Effect of Moringa Leaf *(Moringa oleifera)* Liquid Fertilizer on Growth of *Spirulina platensis*

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Abstract

Spirulina platensis is a microalga widely cultivated and high in protein, making it a potential natural feed. Moringa leaves (*Moringa oleifera*) have a nutritional value greater than that of plants in general and are widely used as a plant fertilizer. This study aims to analyze the effect of Moringa leaf liquid fertilizer on the growth of *S. Platensis* and to determine the optimal fertilizer concentration. The research method used was an experimental design with direct observation during data collection. The Completely Randomized Design (CRD) used consisted of 5 treatments, namely the addition of 3 mL/L (A), 5 mL/L (B), and 7 mL/L (C) Moringa leaf liquid fertilizer, positive control (D), and negative control (E), where each treatment was performed for 3 repetitions. Culture *of S. platensis* was carried out for 7 days, with water quality parameters, including salinity, pH, and temperature, measured. Cell density and chlorophyll-a content were measured daily. The research results showed that water quality was good. The highest cell population density was observed in treatment C (7 mL/L), with a density of 754,000 cells/mL. The chlorophyll-a content did not significantly increase when liquid fertilizer from Moringa leaves was applied ($p>0.05$). Based on the results, applying Moringa leaf liquid fertilizer positively affects the cell population density of *S. platensis*, with an optimal concentration of 7 mL/L.

1. Introduction

Spirulina platensis is a blue-green microalgae (blue-green algae) that is widely cultivated commercially, has the highest protein content among other sources, and has the potential to be developed as a natural feed (Nur, 2014). Its protein content reached 68%, higher than that of meat, soybeans, fish, and eggs (Hadiyanto & Azim, 2012). This nutritional value offers opportunities to develop *S. platensis* for optimal use in the fishing sector.

One factor influencing the growth of *S. platensis* is nutrient availability, which is usually supplied by chemical fertilizers, such as Walne fertilizer. The price of Walne fertilizers was high and could pollute the environment, creating an increasing need for alternative fertilizers that were more readily available, more economical, and safer for the environment. One of these was organic fertilizer, such as Moringa leaf liquid fertilizer.

Several previous studies have reported the use of Moringa leaf liquid fertilizer for plants (Wahyuni et al., 2019; Suhastyo & Raditya, 2021; Tomia & Pelia, 2021). Krisnadi (2015) stated that Moringa leaf extract was the best organic fertilizer for all types of plants due to its nutritional value. However, no one has reported using Moringa leaf liquid fertilizer for growing microalgae *S. platensis.*

In this study, we analyze the effect of Moringa leaf liquid fertilizer (*Moringa oleifera*) on S. platensis growth and determine the optimal concentration of Moringa leaf liquid fertilizer for *S. platensis* cell growth. Chlorophyll-a

content was also measured as an indicator of the fertility of the *S. platensis* culture.

2. Methodology

2.1. Time, Place, and Materials

This study was conducted in October - November 2023 at the Marine Microbiology Laboratory, Department of Marine Science, Faculty of Fisheries and Marine, Universitas Riau. Pure culture *S. platensis* obtained from BBPBAP Jepara Natural Feed Laboratory. Seawater, freshwater, Walne fertilizer, and absolute methanol were obtained from commercial suppliers. Moringa leaf liquid fertilizer was made by fermentation of Moringa leaves.

2.2. Method

This research used an experimental method with direct observation of the data collection. The Completely Randomized Design (CRD) used consisted of 5 treatments and 3 repetitions. The treatments tested were: applying Moringa leaf liquid fertilizer at concentrations of 3 mL/L (A), 5 mL/L (B), and 7 mL/L (C); applying 1 mL/L Walne fertilizer as a positive control (D); and a negative control without fertilization (E).

2.3. Procedures

Preparation of Fertilizer

Liquid Walne fertilizer was used as a positive control. Meanwhile, liquid fertilizer from Moringa leaves was prepared according to the procedure described by Suhastyo & Raditya (2021), using 10 L of rice water and 250 g of brown (Javanese) sugar, with the amount of fresh Moringa leaves adjusted to 1 kg. Moringa leaves were finely mixed and then placed in a bucket. Then the chopped brown sugar and rice water were added, mixed until smooth, and covered. Every day, the bucket was opened to release the gas produced. Fermentation lasts 14 days until it smells good. Then the fermented liquid was filtered and stored in a clean bottle. Fermented Moringa leaf liquid fertilizer was added to the growing medium at concentrations of 3 mL/L, 5 mL/L, and 7 mL/L.

Environment and Growing Media of *S. platensis*

The growing medium is a 1:2 mixture of seawater and freshwater, totaling 1000 mL. Walne fertilizer and Moringa leaf liquid fertilizer were applied at the concentrations determined. Aeration was provided to provide air to the *S. platensis* growing medium. The environment was identical across treatments. The desired growing media environment is a temperature of 25-29 ℃, salinity of 25 ppt, and light intensity of ±2000-3500 lux or equivalent to a 40-watt fluorescent lamp ± 10 cm on the surface of the *S. platensis* growing medium (Sari et al., 2012). At the same time, the optimal pH range for *Spirulina* sp growth is 7.5-9.5 (Putri, 2019).

Inoculation of *S. platensis* Culture

The culture medium was prepared in advance, then liquid Moringa leaf and Wane fertilizer were added according to the treatment. Pure cultures of *S. platensis* at a density of 100,000 cells/mL were placed in aerated culture media for 5 minutes. Calculation of the *S. platensis* seeds required for inoculation using the following formula (Edhy & Kurniawan, 2003):

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

where:

V1 = Volume of seeds from initial stock (mL)
V2 = Desired volume of culture medium (mL)
N1 = Seedling density stock *S. platensis* (cells/mL)
N2 = Seedling density *S. platensis* desired (cells/mL)

Population Density Calculation of *S. platensis*

Population density calculations were performed every day for 7 days after initial seed storage of *S. platensis* using the Sedgwick Rafter Counting Cell (SRCC). One milliliter of the S. platensis culture was dropped onto the SRCC using a dropper pipette, then observed under a microscope at 100x magnification. The population density of *S. platensis* was calculated based on one unit/sinusoid (i.e., one wave) using a handheld counter (Sari et al., 2012). Density calculation of *S. platensis* was performed using the following formula (Pramushinta et al., 2012):

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

where:

N = Density of *S. platensis* (cell/mL)
d = Diameter of field of view (mm)

n = Average number of *S. platensis* per field of view (cell/mL)

Measurement of Chlorophyll-a Content

Chlorophyll-a content of *S. platensis* was determined with reference to (Fakhri et al., 2020), namely the methanol extraction method. 10 mL of microalgae culture was centrifuged at 6000 rpm for 10 minutes. Then, 10 mL of absolute methanol was added to the centrifuged pellet. It was then wrapped in aluminum foil and placed in a water bath at 70 °C for 10 minutes. The samples were vortexed and centrifuged at 6000 rpm for 10 minutes. The absorbance of the clear supernatant was measured at 665 nm using a spectrophotometer. The chlorophyll-a content was calculated using the following formula:

Chlorophyll a (µg/mL) = 12.9447 x A665

Where:

Chlorophyll-a (µg/mL)	=	Chlorophyll-a content of *S. platensis* (µg/mL)
12,9447	=	Absorbance coefficient
A665	=	Absorption value of the filtrate at a wavelength of 665 nm.

2.4. Data Analysis

The data were statistically analyzed using one-way ANOVA. If the results show a significant difference, we proceed to the LSD (Least Significant Difference) test to compare the treatments.

3. Result and Discussion

Water Quality and *S. platensis* Culture

The average water quality measurements during the study are shown in Table 1.

Table 1. Results of measuring water quality parameters during the study

Treatment	Water quality parameters		
	Temperature (°C)	pH	Salinity (ppt)
A	29	8	25
B	29	8	25
C	29	8	25
D	29	8	25
E	29	8	25

Fresh water was added to the seawater to achieve homogeneity, and the desired salinity was obtained. In this study, Walne fertilizer was used as a positive control because it is a PA fertilizer (Pro-analyse) that has been standardized and widely used in the culture of *Spirulina* sp. (Hasim et al., 2022). The liquid organic fertilizer used as an alternative in this study is produced by fermenting fresh young and old Moringa leaves.

During the fermentation of Moringa leaf liquid fertilizer for *S. platensis* culture, microorganisms naturally present in Moringa leaves play a role. Rice washing water contains nitrogen and phosphorus, important elements for microalgae growth, and is therefore used as a mixture for making liquid fertilizers. Nitrogen is a source of chlorophyll-a synthesis, and phosphorus is required for cellular metabolism. Brown sugar and rice washing water were also used as additional ingredients as an energy source for the microorganisms in fertilizer (Hutauruk et al., 2023). The end result of fermenting liquid organic fertilizer from Moringa leaves was golden yellow, with a slightly fragrant smell like tapai (fermented cassava or glutinous rice).

Population Density Calculation of *S. platensis*

The population density for all treatments from H0 to H7 (seventh day) is shown in the curve in Figure 1.

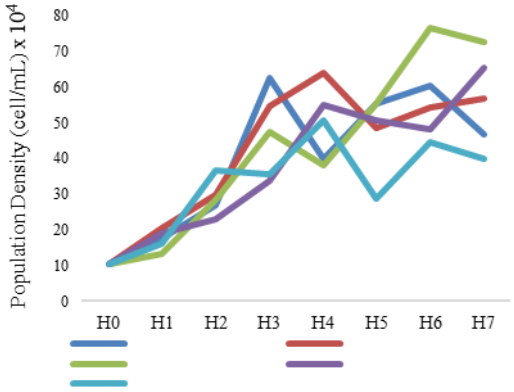

Figure 1. Population density growth of *S. platensis*

In the curve treatment C (7 mL/L) obtained the highest population density, reaching 754,000 cell/mL, compared with treatments A and B; meanwhile, treatment D (positive control) obtained a population density of 642,000 cell/mL. Based on the one-way ANOVA on the 7th day, the results were significantly different ($p<0.05$). Therefore, an LSD test was performed to compare each treatment. The results showed that treatments C and D (positive control) differed significantly from the negative control ($p<0.05$). This demonstrates the potential of 7 mL/L Moringa

leaf liquid fertilizer as an alternative fertilizer for *S. platensis* culture.

Differences in cell density resulted from the application of different fertilizers and dosages across treatments and were influenced by the nutrient content of the culture media. Cell growth of *S. platensis* passes through several phases, as shown in the curve in Figure 1. On days 0 to 1st, there is a lag phase during which the initial stock of *S. platensis* adapts to its new environment. The nutrient concentration in the new media was much higher than in the original media, so *S. platensis* had to adapt at the beginning of the culture (Fithria et al., 2022). In this phase, changes or additions to the population were categorized as remaining the same.

Days 1 to 3 had an exponential phase, during which the increase became more visible as the microalgae divided rapidly, supported by several factors, including the environment, growing media, Moringa leaf liquid fertilizer, and Walne fertilizer. According to Madigan et al. (2011), the exponential phase is characterized by rapid growth driven by increased photosynthetic activity. On the 3rd through 7th days, it experiences a stationary phase characterized by a slightly reduced, constant growth rate. Cell growth of *S. platensis* reached its peak on day 6. In the stationary phase, cell density continues to increase, leading to competition for light and nutrients. The number of living and dead cells was in equilibrium, so the cell concentration tends to be constant (Barsanti & Gualtieri, 2014).

Results of population density calculation of *S. platensis* in each treatment showed that the highest cell population density growth was in treatment C (7 mL/L), with the density value reaching 754,000 cells/mL, and the lowest cell population density was in treatment E (negative control), with population density reaching 498,000 cells/mL. Based on the LSD test, the potential of Moringa leaf liquid fertilizer was comparable to Walne fertilizer ($p>0.05$). This suggests that Moringa leaf liquid fertilizer at a concentration of 7 mL/L can be a potential nutrient source for *S. platensis* cell growth.

Walne fertilizer contains boron, which helps maintain cell pigments. This corresponds to the color of the culture media, which is deep green, and is caused by the green leaf substance (chlorophyll) (Wahyuni et al., 2019). Liquid fertilizer from Moringa leaves contains nitrogen, which is used as a nutrient for the growth of decomposing microorganisms. In addition to these minerals, the Moringa leaf also contains the cytokinin hormone, which can promote cell division and growth, slow cell aging, and stimulate the growth of new cells (Krisnadi, 2012).

Measurement of Chlorophyll-a Content

Chlorophyll-a content measurements were taken every day for 7 days, and the results of the chlorophyll content measurements could be seen based on the curve in Figure 2.

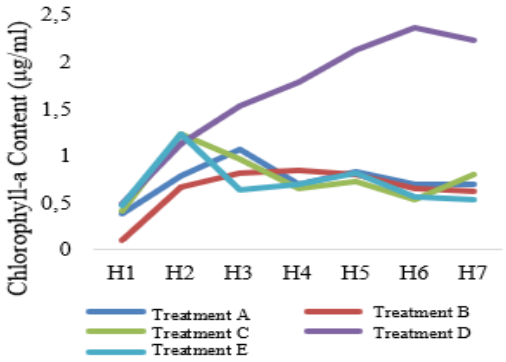

Figure 2. Curve of the chlorophyll-a content of the *S. platensis* culture

Chlorophyll-a content is the highest on the 6th day of treatment D (1 mL/L Walne fertilizer), reaching 2,355 µg/mL. Meanwhile, the addition of Moringa leaf liquid fertilizer resulted in the highest chlorophyll-a content on the 2nd day of treatment C (7 mL/L), reaching 1,230 µg/mL. Based on the one-way ANOVA of the given treatments, which showed significant differences ($p<0.05$), the LSD test was performed. The results showed that treatment C was not significantly different from the negative control ($p>0.05$), and only treatment D was significantly different ($p<0.05$).

Measuring chlorophyll-a content is one of the parameters used to determine water fertility. Measuring chlorophyll-a content could also reflect the biomass of phytoplankton in a water body (Hadiningrum, 2018). The highest chlorophyll-a content was obtained when giving 1 mL/L Walne fertilizer (treatment D), namely 2,355 µg/mL, while giving 7 mL/L Moringa leaf liquid fertilizer (treatment C) resulted in a chlorophyll-a content of 1,230 µg/mL, which is higher than the addition of 3 mL/L (treatment A) and 5 mL/L (treatment B). However, it was lower than the chlorophyll-a content in treatment D.

A *S. platensis* culture supplemented with Moringa leaf liquid fertilizer showed that chlorophyll-a content remained relatively constant from day 1 to day 7, as shown in Figure 2. The low chlorophyll-a content in *S. platensis* cultures given Moringa leaf liquid fertilizer was suggested to be due to an inability to absorb the Moringa leaf extract properly. According to Oktaviani et al. (2017), the synthesis of chlorophyll-a could proceed well if the availability of nitrogen in the growth medium was sufficient. The nutrients in Moringa leaf extract cannot be directly used by *S. platensis* and must undergo a fixation process. Meanwhile, the nutritional value of Walne fertilizer was easily absorbed as it is in ionic form (Wahyuni et al., 2019).

The Correlation of Population Density and Chlorophyll-a Content of *S. Platensis*

Several sources state that salinity affects population density and chlorophyll-a content. The higher the salinity, the higher the population density, but chlorophyll-a content decreases (Anggraeni et al., 2022). However, in this study, the results for treatment D (Walne fertilizer) in S. platensis culture showed a significant increase in chlorophyll-a content ($p<0,05$). Based on this, the low chlorophyll-a content in cultures receiving 7 mL/L Moringa leaf liquid fertilizer may be due to the nutritional content not being optimal for increasing chlorophyll-a in *S. platensis*. The cell population density obtained was the highest, but it was not balanced with chlorophyll-a content; it was known that the cells produced were not productive (not fertile).

4. Conclusion

The addition of liquid fertilizer made from Moringa leaves positively affects *S. platensis* population density, with an optimal concentration of 7 mL/L, and population density reached 754.000 cells/mL on the 6th day. The highest chlorophyll-a content was obtained in treatment D (positive control) at a concentration of 1 mL/L, with a concentration of 2.355 µg/mL.

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