of absorption effectiveness in the P0 treatment occurred because *Chlorella* sp. cells could absorb and utilize phosphate elements for the growth process.

Cow's rumen bokashi enriched with chicken manure in treatments P1, P2, P3, and P4 had a low absorption effectiveness percentage value. The low absorption of phosphate content occurred because microalgae organisms could not utilize the phosphate element optimally. The slow utilization of phosphate could be caused by the phosphate content which had not undergone a complete decomposition process because phosphate required a longer time for the decomposition process compared to other elements. Rosyadi *et al*. (2022) stated that the phosphorus cycle is the slowest compared to other biogeochemical cycles. According to Nurdiana *et al*. (2021), phosphate is utilized by *Chlorella* sp. in culture media to form chlorophyll and cell division. Restuhadi *et al*. (2017) said that phosphate also functions as a producer of metabolic energy for growth and reproduction.

Apart from nutrients, one of the factors that can influence the growth of *Chlorella* sp. is water quality, where *Chlorella* sp. can grow well if it is in a good environment. Temperature is a limiting factor in the distribution of a species. In ecological survival and cell division, temperature changes can cause differences in the composition and cell density of *Chlorella* sp. During the culture process, the temperature obtained was around 26-27 °C. This was still considered optimal for *Chlorella* sp. because according to Boroh *et al*. (2019), the growth of *Chlorella* sp. runs normally in the range of 25-32 °C.

The results of pH observations for each treatment were not much different, namely 6.40-8.45. The pH value is a

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parameter that can determine the solubility and availability of mineral ions in the media so that sufficient nutrient availability will influence nutrient absorption by *Chlorella* sp. Maharsyah *et al*. (2013) reported that a good pH range for the growth of *Chlorella* sp. is in the range of 4.5-9.3.

Dissolved oxygen during the study ranged from 3.6 mg/L to 7.9 mg/L. DO content ranging from 3-5 mg/L indicates less productive conditions, 5-7 mg/L indicates high productivity, and >7 mg/L indicates very high productivity (Rahmawati, 2019). The availability of DO in culture media is an important factor for phytoplankton because DO is directly used as a material to form organic molecules through the photosynthesis process (Rosyadi *et al*., 2022).

CONCLUSION

The use of cow's rumen bokashi enriched with chicken manure had a very significant effect on the cell density of Chlorella sp. where the highest cell density occurred in the P3 treatment (2 g/L cow's rumen bokashi enriched with 3.5 g/L chicken manure) namely 456.1 ± 15.1 × 104 cells/mL on day 10 with a specific growth rate of 0.12 ± 0.0/day. The lowest cell density occurred in treatment P0 (control 2 g/L cow's rumen bokashi), namely 275.0 ± 15.3 × 104 cells/mL on day 6 with a specific growth rate of 0.20 ± 0.0/day.

CONFLICT OF INTEREST

There were no conflicts of interest between all authors when writing and publishing this manuscript.

AUTHOR CONTRIBUTION

The contributions of each author are as follows; Fraja Mukti: conceptualization, methodology, resources, formal analysis, drafting. Rosyadi: conceptualization, methodology, formal analysis, writing, review. Agusnimar: conceptualization, methodology, formal analysis, writing, review. Khairul Hadi: conceptualization, methodology, formal analysis, draft writing, review, and editing. Kurnia Zulfahmi: conceptualization, methodology, resources, and formal analysis. Subramani Nagaraj: conceptualization, methodology, resources, and formal analysis

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