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EFFECT OF ADDITION OF COCONUT WATER AS ENRICHMENT OF *Spirulina platensis* GROWTH MEDIA IN LABORATORY SCALE

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ABSTRACT

Spirulina platensis is a microalga that has high economic value. The growth of *S.platensis* is influenced by environmental conditions and nutrients. The objectives of this study are to determine the best concentration of coconut water for media of *S.platensis* growth. The study was conducted at the Natural Feed Laboratory of the Brackish Water Aquaculture Center (BPBAP) Ujung Batee Aceh. This research is an experimental study using a completely randomized design (CRD) consisting of 3 levels of treatment and control using coconut water of the media with concentrations of 3%, 5%, and 7% with 3 replications. The highest cell density level of *S.platensis* reached 83.102 cells/ml in treatment A (3%), in treatment B (5%) 73.975 cells/ml, the density in the control treatment reached 70.850 cells/ml, and in treatment, C (7%) cell density only reached 54.658 cells/ml. The results of the ANOVA test on day 2, day 3, day 5, and day 6 showed that the addition of coconut water with different concentrations had a significant effect ($p<0.05$) on the growth of *S.platensis.*

Keywords: *Spirulina platensis*, Coconut Water, Density, Growth

1. INTRODUCTION

Microalgae are a group of tiny plants, both single cells and colonies that are very widely used in aquaculture, health, feed, and food industries. One of the microalgae that is widely used in aquaculture is *Spirulina platensis*. *S. platensis* is a blue-green algae (cyanobacteria) that is widely used as raw material for the food industry because it contains 60-71% protein, 8% fat, 16% carbohydrates, and vitamins as well as 1.6% Chlorophyll-a, 18% Phycocyanin, 17% β-Carotene, and 20-30% γlinoleaic acid from total fatty acids[1].

Coconut water is one of the most economically priced and easily available alternative culture media. Coconut water has been used as a growth medium for fungal, macroalgae, and microalgae cultures. Previous research by Putri et al.[2] showed that coconut water is a media enrichment that can produce high population density. In *Tetraselmis* sp. cells can adapt and grow in coconut water media and seawater media. Furthermore, the research of Jadid et al.[3], showed that coconut water media can increase the abundance of microalgae cells *Nannochloropsis* sp.

Based on some of the results of these studies, coconut water media with various concentrations can be used as an enrichment that can increase the cell density of microalgae *Nannochloropsis* sp. and *Tetraselmis* sp. Meanwhile, for the growth of *S.platensis*, there is no documented information about the provision of different concentrations of coconut water on the growth of the *S.platensis* population. For this reason, it is necessary to conduct research with the title "Effect of coconut water addition as media enrichment for *S.platensis* population growth".

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2. RESEARCH METHOD

Time and Place

This research was conducted in January 2022 at the Natural Feed Laboratory of the Brackish Water Aquaculture Centre (BPBAP) Ujung Batee Aceh.

Procedure

The culture of *S.platensis* was carried out in a controlled room influenced by room temperature using an *air conditioner* (AC*)* with a room temperature of 16^{o} C. The coconut water used came from freshly broken coconut water from the type of *Cocos nucifera* L. which was 2 months old. The *S.platensis* seedlings used in the study came from the stock of the Natural Feed Laboratory of the Brackish Water Aquaculture Centre (BPBAP) Ujung Batee Aceh. The study used a completely randomized design (CRD) consisting of 3 different treatment levels of coconut water addition and control, namely treatment A 3% (6 ml coconut water), treatment B 5% (10 ml coconut water), treatment C 7% (14 ml coconut water) and control. Each treatment was repeated 3 times. Calculation of the initial density of *S.platensis* using the formula Edhy et al.[4]:

$$V_1 = \frac{N_2 \times V_2}{N_1}$$

Description:

V_1 : Initial stocking seed volume (ml)
V_2 : The volume of culture vessel used (ml)
N_1 : Pure culture density (cells/ml)
N_2 : The desired density of *S.platensis* (cells/ml).

Data Analysis

The test parameters in this study were population density and absolute growth. Cell counts were conducted every 24 hours starting from day 1 to day 7. Cell count was done under a microscope using SRCC (Sedgwick Rafter Counting Cell) with the formula Sari et al.[5]:

$$N = \frac{1000}{3{,}14 \times \left(\frac{d}{2}\right)^2 \times p} \times n$$

Description:

N : *S.platensis* density (cells/ml)
1000 : area in SRCC (*Sedgewick Rafter Counting Cell)*
d : Diameter of field of view (mm)
p : Number of fields of view in the entire field (10)
n : Total number of cell

In addition to calculating cell density, water quality measurements were also taken including temperature, salinity, and pH every day. Absolute growth is determined by the formula Sopian et al.[6] 2019)

$$G = Wt - Wo$$

Description:

G : Absolute Growth Rate (cells/ml)
Wt : Final cell density (cells/ml)
Wo : Initial cell density (cells/ml)

The data obtained in this study are presented in the form of tables and graphs. To compare population growth between treatments, a one-way Analysis of Variance test was conducted. If from the results of the analysis of variance, it is known that the treatment shows significantly different results, followed by the LSD (Least Significant Different) test.

3. RESULT AND DISCUSSION

The results of the measurement of water quality parameters show water conditions that are following the conditions suitable for the growth of *S.platensis,* namely the average water temperature ranging from 24-25 C^{o} and pH values ranging from 7.4 with salinity at a value of 26 ppt. The results of the calculation of the average population density can be seen in Table 1.

Based on Table 1, it can be seen the difference in the average cell density of *S.platensis* in each treatment with a maintenance time of 7 days. The highest *S.platensis* cell density on day 4 of

treatment A (3%) was 83,102 cells/ml, and the lowest in treatment C (7%) reached 54,658 cells/ml. The growth of *S. platensis* cell density can be compared in each treatment. The density of *S.platensis* increased from day 0 to day 4, after which the population density decreased from day 5 to day 7.

Table 1. Average Population Density of *S.platensis*

Day to	*S. platensis* density (cells/ml)			
	Treatment A (3%)	Treatment B (5%)	Treatment C (7%)	Control
H0	10.000	10.000	10.000	10.000
H1	28.125	27.659	24.092	27.595
H2	30.354	44.098	28.868	29.770
H3	53.862	53.544	41.073	42.984
H4	83.102	73.975	54.658	70.850
H5	48.025	39.587	33.051	26.586
H6	34.917	21.757	23.455	23.455
H7	20.377	17.777	18.254	20.271

The growth of *S.platensis* experienced the highest density peak on day 4. The results obtained from the calculation of *S.platensis* population density in each treatment were analysed with the Analysis of Variance test. The test was conducted on the density level data from day 1 to day 7. Based on the results of the ANOVA test, the results were significantly different on days 2, 3, 5, and 6 because the different doses in each treatment had a significant value of $p < 0.05$, which means that the results of this study reject H_0 so that there is an effect of the addition of different coconut water on the growth of *S. platensis* cell density.

Based on the results of the calculation of *S. platensis* cell density in each treatment during the study, it shows that the total population density of *S. platensis* cells has increased differently due to the different concentrations of coconut water. From the results of this increase in cell density, it can be stated that in the growth period of *S.platensis* cells pass through several phases, namely the lag phase (adaptation phase), exponential phase, growth rate decline phase, stationary phase, and death phase.

The population density of *S. platensis* cells on the first day and the second day showed a rather slow growth of the cell population, this is thought to be because at this stage the *S.platensis* cells are in the lag phase (adaptation phase) to the new environment. On the first day, the highest cell population density growth was found in treatment A (3%) at 28,125 cells/ml, and the lowest growth was found in treatment C (7%) at 24,092 cells/ml. On the second day, the highest cell density growth was found in treatment B (5%) at 44,098 cells/ml, and the lowest growth was found in treatment C (7%) at 28,868 cells/ml. In this phase photosynthesis is still actively taking place and organisms are metabolizing but there has not been high cell division so the density has not increased drastically[7].

On the 3rd day, the growth of *S.platensis* cell population density continued to increase, because *S.platensis* cells began to utilize nutrients in the culture medium to increase cell density. The highest cell density growth was obtained in treatment A (3%) which was 53,862 cells/ml and the lowest was in treatment C (7%) which was 41,073 cells/ml. The available nutrients are still many in the culture media, allowing *S. platensis* cells to perform cell division repeatedly[2].

Furthermore, to determine the difference between each treatment, the LSD (Least Significant Different) further test was carried out, from the output it was

found that several treatments had significantly different results because they received a significant value <0.05. Data from LSD test results can be seen in Table 2.

Table 2. LSD (least significant difference) further tests symbols

Day 2	Treatment	Average (cells/ml)	Symbol
	C	28.868	a
	Control	29.770	a
	B	44.098	b
	A	53.862	a
Day 3	Treatment	Average (cells/ml)	Symbol
	C	41.073	a
	Control	42.984	a
	B	53.544	b
	A	53.862	b
Day 5	Treatment	Average (cells/ml)	Symbol
	Control	26.586	a
	C	33.051	ab
	B	39.587	bc
	A	48.025	c
Day 6	Treatment	Average (cells/ml)	Symbol
	B	21.757	a
	Control	23.455	a
	C	23.455	a
	A	34.917	b

Notes: Treatments followed by the same symbol are not significantly different; Treatments followed by different symbols are significantly different.

The observation of *S.platensis* cells showed different growth results. The highest average absolute growth was in treatment A (3%) with 73,102 cells/ml and the lowest was in treatment C (7%) with 44,658 cells/ml. The absolute growth rate of *S.platensis* can be seen in Figure 2.

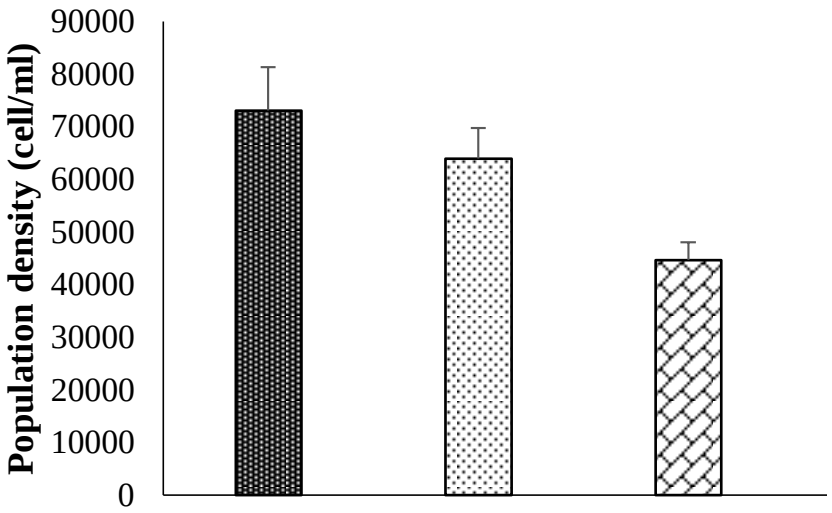

Figure 1. Absolute growth of *S.platensis* culture

Day 4 is the peak density (exponential phase) of *S.platensis* cells, where in this phase there is a very drastic increase in density due to cell division. According to Mukhlis et al.[8], the peak density is marked when the cell population density is one or

more times the initial density. The exponential growth phase occurs due to increased photosynthetic activity that produces high biomass[9]. In this phase, the cells of *S.platensis* undergo continuous division. The highest peak density was obtained in treatment A (3%) which was 83,102 cells/ml and the lowest was in treatment C (7%) which was 54,658 cells/ml. From the results of cell density growth, it can be seen that the lowest concentration of coconut water produces higher population density growth compared to the highest concentration of coconut water, this is thought to be due to the addition of excess nutrients that can inhibit microalgae growth, this is supported by the opinion of Sayedin et al.[10], namely the addition of excess nutrient levels can inhibit microalgae growth. After reaching the peak density, *S.platensis* experienced a decrease in cell density.

The stationary phase in this study was not seen, this is thought to be because the stationary phase occurred when no observations were made, the stationary phase is a phase where cell density decreases relatively low due to the balance between growth rate and death rate. The increase in density stops in the stationary phase which is characterized by a balance between growth and death rates caused by the amount of nutrients in the culture medium decreasing, but even so, *S.platensis* cells can still divide but not as much as in the exponential phase[11].

This phase is characterized by cell division still occurring but not as intensively as in the previous phase so the growth rate decreases[12]. The decrease in density occurred in all treatments. In this phase, the highest cell density was still obtained in treatment A (3%) on day 5 at 48,025 cells/ml and decreased on day 6 to 34,917 cells/ml, and the lowest cell density in the control treatment at 26,586 cells/ml and decreased on day 6 to 23,455 cells/ml. In this phase, cell density decreased due to the reduction of nutrients in the culture medium[3].

The death phase is characterized by a death rate that is greater than the growth rate, resulting in a decrease in cell density in the culture vessel. This phase is marked by changes in media conditions such as color, water temperature, and reduced nutrients in the culture media[12]. This death phase occurred on day 7 where the highest cell density was still obtained by treatment A (3%) at 20,377 cells/ml and the lowest in treatment B (5%) at 17,777 cells/ml.

Based on the results of research that has been carried out for 7 days, it shows that the provision of coconut water with different concentrations can produce a population density of *S.platensis.* The highest cell density (peak density) of each treatment was achieved at relatively the same time on day 4. The peak density occurred on the 4th day following the opinion of Hu and Gao[13], where the optimum density that can be achieved for laboratory scale occurs in the 4-7 day culture period. The results showed that coconut water is a media enrichment that can produce high cell population density. The highest density was found in treatment A (3%) which reached 83,102 cells/ml, then in treatment B (5%) reached 73,975 cells/ml, then the control treatment reached 70,850 cells/ml, and treatment C (7%) reached 54,658 cells/ml.

The increase in average *S platensis* cell density indicates that S.*platensis* cells can adapt and grow in coconut water and seawater media. This indicates that the nutrients in coconut water can be absorbed and utilized by *S.platensis* cells for growth. This is following the opinion of Chiu et al.[14] which states that Nutrients can increase the rate of microalgae productivity because microalgae utilize nutrients as a source of metabolism. Afriza et al.[15] also stated that the limited amount of nitrogen in the growth medium will inhibit the photosynthesis process which will affect microalgae cell density. Coconut water treatment media contains organic nutrients such as carbohydrates, proteins, and fats which are needed as a source of energy for

S.platensis. According to Irhamni et al.[16], microalgae can grow well in conditions where there are nitrogen sources. The energy is used for growth and cell division for microalgae, this is by the statement that the growth of a type of phytoplankton is closely related to the availability of macro and micronutrients and is influenced by the conditions of microalgae culture media[2]. Nitrogen plays a role in the growth and formation of DNA, RNA, enzymes, and photosynthesis. Nitrogen is a macronutrient that affects the growth of *S. platensis.* According to Fakhri et al.[17], carbon, nitrogen, and phosphorus are the main factors that can affect microalgae growth.

The results of cell density calculations that have been carried out show that treatment C (7%) with the highest concentration of coconut water produces the lowest density compared to treatment A (3%), B (5%), and control. However, in previous research by Putri et al.[2] (2013), namely the addition of coconut water as an enrichment of *Tetraselmis* sp. growth media with concentrations of A (0%), B (1%), C (2%), D (3%), E (4%) and F (5%) found the highest density results in treatment F (5%) with cell density reaching $54.75.10^4$ cells/ml.

The difference in the best concentration in this study is thought to be caused by differences in the type of microalgae in absorbing nutrients, this is supported by the opinion of Arfah et al.[18], namely the difference in density growth in each treatment is caused by differences in the ability of cells to absorb nutrients contained in the culture medium. In addition, it can be assumed that the culture medium becomes more turbid due to the higher dose of coconut water, which causes inhibition of photosynthesis. According to Umainana et al.[19], stated that fertilizer concentrations that are too high or concentrated can cause water in the culture medium to become turbid so that light is difficult to penetrate and phytoplankton growth becomes slow.

4. CONCLUSION

The addition of coconut water as a media enrichment for *S.platensis* population growth affects the growth of *S.platensis* cell density. The best concentration of coconut water for *S.platensis* population growth is in treatment A (3%) coconut water with the highest density reaching 83,102 cells/ml.

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