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RESEARCH ARTICLE

The Effect of IAA Phytohormone (Indole-3-Acetic Acid) on the Growth, Lipid, Protein, Carbohydrate, and Pigment Content in *Euglena* sp.

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Abstract Nowadays, energy consumption is massively increasing in the world. The production of biofuels from microalgae has received considerable attention recently and has the potential to supplant fossil fuels. Recent research focuses on developing the potential of microalgae as an alternative fuel. This research will focus on evaluating the effect of indole-3-acetic acid (IAA) on the growth and metabolite production of *Euglena* sp. The methods used in this study started with a medium preparation and cultivation using IAA treatment, where the treatment used controlled IAA 5 g/L, IAA 10 g/L, and IAA 15 g/L with three biological repetitions. Optical density (OD) was measured using a spectrophotometer (OD_{680}), biomass was measured using the gravimetry method, lipid was calculated using Bligh and Dyer (1995), the protein was measured using Bradford solution, carbohydrates were measured using phenol sulfuric acid, and pigments were extracted using methanol and measured using a spectrophotometer. According to the findings of this study, IAA 5 g/L can enhance the growth rate. For biomass, the best result was at 10 g/L of IAA (2.216 g/L ± 0.284). Meanwhile, carbohydrates, proteins, and lipids were higher in IAA 15 g/L. Chlorophyll a, b, and total carotenoid were higher in 5 g/L of IAA. The results obtained in this study showed that the IAA hormone increased the growth and metabolite content of *Euglena* sp.

Keywords: Biofuels, Biomass, IAA (Indole-3-acetic acid) Lipid, Microalgae.

Introduction

The world is becoming dependent on fossil fuels due to the growth of the world's population, urbanization, and industrialization [1]. At this time, fossil fuels have become an essential fuel for the development of the world as fuel for vehicles, power plants, industrial fuels, and much more. The exhaustion of fossil fuels is only a matter of time until fossil fuels can no longer be used. Fossil fuel energy produces carbon oxides (CO_x) and nitrogen oxides (NO_x), and some hydrocarbons; these gas emissions are known as greenhouse gas emissions (Carcinogenic)[2], [3], [4]. The industrial world started, and energy demand has increased rapidly, leading to the growth rate of carbon dioxide (CO_2) emissions. According to global growth data, CO_2 emissions in the 2010s were 0.9%; in 2020, it increased to 38% [5], [6] . A significant increase in energy needs will affect natural conditions, such as climate change, pollution, and world temperature [7]. Alternative energy sources are needed that can replace when fossil fuels are depleted. Alternative fuels available today include palm oil, soybean oil, jatropha oil, sunflower oil, rapeseed oil, spent cooking oil, and palm fatty acid distillate; however, these require considerable land to be produced [8]. Biofuels derived from photosynthetic microbes are a solution that can become renewable energy, one of which is microalgae[6], [9]. Microalgae is a fourth-generate biofuel solution, which the fourth generation will focus on carbon sequestration. Biofuel from microalgae can reduce CO_2 emissions, but the use of microalgae for environmental sustainability is still questionable because of unavailable data

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and the unavailability of Life Cycle Assessment (LCA), and the market distribution of microalgae in the community is still unclear [10], [11]. Microalgae are autotrophic organisms producing primary in waters capable of synthesizing complex organic bonds from simple organic compounds [12]. Photosynthesis is a microalgae activity that converts and absorbs CO_2, which can be converted to renewable biomass and chemical energy [2], [5]. Microalgae have the potential because it reduces a large amount of carbon dioxide. According to recent studies, it takes about 1.83 kg of CO_2 to produce 1 kg of biomass, and carbon affects almost half the biomass yield on microalgae [13]. The productivity of microalgae is greater than that of higher plants, and this is because the distribution of microalgae is widely distributed in the waters. Microalgae are also benthic (attached to the substrate) and planktonic (floating in water bodies). The efficiency of photosynthesis in microalgae is 50 times greater than that of terrestrial plants [14].

Microalgae have a cell chemical composition consisting of carbohydrates, lipids, proteins, carotene, and nucleic acids. Besides, it contains several organic ingredients such as vitamins, minerals, and other secondary metabolite compounds. The growth and development of microalgae influence the composition of the microalgae. Therefore, microalgae species have different biomass content from each other according to their compositions. Lipids are the widest metabolites produced by microalgae; 60% of lipids are extracted from the dry weight of microalgae, and they also have a shorter life cycle than terrestrial plants [15], [16]. Some microalgae species, like *Botryococis braunii*, had cellular lipid contents that reached 75% [26]. The lipids produced by microalgae have the same characteristics as the lipid in higher plants. Microalgae can live in various conditions and extreme environments by accumulating lipids in cells [17], [18]. Several biotic and abiotic factors influence the accumulation of microalgae biomass content. For instance, the need for nutrients, pH, salinity, hormones, and so on will stimulate the growth of metabolite production in microalgae. Some studies focus on lipid production in microalgae using various methods and treatments. The addition of environmental parameter treatment will affect physiological functions, and the physiological role of microalgae will adjust to the treatment given. The lipid content produced by microalgae is essential as the primary material for producing biodiesel. Microalgae are divided into two based on their lipid content. Some microalgae have high lipid content but low cell growth and low lipid content but high cell growth [16].

Euglena sp. was microalgae species whose metabolites have been studied in depth in recent years. *Euglena* sp. could easily produce many compounds on its metabolism processes [19]. Also, *Euglena* sp. made a lot of biomasses and had bigger cells compared to other microalgae. Several studies had been reported that *Euglena* had higher lipid content than other microalgae. There were 12 different types of fatty acids, of which 50% were unsaturated fatty acids [20]. In order to increase productivity of *Euglena*, it was necessary to add several treatments to optimize cultivation, one of which was the addition of phytohormones. Phytohormones were chemicals that can enhance the metabolic system of plants or microalgae. On the other hand, phytohormones played a role in plant adaptation to unfavorable environmental conditions such as temperature, humidity, substance concentration, etc [21].The hormone used in this study was the growth hormone or IAA hormone (Indole-3-Acetic Acid). Plant hormones (phytohormones) were carriers of natural chemicals or synthesis that regulate growth and development. Phytohormones affected higher plants, fungi, and microalgae [21], [22]. The main hormones needed by plants for growth include auxin (IAA), cytokinin (CK), gibberellin (GA), and abscisic acid (ABA). The use of phytohormones in the development of different microalgae and the production of metabolites were also different [21].

This study has evaluated the effect of giving IAA phytohormone (indole-3-Acetic Acid) as a treatment on the growth and biomass content of *Euglena* sp. Also, other parameters had been evaluated including primary and secondary metabolites such as lipids, carbohydrates, proteins, carotenes, as well as chlorophyll a and b.

Materials and Methods

Sample and Medium Preparation

Euglena sp. was isolated from Dieng Plateau, Central Java, Indonesia. The isolation process used a capillary pipette. The isolation stage was performed in the Biotechnology Laboratory, Faculty of Biology, Gadjah Mada University. The microalgae were cultured in a 500 mL bottle containing Cramer Myers (CM medium) [23] with a pH condition of 3.5 and placed at a temperature of 22ºC and a light intensity of 2100 lux. The microalgae were cultured in a 500 mL bottle containing Cramers-Myers medium with a composition 1.000 mg/L KH_2PO_4, 1.000 mg/L $(NH_4)_2SO_4$; 200 mg/L $MgSO_4.7H_2O$, 20 mg/L $CaCl_2.2H_2O$, 1.8 mg/L $MnCl_2.4H_2O$, 3 mg/L $Fe_2(SO_4)_3.7H_2O$, 1,5 mg/L $CoSO_4.7H_2O$, 0,4 mg/L $ZnSO_4.7H_2O$, $CuSO_4.7H_2O$; 0,2 mg/L; $Na_2MoO_4.2H_2O$; 0,1 mg/L vitamin B1, 0,00005 mg/L Vitamin B12 and then dissolved in 1 L distilled water (Cramer & Myers, 1952). The treatment used in this study is the treatment

of IAA hormones by Merck (United States). in powder form, obtained from the FALITMA laboratory, Faculty of Biology, Gadjah Mada University. First, 50 mg of IAA powder was dissolved in 100 ml of sterile aqueous based on the treatment to be used and dripped 2-3 drops of KOH. The dissolved IAA was then filtered using a syringe and Millipore. The treatments used include the following Table 1.

Table 1. Combination of Research Treatment Using IAA

Treatments	Compositions
Control	200 mL inoculant + 300 mL Medium
IAA 5 g/L	200 mL inoculant + 5 mL IAA + 295 mL Medium
IAA 10 g/L	200 mL inoculant + 10 mL IAA + 290 mL Medium
IAA 15 g/L	200 mL inoculant + 15 mL IAA + 285 mL Medium

Microalgae cultured in Cramer and Myers (CM) medium Sampling was carried out daily to see the growth of *Euglena* sp. The number of samples taken varies depending on the content to be tested. Sampling was carried out for 18 days, starting from D_0 to D_{18}.

Cell Density Measurement

The calculation of Optical Density (OD) and the number of cells was calculated daily during the study. Samples were taken as much as 2 mL per treatment, and samples were taken sterile in LAF (Laminar Air Flow) and placed in a 2 mL microtube. For measuring the growth, we choose 3 parameters including; optical density (OD), cell density, and biomass (dry weight). Optical Density calculations were performed using spectrophotometry with a wavelength of 680 and 860 nm, according to Chl a fluorescence (600, 680, 750 nm). The samples were placed in a cuvette, and spectrophotometry was used to measure the OD number. The number of cells was manually calculated using a microscope, hemocytometer, and optiLab. According to research [24], cell density can be calculated using the equation below:

$$\frac{Cells}{mL} = \frac{Total\ Cells}{Total\ Quadrants\ Counted} \times 10^4 \quad (1)$$

Biomass Measurement

Biomass calculation was carried out every three days, starting from D_0, D_3, D_6, D_9, D_{12}, D_{15}, and D_{18}. First, a total of 2 mL of samples were taken in a sterile state and then placed in a 2 mL microtube; the microtube to be used was previously weighed in empty weight, and the sample was then centrifuged at 3300 rpm for 10 minutes until it was able to distinguish which pellets and supernatant were. The supernatant was then taken using a micropipette. The remaining pellets were then dried in the oven to dry for 24 hours. The Biomass content was calculated following this formula:

$$Biomass\ Content = \frac{D_t - D_0}{V} \quad (2)$$

Note:
D_t : Petri dish and sample (biomass) weight after drying (Day-t) (mg)
D_0 : Initial weight of Petri dish (mg)
V : Volume sample (mL)

Determination of Carbohydrate Content

Carbohydrate measurement was carried out every three days, starting from D_0, D_3, D_6, D_9, D_{12}, D_{15}, and D_{18}. First, calculate carbohydrate levels using the phenol-sulfuric acid (H_2SO_4) method. Samples were taken (5 mL) under sterile conditions and placed in a 15 mL tube. The tube is centrifuged at 4000 rpm for 10 minutes and then separated by supernatant. Afterwards, a sample containing pellets was added with 1 mL of concentrated sulfuric acid (H_2SO_4) and 0.5 mL of 5% phenol. Tube was then incubated at room temperature for 30 minutes. The sample evaluated was then measured using spectrophotometry with a wavelength of 490 nm, and the absorbance value was obtained. For the standard solution, we used D-glucose with the concentrations 8.000 µg/mL, 7.000 µg/mL, 6.000 µg/mL, 5.000 µg/mL, 4.000 µg/mL, 3.000 µg/mL, 2.000 µg/mL, 1.000 µg/mL, and 500 µg/mL (R^2= 0.99).

Determination of Lipid Content

Lipid calculation using the Bligh and Dyer method (1995) [25] was carried out once every three

days, starting from D_0, D_3, D_6, D_9, D_{12}, D_{15}, and D_{18}. Samples were taken as much as 5 mL under sterile conditions and placed in a 15 mL tube. The tube was centrifuged at 4000 rpm for 10 minutes, and the supernatant was discarded. The remaining pellets were then reacted using 2 mL of methanol and 1 mL of chloroform. The reacted tube was then homogenized using a vortex. Add another 1 mL of chlorophyll and 1 mL of equates and homogenize using a vortex and re-centrifuge at 4000 rpm for 10 minutes until three layers were formed. The lipid content was calculated using following formula:

$$Lipid\ Content = \frac{D_t - D_0}{V} \quad (3)$$

Note:
D_t : Petri dish and sample (Lipid) weight after drying (Day-t) (mg)
D_0 : Initial weight of Petri dish (mg)
V : Sample volume (mL)

Determination of Protein Content

The sample protein was measured for three days. First, samples were taken as much as 5 mL into a 2 mL microtube, the sample was centrifuged at 12,000 rpm for 5 minutes, and pellets were then added with 1 mL of 10% SDS solution and heated at 95ºC for 5 minutes using a water bath. The sample was then incubated at 4ºC for 5 min; the incubated sample was then taken as much as two µL and put into a 500 µL microplate. The sample was then added with 200 µL of Bradford reagent. Sample absorbance was measured with eLISA Reader BioTechnology at a wavelength of 595 nm. Proteins were analysed using the Bradford (1976) method [15]. Protein analysis was analysed using standard curves, and standard curves were created with Bradford solution. Standard protein solutions were Bovine serum albumin (BSA) with stratified concentrations of 250, 500, 750, 1.000, 1.250, 1.500, 1750, 2000, and 2500 µg/mL.

Chlorophyll and Carotenoid Measurement

The calculation of carotene and chlorophyll a and b content was carried out in 3 days, starting from D_0, D_3, D_6, D_9, D_{12}, D_{15}, dan D_{18}. Samples were taken as much as 2 mL under sterile conditions and placed in a 2 mL microtube. Samples were centrifuged and separated from the supernatant. The remaining pellets were then reacted with 2 mL of methanol and homogenised using a vortex. The sample was incubated in a refrigerator for 24 hours, and then absorbance measurements were performed using UV-vis spectrophotometry with wavelengths of 480 nm, 652 nm, and 665 nm. The absorbance results were calculated using an equation based on research as follows:

$$\text{Chlla} - \text{a}\ (\mu g/L) = 11{,}85\ \times (\lambda 664) - 1{,}54\ \times (\lambda 647) - 0{,}08 \times (\lambda 630) \quad (4)$$
$$\text{Chlla} - \text{b}\ (\mu g/L) = 21{,}03\ \times (\lambda 647) - 5{,}43 \times (\lambda 664) - 2.66 \times (\lambda 630) \quad (5)$$

Note:
Chla-a : Chlorophyll – a content (µg/L)
Chla-b : Chlorophyll – b content (µg/L)
$\lambda 664$: Absorbance value at a wavelength of 664 nm
$\lambda 647$:Absorbance value at a wavelength of 647 nm
$\lambda 630$: Absorbance value at a wavelength of 630 nm

$$Carotene\ (\mu g/L) = 4\ \times (\lambda 480nm) \quad (6)$$

Note:
Carotene: Carotene Content (µg/L)
$\lambda 480$: Absorbance value at a wavelength of 480 nm

Data Analysis

The research results in the form of growth rate, biomass, carbohydrate, lipid and protein, and pigment content of *Euglena* sp. were analyzed using ANOVA and mean differences were analyzed using Duncan Multiple Range Test (DMRT) through IBM's Statistical Product and Service Solutions (SPSS) software (Version 26, IBM Corporation, USA). DMRT was used for multiple comparisons among averages from significant ANOVA tests. The significance of the data was determined by the *p*-value <0.05 at the 5% level.

Results and Discussion

Morphology and Growth Rate of *Euglena* sp.

Euglena sp. was microalgae found in freshwater environments. Group grouping *Euglena* sp. such as *E. adaerens, E. carterae, E. deses, E. mutabilis* were usually grouped by the characteristics of the shape and number of chloroplasts or related forms or the presence or absence of pyrenoids in chloroplasts [26]. *Euglena* sp. changed cell morphology in response to environmental stress factors such as changes in temperature, light, ion concentration, etc. Cells of *Euglena* sp. produced several products, such as paramylon wax esters and organic acids [38]. Paramylon degraded by light and produce wax esters. Wax esters consisted of saturated fatty acids, myristic acid, and myristic alcohol [38]. The application and the use of wax esters has recently been developed for use as biodiesel fuel [26].

The result of this study determined the growth curve and each growth phase of *Euglena* sp. The growth curve can be seen based on density *Euglena sp.* observed every three days in each sample treatment (n=3). The optical density was calculated using spectrophotometer at 680 nm.

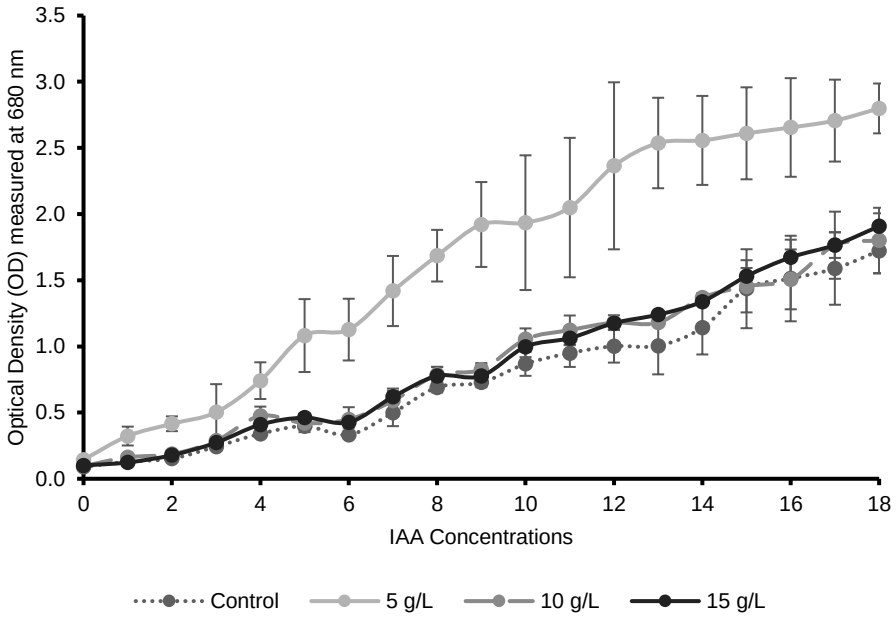

Figure 1. described the growth curve of *Euglena* sp. during cultivation

Based on the chart data above, the IAA treatment of 5 g/L was the most effective treatment for increasing the cell density of *Euglena* sp. followed by IAA treatments of 15 g/L, IAA 10 g/L, and control treatments. The addition of the IAA hormone resulted in a significant increase (p<0.002) and influenced the growth of *Euglena* sp. The addition of IAA phytohormones affected and increased the growth rate of *Euglena* sp, because it stimulated cell division in *Euglena* sp. IAA hormone played a role in cell enlargement, cell differentiation, cell division, and cell response to light and gravity [21], [22]. Based on research belonging to [27] that the addition of IAA phytohormones affected the growth rate and increased the diameter of *Chlorella vulgaris* cells up to 12 µg. Other research by [38] also reported that the addition of IAA phytohormone concentrations induced several genes that regulated the growth. In contrast, high doses of IAA concentrations inhibited the growth and played role as herbicides. The addition of various diverse phytohormones with the optimum concentration had a beneficial effect on the growth of microalgae, and the results that phytohormones had on multiple species of microalgae had different effects [28].

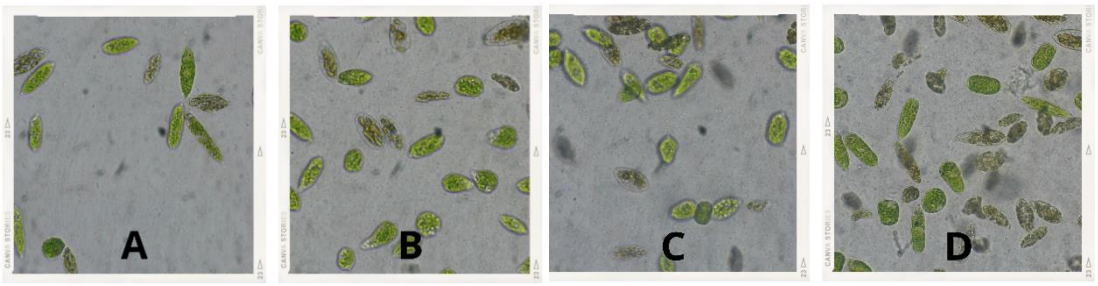

Figure 1. Morphology of Euglena sp., in the last day of cultivation (A) In control treatment; (B) IAA 5g/L; (C) IAA 10g/L, and (D) IAA 15 g / L

Figure 1 showed a pictures of the cell shape of *Euglena* sp. on the last day of cultivation. Based on Figure 1, there were no differences between the IAA treatment and control. *Euglena* sp. had the shape of a spindle cell, rounded, and elongated. *Euglena* sp. changed morphological conditions depending on growing conditions. *Euglena* sp. cells formed cysts when their environment stresses them. *Euglena* sp. accumulated amino acids at superior levels, such as methionine and other essential amino acids, essential vitamins, and unsaturated fatty acids. Based on the observations in Figure 1, the addition of the IAA hormone at 15 g/L produced the highest cell density compared to other treatments. Therefore, in figure (1.D), the number of cells was higher on the last day of observation. After reviewing the morphological shape of *Euglena* sp. cells in Figure 1, it can be observed that the color of *Euglena* sp. was predominantly green in all treatments. The content of photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoids caused the green color in *Euglena* sp [29], [30], [31].The cell morphology of *Euglena* sp. served as an indicator of metabolic regulation, photosynthesis, cell cycle, and cell response to environmental stress [32]. Therefore, by engineering and controlling the morphology of *Euglena* sp., it will be able to know the metabolism in cells and control the production of biomass, which will later be used as an alternative fuel in the future.

Biomass and Primary Metabolites (Carbohydrate, Lipid, Protein)

Measurement of the content of biomass, lipids, carbohydrates, and proteins was carried out once every three days by various methods used. The value of lipid content was calculated using the Bligh and Dyer method [33]. In addition, the value of carbohydrate content was measured using the phenol-sulfuric acid method and continued calculating the absorbance value using UV-vis spectrophotometry. Nowadays, microalgae are considered cell factories to produce many biomass products such as lipids, carbohydrates, proteins, and pigments.

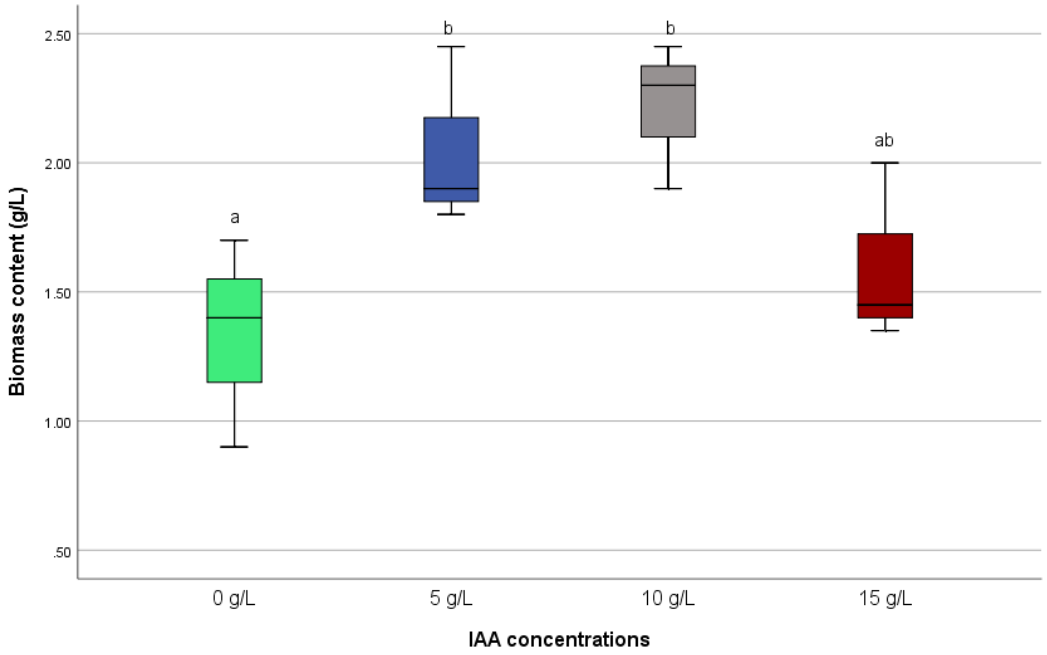

Figure 2. Biomass produced by *Euglena* sp. after the addition of IAA. Data were means ± SD (n=3). Different small and capital letters indicated significant differences between treatments and were calculated by one-way ANOVA followed by Duncan Multiple Range Test (DMRT) ($p < 0.05$)

Figure 2 showed the value of the amount of biomass content in *Euglena* sp. The addition of the hormone IAA (Indole-3-acetic acid) in the accumulation of IAA 10 g/L had the highest biomass content (2.216 g/L ±0.284) compared to other treatments. The biomass content in microalgae was determined by many factors such as microalgae species, nutrient composition (micronutrients and macronutrients), light intensity, carbon dioxide, etc.

Table 2. The productivity of Carbohydrates and Lipids of *Euglena* sp.

Treatments	Carbohydrates (g/L)
Control (IAA 0 g/L)	0.456 ± 0.018[a]
IAA 5g/L	0.498 ± 0.049[a]
IAA 10g/L	0.481± 0.098[a]
IAA 15g/L	0.490± 0.053[a]

Note: A number in the same column followed by the same letter (a) indicates an insignificant difference in the production of carbohydrates and lipids in *Euglena* sp. With or without the addition of IAA hormones and analyzed using standard test and one-way ANOVA through Duncan Multiple Range Test (DMRT) (*p value* <0.05).

According to Table 2 described the results of carbohydrate production in *Euglena* sp. The results in Table 2 showed the productivity of carbohydrate production in *Euglena* sp. It was observed that IAA 5 g/L produced the highest carbohydrates around 0.498 g/L ± 0.049, followed by the IAA treatment of 15 g / L (0.490 g/L ± 0.053), IAA treatment of 10 g / L (0.481 g/L ± 0.098) and control (0.456 g/L ± 0.018). The results of carbohydrate production with the addition of the IAA hormone to *Euglena* sp. were no insignificant deference ($p<0.05$), so the addition of the IAA hormone did not affect the carbohydrate production of *Euglena* sp. This result was in contrast to the research presented in the study by Singh *et al*. (2016) [22] that IAA phytohormones played an important role in triggering cell enlargement, cell differentiation, cell division, cycles of various metabolic disorders, and cell responses to environmental stress. Carbohydrates in microalgae were the main result of photosynthesis pathways and were found in plastids and other components, such as cell walls, in the form of

polysaccharides [34]. Many factors in the environment and in cells, both macronutrients and micronutrients, affected whether carbohydrates were made. The presence of macronutrients such as nitrogen, carbon, and phosphorus was a determining factor in biomass production [34]. When nitrogen was limited, the metabolism of protein photosynthesis changed, therefore, more carbohydrates and lipids were synthesized [35], [34]. Carbohydrates were affected by many factors, such as the lack of nitrogen content in low IAA concentrations. Therefore, more research needs to be conducted to find out the IAA concentrations for producing the highest carbohydrates in *Euglena* sp. In addition, inappropriate results influenced by the type of organism being tested, the addition of auxin hormone (IAA) had different effects on microalgae, and the metabolites produced were also different in each microorganism [21].

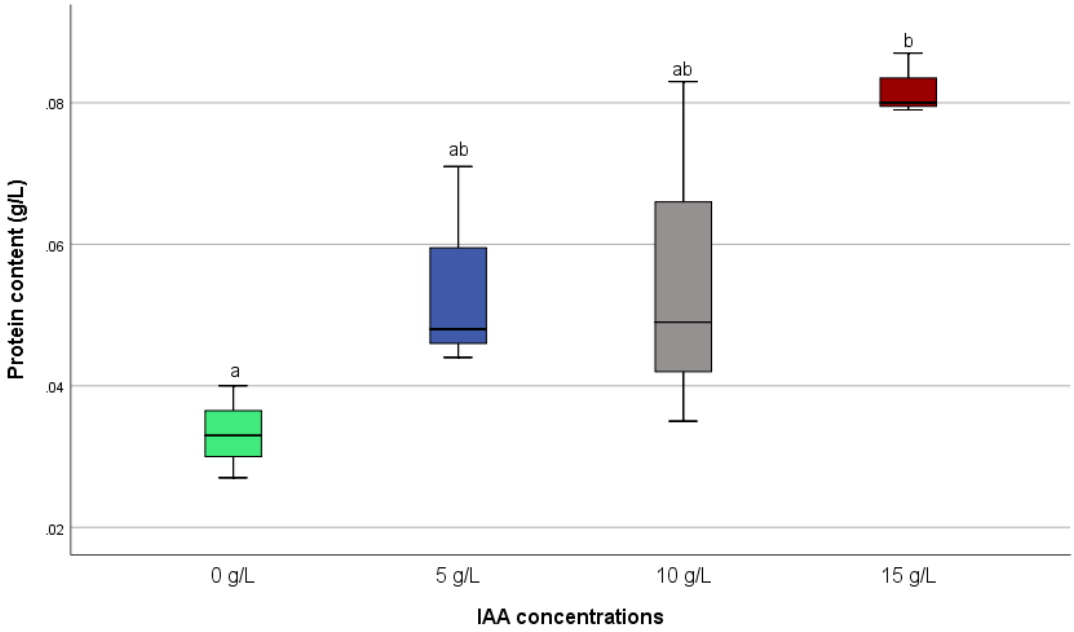

Figure 3. The Protein production of Euglena sp. on the last day of cultivation. Data were means ± SD (n=3). Different small and capital letters indicated significant differences between treatments and were calculated by one-way ANOVA followed by Duncan Multiple Range Test (DMRT) ($p < 0.05$)

Figure 3 showed the value of protein production in *Euglena* sp. According to Figure 3, the treatment of 15 g/L had the highest protein productivity value (0.082 g/L± 0.004), then the treatment of 10 g/L (0.055 g/L ± 0.025), followed by the treatment of 5 g/L (0.054 g/L ± 0.015) and control treatment (0.033 g/L ± 0.006). The addition of IAA produced a significant value ($p<0.05$) and exerted an influence on protein productivity. *Euglena* sp. utilized several proteins as a source of nitrogen which was used for growth and other metabolism. Amino acids such as methionine acid, and leucine can inhibit growth, and glutamate acid was used as a source of carbon and nitrogen [36].

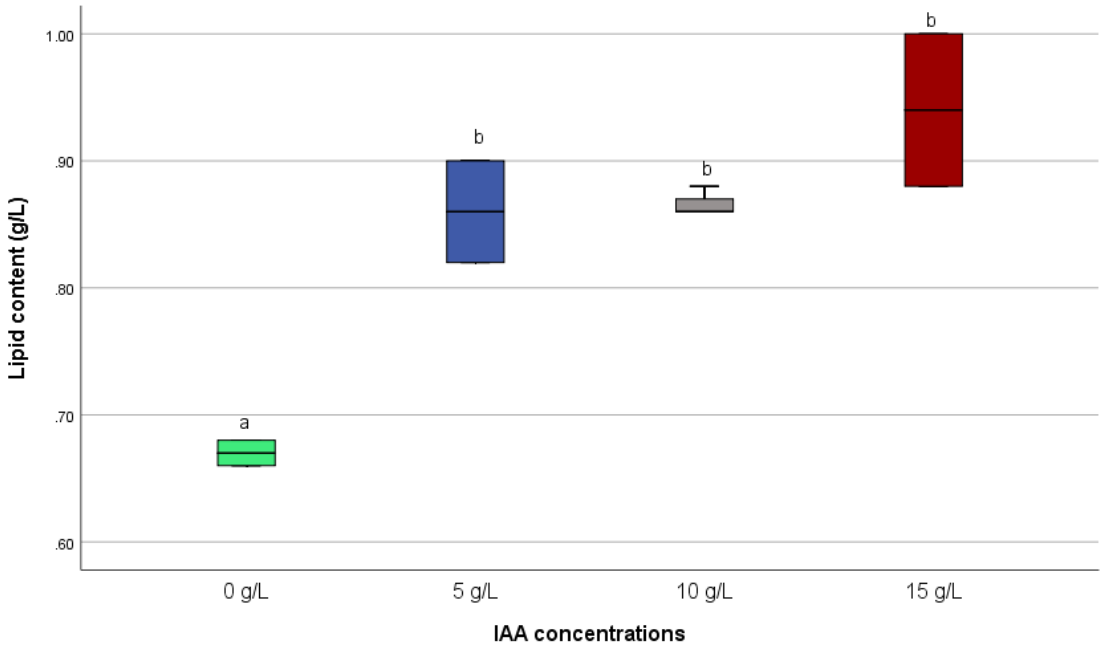

Figure 4. Lipid content of *Euglena* sp. under various IAA concentration. Data were means ± SD (n=3). Different small and capital letters indicated significant differences between treatments and were calculated by one-way ANOVA followed by Duncan Multiple Range Test (DMRT) ($p < 0.05$)

Table 2 showed the value of lipid productivity in *Euglena* sp. after adding IAA treatment of 5 g/L was the most optimal treatment to produce the highest lipid content with a value of 0.940 g/L ± 0.084, followed by a treatment of 10 g/L and 5 g/L reached 0.86 g/L ± 0.011, 0.860 g/L ± 0.056, respectively. Control treatment produced 0.670 g/L ± 0.014. Lipid synthesis was influenced by many factors, both environmental factors and factors in *Euglena* sp., in addition to the nutritional content in *Euglena* sp., such as nitrogen and silica. Nitrogen content affected lipid biosynthesis in microalgae [37]. The lipid content of microalgae was also influenced by the growth rate and the growth period of the kultur. The results of lipid productivity values according to research belonging to [37] which stated that microalgae with a high growth rate had low lipid levels while microalgae with a low growth rate had high lipid levels. According to ANOVA, the result was significantly different between the control group and treatments.

Pigment Content

Microalgae are autotrophic organisms that carried out the process of photosynthesis in their cell metabolism. Photosynthetic started from light absorption by a light-capturing complex, which contained pigments that absorb, and capture light based on the wavelength each photosynthetic pigment can absorb. The pigments in microalgae absorbed different light differently due to the diverse light absorption characteristics. The light absorbed by this pigment used as a material for the photosynthesis process. This pigment showed a distinctive colour when absorbing a specific light spectrum, for instance, in chlorophyll which was the primary pigment for photosynthetic oxygen synthesis. At the same time, carotenoids function in white, green, and blue trans-light in microalgae suspense. Phycobilin served in assisting in the absorption of light with different light spectra in microalgae cells in low-light states [38].

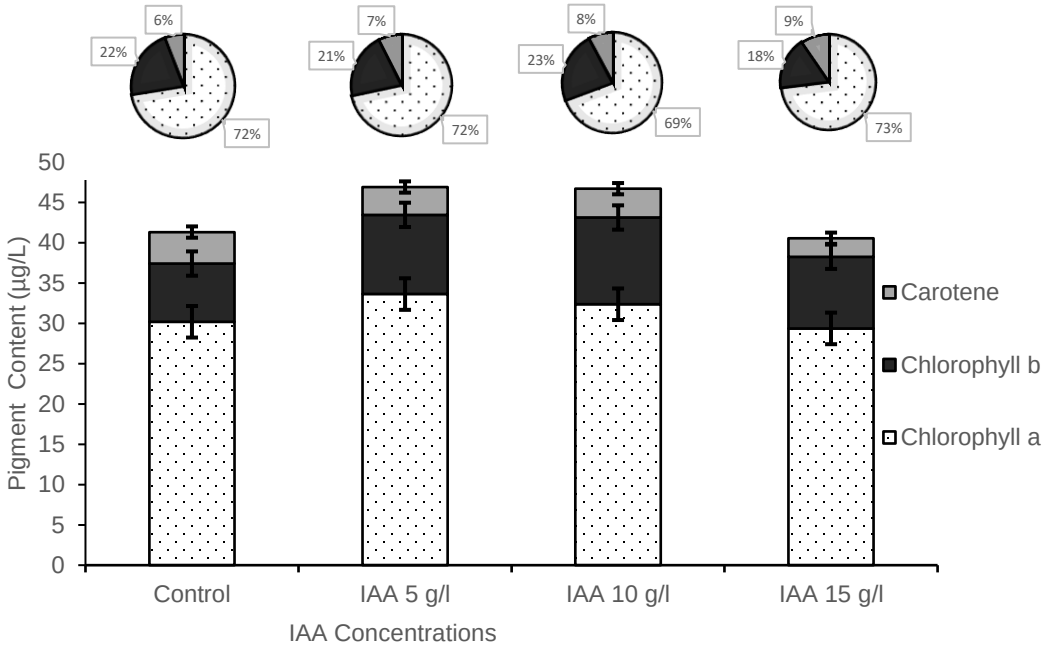

Figure 5. The production of Carotene, Chlorophyll a, and Chlorophyll b Euglena sp. on the last day of cultivation

Based on the data from Figure 5, It showed that the control treatment produced a pigment content of 72% chlorophyll-a, 23% chlorophyll-b, and 6% carotene. In comparison, the addition of IAA 5 g/L resulted in a range of 72% chlorophyll-a, 21% chlorophyll-b, and 7% carotene; the addition of IAA 10 g/L produced 69% chlorophyll-a, 23% chlorophyll-b, 8% carotene and the addition of IAA 5 g/L resulted in 73% chlorophyll-a, 18% chlorophyll-b, 9% carotene. An IAA addition of 5 g/L produced the highest chlorophyll-a, while the addition of 10 g/L IAA had the most chlorophyll-b levels, and the addition of 15 g/L IAA made the highest carotene content. The addition of IAA produced an insignificant result ($p<0.05$); therefore, the addition of IAA to *Euglena* sp. did not affect the pigment composition. Chlorophyll-a content dominated the total pigment content because chlorophyll-a plays an essential role in supporting the process of photosynthesis rate. It also showed on the study belongs to [39][30], where microalgae needed the energy to perform photosynthesis; besides that, some nutrients were also required to increase the rate of photosynthesis. Nutrients needed such as nitrogen, carbon, and phosphorus.

The addition of the hormone IAA (indole-3-acetic acid) triggered faster cell growth and differentiation, but excessive treatment inhibited the rate of division and differentiation of existing cells. The addition of IAA levels of 5 g/L and IAA of 10 g/L provided a faster growth effect than the control treatment without the addition of IAA. Still, the treatment of IAA 15 g/L inhibited the growth of chloroplast cells. Previous research reported that the trend of subtraction of light intensity was non-linear with pigment content in microalgae; intracellular pigment content increased but cell size and light absorption did not increase significantly. Current research focused on characterizing chlorophyll-binding proteins associated with photosystem I (PS1) and genes that encoded these proteins to increase microalgae biomass production [31]. One of the most significant factors in the process of light absorption by this pigment was sunlight, including weather changes, day and night cycles, seasonal changes, and so on will change the light intensity and its spectrum [40]. The light and dark cycle was an essential factor in the growth, differentiation, and production of biomass in microalgae. Changes in the light and dark cycles influenced variations in the biomass composition of microalgae. A balanced light/dark cycle led to maximum lipid production, whereas an unbalanced light/dark cycle led to lower lipid production [41]. Microalgae changed their chemical composition, pigment content, and photosynthetic activity when cultivated under various light and dark cycles [36]. The treatment of IAA phytohormones triggered the growth and cell division of microalgae cell cells, not the optimization of pigments and carotene regulation.

Conclusions

In conclusion, the optimal IAA concentration for enhancing the growth rate was in the 5 g/L. For the metabolite content, IAA 15 g/L was the best treatment, particularly for increasing carbohydrates, proteins, and lipids. The addition of IAA phytohormone as a treatment on *Euglena* sp. significantly affected the growth rate in *Euglena* sp. and its metabolite content. On the other hand, several parameters, such as carbohydrates did not have a significant effect after giving the IAA hormone. In this study, Phytohormone IAA (indole-3-acetic acid) was a growth hormone that stimulated and improved cell growth and differentiation. Research related to the optimization of cultivation in *Euglena* sp. using IAA phytohormones at appropriate doses still needs to be carried out to increase the production of biomass and primary and secondary metabolites from microalgae, especially on *Euglena* sp.

Conflicts of Interest

There is no conflict of interest regarding the publication of this paper.

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