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The Combination Effect of Bean Sprouts Extract Concentration with Coconut Water on the Growth of the Density of *Chlorella* sp

Pengaruh Campuran Konsentrasi Ekstrak Tauge dengan Air Kelapa Terhadap Pertumbuhan Kepadatan Chlorella sp

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Abstract

Chlorella sp is a kind of phytoplankton used as a natural food source for zooplankton, such as fish larvae and shrimp. *Chlorella* sp contains various nutrients that are good for the growth of fish larvae and shrimp. The success in cultivating Chlorella sp is closely related to the nutrient content at the time of cultivation. One alternative cultivation of *Chlorella* sp is using bean sprout extract and coconut water as the medium. Bean sprouts and coconut water extract can be used as a substitute for inorganic fertilizers. This research aims to determine the effect of bean sprout extract and coconut water on the growth of the density of *Chlorella* sp, as well as grasping the best dose that can be used to cultivate *Chlorella* sp. This research was conducted in July 2022 at the Aquaculture Environmental Quality Laboratory, Faculty of Fisheries and Marine, Universitas Riau. This research used a one-factor, Completely Randomized Design (CRD) with four treatments and three replications. The treatments as defined in this research were P0 (Walne fertilizer 1 mL/L as control), P1 (8% bean sprout extract + 10% coconut water), P2 (10% bean sprout extract + 12.5% coconut water) and P3 (bean sprout extract 12% + coconut water 15%). These results indicate that P3 (12% bean sprout extract + 15% coconut water) is the best treatment for the growth of *Chlorella* sp with a density of 1091.00 $x10^4$ cells/mL and a specific growth rate of 0.9607 sel/mL, which occurred on the sixth day.

Keywords: Cell density, *Chlorella* sp, Coconut water.

Abstrak

Chlorella sp adalah fitoplankton yang digunakan sebagai pakan alami bagi zooplankton, larva ikan ataupun udang. *Chlorellla* sp mengandung berbagai nutrient yang baik untuk pertumbuhan larva ikan ataupun udang. Keberhasilan dalam melakukan kultur *Chlorella* sp tentunya berkaitan erat dengan kandungan nutrien yang diberikan pada saat kultur. Salah satu alternatif kultur *Chlorella* sp menggunakan Media Ekstrak Tauge dan air kelapa. Media Ekstrak Tauge dan air kelapa dapat dimanfaatkan sebagai pengganti pupuk anorganik. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian ekstrak tauge dan air kelapa terhadap pertumbuhan kepadatan *Chlorella* sp serta mengetahui dosis terbaik yang dapat digunakan untuk kultur *Chlorella* sp. Penelitian ini dilakukan pada bulan Juli 2022 di Laboratorium Mutu Lingkungan Budidaya, Fakultas Perikanan dan Kelautan, Universitas Riau. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) satu faktor dengan empat perlakuan dan tiga ulangan. Perlakuan yang ditentukan dalam penelitian ini adalah P0 (Pupuk walne 1 mL/L sebagai kontrol), P1(ekstrak tauge 8% + Air kelapa 10%), P2 (ekstrak tauge 10% + Air

kelapa 12,5%), dan P3 (ekstrak tauge 12% + Air kelapa 15%). Hasil penelitian menunjukkan bahwa P3 (ekstrak tauge 12% + Air kelapa 15%) merupakan perlakuan terbaik untuk pertumbuhan *Chlorella* sp dengan kepadatan sebesar 1091,00 x10^4 sel/mL dan dan laju pertumbuhan spesifik sebesar 0,9607 sel/mL yang terjadi pada hari keenam.

Kata kunci: Kelimpahan sel, *Chlorella* sp, Air kelapa.

1. Introduction

Chlorella sp is phytoplankton, which is used as natural food for zooplankton and fish larvae, and *Chlorella* sp contains various nutrients that are good for the growth of fish or shrimp larvae success in culturing *Chlorella* sp. Of course, it is closely related to the nutrient content provided during culture. Generally, *Chlorella* sp is cultured using inorganic fertilizers, but inorganic fertilizers are relatively expensive. Taradifa et al. (2022) stated that using inorganic fertilizers has the weakness of producing waste that can pollute and harm aquatic organisms. For this reason, we need an alternative way to reduce the use of inorganic fertilizers by using organic fertilizers.

One type of organic fertilizer that can be used for culturing *Chlorella* sp. is by administering bean sprout extract and coconut water. Providing a mixture of bean sprout extract with headwater in the culture media has a good influence on the growth of phytoplankton density (Ilahi, 2019). Dianita et al. (2020) stated that mung bean sprout fertilizer can influence microalgae's density and growth rate. The treatment medium using mung bean sprout fertilizer contains organic nutrients such as carbohydrates, protein and fat, which are needed as an energy source. Then coconut water contains organic nutrients such as carbohydrates, proteins and fats, which are required as an energy source for phytoplankton during the culture period (Jadid et al., 2017).

The many benefits and ingredients contained in bean sprout extract fertilizer and coconut water can be used as an alternative fertilizer substitute for *Chlorella* sp culture media. Therefore, to find out the best dose of bean sprout extract fertilizer and coconut water for the growth of *Chlorella* sp. Researchers are interested in researching the effect of a mixture of bean sprout extract concentrations and coconut water on the growth of *Chlorella* sp density.

2. Material and Method

2.1. Time and Place

This research was carried out on 29 May-14 June 2022, observing the density growth of *Chlorella* sp carried out for 12 days at the Aquaculture Environmental Quality Laboratory, Aquaculture Department, Faculty of Fisheries and Marine, Universitas Riau, Pekanbaru.

2.2. Methods

The one-factor Completely Randomized Design (CRD) method was used with four treatments and three replications. The treatment applied is as follows:

P0: Using Walne fertilizer 1 mL/L as a control.
P1: Using mixed fertilizer 1 (8% bean sprout extract + 10% coconut water)
P2: Using mixed fertilizer 2 (10% bean sprout extract + 12.5% coconut water)
P3: Using mixed fertilizer 3 (12% bean sprout extract + 15% coconut water)

2.3. Procedure

2.3.1. Container Preparation

The equipment (culture containers and test tubes) used in this research must be sterilized first by washing with soap, then rinsing and drying in the sun. Other tools, such as hemocytometers, can be fixed using 70% alcohol and dried with tissue. Meanwhile, the materials used need to be washed thoroughly with water.

2.3.2. Preparation of Bean Sprout Extract and Coconut Water

The beans used as media are fresh (mung bean sprouts) from purchases at the “Pasar Selasa Panam”. Wash the bean sprouts using water until clean, then drain, then blend 200g of bean sprouts with 1000 mL of distilled water until smooth, then filter them using a sieve. The resulting 500 ml filter is then put into a sterile element and covered using cotton wrapped in gauze and aluminum foil. The bean sprout extract is heated at a temperature of 100 to form two layers (precipitate and solution). After 2 hours, it is filtered again using cotton placed in the funnel, so the filtering results are spotless. The 250 mL filtered results are reheated until boiling and then cooled. The bean sprout extract that has been made has a clear yellow color. Then, the bean sprout extract is mixed with young coconut water and is ready to be applied to the culture medium.

2.3.3. Chlorella sp Culture

The inoculum used in this research was obtained from the Algae (*Chlorella* sp) Laboratory, Department of Aquatic Resources Management. Culturing *Chlorella* sp is carried out in a container as a jar measuring 10x10x20 with a culture media volume of 1000 mL. Febtisuharsi (2016) states that the density of the inoculum that is spread first is calculated using a hemocytometer with the formula.

$$\text{Cell density (cells/ml) N} = \text{Total number of cells x } 10^4$$

The initial inoculum density used in this study was 9,000,000 cells/ml, with the desired initial inoculum density in the culture media being 900,000 cells/mL (Napitupulu et al., 2019). According to Kwangdinata et al. (2010), to calculate the volume of inoculum needed for inoculation, the following formula can be used:

$$V1 = \frac{V2 \; x \; N2}{N1}$$

Information:

V1 : Volume of inoculum used (mL)
N1 : *Chlorella* sp inoculum cell density (cells/mL)
V2 : Volume of media to be used (mL)
N2 : Cell density of *Chlorella* sp inoculum required (cells/mL)

The inoculum volume used in this study was 100 mL in each container. Then distilled water is added until it reaches a volume of 1000 ml, and fertilizer is given according to the treatment. Next, the culture container is placed on the culture table, and three 40-watt lamps are given.

2.3.4. Population Observations o Chlorella sp

Observation of the population density of *Chlorella* sp was carried out every 24 hours for 12 days by taking a sample using a dropper of 1 mL, dropping it on a hemocytometer, then observing with a binocular microscope using 10x10 magnification and calculating the cell density of *Chlorella* sp on 25 boxes visible on the Hemocytometer using a hand counter. The number of *Chlorella* sp is calculated by multiplying by 10^4. Specific growth rate observations are observed every two days, using the formula (Vonshak in Napitupulu et al., 2019):

$$\mu = \frac{\ln N_t - \ln N_0}{t}$$

Information:

μ : Specific growth rate (%/ day)
Nt : Final cell density (cells/mL)
N0 : Initial cell density (cells/mL)
t : Time (days) from N0 to Nt

2.3.5. Water Quality

Water quality measurements (temperature, pH and DO) were observed every two days (Napitupulu et al., 2019), nitrate and orthophosphate) were observed three times during the research, namely at the beginning, middle and end.

2.4. Data Analysis

The Data obtained from the measured parameters are presented as tables and graphs. To determine whether or not different fertilizers affect *Chlorella* sp growth, a statistical test was conducted using Analysis of Variance (ANOVA) using the F statistical test. If $p<0.05$, then there was an effect of giving different fertilizers on the growth of *Chlorella* sp. Furthermore, Dianita et al. (2020) stated that to determine the differences between each treatment, the Newman-Keuls test range was carried out.

3. Result and Discussion

3.1. Population Cell Density of Chlorella sp

Microalgae density is one of the growth parameters that can determine whether the microalgae is growing (Nisa et al., 2020). Providing a fertilizer mixture with a concentration of bean sprout extract and coconut water on the cell density of *Chlorella* sp showed that the cell density of *Chlorella* sp has increased from time to time *Chlorella* sp cell density. Observed at each growth phase (lag, exponential, stationary and death) and presented in Table 1.

Data in Table 1 shows that in the lag phase, the cell density of *Chlorella* sp increased. It was very low or tended not to grow. This is because *Chlorella* sp cells are still adapting to the culture medium. Novianti et al. (2017) stated that the lag phase usually occurs on days 1 and 2, depending on how quickly the inoculum can adapt. The average cell density of *Chlorella* sp in the lag phase tends to be the same as the amount of inoculum at the start of the culture. P0 is a comparison control for other treatments. The total cell density of *Chlorella* sp at P0 in the lag phase was 124.00 ± 1.00 cells/mL, lower than the whole cell density of *Chlorella* sp on P3, P2, and

P1. The total cell density of *Chlorella* sp at P3, P2 and P1, respectively, was 131.66 ± 1.52 cells/mL, 127.66 ± 1.52 cells/mL, and 125.33 ± 2.08 cells/mL.

Table 1. The average cell density of *Chlorella* sp

Phase growth	Treatment [density (cells x 10^4 cells/mL) ± std. deviation]			
	P0	P1	P2	P3
Lag	124.00±1.00^a	125.33 ± 2.08^a	127.66 ± 1.52^a	131.66±1.52^b
Exponential	754.00±13.11^a	862.00±45.43^b	913.33±49.96^b	1091.00±37.98^c
Stationary	596.66±32.31^a	723.33±41.93^b	802.33±37.01^c	958.33±11.15^d
Death	107.66 ± 2.51^a	113.00 ± 4.58^a	118.33 ± 4.16^a	147.00±17.52^b

Note: Superscript letters that are on the same line indicate significant differences between treatments ($p<0.05$)

The density of *Chlorella* sp experienced a significant increase in the exponential phase, which occurred on day 3, with the highest density peak on day 6. This is because cell division occurs in the reproductive process, so the cell density of Chlorella sp increases. Rachmawati (2019) added that *Chlorella* sp cells have a high reproductive rate, and each cell of *Chlorella* sp can grow into 10,000 cells within 24 hours. According to Prayitno (2016), the length of the exponential phase depends on the nutrient content in the culture media. Theoretically, if environmental conditions and nutrients are in sub-optimum conditions, the exponential phase will last a short time or even not be reached. Theoretically, the exponential phase will continue continuously if environmental conditions and nutrients are maintained in optimum conditions. *Chlorella* sp cell density in the exponential phase, the highest occurred at P3, namely 1091.00 ± 37.98 cell/mL, followed by P2, P1, and P0, respectively, at 913.33 ± 49.96 cell/mL, 862.00 ± 45.43 cells/mL, and 754.00±13.11 cells/mL (Figure 1).

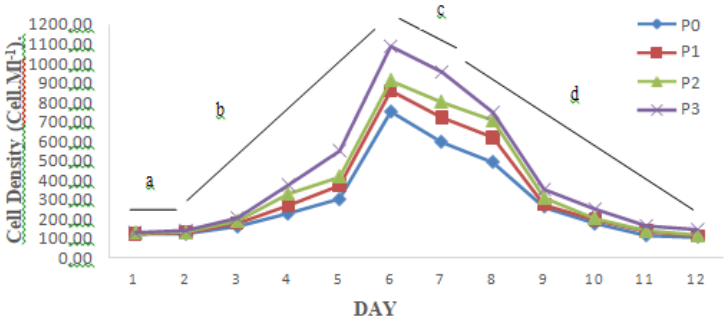

Figure 1. The average cell density of *Chlorella* sp. during each treatment12 days of research

Figure 1 shows that the stationary phase occurs on the 7th day. Microalgae cells generally enter the stationary phase on days 5-10 (Prayitno, 2016). In this phase, the cell density of *Chlorella* sp decreased, but not significantly *Chlorella* sp cell density. The highest in this phase occurred in P3, namely 958.33 ± 11.15 cells/mL, followed by P2 at 802.33±37.01 cells/mL, and P1 at 723.33 ± 41.93 cells/mL. The lowest density occurred at P0 at 596.66 ± 32.31 cells/mL.

The final phase of *Chlorella* sp culture activities is the death phase. This phase occurs on day nine, which is marked by the rate of *Chlorella* sp cell death being higher than the growth rate, so Novianti et al. (2017) stated that when the culture is more than one week old, microalgae have entered the death phase. Cell death is caused by nutrient exhaustion and the accumulation of metabolic waste or specific toxic substances. The absence of adding new nutrients from outside the culture media causes many cells to die. *Chlorella* sp cell density at P3, P2, P1, and P0, respectively, were 147.00±17.52 cells/mL, 118.33±4.16 cells/mL, 113.00±4.58 cells/mL, 107.66±2.51 cells/mL.

Chlorella sp cell density data at each phase was obtained in this study, and analysis of variation (ANOVA) was tested. The results showed that applying fertilizer mixed with bean sprout extract and coconut water significantly affected ($p<0.05$) the density growth of *Chlorella* sp. These results indicate that P3 is the best treatment for the growth of *Chlorella* sp, namely with a mixture of 3 fertilizers (12% bean sprout extract + 15% coconut water). This shows that adding coconut water to the culture media has a positive influence because young coconut water contains organic nutrients such as carbohydrates, proteins, and fats, which function as an energy source for microalgae during the culture process (Jadid et al., 2017). Suryati et al. (2019) added that coconut water, rich in the hormones auxin and cytokinin, is very suitable as a plant growth stimulant. Besides that, coconut water is also rich in mineral elements such as K, N, Ca, Mg, Fe, Cu, P, and S, often used as ingredients in making liquid organic fertilizer in almost all products.

3.2. The Rate of Sediment Accumulation

Specific growth rate (LPS) is directly proportional to *Chlorella* sp cell density growth. LPS describes the rate of increase of individual algae per unit time (Soewardi et al., 2015). The LPS graph for *Chlorella* sp during the research can be seen in Figure 2.

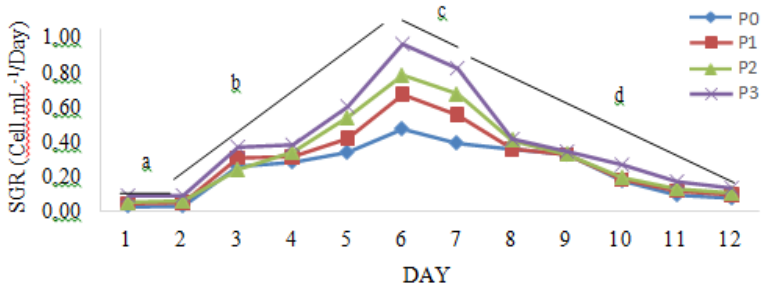

Figure 2. Specific growth rate of *Chlorella* sp

Description: P0 (walne 1 mL/L), P1 (mixed fertilizer 1), P2 (mixed fertilizer 2), P3 (mixed fertilizer (3). a= Lag Phase, b= Exponential Phase, c= Stationary Phase, d= Phase Death.

Figure 2 shows that the LPS value in the lag phase on days 1 and 2 did not increase. The LPS value increased significantly in the exponential phase on day 3, with the highest LPS value on day 6. On the 7th day, the LPS value decreased, but it was insignificant. The LPS value significantly reduced on the 8th day because, in this phase, *Chlorella* sp experienced the death phase. The highest LPS in the lag phase occurred in P3 with a value of 0.0927 ± 0.0116 cell/mL/day, followed by P2, P1 and P0, respectively, with an LPS value of 0.0619 ± 0.0119 cell/mL/day, 0.0434 ± 0.0166 cells/mL/day and 0.0328±0.0080 cells/mL/day. LPS in the highest exponential phase also occurred at P3 at 0.9607 ± 0.0480 cells/mL/day, followed by P2 of 0.7822±0.0992 cells/mL/day, P1 of 0.6711±0.2543 cells/mL/day and P0 of 0.4723± 0.0493 cells/mL/day.

The LPS value begins to decrease in the stationary phase, and this is due to the density of *Chlorella* sp cells also reducing. The highest LPS value in the stationary phase occurred at P3 at 0.8125 ± 0.0646 cells/mL/day, followed by P2 at 0.6760±0.0245 cells/mL/day, P1 at 0.5531 ± 0 .2344 cells/mL/day and P0 of 0.3893±0.1799 cells/mL/day. The LPS value experienced a significant decrease in the death phase, with the LPS value at P3 of 0.1335±0.0143 cells/mL/day, P2 of 0.1051±0.0178 cells/mL/day, P1 of 0 .0946±0.0087 cells/mL/day and P0 of 0.0747±0.0294 cells/mL/day. Low LPS values can occur due to sub-optimal nutrients and culture media conditions (Kawaroe et al., 2012). Average LPS value in each growth phase of *Chlorella* sp more details can be seen in Table 2.

Table 2. Average specific growth rate of *Chlorella* sp in each treatment in each growth phase during the study

Phase growth	Treatment [LPS (cell/mL/day) ± std. deviation]			
	P0	P1	P2	P3
Lag	0.0328±0.0080^{a}	0.0434±0.0166^{ab}	0.0619±0.0119^{b}	0.0927±0.0116^{c}
Exponential	0.4723 ± 0.0493^{a}	0.6711±0.2543^{ab}	0.7822±0.0992^{ab}	0.9607±0.0480^{b}
Stationary	0.3893 ± 0.1799^{a}	0.5531±0.2344^{ab}	0.6760±0.0245^{ab}	0.8125±0.0646^{b}
Death	0.0747±0.0294^{a}	0.0946±0.0087^{ab}	0.1051±0.0178^{ab}	0.1335±0.0143^{b}

Note: Superscript letters on the same line indicate significant differences between treatments ($p<0.05$).

3.3. Water Quality

Water quality influences the growth of *Chlorella* sp, where *Chlorella* sp can grow optimally in an optimal environment. The parameters measured in this study included temperature, pH, and DO, estimated every two days, as well as nitrate and phosphate, measured three times during the study. The results of measuring water quality parameters can be seen in Table 3.

Table 3. The results of water quality parameters

Parameter	P0	P1	P2	P3	Optimal
Temperature (^{o}C)	26-30	26-30	26-30	26-30	25-30 (Mufidah et al., 2017)
pH	7,5-8,5	7,9-8,3	7,9-8,4	7,9-8,4	7,2-8,4 (Shaflo & Fayek, 2013)
DO (mg/L)	6,4-8,4	6,5-8,8	6,5-8,9	6,5-9,6	>7 (Rahmawati & Nadya, 2020)
Nitrate (mg/L)	2,035-17,963	1,822-9,477	1,597-13,953	0,870-15.027	3-15,5 (Boroh et al., 2019)
Phosphate (mg/L)	1,083-4,580	0,986-3,674	0,782-3,946	0,539-4,153	0,27-5,51 (Boroh et al., 2019)

The results of measuring water quality parameters during the research were still within the optimal range to support the growth of *Chlorella* sp. The results of water quality measurements are a temperature of 26-30^{o}C. According to Mufidah et al. (2017), *Chlorella* sp grows optimally at 25^{o}C, and pH levels during the study in each treatment ranged from 7.5 to 8.5. According to Shaflo & Fayek (2013), the optimum pH value range for the growth of *Chlorella* sp ranges from 7.2 to 8.4. *Chlorella* sp can still grow to a pH of 9 (Mufidah et al., 2019).

Chlorella sp carries out the photosynthesis process for growth to produce dissolved oxygen in water. The DO values for each treatment in this study ranged from P0 6.3-8.4 mg/L, P1 6.5-8.8 mg/L, P2 6.5-8.9 mg/L, and P3

6.5 -9.6 mg/L. DO levels of 3-5 mg/L are less productive, 5-7 mg/L are high productivity, and >7 mgL are very high productivity (Rahmawati & Nadya, 2020). The highest DO value occurred at P3 at 9.6 mg/L on day 6, which was the peak density of Chlorella sp. Furthermore, on the 8th day, the DO value gradually decreased in proportion to the decrease in the *Chlorella* sp cell productivity level, with the lowest value occurring at P0 of 6.3 mg/L. Saragih et al. (2018) stated that the cell density of *Chlorella* sp influences the photosynthesis process, which affects DO levels and the photosynthesis process itself, so the higher the cell density in a medium, the higher the DO level in the medium.

The nitrate and phosphate content in the culture media container varied between treatments. This is because the fertilizer dose given to each treatment is different. According to Boroh et al. (2019), Optimum nitrate and phosphate levels in culture media for the growth of *Chlorella* sp ranged between 3-15.5 and 0.27-5.51 mg/L. The nitrate and phosphate content at the beginning of the culture was quite high, namely at P0, it was 17.963 mg/L and 4.580 mg/L, P1 was 9.477 mg/L and 3.674 mg/L, P2 was 13.953 mg/L and 3.946 mg/L while P3 was 15.027 mg/L and 4.153 mg/L. This shows that the nitrate and phosphate content in P1, P2, and P3 is in the optimum range required for *Chlorella* sp to grow. The nitrate and phosphate content on the 6th day (exponential phase) began to decrease. This is because nitrate and phosphate have been utilized by *Chlorella* sp for growth so that on the 6th day, peak density occurs. The nitrate and phosphate content at P0 was 8.307 mg/L and 2.795 mg/L, P1 5.916 mg/L and 2.568 mg/L, P2 7.052 mg/L and 2.492 mg/L then at P3 it was 7.918 mg/L and 1.994 mg/L. Furthermore, on the 12th day, the nitrate content in the culture media was below the optimum limit required by *Chlorella* sp to grow. Hence, the cell density of *Chlorella* sp decreased, while the phosphate content in the culture media was still in the optimum range for the growth of *Chlorella* sp. The nitrate and phosphate content on day 12 was P0 2.035 mg/L and 1.083 mg/L, P1 1.822 mg/L and 0.986 mg/L, P2 1.597 mg/L and 0.782 mg/L and P3 0.870 mg/L, and 0.539 mg/L.

4. Conclusions

Providing fertilizer with a concentrated mixture of bean sprout extract and coconut water influences the growth of *Chlorella* sp cell density. Treatment at P3 with a dose of 12% bean sprout extract + 15% coconut water was the best treatment in this study, with the highest cell density at the peak of the exponential phase on day 6 of 1091.00×10^4 cells/mL and a specific growth rate of 0.9607 cells/mL/day.

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