Effect of Indole 3-Acetic Acid (IAA) and 6-Benzyl Amino Purine (BAP) on *Nannochloropsis* sp. culture growth

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Abstract. One of the factors influencing the growth of *Nannochloropsis* sp. is the composition of culture media. The addition of growth regulators in the form of auxins and cytokines in culture media can increase the growth of microalgae. This study aims to examine the effect of IAA (auxin) and BAP (cytokinin) with various concentrations on biomass, chlorophyll content and carbohydrate content of *Nannochloropsis* sp. culture. *Nannochloropsis* sp. culture was treated with IAA and BAP in concentration variations each consisting of 0, 0.1, 1, 10 ppm with 3 replications. Data were analyzed with two way ANOVA at 95% confidence level and Tukey follow-up test. The results showed that the combination treatment of IAA and BAP did not affect the chlorophyll-a content of *Nannochloropsis* sp. culture, but it affected the biomass with the highest P16 (I10B10) of 3.65 g/L and carbohydrate content with the highest content in P4 (I10B0) of 0.30 mg/L. The highest chlorophyll-a content was found in P15(I1B10) of 5.574 mg/L, increased by 6 % compared to controls. Whereas the lowest chlorophyll-a content was found in P12(I10B1) of 1.563 mg/L, decreased by 70 % compared to the control.

1. Introduction

Microalgae are organisms belonging to protists that are similar to plants. They are unicellular or multicellular [1]. Most microalgae live in water [2]. Microalga are classified as a potential microorganism and have many benefits because they are cosmopolitan and can do photosynthesis efficiently [3]. *Nannochloropsis* sp. is a unicellular green microalga that was commercially utilized as an animal feed, supplement, energy biomass, and pharmaceutical industry because it contains protein, carbohydrates, lipids, and various minerals [1,4]. Besides, microalga have the advantage of being easily cultivated and containing high nutrients compare to other microalgae. Nutritional value of *Nannochloropsis* sp. consist of 52.11% protein; 27.65% fat; 16.00% carbohydrates; 42.70% total ω3-HUFA; 30.50% EPA; 0.85% vitamin C; and 0.89% chlorophyll-α [4]. Growth of *Nannochloropsis* sp. is affected by many factors, one of the main factors is the composition of the culture media. Culture media with right composition can increase biochemical content and the growth of microalgae [5]. Previous studies have suggested that the addition of auxin and cytokinin in culture media can enhance microalgae growth by controlling internal biochemical pathways [6]. Plant growth regulators (PGRs) are synthetic organic compounds that have the same structure and effect as phytohormones. PGRs are not active nutrients but when in small amounts it gave rise to biochemical, physiological, and morphological responses [7]. The addition of auxin and cytokinin to microalgae culture media can enhance biomass and biochemical content.
Indole 3-acetic acid (IAA) is an auxin type of PGR. IAA play a role in regulating growth and cell elongation. IAA can stimulate cell division, pigment biosynthesis, and biomass production in microalgae [8]. Previous research showed that IAA was effective in increasing the growth and cell composition of Chlorella pyrenoidosa and Scenedesmus quadricauda. Cultivation of Chlorella sorokiniana IAM C212 with the addition of IAA 10 ppm in the medium produced the highest dry weight of 4.68 g/L [9]. IAA concentrations of 10-8 to 10-5 M also increase growth, biomass, carbohydrate, and lipid content of Scenedesmus obliquus [10].

6-Benzyl Amino Purine (BAP) is a cytokinin of PGR. BAP play a role in stimulating cell division and accumulation of photosynthetic pigments [11, 12]. Previous studies have shown that the addition of BAP 1 ppm can increase Chlorella pyrenoidosa growth with a cell density of 23.58 x 10⁶ on the 12th day of cultivation. BAP 1 ppm also increased its lipid content of 14.70 mg/L [13]. The addition of cytokinins to Chlorella vulgaris affect the accumulation of photosynthetic pigments [14]. In another study, the addition of BAP 4 ppm in culture media can increase lipid and biomass content 1.26 times higher in microalgae [15].

Therefore, this study aims to examine the effect of IAA (auxin) and BAP (cytokinin) with various concentrations on biomass, chlorophyll content and carbohydrate content of Nannochloropsis sp. culture.

2. Materials and Methods

2.1 Materials

Nannochloropsis sp. with a density of 107 cells/ml was obtained from the culture collection of microalgae at the Natural Feed Laboratory of the Brackish Water Aquaculture Center Situbondo, East Java. Plant growth regulators (IAA and BAP) were purchased from Merck and Sigma-Aldrich, respectively. Stock solutions of plant growth regulator were prepared in 1 N NaOH for IAA and 1 N HCl for BAP.

2.2 Preparation and Sterilization of Tools and Culture Medium

Seawater as a culture media was adjusted the salinity at 30-32 ppt using a refractometer and the pH 7-8 using pH meter. All glassware and seawater were sterilized using autoclave at 121°C with a pressure of 1 atm for 15 minutes. While the plastic hose was sterilized by immersion in sodium hypochlorite (NaClO) solution for 10-15 minutes, then washed and put in heat-resistant plastic to be sterilized using autoclave. IAA and BAP were sterilized with a syringe filter (0.22 μm) in Laminar Air Flow.

2.3 Making of Conway Fertilizer

Conway fertilizer was prepared with a composition of Na₂EDTA (45 g/L), NaNO₃ (100 g/L), H₂BO₃ (33.6 g/L), Na₂HPO₄ (20 g/L), MnCl₂.4H₂O (0.36 g / L), FeCl₃.6H₂O (1.3 g/L), ZnCl₂ (2.1 g/L), CoCl₂.6H₂O (2 g/L), (NH₄)₆Mo₇O₂₄.4H₂O (0.9 g/L), CuSO₄.5H₂O (2 g/L), and vitamin (1 ml/L) consist of vitamin B12 (10 mg/200 ml) and vitamin B1 (200 mg/200ml) [16,17]. 1 ml of Conway fertilizer was used for 1 liter of culture medium.

2.4 Determining Growth Curve and Harvest Time of Nannochloropsis sp.

The growth curve in normal condition was used to find out the starter time of Nannochloropsis sp. Starter time was the time of growth of microalgae at half the exponential phase [18]. Nannochloropsis sp. at starter age was cultured in the treatment medium as much as 10% of the total culture medium. The treatment medium consisted of a combination of IAA (0; 0.1; 1; 10) ppm and BAP (0; 0.1; 1; 10) ppm. Furthermore, the value of OD (optical density) was measured every 24 hours starting from the first day until the death phase using a UV Vis spectrophotometer with a wavelength of 680 nm. Determination of the harvest time of Nannochloropsis sp. was the final of the exponential phase [19].

2.5 Culture Conditions in IAA and BAP Treatment
55 ml of Nannochloropsis sp. at starter age was cultivated into the treatment media. The total volume of Nannochloropsis sp. culture in the bottle was 550 ml each treatment. IAA (0; 0.1; 1; 10) ppm and BAP (0; 0.1; 1; 10) ppm treatments were repeated 3 times (Table 1). Microalgae were cultivated with aerator, the light intensity of 1,000 lux, and the ratio of light: dark photoperiod 24:0 in a sterile culture room.

Table 1. Treatment of IAA and BAP in Nannochloropsis sp. Culture

| BAP (ppm) | IAA (ppm) |
|-----------|-----------|
| 0         | 0         |
| 0.1       | 0.1       |
| 1         | 1         |
| 10        | 10        |
| 0         | P1(I0B0)  |
| 0.1       | P5(I0B0.1)|
| 1         | P9(I0B1)  |
| 10        | P13(I0B10)|
| 0.1       | P2(I0.1B0)|
| 0.1       | P6(I0.1B0.1)|
| 1         | P10(I0.1B1)|
| 10        | P14(I0.1B10)|
| 1         | P3(I1B0)  |
| 1         | P7(I1B0.1)|
| 1         | P11(I1B1) |
| 10        | P15(I1B10)|
| 10        | P4(I10B0) |
| 0.1       | P8(I10B0.1)|
| 1         | P12(I10B10)|
| 10        | P16(I10B10)|

Annotation:
P1(I0B0) is a treatment that combines the concentration of 0 ppm IAA with 0 ppm BAP
P2(I0.1B0) is a treatment that combines the concentration of 0.1 ppm IAA with 0 ppm BAP
P3(I1B0) is a treatment that combines the concentration of 1 ppm IAA with 0 ppm BAP
P4(I10B0) is a treatment that combines the concentration of 10 ppm IAA with 0 ppm BAP
P5(I0B0.1) is a treatment that combines the concentration of 0 ppm IAA with 0.1 ppm BAP
P6(I0.1B0.1) is a treatment that combines the concentration of 0.1 ppm IAA with 0.1 ppm BAP
P7(I1B0.1) is a treatment that combines the concentration of 1 ppm IAA with 0.1 ppm BAP
P8(I10B0.1) is a treatment that combines the concentration of 10 ppm IAA with 0.1 ppm BAP
P9(I0B1) is a treatment that combines the concentration of 0 ppm IAA with 1 ppm BAP
P10(I0.1B1) is a treatment that combines the concentration of 0.1 ppm IAA with 1 ppm BAP
P11(I1B1) is a treatment that combines the concentration of 1 ppm IAA with 1 ppm BAP
P12(I10B1) is a treatment that combines the concentration of 10 ppm IAA with 1 ppm BAP
P13(I0B10) is a treatment that combines the concentration of 0 ppm IAA with 10 ppm BAP
P14(I0.1B10) is a treatment that combines the concentration of 0.1 ppm IAA with 10 ppm BAP
P15(I1B10) is a treatment that combines the concentration of 1 ppm IAA with 10 ppm BAP
P16(I10B10) is a treatment that combines the concentration of 10 ppm IAA with 10 ppm BAP

2.6 Biomass Analysis

100 ml of Nannochloropsis sp. culture in the final exponential phase was filtered using Whatman filter paper (41). Filter paper and yield were dried in the oven for 24 hours at 60°C then weighed with an analytical balance [20]. The results of the weighing filter paper before and after the screening was calculated the difference for biomass weight calculation. The biomass weight equation was calculated using the following formula [21].

\[
\text{Biomassa} \left( \frac{\text{gr}}{1} \right) = (A - B) \frac{100}{\text{ml sample}}
\]

where:
A: filter paper weight after filtering (gr)
B: filter paper weight before filtering (gr)

2.7 Chlorophyll Analysis

Analysis of Nannochloropsis sp. chlorophyll a content consisted of three steps. There were sample preparation, extraction, and quantification of chlorophyll. 3 ml of microalgae samples were centrifuged at 3000 rpm for 10 minutes. The results of the centrifugation were taken pellets, then resuspended in 3 ml of distilled water for washing salt solution and centrifuged again. This washing process was repeated twice. Furthermore, the microalgae cells were suspended in 3 ml of 96% ethanol and homogenized with vortex for 15 seconds then centrifuged 4000 rpm for 5 minutes [22]. The solution was incubated for 24 hours and the absorbance was analyzed with UV Vis spectrophotometer with wavelengths of 665, 645
and 630 nm [23]. Calculation of chlorophyll content in *Nannochloropsis* sp. based on the Strickland and Parson equation as follows [23]:

\[
\text{Chlorophyll } a \left( \frac{mg}{L} \right) = 11.6E_{665} - 1.31E_{645} - 0.14E_{630} \tag{2}
\]

2.8 Carbohydrate Analysis
Carbohydrate content analysis using the colorimetric method (phenol-sulfuric acid). 10 mg of dry biomass was put into 10 ml of deionized water. 2 ml of a solution containing carbohydrates was mixed with 1 ml of 5% phenol (w/v) in a test tube, then reacted with 5 ml of sulfuric acid at room temperature. After 10 minutes, the solution was homogenized with vortex for 1 minute then placed in a water bath at 30°C for 20 minutes for color development. Blank was also prepared as the above method by replacing the carbohydrate solution with dH₂O. After color development, carbohydrate content was measured using a UV Vis spectrophotometer with a wavelength of 490 nm. Carbohydrate content referred to a standard glucose curve with a concentration of 0; 0.02; 0.04; 0.06; 0.08; 0.10 mg/ml [24].

2.9 Data Analysis
Data were subjected by two-way analysis of variance (ANOVA) at 95% confidence level and the means were compared using Tukey follow-up test (p<0.05). All data were presented as means ± standard deviation (SD) of three replications.

3. Results and Discussions

3.1 Growth Curve and Harvest Time of *Nannochloropsis* sp. on IAA and BAP Treatments
Growth was an increase in the number or volume of cells influenced by internal and external factors. *Nannochloropsis* sp. produced double biomass at the 24th hour (doubling time) [25]. Therefore, the growth curve was determined every 24 hours by measuring the value of OD (Optical Density) of microalgae culture. OD values on the spectrophotometer correlated with the number of cells in the media so that it can be used to monitor microalgae growth [26]. The results showed that the growth curve of *Nannochloropsis* sp. consisted of 4 phases: lag phase, exponential phase, stationary phase, and death phase (data not shown). The lag phase occurred very quickly on 1st day to 2nd day. The exponential phase occurred on the 2nd day until the 10th day and the stationary phase occurred on the 10th day until the 11th day. While the death phase started from the 11th day. Based on the growth curve, the starter time of *Nannochloropsis* sp. occurred on the 6th day which was half an exponential phase. In this phase, microalgae cells actively divided and optimally metabolized before entering the stationary phase [27,28]. Starter culture was used for the next culture [29,30].

The harvest time of microalgae was important because it related to the amount of biomass and biochemical content produced [31]. Harvest time for microalgae can be determined through the growth curve in the combined treatment of IAA and BAP concentrations (data not shown). The results showed that the harvest time of *Nannochloropsis* sp. in the treatment of a combination of IAA and BAP concentrations that are varied. P1 (I0B0), P2 (I0.1B0), P3 (I1B0), P4 (I10B0), P5 (I0B0.1), P6 (I0.1B0.1), P8 (I0B0.1), P10 (I0B0), P5 (I0B0.1), P6 (I0.1B0.1), P8 (I10B0.1), P10 (I0.1B1), P12 (I10B1), P13 (I0B10), and P14 (I0.1B10) showed the longest harvest time on the 11th day. Treatments P11 (I1B1) and P16 (I10B10) indicated harvest time on the 10th day. Treatments P7 (I1B0.1) and P15 (I1B10) indicated harvest time on the 9th day. Whereas, treatment P9 (I0B1) showed the fastest harvest time, which was on the 7th day. Microalgae harvest time was determined at the final of the exponential phase [19]. The OD680 value related to cell density in the media was highest at P10 (I0.1B1) of 1.358 and lowest at P8 (I10B0.1) of 0.321 at harvest time. Differences in harvest time and cell density in *Nannochloropsis* sp. suspected due to the influence of a combination of IAA and BAP with various concentrations in the medium. Variations in the concentration of growth regulators can stimulate or inhibit growth and metabolism [12].

3.2 Effect of IAA and BAP on *Nannochloropsis* sp. Biomass
Microalgal biomass was composed of 45 to 50% carbon-based on dry weight measurements. Increased biomass can also affect the biochemical content of microalgae including lipid, starch, carbohydrate, protein, and total caloric value of the biomass [32].

### Table 2. Biomass of *Nannochloropsis* sp. on IAA and BAP treatments

| BAP (ppm) | IAA (ppm) | 0     | 0.1   | 1     | 10    |
|-----------|-----------|-------|-------|-------|-------|
| 0         | 0.1       | 2.19±0.08 | 2.42±0.00 | 2.32±0.00 | 3.33±0.00 |
| 1         | 2.13±0.05 | 2.47±0.00 | 2.59±0.00 | 3.32±0.00 |       |
| 10        | 2.37±0.00 | 2.47±0.00 | 2.54±0.00 | 3.21±0.00 |       |

Note: Biomass content (mean of three replicates± standard deviation) in *Nannochloropsis* sp. grown on IAA and BAP treatments. Number with the same letters are not significantly different (p<0.05) according to the Tukey’s test for mean comparisons.

Based on the results (Table 2) of the two way ANOVA treatment of the combination of IAA and BAP concentrations affected (p <0.05) on the cell biomass of *Nannochloropsis* sp. with p-value in the IAA treatment of 0.00, BAP of 0.00, and interaction between IAA and BAP of 0.00. The biomass in P1 (I0B0) as control was not significantly different compared to P5 (I0B0.1) and was significantly different than other treatments. The highest biomass was found in P16 (I10B10) of 3.62 g/L, increased by 16% compared to controls. Whereas the lowest biomass was found in P5 (I0B0.1) of 2.13 g/L, decreased by 3% compared to controls. The increase and decrease in biomass were thought to be due to the influence of a combination of IAA and BAP. The combination of IAA concentration of 10 ppm and BAP 10 ppm was thought to be optimal for increasing the biomass of *Nannochloropsis* sp.

IAA was classified as auxin, whereas BAP was classified as cytokinin. The combination of both growth regulators can be more effective in increasing the growth and metabolism of microalgae [12]. IAA can stimulate cell division, biomass production, and pigment biosynthesis in microalgae [8]. The addition of IAA 10 mg/L in the cultivation medium of Chlorella sorokiniana IAM C212 produced the highest dry weight of 4.68 g/l [9]. IAA concentrations of 10⁻⁸ to 10⁻⁵ M can increase growth, biomass, lipid content, and carbohydrate Scenedesmus obliquus [10]. The addition of BAP 1 mg/L can increase the growth and lipid content of Chlorella pyrenoidosa [13]. In another study, the addition of BAP 4 mg/L to culture media also increased lipid and biomass content 1.26 times higher in microalgae [15].

Plant growth regulators such as IAA and BAP acted as chemical messengers that had functions in various physiological processes of microalgae [33]. Both of them can increase carbohydrate, lipid, protein, and another bioactive compound. The increase of carbohydrates was suspected because of auxin and cytokines play a role in the microalgae photosynthetic apparatus through increased chlorophyll, carotene, and xanthophyll content [12,14]. The existing evidence suggested that exogenous auxins and cytokines effectively regulate the biosynthesis and fatty acid profiles in green microalgae, which might be directly or indirectly related to lipid biosynthesis [8,14,34,35]. The accumulation of soluble proteins occurred when microalgae cells displayed the maximal microalgal metabolism and mitotic activity undergrowth regulator treatment as shown in the previous studies performed on Chlorella pyrenoidosa [14].

#### 3.3 Effect of IAA and BAP on Nannochloropsis sp. Chlorophyll a Content

Chlorophyll a was the most important pigment for photosynthesis in microalgae because it acted to convert photons into chemical energy [36].

Based on the results (Table 3) of the two way ANOVA test for IAA treatment and the interaction between IAA and BAP at various combinations of concentration did not affect (p>0.05) on the chlorophyll-a content of *Nannochloropsis* sp. with p= 0.448 and p= 0.421, respectively. Whereas the
BAP treatment affected (p<0.05) on the chlorophyll-a content of *Nannochloropsis* sp. with p=0.000. The chlorophyll content in P1 (I0B0) as control was significantly different compared to P12 (I10B1) and was not significantly different than other treatments. The highest chlorophyll-a content was found in P15 (I1B10) of 5.574 mg/L, increased by 6% compared to controls. Whereas the lowest chlorophyll-a content was found in P12 (I10B1) of 1.563, decreased by 70% compared to the control. Increased and decreased chlorophyll-a content of *Nannochloropsis* sp. suspected due to the influence of a combination of IAA and BAP. The combination of 1 ppm IAA concentration and 10 ppm BAP was thought to be optimal for increasing the chlorophyll content of Nannochloropsis sp.

### Table 3. Chlorophyll a content of *Nannochloropsis* sp. on IAA and BAP treatments

| BAP (ppm) | IAA (ppm) |
|-----------|-----------|
| 0         | 0.1       | 1         | 10        |
| 0         | 5.26±0.93 | 4.19±0.86 | 4.78±1.19 | 5.45±0.94 |
| 0.1       | 5.00±0.36 | 3.85±0.91 | 3.53±2.25 | 4.87±1.84 |
| 1         | 2.87±0.22 | 2.20±1.15 | 2.16±0.53 | 1.56±0.56 |
| 10        | 4.14±1.56 | 4.30±0.56 | 5.57±0.54 | 4.79±0.15 |

Note: Chlorophyll a content (mean of three replicates± standard deviation) in *Nannochloropsis* sp. grown on IAA and BAP treatments. Number with the same letters are not significantly different (p<0.05) according to the Tukey’s test for mean comparisons.

IAA was classified as auxin, whereas BAP was classified as cytokinin. The combination of both growth regulators can be more effective in increasing the growth and metabolism of microalgae [12]. Auxin increases the chlorophyll content which will be used in photosynthesis and growth [12]. However, the effect of auxin on chlorophyll a biosynthesis depends on the dose given. IAA showed the effect of stimulation of metabolism at low concentrations and inhibition at high concentrations in green microalgae [12,35]. The most effective IAA concentration was between 10⁻⁹ and 10⁻⁴ M [8]. In another study also showed the highest chlorophyll-a content of 0.93 mg/L in *Nannochloropsis oculata* with IAA treatment of 1 mg/L [33]. Cytokines also play a role in the accumulation of photosynthetic pigments in microalgae. The addition of BAP with a concentration of 10⁻⁷ M in green microalgae, *Chlorella vulgaris* affected on chlorophyll accumulation [14]. The optimum concentration of cytokinins for increasing chlorophyll content in each microalgae species was different because of the morphological and physiological differences of each species and the conditions of microalgae growth [8].

Plant growth regulators such as IAA and BAP acted as chemical messengers that had functions in various physiological processes of microalgae [33]. Auxin and cytokinin played a role in stimulating the accumulation of photosynthetic pigments such as chlorophyll, caroten, and xanthophyll. Cytokinins were more effective than auxins in increasing the chlorophyll content of microalgae [14]. BAP had an inhibitory effect on damage to chlorophyll by chlorophyllase, Mg-dechelatase, and peroxidase which can degrade chlorophyll in vascular plants [37]. A similar mechanism was thought to occur in green microalgae due to increased chlorophyll-a content which was influenced by BAP growth regulators. Furthermore, the addition of growth regulators also played a role in protecting chloroplasts against ROS (Reactive Oxygen Species) through the accumulation of carotenoids [14]. However, the addition of growth regulators can also cause inhibition of microalgae metabolism. The addition of growth regulators that were too high can inhibit growth and metabolism in vascular plants because the plants already contain endogenous phytohormones so that they become unbalanced [38]. A similar mechanism was suspected to occur in *Nannochloropsis* sp. *Nannochloropsis oceanica* was found to contain cytokinin phytohormone [39].

### 3.4 Effect of IAA and BAP on Nannochloropsis sp. Carbohydrate Content
Carbohydrates were the main nutrients in microalgae as a result of photosynthesis. Glucose was one of the monosaccharides from carbohydrates contained in microalgae cells [40].

Based on results (Table 4), the two way ANOVA test results of IAA treatment did not affect (p>0.05) on the carbohydrate content of *Nannochloropsis* sp. with p= 0.445. Whereas the BAP treatment and the interaction between IAA and BAP influence (p<0.05) on the carbohydrate content of *Nannochloropsis* sp. with p= 0.000 and p=0.030, respectively. The carbohydrate content in P1 (I0B0) as control was significantly different compared to P10 (I0.1B1) and was not significantly different than other treatments. The highest carbohydrate content was found in P4 (I10B0) of 0.302 mg/L, increased by 21% compared to controls. Whereas the lowest carbohydrate content was found in P10 (I0.1B1) of 0.185, decreased by 22% compared to controls. The increase and decrease in carbohydrate content were thought to be due to the influence of a combination of IAA and BAP. The combination of IAA concentration of 10 ppm and BAP 0 ppm was thought to be optimal for increasing the carbohydrate content of *Nannochloropsis* sp.

Table 4. Carbohydrate content of *Nannochloropsis* sp. on IAA and BAP treatments

| BAP (ppm) | IAA (ppm)     |
|-----------|--------------|
|           | 0            | 0.1          | 1            | 10           |
| 0         | 0.24abc±0.02 | 0.27abc±0.05 | 0.23abc±0.02 | 0.30abc±0.04 |
| 0.1       | 0.22ab±0.02  | 0.27ab±0.04  | 0.28ab±0.04  | 0.22abc±0.01 |
| 1         | 0.20abc±0.02 | 0.19ab±0.01  | 0.20abc±0.00 | 0.20abc±0.02 |
| 10        | 0.23abc±0.05 | 0.24abc±0.03 | 0.22abc±0.02 | 0.21abc±0.01 |

Note: Carbohydrate content (mean of three replicates± standard deviation) in *Nannochloropsis* sp. grown on IAA and BAP treatments. Number with the same letters are not significantly different (p<0.05) according to the Tukey’s test for mean comparisons.

IAA was classified as auxin, whereas BAP was classified as cytokinin. Auxin and cytokinin can increase carbohydrate content at different concentrations in each species. In the previous study showed that auxin can stimulate carbohydrate increase in Scenedesmus obliquus, while cytokinin can stimulate carbohydrate increase in Acutodesmus obliquus [12]. The increase of carbohydrates was suspected because of auxin and cytokines play a role in the microalgae photosynthetic apparatus through increased chlorophyll, carotene, and xanthophyll content [12,14]. Carbohydrate was the result of photosynthesis. Chlorophyll, carotene, and xanthophyll played an important role in the process of photosynthesis of microalgae to capture photons. Increased carotene pigments can also protect chloroplasts as photosynthesis sites from ROS (Reactive Oxygen Species). Cytokinins also had an inhibitory effect on damage to chlorophyll by chlorophyllase, Mg-dechelatase, and peroxidase which can degrade chlorophyll. Protection of chloroplasts as photosynthetic apparatus by cytokines also by delaying oxidative damage through increased levels of non-enzymatic antioxidants (ascorbate, glutathione) and antioxidant enzyme activities (catalase, glutathione reductase, ascorbate peroxidase). Besides, auxin also stimulated the activity of superoxide dismutase in green microalgae *Acutodesmus obliquus* [14].

In addition to stimulating metabolism in the form of carbohydrate content, the addition of growth regulators can also cause inhibition of microalgae metabolism. The addition of growth regulators that were too high can inhibit growth and metabolism in vascular plants because the plants already contain endogenous phytohormones so that they became unbalanced [38]. A similar mechanism was suspected to occur in *Nannochloropsis* sp. *Nannochloropsis oceanica* was found to contain cytokinin phytohormone [39].

Based on Tables 2 and 3, the chlorophyll content and carbohydrate content at the certain concentration of *Nannochloropsis* sp. positively correlated. The higher the chlorophyll content, the higher the carbohydrate content. The P4 (I10B0) treatment had high chlorophyll and carbohydrate content. It was because chlorophyll and carbohydrates had related to the process of photosynthesis.
Chlorophyll-a was the most important pigment for photosynthesis in *Nannochloropsis* sp. because it played the role of capturing and converting photons into chemical energy [36]. Carbohydrate was chemical energy that results from photosynthesis [14].

4. Conclusions
The combination treatment of IAA and BAP did not affect the chlorophyll-a content of *Nannochloropsis* sp. culture, but it affected the biomass with the highest P16 (I10B10) of 3.65 g/L and carbohydrate content with the highest content in P4 (I10B0) of 0.30 mg/L. Furthermore, based on the results of this study, the treatment of P16(I10B10) can be developed as a method of propagating the biomass of *Nannochloropsis* sp. in biodiesel production techniques by adding metabolism manipulation treatment in the form of abiotic stress to increase the content of triacylglycerol and its fatty acids.

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