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Growth of *Arthrospira platensis* with Different Nitrogen Sources

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Abstract: Microalgae is a promising and innovative biological resource. Indonesia has the potential for the development of microalgae production. One of the microalgae that have high economic value is *Arthrospira platensis*. In the production of microalgae, the development of suitable media and environmental conditions need to be factors that must be considered. This study aimed to analyze the effect of different nitrogen sources and nitrogen doses and their interaction on the growth of microalgae *Arthrospira platensis* cultured in fresh water. In addition, analyzing the water quality of *Arthrospira platensis* culture media. This study used an experimental method of Completely Randomized Factorial Design) with two factors. The first factor is the nitrogen source (Sodium nitrate, Potassium nitrate, and Calcium nitrate). The second factor is the dose of nitrogen source (2 g/l.2.5 g/l.3 g/l). The main parameter observed was microalgae growth, while the supporting parameter was the water quality which affected the growth of *Arthrospira platensis*. The highest density results were found in the provision of a nitrogen source of $Ca(NO_3)_2$ 3 g/l, which was 370,000 cells/ml. The results of the ANOVA test showed that the type of nitrogen source, the dose, and the interaction between the two affected the specific growth rate of *Arthrospira platensis*. The environmental conditions during culture ranged from 20.2°C-26.2°C, pH values 8.95 – 10.18, and dissolved oxygen 9.17 – 9.77 mg/L.

Keywords: *Arthrospira platensis*; Growth; Nitrogen source; Water quality

Introduction

Microalgae are biological resources that have become promising and innovative new food sources and functional products in the 21st century (Ye et al., 2018). Indonesia is a tropical country crossed by the equator and only has two seasons, namely, the rainy and dry seasons. These conditions allow autotrophic organisms, including microalgae, to thrive with a light intensity of 12 hours per day (Nur, 2014). Risjani et al. (2021) indicate Indonesia's diversity of microalgae and diatoms has a higher index than tropical oceanic islands such as the Galapagos and Martinique. As a tropical country, Indonesia has prospects for developing microalgae culture and production on a larger scale (Prasadi, 2018).

Arthospira (Spirulina) is a microalga with considerable popularity in the health, food, and aquaculture sectors (Soni et al., 2019). The commercial production of *Arthrospira* has received worldwide attention for use in human food supplements, animal feeds and pharmaceuticals. In aquaculture, *Arthrospira* is used as a feed additive to increase growth, feed efficiency, carcass quality, and physiological response to disease in several fish species.

Arthrospira platensis is multicellular blue-green microalgae (prokaryotes) belonging to the Cyanophyta phylum. *A. platensis* is 50-500 µm long and 3-4 µm wide (Jung et al., 2019). *Arthrospira platensis* contains high protein, gamma-linolenic acid, and phycocyanin pigments (Fradinho et al., 2020). According to Suharyanto et al. (2014), *A. platensis* contains 55-70% protein, 17-25% carbohydrates, and 4-6% lipids. *A. platensis* also contains unsaturated fatty acids, namely linoleic acid and gamma linoleic acid, several vitamins, including nicotinic acid, riboflavin, thiamin, cyanocobalamin, minerals, amino acids, and active ingredients such as chlorophyll, phycocyanin, and carotenoids.

Several factors must be considered before undertaking microalgae production, including the

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selection of culture methods, the development of suitable media, and environmental variables (Veza et al., 2021). The main factor that can affect growth is nutrient (nitrogen) (Juneja et al., 2013). Nitrogen plays a vital role in cellular metabolism through energy transfer efficiency in photosynthesis (Rahayu et al., 2022). Microalgae can use organic nitrogen sources (urea and amino acids) and inorganic nitrogen (ammonia, nitrite, and nitrate) (Li et al., 2019). In addition, the growth of *Arthrospira* sp. is also influenced by environmental factors, namely temperature, pH, and dissolved oxygen (Muliani et al., 2018).

This study aimed to analyze the effect of adding different types of nitrogen sources, different doses, and the interactions between the two on the specific growth rate of *Arthrospira platensis* cultivated in fresh water. In addition, analyzing the water quality of *Arthrospira platensis* culture media. Determination of the proper nutrients and water quality is expected to maximize growth and become information for freshwater *Arthrospira platensis* production activities.

Method

Sterilization of Tools and Materials

First, *A.platensis* culture activities are carried out by sterilizing tools and materials to free them from living microorganisms that interfere with the research process (Suyoso et al., 2022). In this study, sterilization of 5L jars used chlorine and Na-Thiosulfate. At the same time, the aeration stone, hose, and zarrouk media were sterilized using an autoclave (1 atm and a temperature of 105°C for 15 minutes). Fresh water used as a culture medium comes from the Faculty of Fisheries and Marine Sciences, Brawijaya University. Freshwater sterilization by filtering using a filter cloth.

Preparation of A.platensis Culture

In this study, *A.platensis* was cultured using fresh water from Topspira Spirulina Figure 1. The strain used by *A.platensis* with Accession No.MG912588.1. Stock culture of *A.platensis* was grown with 1 ml/l zarrouk medium. *A.platensis* inoculant aged four days was cultivated with a volume of media and 3.5 L of inoculant. The culture conditions were with the light intensity of 2.500 lux, lighting of 24:0 light/dark cycle, and continuous aeration.

Cultures of *A. platensis* have various types of nitrogen sources, namely sodium nitrate ($NaNO_3$), potassium nitrate (KNO_3), and calcium nitrate ($Ca(NO_3)_2$) in zarrouk media. Treatment of each type of nitrogen source using three doses (2 g/l, 2.5 g/l, and 3 g/l).

Figure 1. *A. platensis* under a microscope with a magnification of 400x (Research documentation, 2022)

Experimental Design

The experimental design used in this study was a factorial complete randomized design (FCRD) with twelve treatments and three replications. The following is a research design which can be seen in Table 1.

Table 1. Research Design

Factor A	Factor B	Repeat		
		1	2	3
K (without nutrient)	-	K1	K1	K1
	-	K2	K2	K2
	-	K3	K3	K3
$NaNO_3$	2 g/L	A	A	A
	2.5 g/L	B	B	B
	3 g/L	C	C	C
$Ca\ NO_3)_2$	2 g/L	D	D	D
	2.5 g/L	E	E	E
	3 g/L	F	F	F
KNO_3	2 g/L	G	G	G
	2.5 g/L	H	H	H
	3 g/L	I	I	I

The dose determination is based on the standard composition of the nitrogen source in zarrouk media, namely 2.5 g/l.

Density Calculation of A.platensis

The density of *A.platensis* was calculated using a haemocytometer using the Equation 1 (Erdawati et al., 2020).

$$D = \left\{\frac{N_1 + N_2}{2} \times \frac{(25\ X\ 10^4)}{n}\right\} \times DF \quad (1)$$

Where D is the density of *A.platensis* (cells/ml), N_1 is the number of cells of a particular species in the upper field of view, N_2 is the number of certain species in the lower field of view, n is the number of fields of view, $25x10^4$ is the haemocytometer constant, and DF is the dilution factor.

Calculation of the Specific Growth Rate of A.platensis

Calculating the specific growth rate was carried out from the growth at the start of the culture to the exponential phase. To find out the specific growth rate (K') can be calculated by the Equation 2 (Moheimani et al., 2013).

$$K' = \frac{ln(N_t - N_0)}{t} \quad (2)$$

Where K' is the specific growth rate (day^{-1}), N_0 and N_t are the initial density at time 0 and density at time t (cells/mL), and t_2 and t_1 are time intervals (days).

Measurement of Water Quality Parameters

Temperature and dissolved oxygen (DO) measurements were carried out using a DO meter (Lutron PDO-520). pH measurements were carried out with a pH meter (ATC PH-009). Light intensity was measured using a lux meter (Benetech-GM1010). Water quality parameters were measured every day during the trial period.

Data analysis

Density results and parameters supporting the growth of A.platensis were analyzed descriptively. Data from observations of the specific growth rate of A.platensis were processed statistically using ANOVA with a significance level of 5%. If they are significantly different, the least significant difference test is performed. The data obtained was analyzed using Microsoft Excel 2021.

Result and Discussion

Growth of A.platensis

Growth of *A. platensis* given different nitrogen sources, namely $NaNO_3$, KNO_3, and $Ca(NO_3)_2$ at doses of 2 g/l, 2,5 g/l, and 3 g/l, showed different logarithmic cell numbers during the culture period. The growth of *A. platensis* can be observed visually by changing the color of the medium from green to yellow. The growth of *A. platensis* is described by calculating microalgae cell density and specific growth rate. Cell density is one of the parameters used to determine the success rate of microalgae production. Figure 2 shows the results of observations of *A. platensis* cell density.

Based on Figure 2 the initial density of *A. platensis* microalgae at 50.000 cells/mL increased with increasing culture time. At the beginning of the *A. platensis* culture, all treatments experienced a lag phase (adaptation) that lasted less than 24 hours. According to Hassim et al. (2022), adaptation time for microalgae is influenced by environmental factors and the amount of inoculant. During this phase, *Arthrospira* sp. remains active, carrying out photosynthesis and undergoes metabolism, but cell division does not occur. *Arthrospira* sp. makes adjustments to the new environment. In this lag phase, *Arthrospira* sp. has an enzyme or coenzyme deficiency. Cell biochemical activity will take place when enzymes or coenzymes are synthesized. The synthesized enzymes can be enzymes that can absorb nutrients (Lesmana et al., 2019).

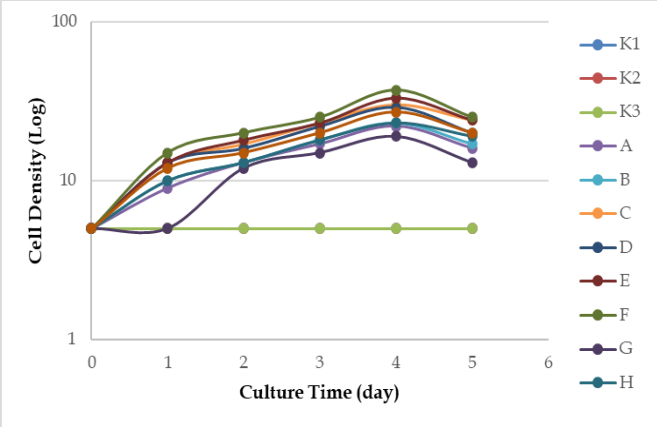

Figure 2. Cell density *A. platensis*

Arthrospira platensis culture from day 1 to day 4 underwent a log (exponential) phase with an increase in the number of cells in each treatment. During this period, microalgae experience peak growth. Due to the utilization of nutrients in the media, the amount of nitrogen available in the media is large enough to be used for biosynthesis and fast cell metabolism (Piu et al., 2018). During the exponential phase, microalgae experience rapid growth, and cell division occurs when there are many cells (Yousuf, 2020).

After the exponential phase of *A. platensis*, it enters the stationary phase. At this stage, the reproduction rate equals death (Viqram et al., 2018). The stationary phase in this study took place quickly and was not observed at 24-hour intervals, so it was not visible. The density of A. platensis decreased on the 5th day, so it entered the death phase. Microalgae cell death is caused by a lack of nutrients in the medium. However, still-alive cells cannot grow and only survive (Ukhty, 2018).

The highest mean maximum cell density was obtained in the treatment of adding 3 g/l $Ca(NO_3)_2$ nitrogen source, namely 370.000 cells/ml, and the lowest in the 2 g/l KNO_3 treatment for 191.667 cells. /ml. It is suspected that the administration of $Ca(NO_3)_2$ at a dose of 3 g/l contains Ca^{2+} and NO_3^- ions and has a higher nitrogen-nitrate ratio. The element nitrogen itself plays an important role in the growth process. Appropriate addition of nitrogen and environmental factors that support can induce optimal photosynthesis to increase the growth of *Arthrospira* sp. Calcium ions also respond to cyanobacteria heterocysts that bind nitrogen (Alghanmi et al., 2019).

Table 2. The Average Specific Growth Rate of *A.platensis*

Treatments	Specific Growth Rate (Day^{-1})
K1	0.058±0.017^{ab}
K2	0.056±0.020^{a}
K3	0.061±0.031^{c}
A	0.292±0.026^{de}
B	0.301±0.002^{g}
C	0.356±0.001^{j}
D	0.348±0.000^{hi}
E	0.380±0.001^{k}
F	0.400±0.000^{l}
G	0.261±0.071^{d}
H	0.308±0.006^{f}
I	0.334±0.004^{h}

The specific growth rate describes the speed of algal cell growth per unit of time as a benchmark to determine the carrying capacity of the media for microalgae growth (Viqram et al., 2018). Based on Table 1 from the statistical analysis results, some treatments were not significantly different and significantly different from the specific growth rate. The specific growth rate is caused by the different or the same ability of *A. platensis* to absorb nutrients. According to Tangguda et al. (2019), the specific growth rate is influenced by the ability of microalgae to utilize nutrients in different media every day. Sometimes the concentration of the material that is too high makes it difficult for the material to be absorbed by the cells (Afriza et al., 2015).

The highest average specific growth rate of *A. platensis* was 0,400 day^{-1} given a $Ca(NO_3)_2$ 3 g/l nitrogen source. The lowest average specific growth rate was 2 g/l KNO_3 of 0,261 day^{-1}. $Ca(NO_3)_2$ 3 g/l has a higher nitrate percentage than other nitrogen sources. Oktaviani et al. (2017) state that the higher the nitrate content in the culture media, the higher the microalgae growth rate. Since nitrate is an essential macronutrient used to form chlorophyll, a high nitrate intake promotes cell division and photosynthesis.

Parameters Supporting the Growth of A.platensis

The growth of *A.platensis* depends on several factors, namely light intensity, pH, temperature, dissolved oxygen, and nutrient availability (Jesus et al., 2018). Following are the results of water quality during *A.platensis* culture.

The temperatures of all treatments are presented in Figure 3. The temperatures ranged from 20.2°C-26.2°C. This range is still in the optimal temperature category for the growth of *A. platensis*. This follows the statement of Mutia et al. (2021) that the optimal media temperature for the growth of *A. platensis* ranges from 20°C– 30°C. Temperature is an important factor affecting microalgae growth, photosynthetic rate, and biomass composition (Carneiro et al., 2020). The optimum temperature will positively affect the process of photosynthesis and cell division with enzymatic activity such as the Calvin cycle (Serra-Maia et al., 2016). An increase in temperature causes an increase in cell activity so that metabolism takes place more quickly. High temperatures that exceed the maximum temperature will disrupt metabolism (Addini et al., 2017). When the temperature reaches 20°C, the culture of *Arthrospira* sp. will experience a decrease in growth (Arahou et al., 2021). A decrease in temperature can cause a decrease in growth rate and death (Ilhami et al., 2015).

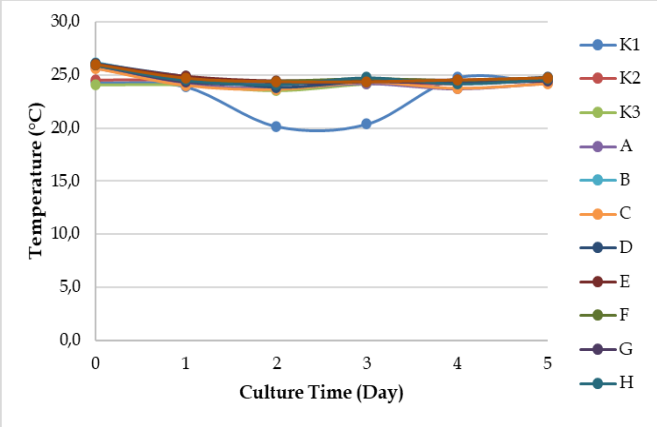

Figure 3. The temperature in an *A. platensis* culture

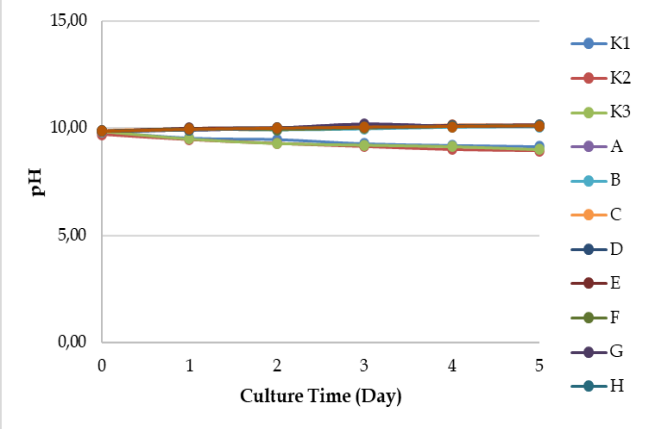

Figure 4. pH in A *A. platensis* culture

The average pH observed during the study Figure 4 ranged from 8.95 to 10.18, which is in the optimal range. This is in line with Soni et al. (2019), *Arthrospira* sp. will grow optimally at a pH value of 8.5-10.5. pH directly influences the physiological properties of microalgae and nutrient availability (Usharani et al., 2012). This increase in the pH of the culture media is caused by free CO_2 (inorganic carbon), which is used as the main raw material in the photosynthesis process (Ilhami et al., 2015). In addition, the absorption of bicarbonate ions (HCO_3^-) by microalgae will also increase the pH to become alkaline (Choi et al., 2017). Extreme pH in microalgae culture media will inhibit the availability of CO_2 and the growth of microalgae (Brindhadevi et al., 2021).

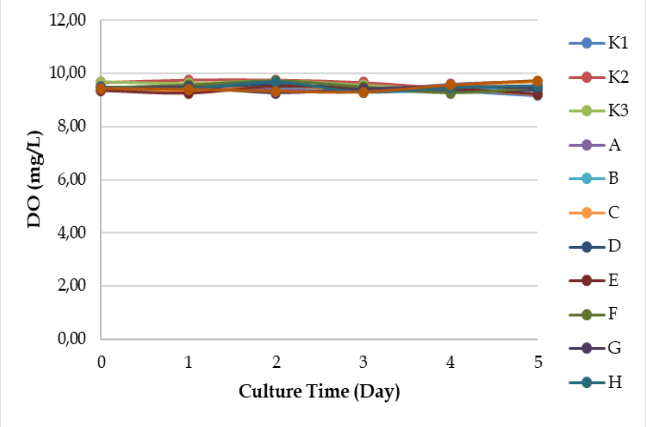

Figure 5. Dissolved oxygen (DO) in a *A. platensis* cultur

The average DO value in *A. platensis* culture media during the study ranged from 9.17 to 9.77 mg/L. DO value is still in the optimal range for the growth of *A. platensis*. According to Buwono et al. (2018), the DO value for microalgae growth ranges from 3-10. Dissolved oxygen is an indicator of photosynthetic activity by microalgae. The increase and decrease in DO are thought to be influenced by population growth, and the larger the microalgae population in culture, the more O_2 is produced through photosynthesis. Low dissolved oxygen will affect the growth of microalgae (Morales et al., 2018). According to Arsad et al. (2020), the decrease in DO was due to a decomposition process by many microalgae experiencing a death phase.

Conclusion

The results showed that the type of nitrogen source, the dose of nitrogen, and the interaction between the two affected the specific growth rate. *A. platensis* cultivated in freshwater produced more optimal density and growth rate using a $Ca(NO_3)_2$ 3g/l nitrogen source, namely 370.000 cells/ml and 0,400 day^{-1}. Environmental conditions with temperatures ranging from 20.2°C-26.2°C, pH value 8.95–10.18, and dissolved oxygen 9.17–9.77 mg/L are still within the optimal range for the growth of *Arthrospira platensis*.

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