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Effect of Light Quality, Light Intensity, and Cell Inoculum Arrangement on Growth, Pigment and Carbon Content from *Spirulina platensis* using LED Light

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ABSTRACT

This study evaluates the effect of lighting and cell inoculum in *Spirulina platensis* cultivation on its growth, pigment composition, and ability to capture CO_2. Different light intensities (1,000, 3,000, and 5,000 lux), light quality (white, blue, and red), and cell inoculum which are shown as OD values (0.2, 0.3, and 0.5) were assessed. The highest growth rate was obtained from red light, followed by white and blue light. The maximum biomass concentration (0.0711 mg/ml) was obtained when red light was used under 5,000 lux light intensity. The highest carbon content (5.1274 mg/ml algae) was also obtained during red light cultivation under 5,000 lux light intensity. Meanwhile, the highest chlorophyll (1.4365 mg/mg algae) content was obtained at blue light cultivation under 5,000 lux intensity and OD 0.5 cell inoculum. The highest phycocyanin (0.0309 mg/mg algae) was obtained under red light with 3,000 lux light intensity and OD 0.5 cell inoculum. It was found that the cultivation conditions to achieve high biomass and high pigment concentration were different.

1. Introduction

Microalgae are potential sources of biomass production, where they have various applications in the industry, including food, feed, cosmetics, and pharmaceuticals. Among the microalgae, *Spirulina* is considered one of the most valuable types of microalgae, which contain 60 to 70% of proteins in dry weight (López-Rodríguez *et al.* 2021). It also contains vitamins and minerals that usually can be used to incorporate food to increase its nutritional value. *Spirulina* is an important source of valuable pigments such as chlorophyll-a and phycobiliprotein (one of them was phycocyanin). Chlorophyll is one of the valuable natural green pigments where it can be used as a coloring and has health benefits, such as antioxidant, anti-inflammatory, and antimicrobial (Seo *et al.* 2018; Zhang *et al.* 2022). As for Phycocyanin, it is a blue pigment that can be used in industry for natural food coloring and in the biotechnology field as a fluorescent marker. It also has health benefits such as neuroprotective, anti-inflammatory, and antioxidant properties (Liao *et al.* 2016; Hao *et al.* 2018; Jiang *et al.* 2019). As a photosynthetic microorganism that needs CO_2 for its growth, microalgae can also be advantageous in capturing CO_2 and lowering the CO_2 concentration in the environment.

Microalgae can be cultivated by different kinds of methods, such as autotrophic, heterotrophic, mixotrophic, and photoautotrophic, which are differentiated by their nutrient supply (Verma *et al.* 2020). Photoautotrophic and mixotrophic cultivation methods require CO_2 and light for the photosynthesis process. Light serves as one of the most important factors during microalgae and cyanobacterial cultivation, where the light energy is captured and converted to carry out the photosynthesis process (Prates *et al.* 2018). During large-scale cultivation, generally, the production uses the sun as

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a light source. However, cultivating outdoors in large-scale production has its limitations, one of which is light irradiance which fluctuates based on season and might affect algal growth and its biocomponent content (Holdmann *et al.* 2019). Therefore, indoor cultivation is used to solve the limitation. Indoor cultivation usually uses artificial light as a light source. One of the artificial lights that is usually used is LED, which has various advantages. LEDs' main advantages are high efficiency of electricity conversion, reduced energy consumption, smaller material mass and volume, lower heat dissipation, and a single wavelength (Atta *et al.* 2013).

Some factors need to be considered, such as light sources, nutrition, and cell inoculum, to achieve high yields of biomass and high value-added products. Light has a great impact on spirulina growth, where light is the main source of energy for *Spirulina* to perform photosynthesis. Photosynthesis is performed through the light-harvesting complex, which consists of photosynthesis pigments such as chlorophyll, phycobilisomes, and carotenoids (Liu and Blankenship 2019). Each pigment has a different absorption range of light wavelength, where phycocyanin from phycobilisomes absorbs green-yellow (550-630 nm) and orange-red (650-670 nm). In comparison, chlorophyll absorbs violet light (~430 nm) and red light (660 nm) (Devaraja *et al.* 2017). Several studies have been carried out to evaluate the effect of light colors on the cell growth and pigment production of *S. platensis*. Prates *et al.* (2018) found that the highest biomass concentration was obtained with red light in *Spirulina*. Jung *et al.* (2021) also show that the highest cell growth and highest cell densities in *Arthorspira platensis* are in the order of red > white > green > blue LED light. It was also found that the highest phycocyanin concentrations were found in the order of blue > white > red > green LED light (Jung *et al.* 2021).

Light intensity has also a great impact on spirulina growth. Some evidence proves the highest spirulina biomass concentration was obtained at the highest light intensity (González-Camejo *et al.* 2019; Chaiklahan *et al.* 2022). Microalgae growth is proportional to light intensity until reaching a saturation point at which the photosynthetic activity of microalgae achieves its maximum value (Liu and Blankenship 2019). When microalgae are cultivated at low intensity, their growth will be limited. On the other hand, if the microalgae are cultivated with high intensity and exceed the optimum value, the photosystem will be damaged, causing photoinhibition (Ramanna *et al.* 2017). Cultivating microalgae in high cell density may result in light-shading in the culture, which may lead to lowering their growth rate. The past research of Chaiklahan *et al.* (2022) found that during the OD of 0.4 the specific growth rate is higher compared to the higher OD such as 0.6 and 0.8. However, this study did not inform the growth rate if we use OD 0.5 and lower that 0.4. Therefore, in this study we use inoculum with optical density 0.2, 0.3, and 0.5.

In this study, we investigate the relationship between light intensity, light quality, and cell concentration on biomass growth, chlorophyll content, phycocyanin content, and carbon content of *S. platensis*. The cultivation was conducted in a photobioreactor with a batch process. To analyze the effects of light type, light intensity, and cell inoculum on the growth rate, Carbon organic content, pigment content, and biomass content of *S. platensis* microalgae, measurements of optical density, carbon content, chlorophyll content, and phycocyanin content will be conducted.

2. Materials and Methods

2.1. Pre-Culture Preparation *Spirulina platensis*

The microalgae used in the experiment is *S. platensis*, which has been used widely in many performance studies. The culture was inoculated in a 2 L cylindrical tube with Zarrouk media. Zarrouk media was prepared to culture the cyanobacterium, which is composed of (per liter of distilled water) 16 g $NaHCO_3$, 2.5 g $NaNO_3$, 1 g NaCl, 0.5 K_2HPO_4, 0.04 g $CaCl_2 \cdot 2H_2O$, 1 g K_2SO_4, 0.2 g $MgSO_4.7H_2O$, and 0.01 $FeSO_4$. The prepared medium pH was adjusted to 8-9 and maintained at room temperature (25 + 2°C) and under white continuous light exposure. The pre-culture was carried out for more than a week to make a culture stock until the desired amount of culture was achieved.

2.2. Photobioreactor (PBR) Setup and Settings

Microalgae were cultivated in 2 L photobioreactors. Photobioreactors material used in this research is glass photobioreactors. Each of the photobioreactors was equipped with silicon pipe and a plastic pipe that connected to an air pump. Each of the PBR will be placed at a rack with light source. The light source used in this research is a light panel with different light colours for each panel, which are red, blue, and white LED lights, as shown in Figure 1. The distance between the reactors and the illumination source will be adjusted based on the requirement to get the desired light intensity.

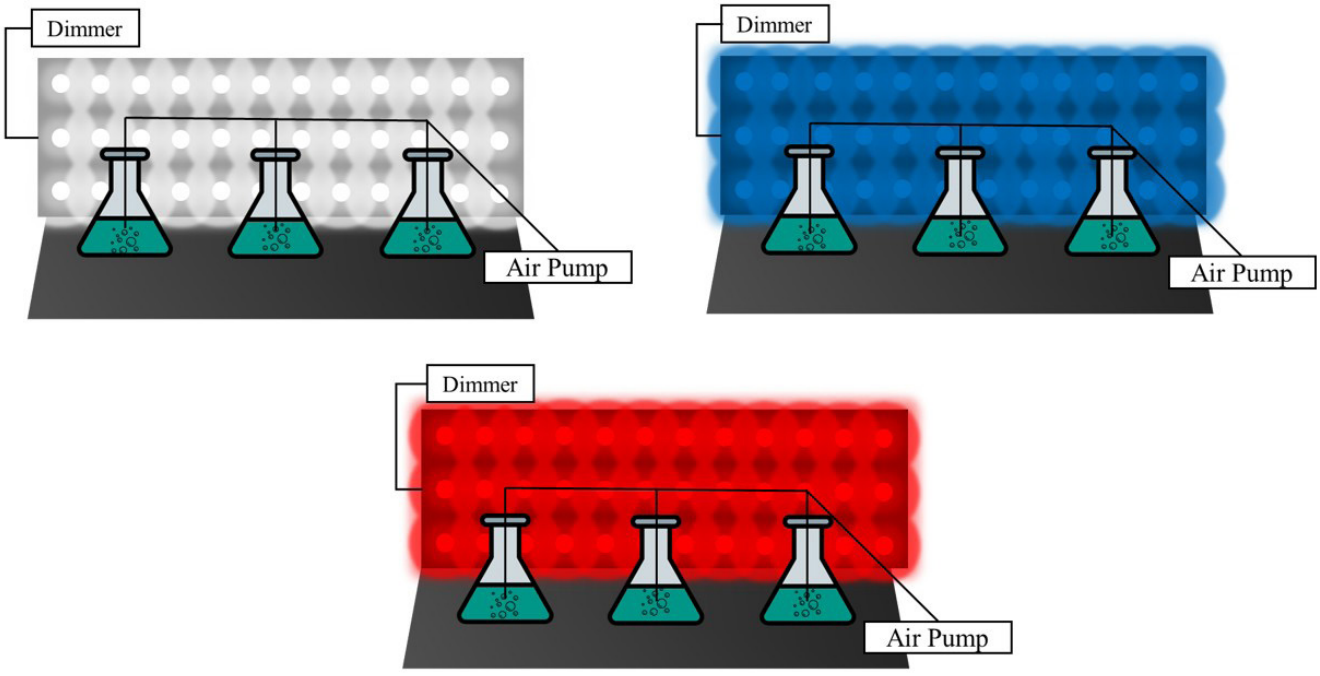

Figure 1. *Spirulina platensis* cultivation in Photobioreactor under white light (control), blue light, and red light on different inoculum densities (0.2, 0.3, and 0.5)

The light panels size was 60 cm × 15 cm (length × width). This panels consist of 36 LED light, where each LED light was 1 watt. This panels are equipped with dimmer to control light intensity.

2.3. Experimental Design

Each PBR flask was inoculated with *S. platensis* with different optical density values of 0.2; 0.3; and 0.5. The OD value was obtained by diluting culture stock with fresh medium until the ratio is 1:2. Each flask was then cultivated under different light intensities which are 1,000 lux, 3,000 lux, and 5,000 lux for blue light, red light, and white light (control). Continuous lighting (24h light: 0h dark) and continuous aeration were provided during the cultivation process. The aeration was provided through an air pump that connected with plastic flexible pipe. The cultivation was conducted for 54 hours at room temperature (27°C ± 2). Optical density and pH were measured periodically every 3 hours for 6 hours every day during the cultivation process. The optical density was measured by spectrophotometer UV-Vis, while pH was measured by pH meter. This experiment was done duplicate. The condition in which obtained the highest biomass then subjected to quantification of their chlorophyll, phycocyanin, and carbon content. Figure 1 presents a schematic diagram of the system used.

2.4. Determination of Biomass Concentration and Productivity

The growth of *S. platensis* was determined by measuring the optical density at 680 nm via spectrophotometer UV-VIS. 5 ml was taken from each cultivation and measured. If the absorbance was over the value of 1, then the sample will be diluted with distilled water and re-measured. The dilution is to obtain an OD value between 0.2-1. Measurement of cell dry weight was conducted by centrifuging and rinsing the culture sample. The supernatant was then taken to dry in the oven at 40°C until the biomass was fully dried. The dried biomass was then weighed using an analytical balance. The biomass dry weight is then calculated with the equation:

$$\text{Biomass concentration}\left(\frac{\text{mg}}{\text{L}}\right)=\frac{\text{W1 - W0}}{\text{volume sample}} \quad (1)$$

Where W1 is the weight of a drying container with algal biomass and W0 is the weight of a drying container without algal biomass. Meanwhile, the productivity of biomass was calculated according to equation (Gonçalves *et al.* 2014):

$$P\left(\frac{\text{mg}}{\text{L}}\right)=\frac{X_1 - X_0}{t_1 - t_0} \quad (2)$$

Where P is productivity (mg L^{-1} d^{-1}) and X_1 and X_0 are the biomass concentration (mg L^{-1}) at times t_1 and t_0 (days), respectively.

2.5. Measurement of Chlorophyll

The extraction of chlorophyll was conducted by adapting a method from Khairunnisa *et al.* 2024). Chlorophyll was measured by reading the absorbance of the solution at 663 nm and 645 nm via spectrophotometer UV-VIS. The concentration of chlorophyll was calculated using the equation:

$$\text{Chlorophyll concentration}\left(\frac{\text{mg}}{\text{ml}}\right) = 12.7 \times OD_{663} - 2.69 \times OD_{645} \quad (3)$$

$$\text{Chlorophyll content in alga}\left(\frac{\text{mg}}{\text{alga}}\right) = \frac{\text{Chlorophyll concentration}}{\text{dry weight biomass used}} \quad (4)$$

Where $OD_{663\ nm}$ has an absorbance value of 663 nm and $OD_{645\ nm}$ has an absorbance value of 645 nm.

2.6. Measurement of Phycocyanin

The extraction method of phycocyanin was conducted using a combination of maceration and ultrasonication (Tavanandi *et al.* 2018). A sample of 0.1 g was suspended with 5 ml of 0.1 M phosphate buffer pH 6.8 and incubated for 120 min at room temperature. The sample was then ultrasonicated with 50% amplitude for 2.5 min in an on-and-off cycle of 1/1s. The sample was then centrifugated at a low temperature of 4°C for 30 minutes at 5,000 rpm. The supernatant was then measured at 620 nm and 652 nm with a spectrophotometer UV-Vis. The phycocyanin concentration was then calculated using the equation:

$$\text{Phycocyanin concentration}\left(\frac{\text{mg}}{\text{ml}}\right) = \frac{OD_{620} - 0.70 \times OD_{650}}{7.38} \quad (5)$$

$$\text{Phycocyanin content in alga}\left(\frac{\text{mg}}{\text{mg alga}}\right) = \frac{\text{phycocyanin concentration}}{\text{dry weight biomass used}} \quad (6)$$

Where $OD_{620\ nm}$ has an absorbance value of 620 nm and $OD_{650\ nm}$ has an absorbance value of 650 nm.

2.7. Quantification of Carbon content

The quantification of carbon content was conducted using the colorimetric method provided by Black-Walkley (Walkley and Black 1934). The solution was measured at 600 nm with a spectrophotometer UV-VIS. Carbon content in microalgae *S. platensis* was calculated using the equation:

$$C_{\text{organic carbon}}\left(\frac{\text{mg}}{\text{mg mg alga}}\right) = \frac{\text{intercept} + \text{slope}}{\text{dry weight sample}} \quad (7)$$

Where $C_{\text{organic carbon}}$ has a carbon organic content value, and $OD_{600\ nm}$ has an absorbance value of 600 nm.

2.8. Data Analysis

The significance of obtained data is represented by performing ANOVA test then further analysed by Tukey's test. The statistical significance (the probability value; p) analysis of all parameters are observed according to $p<0.05$. The ANOVA tests are performed using Origin software.

3. Results

3.1. Effects of Light Arrangement and Cell Inoculum on Growth Rate

Figure 2 shows the growth rate of *Spirulina platensis*, which is cultivated under different conditions. In Figure 2, the growth rate was shown when *S. platensis* was cultivated under 1,000, 3,000, and 5,000 lux light intensity. Generally, it is found that the highest growth rate for each light intensity was found in cultivation under red light, followed by white and blue light. While red-light cultivation showed an increasing growth rate during the cultivation period, blue-light, on the contrary, showed a decreasing growth rate at every light-intensity cultivation. It is shown that *S. platensis* was having difficulties growing under blue light. The highest growth rate was obtained during cultivation under red light with 5,000 lux light intensity and 0.5 cell inoculum, in which the growth rate was up to 0.0366/ hour at 24h cultivation period (Figure 2C). While the lowest was found under blue light cultivation at 1,000 lux with 0.2 cell inoculum.

Based on Figure 2A and B, it showed that under the same light intensity and light qualities, cultivation with cell inoculum of 0.3 showed to have the highest growth rate compared to 0.2 and 0.5. On the other hand, when *S. platensis* was cultivated with red light and white light under 5,000 lux light intensity, the highest growth rate was obtained when cultivated with 0.5 cell inoculum.

3.2. Effects of Light Arrangement and Cell Inoculum on Biomass Dry Weight

As shown in Figures 3A, B, and C, the biomass was shown to increase with the increase of optical density (OD) value in every light quality and light intensity. The highest biomass obtained from white light was at an OD

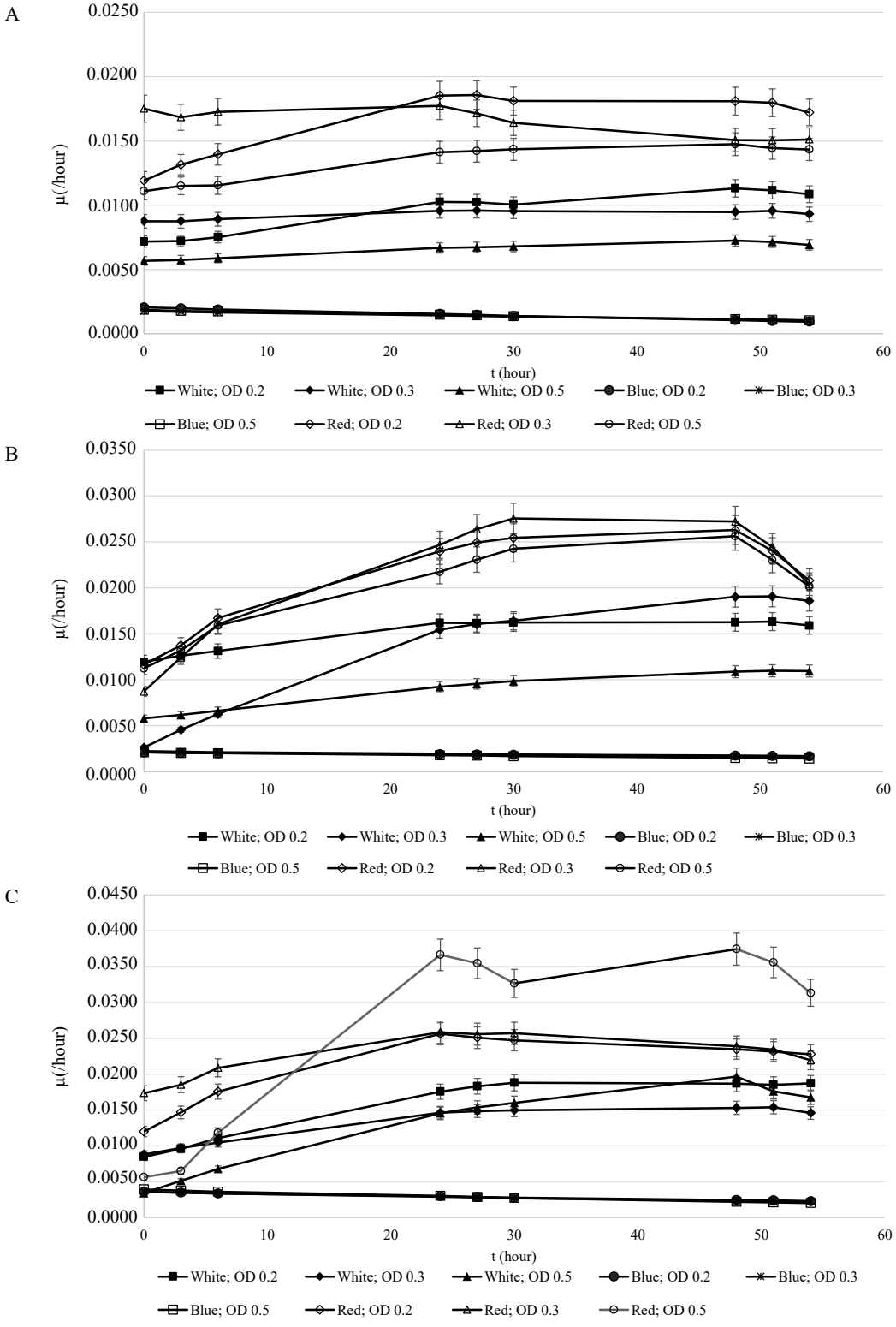

Figure 2. Growth rate of *Spirulina platensis* cultivated under different light qualities (white, blue, and red) and different cell inoculum (0.2, 0.3, and 0.5) at (A) 1,000 lux, (B) 3,000 lux, and (C) 5,000 lux

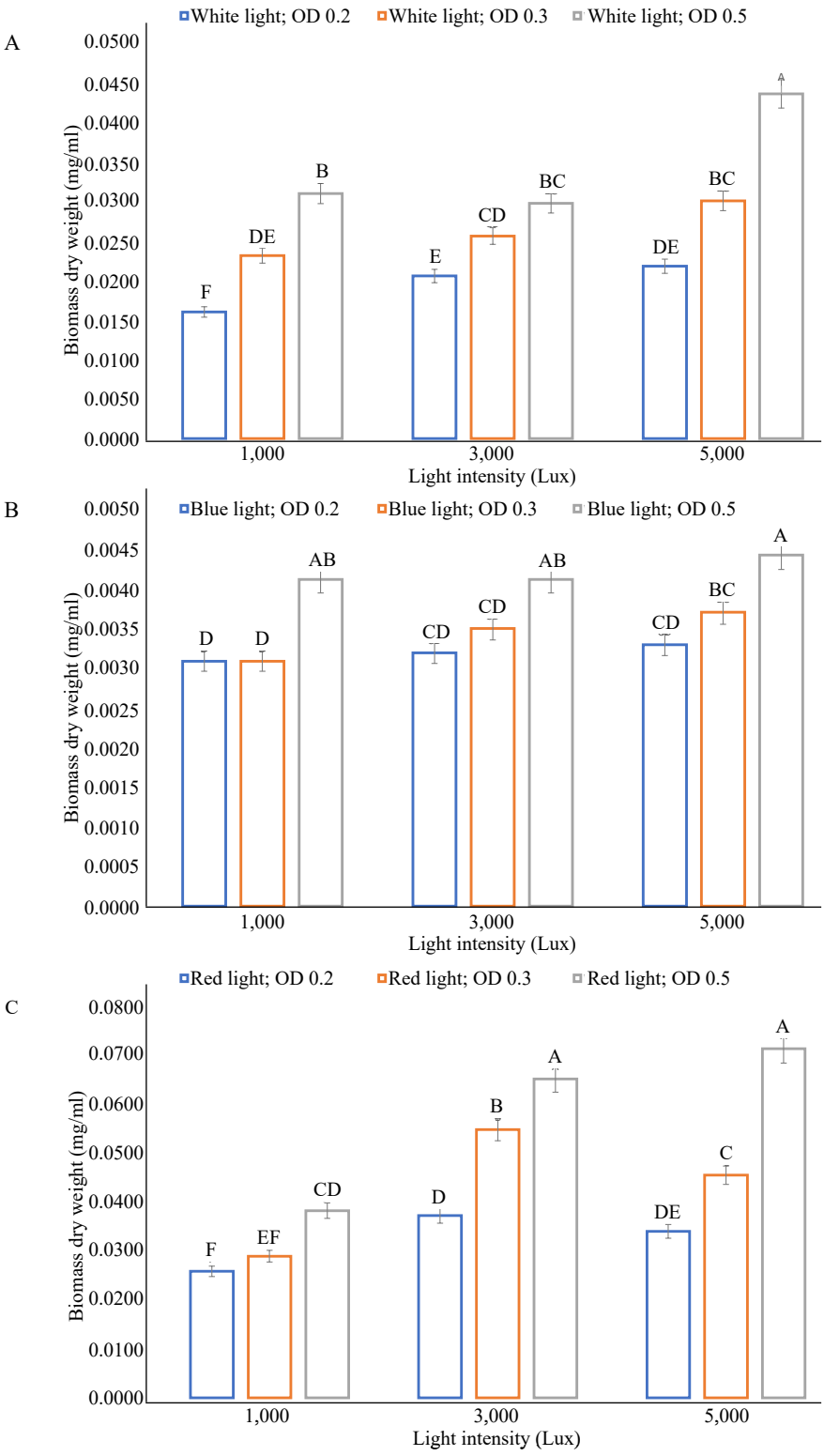

Figure 3. Final biomass concentration of *Spirulina platensis* cultivated under different light intensity and cell inoculum at (A) white light, (B) blue light, and (C) red light

value of 0.5 (Figure 3A), where 0.0422 mg/ml from 5,000 lux. Under blue light cultivation (Figure 3B), the highest biomass was also obtained from an OD value of 0.5, where 0.0043 was found from 5,000 lux. Under red light cultivation (Figure 3C), the highest biomass obtained was also from OD value of 0.5, where 0.0690 mg/ml from 5,000 lux. Therefore, it is found that the highest biomass was obtained from an OD value of 0.5 for every wavelength and every light intensity. Meanwhile, it is shown that under red light cultivation with cell inoculum OD values of 0.2 and 0.3, the biomass value seems to be decreasing when the light intensity was increased from 3,000 lux to 5,000 lux.

Figure 4 shows the highest biomass when *S. platensis* was cultivated under red light, where the biomass dry weight is 0.0690 mg/ml for each light intensity, followed by white and blue light. The biomass concentration was shown to have a growth limitation when cultivated under blue light, which corresponds to the growth rate shown in Figure 2. It is shown from the statistical analysis where consistently red light results in the highest biomass dry weight at each intensity level, where it is labelled A during 3,000 and 5,000 intensity. However, the biomass which obtained from red light during 1,000 lux light intensity is not significantly different from white light in 5,000 lux light intensity. The statistical analysis shows that there's no significant difference between biomass obtain from blue light with the increasing of light intensity, where the value was labelled D across all intensities.

3.3. Effects of Light Arrangement on Carbon Content

Based on Figure 5, it is shown that the light intensity was shown to have a positive linear effect with carbon content. The highest carbon content was obtained under red light cultivation with 5,000 lux, where the carbon content was 5.1274 mg/ml algae. However, at 5,000 lux, the carbon content between red and white light was not significantly different (labelled A), where under white light the carbon content was 5.0426 mg/ml algae. Under blue light, the carbon content was lower compared to red and white light for every light intensity.

3.4. Effects of Lights Arrangement on Chlorophyll Content

The study was conducted with cell inoculum (OD) of 0.5, which has the highest biomass accumulation. The effects of lights on chlorophyll content are shown in Figure 6. As shown in Figure 6 it is shown that the chlorophyll content of *S. platensis* is increasing with the increase of light intensity from 1,000 to 3,000 lux. Where the highest chlorophyll content in 3,000 lux was obtained from red light with 0.9094 mg/mg algae, but it wasn't significantly different from white light chlorophyll content which is 0.8578 mg/mg algae, where it shares the same label B. Furthermore, it is shown that 5,000 lux light intensity increases the chlorophyll content from in blue light cultivation from 0.406 to 1,395 mg/mg. However, it is also shown

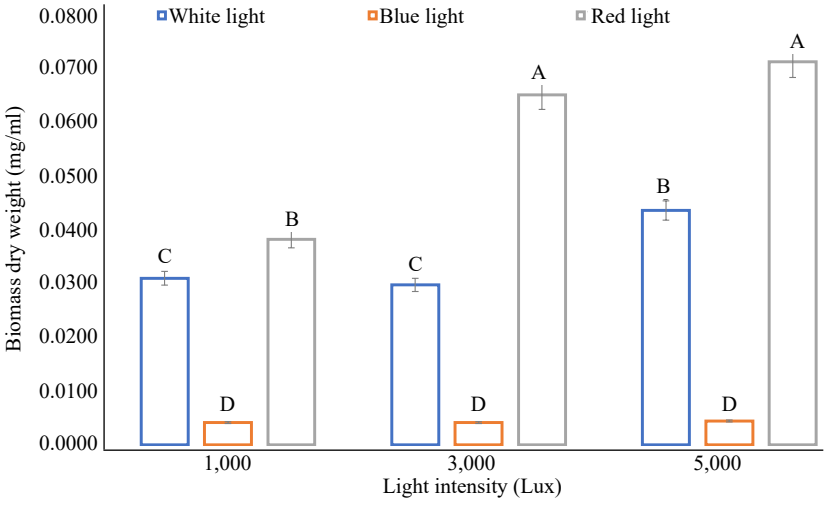

Figure 4. Final biomass concentration (dry weight) of *Spirulina platensis* during cultivation under 0.5 cell inoculum and different light intensity and light quality

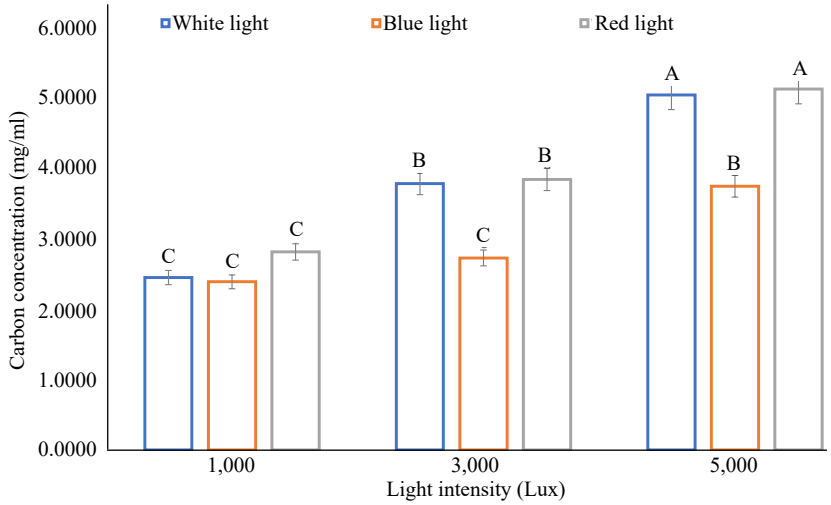

Figure 5. Total carbon organic concentration of *Spirulina platensis* under various light intensity and light colours

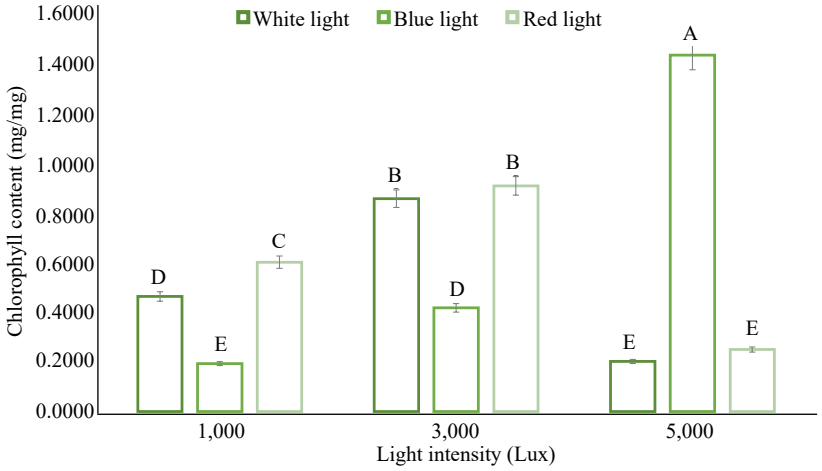

Figure 6. Chlorophyll content on *Spirulina platensis* cultivated on different light intensity and light colours

that the chlorophyll content is decreases at 5,000 lux during cultivation under white and red light.

3.5. Effects of Light Arrangement on Phycocyanin Concentrations

The study was conducted with cell inoculum (OD) of 0.5, which has the highest biomass accumulation. The effects of lights on phycocyanin content are shown in Figure 7. It is shown that the phycocyanin content of *S. platensis* is increasing with the increase of light intensity from 1,000 to 3,000 lux. Where the highest phycocyanin content in 3,000 lux was obtained from red light with 0.0309 mg/ml algae. Furthermore, it is shown that increasing light intensity up to 5,000 lux, lower the phycocyanin content for red light (0.0247 mg/ml) and increasing phycocyanin concentration in blue light from 0.0175 mg/ml to 0.0245 mg/ml. However, both blue and red light in 5,000 lux light intensity show similar effects and marked as B.

4. Discussion

This study provides insights into the cultivation of *S. platensis* under different illumination arrangements and their cell inoculum with artificial LED as a light source. This study aims to investigate the effects of light intensity, light quality, and initial inoculum concentration on the growth, pigment production, and carbon content of *Spirulina platensis*; this cyanobacterium was cultivated under diverse experimental conditions.

As a photosynthetic microorganism, *Spirulina platensis* uses light as a main source of energy, where light intensity and light quality have been proven to have a significant impact on cyanobacterial growth and

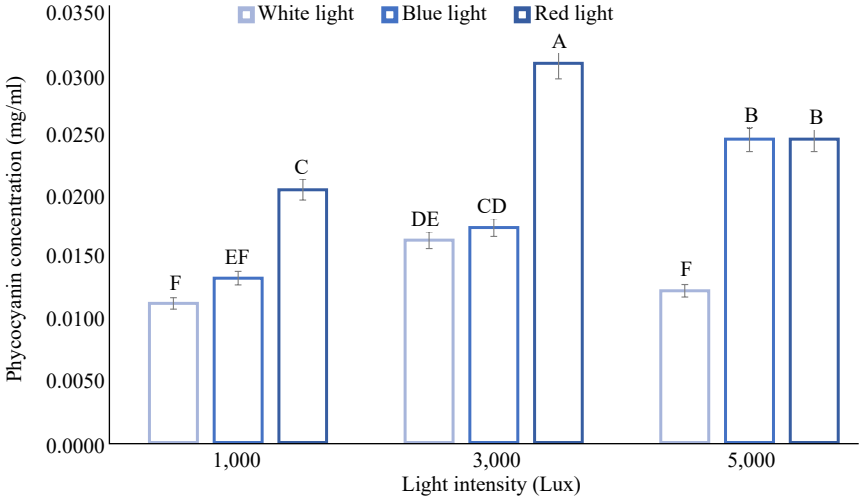

Figure 7. Phycocyanin content on *Spirulina platensis* cultivated under different light intensities and light colors

metabolism. In order to utilize the light as an energy, the light was capture by their photosynthetic pigments including chlorophyll, phycocyanin, and carotenoids which located in chloroplast. Each of those photosynthetic pigments absorb light in specific wavelengths, where phycocyanin effective to absorbs 550-630 nm and 650-670 nm and chlorophyll is 430 nm and 660 nm (Devaraja *et al.* 2017). Therefore, light conditions affect the production of biomass and their metabolite production. Red light and blue light were two of those light that are widely used in research, where red light cover 620-645 nm light spectrum and blue light covers ~440 nm light spectrum (Prates *et al.* 2018). Therefore, in this study the light qualities used in this study were red, blue, and white, where white light was used as control. As in light intensity, he intensity of the light intensity used in this study were 1,000, 3,000, and 5,000 lux. There's certain light intensity that allowed to be absorb that are those light intensity in which under the light saturation area. Further increase intensity than light saturation area may cause photoinhibition, in which will cause stress to the microalgae cell which leads to lower biomass production and death. It has been proven that for microalgae Spirulina sp. that light intensity between 1,000 up to 4,000 lux shows a positive effect towards cell productivity (Bhat et al., 2023).

In this study, from Figure 2 shows that increasing the light intensity shows to increase the biomass dry weight for each light quality and inoculum. This is possible due to increase of light exposure, therefore enhance their photon absorption which leads to accelerating photosynthetic rates and carbon fixation process. This statement was supported by Niangoran *et al.* (2021) where high light intensity (160 µmol/m^2/s) produces higher dry biomass compared to those who cultivated under low light intensity (80 µmol/m^2/s). Furthermore, the cell's highest biomass dry weight was obtained from the highest cell inoculum (OD 0.5) at every light intensity, but the highest was obtained from 5,000 lux light intensity. Meanwhile, it was also found that there was a visible decrease in biomass dry weight and growth obtained during cultivation under 5,000 lux with cell inoculums 0.2 and 0.3 compared to those who cultivated under 1,000 lux. This increased light intensity may be caused by photolimitation from cell concentration. Intense light exposure can induce photoinhibition in algae, which might lead to damage or inhibition of photosynthetic processes. This can result in decreased growth rates and metabolite production and even lead to cell death. Meanwhile, it is shown that the higher inoculum (OD 0.5) did not have a decrease biomass effect compared to those with OD 0.2 and 0.3. This happens due to the greater biomass contain on the liquid medium, which means denser cell populations. This denser population may create self-shading which can reduce the light exposure and therefore mitigate the photoinhibition effects. This was in line with the founding by Schipper *et al.* (2021), where using 50% inoculum can minimized photodamage even when using light intensity up to 5,600 µmol photons/m^2/s. On the other hand, Chaiklahan *et al.* (2022) stated that a higher OD value can also decrease the growth rate and may lead to lower biomass production, where it is found that OD 0.6 has a higher growth rate than OD 0.8. This may cause increasing self-shading and decreasing transparency of the algal culture; therefore, the light cannot penetrate well into the culture.

It is also found that the highest cultivation was obtained during red illumination. In contrast, blue LED illumination provides the lowest significant amount of biomass compared to red and white light. This was also shown in Figure 2, where the red light has the highest growth rate compared to white and blue light. It is well known that *S. platensis* could grow to produce biomass by absorbing energy from light using a photosynthetic apparatus (Chini Zittelli *et al.* 2022). This result proves that the photosynthetic pigments in *Spirulina platensis* can absorb the efficient use of red light. *Spirulina platensis* consists of photosynthetic pigments such as chlorophyll and phycobilin. These pigments absorb light and are used for photosynthesis. Higher light intensity can enhance the production of these pigments, which may eventually enhance photosynthetic efficiency. There are three possible fates for the absorption of light by chlorophyll pigments. First, it is emitted as heat or fluorescence through redistribution into atomic vibrations within the molecule. Second, light energy is transferred to nearby photopigments via resonance; third, photochemical reduction/oxidation, and the electron is transferred to a new molecule. The first one occurs during saturating light conditions and may potentially damage chlorophyll excitation energy, which leads to the production of free radicals. The second and third one occurs under optimal conditions and leads to photosynthetic light reactions (Wang 2020).

The highest biomass that was obtained from OD 0.5, was then further analyse the content of chlorophyll, phycocyanin, and their carbon content. The highest chlorophyll concentration levels were observed under blue and red-light cultivation, suggesting that the microalgae absorb these wavelengths most efficiently. The higher chlorophyll content under blue light may be linked to slower growth rates, allowing the cells to have more time to synthesize valuable compounds (Tayebati *et al.* 2021). These results are consistent with the findings from Kim *et al.* (2014), which stated that the microalgae cells have the highest absorption in the peak of red and blue light wavelength, and the high amount of chlorophyll belongs to blue light due to larger cells produced. The cultivation under blue light conditions may leads to stress growth conditions which trigger microalgae to produce phycocyanin higher as an adaptive response. When microalgae cultivated under a stress conditions, such as light deficiency or nutrition excess, Spirulina tends to diver their energy to the productions of secondary metabolites, including phycocyanin as a survival strategy. The energy produced from blue light is used more for pigment synthesis rather than cell growth processes such as cell division and multiplication (Sohani *et al.* 2023). This may explain why during *Spirulina* sp. cultivation under the blue light, the increase of phycocyanin is higher even though the biomass concentration is low. Furthermore, it is shown that red light promotes the production of phycocyanin higher than using blue light and white light. It is in line with the finding from Tayebati *et al.* (2021), where the highest phycocyanin concentration was found when cultivated under red light. Also, it is found that despite promoting biomass growth, when *Spirulina platensis* was cultivated under high red-light intensity, the chlorophyll and phycocyanin content was reduced. Red light is strongly absorbed by chlorophyll and phycocyanin pigments, increasing the risk of giving overload energy within high light intensity. This energy overload might accelerate photodamage to photosystem II (PSII) and lower the pigment biosynthesis pathways (Maali *et al.* 2024), also triggering carotenoid synthesis which divert resources from chlorophyll and phycocyanin production. Niangoran *et al.* (2021) mentioned that an increase in light intensity induces a decrease in the photosystem concentration of the thylakoid membrane and a decrease in the size of the photosystem II (PSII). Therefore, there is a trade-off between chlorophyll and phycocyanin pigment biosynthesis and biomass accumulation. High growth condition conditions can suppress pigment production due to resource allocation and metabolic shifts (Depraetere *et al.* 2015).

It is known that during the photosynthesis process, microorganisms use energy from light to convert CO_2 into chemical energy and sugar, which is then called a carbon fixation process. Microalgae are no exception to this phenomenon. In this study, it was found that the highest carbon content was obtained from the highest biomass dry weight, which was obtained during cultivation under red light with 5,000 lux and cell inoculum 0.5. A finding from (Zhu *et al.* 2021) stated that the ability of microalgae to fixate CO_2 is linearly related to biomass production and growth rate. This phenomenon can be explained by the fact that photosynthesis has two phases, which are light reactions and dark reactions. The process of carbon fixation occurs in the Calvin cycle, specifically in the C3 cycle. This cycle requires ATP energy to reduce energy levels and consumes NADPH to convert carbon dioxide into glucose (Lokstein *et al.* 2021). This indicated that the ATP productivity obtained from the light reaction on red and white light was higher than that of blue light. Lower ATP production that happened in the blue light. Therefore, the carbon fixation activity in blue light was

lower than in red and white light. The lower production of ATP in blue light can be caused by the limited blue light spectrum that is absorbed by the protein pigment, which plays a significant role in capturing light and delivering it to photosystem I.

Meanwhile, red light and white light may have a better spectrum to be captured by the protein pigment, especially red light that have the highest amount of carbon content. The red light is easier to capture by protein pigment because the light spectrum from the red light that ranges from 620-645 nm right covers the spectrum of light absorption of phycocyanin (Jiang *et al.* 2023). Therefore, it efficiently absorbs red-light energy, which contributes to increased biomass accumulation and carbon content.

In conclusion, the results investigated the effects of light intensity, light quality, and initial inoculum concentration on the growth, pigment production, and carbon content of *Spirulina patensis* using artificial LED light sources. This study revealed that increasing light intensity generally enhanced biomass dry weight, likely due to increased photon absorption and photosynthetic rates. However, at high light intensity (5,000 lux), photoinhibition occurred in lower inoculum densities (OD 0.2 and 0.3), resulting in decreased biomass. Red light proved most effective for biomass production and carbon content, as well as in phycocyanin concentration. Meanwhile highest chlorophyll content can be found under blue and red light. However, high intensity in red light might reduce chlorophyll and phycocyanin content, which likely due to photodamage. Overall, this study shows that red light was identified as the most effective light quality, while the optimal light intensity and inoculum density must be carefully balanced to avoid photoinhibiton.

Further research might need to be done in the field to explore other biomass compositions as well as the morphological changes with the arrangements of illumination. Furthermore, further study which conducted with different light spectrum, different light source, as well as regulations of media cultivations can be done to further understand the effect of those alteration towards *Spirulina* growth and their biochemical compositions. In addition, an optimization study must be conducted to obtain the optimal condition to cultivate *Spirulina* sp. to obtained high biomass and their biochemical composition. The energy produced from the light can also be calculated for further understanding. This further research may lead to deepen the understanding of how light and cell concentration made changes to *S. platensis*.

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