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EFFECT OF UREA CONCENTRATION ON THE GROWTH OF CELLS OF *Nannochloropsis* sp. LABORATORY SCALE

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ABSTRACT

This research was conducted from October 2021 to January 2022 in the Natural Feed Laboratory of the Brackish Water Cultivation Fisheries Center (BPBAP) Ujung Batee, Aceh. This study aimed to determine whether urea has an effect and the best urea concentration on the growth of *Nannochloropsis* sp. This study used an experimental method with one-factor RAL (Completely Randomised Design), consisting of 4 levels of treatment and control with three replications so that 15 experimental units were obtained. The treatments tested were the uses of urea 40 ppm (treatment A), urea 60 ppm (treatment B), urea 80 ppm (treatment C), urea 100 ppm (treatment D), and Walne 1 ml (control). The population density of *Nannochlropsis* sp. was found in treatment D on day 4 (2414 x 10^4 cells/mL), and the lowest population growth was shown in treatment A (1177 x 10^4 cells/mL). The highest absolute growth rate occurred in treatment D (2229x 10^4 cells/mL), while the highest relative growth rate occurred in treatment D (1205%), and the highest specific growth rate occurred in treatment B (79% per day).

Keywords: *Nannochloropsis* sp., Urea, Density.

1. INTRODUCTION

Natural phytoplankton food is essential in providing a source of protein and nutrition for larvae. Apart from having complete nutritional value, natural food is also easy to digest, not because of a decrease in water quality in fish cultivation containers, and the pollution level in culture water will be lower than using artificial food. One type of natural feed widely used in aquaculture, especially in fish hatcheries, is *Nannochloropsis* sp. This is because *Nannochloropsis* sp. has nutritional content, namely 50–55% protein, 16% carbohydrates, 28.3% fat, and 0.05% chlorophyll-a[1], as well as fast growth.

The price of pro-analysis fertiliser used in phytoplankton culture is relatively expensive, so alternative fertilisers, including agricultural fertiliser, are needed at more affordable prices[2]. With a more affordable price and easy to obtain, urea fertiliser can be used in phytoplankton culture.

Urea has a function and role as a nitrogen source for *Nannochloropsis* sp cells, which helps accelerate growth. According to Rusyani[3], nitrogen, as the main constituent of protein, can be given to *Nannochloropsis* sp. in the form of urea ($(NH_2)_2CO$). Urea fertiliser is easily soluble in water and is hygroscopic. The N content in urea fertiliser is 46%[4]. Urea fertiliser has also been widely used in microalgae culture research and has been proven to increase population density.

Previous research shows that phytoplankton need nitrogen for their development, both in large and relatively small amounts. Therefore, the nitrogen concentration in the culture medium can determine the growth of the *Nannochloropsis* sp. cell population. So,

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the concentration of urea fertiliser as an alternative fertiliser needs to be studied

2. RESEARCH METHOD

Time and Place

This research was carried out from October 2021 to January 2022. Culture analysis and cell counting were conducted at the Natural Feed Laboratory, Ujung Batee Brackish Water Aquaculture Fisheries Center (BPBAP), Aceh.

Method

The method used in this research is an experimental method using one-factor CRD (Completely Randomised Design), consisting of 4 treatment levels and control with three repetitions so that 15 experimental units were obtained. In this study, all conditions were the same except for the urea fertiliser. The treatment in this study was the administration of different urea concentrations. Each urea fertiliser concentration treatment given was Control (Walne 1mL), A (40 ppm), B (60 ppm), C (80 ppm), and D (100 ppm).

This research uses a lottery randomisation technique with a 3 x 5 layout. This research uses LED lights, available in each row of culture racks for 24 hours every day and are expected to provide the same light to each experimental unit.

Procedure

Preparation of Culture Media

Phytoplankton culture equipment is sterilised by washing it with soap until it is clean and then autoclaving it. Plastic culture equipment is sterilised by boiling in fresh water at 100-150°C for 15-30 minutes, inning until dry, then drying with 70% alcohol. Meanwhile, the glass culture equipment was autoclaved and dried, then sprayed with 70% alcohol *Nannochloropsis* sp. culture media. A laboratory scale using seawater with a salinity of 30 ppt has been sterilised using reverse osmosis and autoclaved. A culture container of 15 Erlenmeyer flasks was prepared.

Fertiliser Formulation

Fertiliser used in culture *Nannocloropsis* sp. for all treatments, namely Walne fertiliser 1 mL (control), vitamin B12 1 mL, and urea with various concentrations.

Nannochloropsis sp. Culture.

This research was conducted in a controlled room (laboratory scale). Before starting a research, first, carry out a pure stock culture. After the pure culture stock reaches the exponential phase, research is then carried out. The stages in culturing *Nannochloropsis* sp. are as follows.

A culture container was prepared with 15 Erlenmeyer flasks with a volume of 1000 ml. The Erlenmeyers are placed randomly. Sterile culture media water with a salinity of 30 ppt was added in 800 mL per Erlenmeyer flask. Add 1 mL of Walne fertiliser to the control and 1 mL of vitamin B12 to all treatments and urea fertiliser with different doses, namely 40 ppm of urea (treatment A), 60 ppm (treatment B), 80 ppm (treatment C), and 100 ppm (treatment D) using a dropper in each culture container.

Nannochloropsis sp. 200 mL was put into the culture container with an initial density of 185 x 10^4 cells/mL originating from pure culture stock with a seed density of 925 x 10^4 cells/mL in a 3000 ml volume. The container is shaken slowly until the *Nannochloropsis* sp., the fertiliser (walne and urea), and the vitamin B12 given are evenly distributed. Calculation of the initial density of *Nannochloropsis* sp. using the formula[5]:

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

Information :

V1 : Initial stocking seed volume (mL)
V2 : The volume of culture media (mL)
N1 : Density of seeds/stock (cells/mL)
N2 : Seedling density (cells/mL)

The aeration hose connected to the aerator is inserted, and the Erlenmeyer is covered using aluminium foil. Make a

treatment label and culture date, then place the Erlenmeyer on a culture rack equipped with light. Next, water quality measurements were carried out for each treatment and control. This water quality measurement is carried out every day before observing the cells in the microscope.

Observation of the Growth of *Nannochloropsis* sp.

Calculation of cell density of *Nannochloropsis* sp. for six days using a microscope with 10x magnification. According to Mudjiman[6], the density of *Nannochloropsis* sp. can be calculated using the formula:

$$D = \frac{n1+n2+\cdots+nx}{X} \text{ X } 25 \text{ X } 10^4 \text{ cells/mL}$$

Information :
D : Phytoplankton density (cells/mL)
n : Number of phytoplankton in the box
x : Number of boxes

Test Parameters

Absolute Growth

According to Sopian et al.[7], absolute growth is determined using the formula:

$$G = Wt - Wo$$

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

Relative Growth Rate

According to Sopian et al.[7], the relative growth rate is determined using the formula:

$$RGR = ((Ct - C0) / C0) \text{ x } 100\%$$

Information :
RGR : Relative growth rate (%)
C0 : Cell population density (cells/mL) at the beginning of the observation period
Ct : Cell population density (cells/mL) at the end of the observation period

Specific Growth Rate

According to Becker[8], the specific growth rate is determined using the formula:

$$SGR = \frac{\text{Ln Nt} - \text{Ln No}}{t} \text{ x } 100\%$$

Information :
SGR : Specific Growth Rate (%/day)
No : Initial population density (Ind/L)
Nt : Final population density (Ind/L)
t : Time (day)

Data Analysis

To compare the growth of *Nannochloropsis* sp. At different urea fertiliser concentrations, analysis was done using a one-way Analysis of Variance (ANOVA). If it shows a real difference, the Least Significant Difference (LSD) test is then carried out. Next, it is compared based on literature related to microalgae growth.

3. RESULT AND DISCUSSION

Population Density

Growth of *Nannochloropsis* sp. those cultured on a laboratory scale experienced the highest peak density in treatment D on day 4 of 2414 x 10^4 cells/mL. Meanwhile, the lowest peak density was in treatment A on day 4, as much as 1177 x 10^4 cells/mL, as seen in Table 1.

Based on observations, urea fertiliser as a nitrogen source can produce cell population density of *Nannochloropsis* sp. The highest was in treatment D (urea 100 ppm). Treatment D was able to compete with the growth of the control population using Walne fertiliser. Meanwhile, in treatments A (urea 40 ppm), B (urea 60 ppm), and C (urea 80 ppm), the growth process still occurred, but it was lower than in treatments D and Control. This is because treatments A, B, and C have lower urea concentrations than treatment D. This aligns with the statement Arfah et al.[9] that the limited amount of nitrogen in the growth medium will inhibit the photosynthesis process, affecting population density.

Next, the ANOVA statistical test was carried out. Based on the results of the ANOVA test, results were significantly

different on the 4th, 5th, and 6th days, which had a significant value of <0.05, which means H0 was rejected and H1 was accepted, so it was known that there was an influence of urea fertiliser concentration on cell population growth. Next, a Least Significant Difference (LSD) test was carried out to determine the differences between each treatment. The observation results show that the population growth of *Nannochloropsis* sp. produces a growth pattern that increases daily until the peak density, as shown in Figure 1.

Table 1. The average population density in *Nannocloropsis* sp culture

Treatment	Population Density x10^4 cells/mL						
	H0	H1	H2	H3	H4	H5	H6
K	185	938	1158	1687	2247	1647	767
A	185	928	990	1092	1177	632	179
B	185	912	900	1467	1072	510	176
C	185	838	993	1787	1363	647	185
D	185	803	998	1752	2414	957	435

Information: H0 (Initial density); H1 (Day 1); H2 (Day 2); H3 (Day 3); H4 (Day 4); H5 (Day 5); H6 (Day 6)

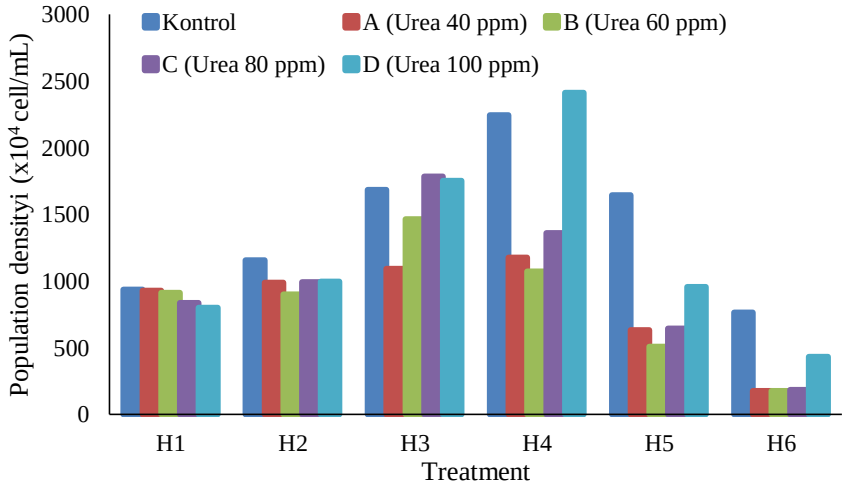

Figure 1. Population density of *Nannochloropsis* sp

Based on the population growth graph of *Nannochloropsis* sp. On day 1, *Nannochloropsis* sp. experiences an adaptation or lag phase because these microalgae cells are still adapting to the culture media, characterised by cells that are not reproducing. According to Utami et al.[10], cells adjust to the culture medium in the adaptation or lag phase.

On day 2, the highest population growth was obtained by control of 1158 x10^4 cells/mL; the lowest was in treatment B, 900x10^4 cells/mL. Population growth continues to increase because the adaptation phase has run typically and is utilising the nutrients in the culture media to increase the number of cells. The nutrients in the culture media are still very abundant and have begun to be utilised by *Nannochlroropsis* sp.[11].

On day 3, the peak cell population density of *Nannochloropsis* sp. occurred in treatment B as much as 1467 x10^4 cells/mL and in treatment C as much as 1787 x10^4 cells/mL. Meanwhile, treatments A, D, and Control experienced peak density on day 4: treatment A was 1177 x10^4 cells/mL, treatment D was 2414 x10^4 cells/mL, and control was 2247 x10^4 cells/mL. On days 3 and 4, *Nannochloropsis* sp. experiences an exponential phase, characterised by the number of cells at peak density reaching

more than double. According to Mukhlis et al.[12], the exponential phase is characterised when density is at its peak, and there is an increase in cell population density by one order of magnitude or more from the initial density.

Nannochloropsis sp experienced a phase of decreasing growth rate on the 4th day in treatments B and C and on the 5th day in treatments A, D, and control, allegedly due to the ability of *Nannochloropsis* sp. to absorb different nutrients. This is in accordance with the statement of Arfah et al.[9] that the difference in daily growth for each treatment is caused by the ability of the cells to absorb the nutrients contained in the culture media. Furthermore, the highest decrease in the population growth rate of 60% occurred in treatment D (urea 100 ppm) to 957 $x10^4$ cells/mL, and the lowest of 24% occurred in treatment C (urea 80 ppm) to 1363 $x10^4$ cells/mL. In this phase, *Nannochloropsis* sp cells. Experiencing a decrease in density, it is thought that the nutrient content in the culture media has decreased or is in limited quantities. In this phase, population density experienced a significant decrease due to the reduced presence of nutrients in the treatment medium[13].

When observing the culture of *Nannochloropsis* sp. This means that there is no visible stationary phase. It is suspected that the stationary phase occurs when no observations are being made. The stationary phase balances the growth rate with the death rate[14].

In this study, *Nannochloropsis* sp. experiencing the death phase on the 6th day is thought to be caused by very little nutrition because *Nannochloropsis* sp. utilises it for growth. At the same time, the addition of nutrients was not carried out during the research, thus triggering competition for nutrients and oxygen. This is to the statement Riduan et al.[15] stating that nutrients dissolved in water bodies are directly utilised by phytoplankton for population growth so that the nutrients in the culture media continue to decrease.

Based on the results of observations that have been made, urea fertiliser as a nitrogen source can produce cell population density of *Nannochloropsis* sp. The highest was in treatment D (urea 100 ppm). Treatment D was able to compete with the growth of the control population using Walne fertiliser. Meanwhile, in treatments A (urea 40 ppm), B (urea 60 ppm), and C (urea 80 ppm), the growth process still occurred, but it was lower than in treatments D and Control. This is because treatments A, B, and C have lower urea concentrations than treatment D. This aligns with the statement of Arfah et al.[9] that the limited amount of nitrogen in the growth medium will inhibit the photosynthesis process, affecting population density.

Nitrogen is the most essential nutrient for microalgae after carbon and has a role in microalgae growth. Optimal nitrogen application can increase microalgae growth, protein synthesis, and chlorophyll formation[16], as well as microalgae metabolism protein and carbohydrate formation, resulting in cell growth and production increase[17].

The amount of nitrogen greatly influences population density; this is in line with Ru'yatin et al.[18], who state that the decline in phytoplankton can be caused by a reduction in nutrients so that they can no longer grow. Urea fertiliser is an artificial nitrogen fertiliser widely used in microalgae cultures. Urea contains nitrogen with the highest levels of 45-46%, which plays a role in microalgae growth.

In this study, all treatments except the control only utilised the nitrogen element from urea fertiliser. This caused microalgae growth in treatments A, B, and C not to be optimal because it was thought there was little nitrogen element, and the media did not have other nutrient reserves besides nitrogen. This also shows that to produce optimal microalgae growth, the culture medium must have high nitrogen content, and apart from nitrogen, microalgae also need other macronutrient reserves.

Phosphorus is one of the main macronutrients that have a vital role in cell metabolic processes. It forms various structural and functional components needed by cells for average growth and development of microalgae, where its content in cells is around 1% of dry weight[12]. Mukhlis et al.[12] state nitrogen's importance, which must be balanced with phosphorus availability for microalgae growth. Fakhri et al.[19], carbon, nitrogen, and phosphorus are the main factors that can influence microalgae growth.

This study used Walne fertiliser as a control. Walne fertiliser is better for increasing green microalgae growth than Guillard media because of its better nutritional content[20]. The use of this fertiliser in microalgae culture is also considered more practical because its nutrient content is ultimately compared to organic fertilizer[21].

Absolute, Relative, and Specific Growth

When observing N*annochloropsis* sp. cells. Show different growth results. The highest absolute growth average was in treatment D at 2229 x10^4 cells/mL, and the lowest was in treatment A at 992 x10^4 cells/mL. The absolute growth rate of *Nannochloropsis* sp. can be seen in Figure 2.

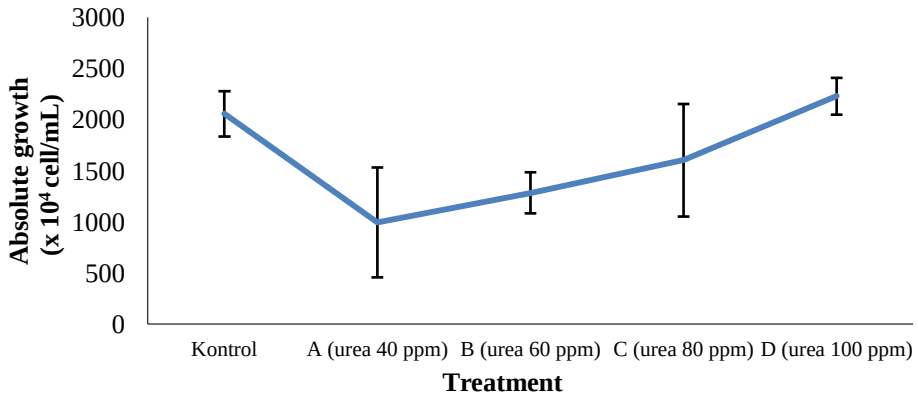

Figure 2. Absolute growth

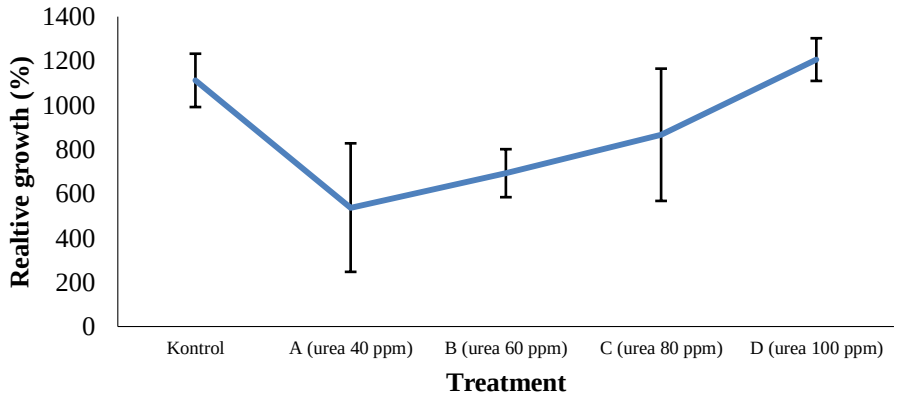

Figure 3. Relative growth

Based on the calculation results, it is known that treatment D produces the highest average relative growth, namely 1205%. Control shows the following order, namely 1115%. Treatment C was 866%, treatment B was 693%, and treatment A was 536%. A graph of the relative population growth rate of *Nannochloropsis* sp. can be seen in Figure 3.

Results of observations of *Nannochloropsis* sp cells. Shows the highest average specific growth rate, namely in treatment B, as much as 79% per day. The following order is treatment C, as much as 75% daily. Treatment D is as much

as 64% per day, control is as much as 62% per day, and Treatment A is as much as 46% per day, as seen in Figure 4.

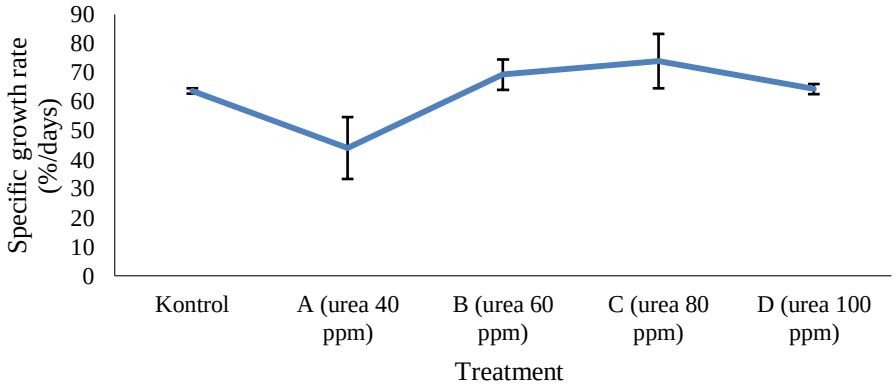

Figure 4. Specific growth

The highest absolute growth occurred in treatment D and the lowest in treatment A. In calculating the relative growth rate, the highest growth rate occurred in treatment D and the lowest in treatment A. The greater the urea concentration, the higher the absolute and relative growth rates. This is supported by the statement that urea contains nitrogen as the main constituent of protein, which is quite large, namely 46%, so that it can accelerate the growth of phytoplankton[22].

Meanwhile, treatment C had the highest specific growth, and treatment C had the lowest. This is because, on day 3, treatment C was already in the exponential phase, while treatment D experienced this phase on day 4. The difference in growth for each treatment is caused by the ability of the cells to absorb the nutrients contained in the culture media. According to the statement of Afriza et al.[23], sometimes the concentration of the material is too high, making it difficult for the material to be absorbed by cells.

Water Quality Parameters

In this study, water quality measurements were carried out every day at 11.00 WIB before sampling to observe the growth of *Nannochloropsis* sp cells. The water quality parameters measured are temperature, pH, and salinity. Results of measuring water quality parameters from 3 repetitions of measurements on *Nannocloropsis* sp culture. Each treatment can be seen in Table 3.

Table 3. Average water quality parameters in Nannochloropsis sp culture

No.	Water quality parameters	Treatment				
		A	B	C	D	K
1.	Salinity (ppt)	30	30	30	30	30
2.	Temperature (°C)	25	25	25	25	25
3.	pH	8.3	8.3	8.3	8.3	8.3

The average temperature observed from the start to the end of maintenance is 25. According to Permata & Abdul[24], the optimum temperature for phytoplankton growth ranges from 25-30. The pH value of the water media measured in this study is relatively constant in the range of 8.0 - 8. .3 according to the statement by Kurniawan et al.[25], explaining that the optimal pH range for the growth of *Nannochloropsis* sp. is 8 - 8.5. Then, the salinity value in this study remains at the optimum value for phytoplankton growth, namely 30 ppt. According to Isnansetyo & Kurniastuty[26], the optimum salinity ranges from 25-30 ppt. All water quality parameters of the culture

media were in the optimum range for the growth of *Nannochloropsis* sp., so it is assumed that the results of population density, absolute growth, relative growth rate, and specific growth rate of *Nannochloropsis* sp. The results obtained in this study were purely influenced by the administration of urea with different concentrations.

4. CONCLUSION

Based on the research results, all urea concentration treatments can be used because they can produce growth in the *Nannochlropsis* sp cell population. A concentration of 100 ppm resulted in the growth of the *Nannochloropsis* sp cell population. The highest was 2414 $x10^4$ cells/mL, obtained on the 4th day after stocking. Treatment D had the highest absolute growth rate, as much as 2229 $x10^4$ cells/mL. The highest relative growth rate occurred in treatment D, as much as 1205%, and the highest specific growth rate in treatment B, as much as 79% per day.

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