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GROWTH RATE OF *Nannochloropsis* sp ACCORDING TO ADDITING CONCENTRATION OF ZINC (ZN) AND COOPER (CU)

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ABSTRACT

Zinc and copper are micronutrients that are the factors that support microalgae growth rate besides macronutrients, CO_2, temperature, salinity, and pH. Growth of *Nannochlopsis* sp was observed to determine the influence of the addition of Zn and Cu concentration. Microalgae cultivation was located outdoors on the semi-mass scale using 800 L medium on mesophilic condition in 3 treatments with the addition of 1 ppm, 3 ppm, and 5 ppm concentrations of Zn and Cu metals. Cells density was calculated using a hemocytometer, and the absorbed metal content was measured by AAS. The study aimed to test the growth response of *Nannochloropsis* sp cells to Zn and Cu metals. The growth rate of microalgae was observed for the addition of Zn and Cu concentration in the cultivation medium. The result showed the highest *Nannochloropsis* sp growth rate for Zn addition was 0.053/day and for Cu addition was 0.279/day. Between Zn and Cu addition, the highest growth rate was observed in the addition of 1 ppm Cu metal (Cu1) which was equal to 0.279/day. This shows that adding micronutrients are not automatically will increase the growth rate of microalgae as well.

Keywords: Growth Rate, Micronutrient, Zinc, Copper, *Nannochloropsis* sp.

I. INTRODUCTION

Nannochloropsis sp. is an alga measuring 2-4 m, greenish in color, not motile, and not flagellated. The cells are spherical in shape and small. *Nannochlropsis* sp. can live at a temperature of 25-35ºC, 25-35 ppt salinity, and pH 8-9.5 [1]. *Nannochloropsis* sp. is better known as marine chlorella because it has very high nutritional value, is easy to cultivate in bulk, does not cause toxins, contains antibiotics, grows relatively fast, and has good accumulation ability. The relatively fast growth process of *Nannochloropsis* and its good accumulating ability allows it to be a biosorbent of heavy metals [2].

Nannochloropsis sp. reproduces asexually by dividing. The multiplication of *Nannocholropsis* sp. cells occurs very quickly. This is due to adequate nutrient sources and supportive environmental factors such as pH, salinity, temperature, and light. Nutrient factors are one of the important factors that need to be considered in the growth of microalgae. The nutrients needed by microalgae consist of macronutrients and micronutrients. Zinc (Zn) and copper (Cu) are some of the micronutrients needed by microalgae for their growth [1].

Zinc (Zn) acts as an enzyme activator and constituent of chlorophyll and increases CO_2 fixation [3] while copper (Cu) acts as part of the phenolase, lactase,

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and ascorbate oxidase enzymes, constituent of plastocyanin which plays a role in electron transport in the process of photosynthesis [4], forming chlorophyll and plays a role in microalgae cells division. Zn and Cu besides being needed as micronutrients are essential heavy metals that are only needed by aquatic organisms in certain concentrations. If the concentration of these metals exceeds the permissible threshold, it can pose a hazard to aquatic organisms. These heavy metals can interfere with the growth of organisms in water, one of which is phytoplankton [5]. Threshold values have been set for Zn 0.05 ppm and Cu 0.008 ppm [6].

The addition of growth nutrients into the microalgae cultivation media is considered an important aspect and the most influential on the quantity and quality of biomass and microalgae growth. Several research results on the effect of the addition of Zn and Cu micronutrients on microalgae noted that the addition of certain concentrations of Zn and Cu metal ions in cultivation media could inhibit and increase the growth of microalgae cells. The interaction of Cu ions with *Chaetoceros calcitrans* showed a trend of decreasing population, which was directly proportional to the increase in Cu concentration in the cultivation medium up to a concentration of 40 ppm [7]. The same was noted in *Scenedesmus incrassatlus* [8]. It was noted that *S.obliquus* and *Desnodesmus pleiomorphus* showed varied trends in the addition of Zn between 1-45 ppm [9]. Soeprobowati and Hariyati [10] noted that the addition of Cu metal concentration in *Spirulina platensis* was different, 1 ppm Cu increased growth, 3 ppm inhibited cells growth and 5 ppm was toxic to *S. platensis*. The growth of *Cyomodecea nodosa* was inhibited by Zn and Cu ions in the cultivation medium [11].

The effect of increasing the concentration of Zn and Cu on microalgae was determined by the growth rate of microalgae by increasing the density of microalgae. It should be noted and remembered that micronutrients such as Zn and Cu are essential metals, so it is necessary to know how much concentration of micronutrients *Nannochloropsis* sp. needs.

2. RESEARCH METHOD

Time and Place

The research was carried out at the Ecology Laboratory, Polytechnic KP Karawang, West Java, and the Integrated Chemistry Laboratory, IPB Baranangsiang Campus, Bogor, West Java. The research period starts from March to June 2022.

Research Methods

Microalgae Cultivation

Research on the growth of microalgae was carried out on a semi-mass scale. The microalgae used were from the Eutigmatophyceae class, namely *Nannochloropsis* sp. The microalgae were cultivated in 16 L containers that had been given WALNE fertilizer as a source of nutrients and placed in the laboratory under controlled conditions for 7 days intended for the restocking of the inoculum used in the study. The microalgae were then cultivated in an 800 L container that had been given TSP, ZA, and Urea fertilizers as a source of nutrients and placed outdoors for 17 days. The density of *Nannochloropsis* sp at initial cultivation was 700 x 10^4 cells/mL. The generalization of the initial density of cultivated microalgae is intended as a research standard because each cell has different characteristics, survival abilities, and growth rates.

Cells Density Observation

Phytoplankton density observations were carried out every day from the beginning to the end of the study. Calculations were carried out using a haemacytometer and to facilitate the calculations, a hand tally counter was used. Microalgae samples were dripped using a dropper as much as 1 drop (0.05 mL) on a hemocytometer and observed under a microscope with a magnification of 100 or 400 times. According to [13] the density of *N. oculata* cells for each mL with a hemocytometer can be calculated using the following formula:

$$N = ni\ xx\ 104\frac{25}{5} \qquad (i),$$

where N is the cells density and ni is the number of cells counted.

Research Procedure

Treatment of Addition of Zn and Cu. Metal Concentrations

The metals or micronutrients used in this study were Zn and Cu. There are 3 treatments, namely control, the addition of Zn metal, and the addition of Cu metal. The concentrations of metal ions and Cu with three different concentrations of 1 ppm, 3 ppm, and 5 ppm were added to each pond without any mixing between the two metals or a single metal system. The addition of metal ion concentrations of Zn and Cu was carried out at the beginning of cultivation.

Data analysis

Growth rate

The growth rate of microalgae (k) was calculated using equation [12;13]:

$$k=\frac{LnNt\text{-}LnN0}{t\text{-}t0}$$

k is the specific growth rate (day-1), Nt is the density of microalgae cells at time t, No is the initial density of microalgae cells, Tt is the initial time of cultivation, and To is the end time of cultivation.

Percentage of *Nannochloropsis* sp Cells Growth Inhibition

The percentage of growth inhibition of *Nannochloropsis* sp. cells in each treatment with the addition of Zn and Cu metal ion concentrations to the control treatment was calculated by equation (9):

$$\%\ Cells\ inhibition = \frac{DC\text{-}DT}{DC}\ x\ 100\ \%$$

DC is the optimum cells density of the control treatment (x 10^4cells/mL) and DT is the optimum cells density of the treatment (x10^4 cells/mL).

Statistical analysis

The effect of different concentrations of Zn and Cu on the growth of microalgae *Nannochloropsis* sp was tested using Analysis of Variety (ANOVA) through SPSS Version 22 with a 95% confidence interval.

3. RESULT

Effect of Addition of Zn Metal Concentration.

The results of the observation of the effect of increasing the concentration of zinc metal ion (Zn) on the cultivation medium are presented in Table 1. The highest density observed in the treatment of increasing the concentration of Zn metal in the cultivation medium on the growth of *Nannochloropsis* sp. There is a Zn concentration of 3 ppm (Zn^3) with an optimum density of 1410 x 10^4 cells/mL with a growth rate of 0.053/day. The lowest density occurred in the treatment with the addition of 5 ppm. Zn metal concentration (Zn5) with an optimum density of 1290 x 10^4 cells/mL with a growth rate of 0.045/day.

Effect of Addition of Cu Metal Concentration

The results of observations of the effect of increasing the concentration of copper metal ions (Cu) on the cultivation medium are presented in Table 2. The highest density observed in the treatment of increasing the concentration of Zn metal in the cultivation medium on the growth of

Nannochloropsis sp. There is a Cu concentration of 1 ppm (Cu1) with an optimum density of 1320 x 10^4 cells/mL with a growth rate of 0.279/day. The lowest density occurred in the treatment with the addition of 5 ppm Cu metal concentration (Cu5) with the optimum density reaching 1100 x 10^4 cells/mL with a growth rate of 0.061/day.

Table 1. The growth rate of *Nannochloropsis* sp. in the addition of Zn metal

Days to-	Growth Rate (k)			
	K	Zn1	Zn3	Zn5
1	0	0	0	0
2	0.035	0.108	0.24	0.396
3	-0.014	0.187	0.091	0.052
4	0.028	0.293	0.252	0.045
5	0.097	0.073	0.016	0.115
6	0.138	-0.004	-0.056	-0.073
7	0.154	-0.3	0.087	-0.078
8	0.105	-0.446	-0.023	-0.167
9	0.121	-0.09	0.053	-0.452
10	0	0.025	0.029	
11	-0.045		0.007	
12	-0.027		-0.074	
13	0.008		-0.08	
14	-0.044		-0.087	
15	0.048			
16	0.019			
16	0.019			

Description: K: Control; Zn1: Addition of 1 ppm Zn metal; Zn3: Addition of 3 ppm Zn metal; Zn5: Addition of 5 ppm Zn metal.

Table 2. Growth Rate of *Nannochloropsis* sp in Cu. Metal Addition Treatment

Day -	Growth Rate (k)			
	K	Cu1	Cu3	Cu5
1	0	0	0	0
2	0.035	0.049	0.164	0.182
3	-0.014	0.040	0.03	0.118
4	0.028	0.094	0.211	0.156
5	0.097	0.058	-0.121	0.061
6	0.138	-0.100	-0.044	-0.084
7	0.154	0.112	0.207	-0.205
8	0.105	0.279	-0.359	-0.265
9	0.121	0.100	-0.013	0.132
10	0	-0.015	0.052	-0.154
11	-0.045	0.004	0.025	-0.063
12	-0.027	-0.068	-0.09	-0.024
13	0.008	-0.104	-0.216	
14	-0.044	0.040		
15	0.048	-0.097		
16	0.019	-0.245		

Description: K: Control; Cu1: Addition of 1 ppm Cu metal; Cu3: Addition of 3 ppm metal Cu; Cu5: Addition of 5 ppm Cu metal

Percentage of Cells Growth Inhibition of *Nannochloropsis* sp.

Table 3 shows the percentage of growth inhibition of Nannochloropsis sp. cells due to the addition of heavy metal concentrations of zinc (Zn) and copper (Cu) in the cultivation medium. The addition of zinc metal concentration (Zn) in cultivation media inhibited about 9.032 -16.774% growth of *Nannochloropsis* sp. In the addition of copper (Cu) concentration treatment, the growth inhibition of *Nannochloropsis* sp. cells reached 14.838-29.0322%. From the results of this calculation, it can be seen that the percentage of growth inhibition of *Nannochloropsis* sp. by copper (Cu) is higher than the addition of zinc (Zn) concentration in the cultivation media of *Nannochloropsis* sp.

Table 3. Percentage of Growth Inhibition of *Nannochloropsis* sp.

Microalgae	Metal	Treatment	% Cells Inhibition
*Nannnochloropsis*sp	Zn	Zn1	12,25806
		Zn3	9.032258
		Zn5	16,77419
	Cu	Cu1	14.83871
		Cu3	29,03226
		Cu5	23.87097

Description: K: Control; Zn1: Addition of 1 ppm Zn metal; Zn3: Addition of 3 ppm Zn metal; Zn5: Addition of 5 ppm Zn metal; Cu1: Addition of 1 ppm Cu metal; Cu3: Addition of 3 ppm Cu metal; Cu5: Addition of 5 ppm Cu metal

4. DISCUSSION

The density of *Nannochloropsis* sp. at each addition of metal concentrations of both Zn and Cu continued to increase until the fourth day. It is estimated that the nutrient content is still sufficient for the growth of *Nannochloropsis* sp. [1] and the ability of *Nannochloropsis* sp. cells to absorb metal concentrations. The specific growth rate (k) is the growth rate of algae during the exponential phase.

The growth rate (k) describes the speed of microalgae cells growth per unit of time to determine the carrying capacity of the medium or nutrients for the growth and division of microalgae cells. The growth rate (k) can be used as a benchmark to determine the effect of adding metal ion concentrations of Zn and Cu to the cultivation media on the growth of *Nannochloropsis* sp. cells. One of the factors that affect the growth rate is the nutrient content or nutrients contained in the cultivation media for cells activity [Myers in 15]. The growth rate of microalgae cells on the cultivation medium must be proportional to the nutrients [15].

The growth rate of *Nannochloropsis* sp. in the control treatment in this study was obtained at 0.121/day, this result was higher than the research conducted [12], which was 0.05/day. On an outdoor scale, this result is higher than the research conducted by [16], which achieved a growth rate of 0.048/day.

The highest growth rate of *Nannochloropsis* sp. obtained in the treatment of the addition of Zn metal in this study reached 0.029/day, this result is lower than the results of the study [17] that reached 0.665/day. In the treatment of the addition of Cu cells of *Nannochloropsis* sp., the highest growth rate was 0.205/day, which was lower than the study [17], which was 0.7469/day. In several similar studies the results shown are not much different from the results obtained in this study, [18] noted that *Chlorella vulgaris* achieved a growth rate of 0.295/day and *S. platensis* reached 0.105/day on cultivation media added with Cu and Cr metals and [19] noted that the growth rate of *S.costatum* reached 0.31/day.

The results of the percentage of growth inhibition in the study were lower than the research conducted by [9] where the addition of Zn inhibited the growth of *D.pleiomorphus* by about 74.0 ± 1.5% and in *S.obliquus* around 97.1 ± 2.0%. When compared with the results of research conducted [20] it was higher where the addition of 0.5 ppm concentration of Cu metal inhibited the growth of *Chlorella vulgaris* by about 1.59%. However, [20] also noted that if the microalgae cultivation medium was added to a combination of 1.5 ppm Cu metal with 2.0 ppm Cd metal ion, it could inhibit the growth of Chlorella vulgaris up to 78.55%. The difference in growth rate and the percentage of growth inhibition of microalgae obtained in this study was due to differences in environmental factors that affect the growth of microalgae. Internal factors can also affect differences in the growth rate of microalgae because the strains or species of *Nannochloropsis* sp used in each study are different. Several studies showed that microalgae death occurred at a maximum metal content of Zn 10 mg/L and Cu 18 mg/L [21].

The results of statistical tests using analysis of variance (ANOVA) at a 95% confidence interval showed that the addition of metal ion concentrations of Zn and Cu to the cultivation media of *Nannochloropsis* sp. had a significant effect on the growth of microalgae *Nannochloropsis* sp. ($p > 0.05$), while in research conducted by [22] showed that the addition of different Zn heavy metals in cultivation media did not significantly affect the growth of microalgae, especially *Dunaliella salina*.

5. CONCLUSSION

The addition of metal ion concentrations of Zn and Cu to the cultivation media of *Nannochloropsis* sp. had a significant effect on the cultivation of *Nannochloropsis* sp. The addition of a Cu concentration of 1 ppm (Cu1) gave a significant effect on the growth rate of *Nannochloropsis* sp. that was characterized by the achievement of the highest density and growth rate. To see a more significant effect of Zn and Cu on *Nannochloropsis* sp. cells, it is necessary to analyze the chlorophyll content, photosynthetic pigment content, and oxygen exchange of these microalgae.

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