Chlorophyll Content of *Chlorella vulgaris* (Beijerinck, 1890) on Different Light Intensity

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Abstract

Chlorella vulgaris is a green microalga (Chlorophyta) known to produce chlorophyll pigment as its primary pigment. Chlorophyll is known for its health benefits because it helps heal wounds and prevent hemophilia and diabetes and asthma. Chlorophyll is one of the pigments targeted as a functional food source. One of the environmental parameters that can affect chlorophyll content is the presence of light. This study aims to determine the effect of differences in light intensity on the chlorophyll content of *C. vulgaris*. The method used in this research is experimental, conducted in the laboratory using a completely randomized design (RAL). ANOVA is the statistical analysis used to analyze the effect of light intensity on chlorophyll content in *C. vulgaris. C. vulgaris* was cultivated with three different light intensity treatments, namely 1500, 3000, and 4500 lux, with three repetitions each. The growth of *C. vulgaris* was observed for 8 x 24 hours and then harvested by centrifugation on the eighth day to obtain the wet biomass. Cultivation wet biomass was extracted using acetone PA solvent. The acetone extract of *C. vulgaris* was analyzed for its chlorophyll pigment content using a spectrophotometer at 645 and 663 nm absorbance. The highest content of chlorophyll-a, b, and chlorophyll produced at a light intensity of 1500 lux was 26.2, 48.5, and 74.7 µg/ml, respectively. According to the results of statistical analysis, it can be concluded that different light-intensity treatments did not show a significant effect ($p>0.05$) on the content of chlorophyll-a, b, and total chlorophyll in *C. vulgaris.*

Keywords : Microalgae, functional food, pigment

INTRODUCTION

Functional food is essential for humans to provide good health nutrients and prevent diseases. Functional food is defined as naturally and technologically containing various compounds beneficial to human health (Granato *et al.*, 2017). One of the natural resources that can be utilized as a functional food is microalgae. Microalgae are microorganisms found in various ecosystems and environmental conditions, and they function as producers in ecosystems; therefore, microalgae are enriched with bioactive compounds (Ferrazzano *et al.*, 2020). Microalgae contain pigments that are necessary for photosynthetic activity. Pigments are stored in specific organelles, whose primary function is to absorb different wavelengths of visible light, thus reflecting color to human vision (Figon & Casas, 2021). The primary pigments in microalgae, such as chlorophyll, carotenoids, and phycobiliproteins (Andrade, 2018). Chlorophyll is one of the targeted pigments as a source of functional food due to its benefits in the health sector. Chlorophyll is known to prevent diabetes, asthma, and hemophilia. Chlorophyll, whose primary role is as a photosynthetic pigment, is a tetrapyrrolic pigment containing magnesium in its molecules, and its green color is obtained because chlorophyll absorbs light in the red and blue regions (Borah & Bhuyan, 2017). *Chlorella vulgaris,* an autotrophic microalgae that belongs to green algae (*Chlorophyta*), is rich in chlorophyll and can be extracted to produce functional foods (Rani *et al*., 2018). Functional food products from *C. vulgaris* are commercialized in the markets as powder, tablets, or even capsules. However, the chlorophyll found in *C. vulgaris* can be affected by several factors such as light, pH, temperature, and salinity, thus affecting the amount of content in *C. vulgaris*.

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Light is the primary energy source for cell growth and the synthesis of various essential compounds in *C. vulgaris* as a photoautotrophic organism (Novianti *et al*., 2019). Light can affect the process of cell metabolism through the process of photosynthesis and affect cell growth as a result. Lack of light will disrupt the photosynthesis process, leading to cell biosynthesis (Hanani *et al*., 2020). Light can be a limiting factor in the growth of microalgae, as well as a major factor during photosynthesis activity. The low light intensity can affect the photosynthesis rate, leading to decreasing growth rate. However, excessive light intensity can also cause photooxidative damage to the photosynthetic function of microalgae, thus decreasing the efficiency and photosynthesis rate, which is called photoinhibition (Erickson *et al.,* 2015).

MATERIAL AND METHODS

The *C. vulgaris* used in this study was pure stock from *Balai Besar Perikanan Budidaya Air Payau* Brackish Water Cultivation Fisheries Center (BBPBAP), Jepara, Central Java. This experiment was conducted in April – May 2023 in Marine Biology and Biotechnology Laboratory at Diponegoro University, Central Java. The experimental design used in this study was a completely randomized design (RAL) with an experimental method consisting of three treatments with three replications each. *C. vulgaris* cultivation was conducted using 3 litre of glass containers. The light source used in this study was an LED lamp with a light intensity treatment of 1500, 3000, and 4500 lux according to Fakhri *et al.*, 2017) that light intensity used were also 1500, 3000, and 4500 lux were found to affect growth and carotenoid content on *Nannochloropsis* sp. *Chlorella* can grow in light intensity ranging from 3000 – 11000 lux (Satriaji *et al.,* 2016)

Preparation

Sterilization was done to sterilize both equipment and materials used from contaminants. Seawater sterilization was done by boiling and filtering seawater until it boiled for approximately 2 hours. Seawater that had been boiled was cooled until it reached room temperature, stored in a plastic container, then covered with plastic wrap (Hartanto *et al.,* 2013). The equipment used in this study was also sterilized by rinsing them with fresh water, spraying with 70% alcohol, and UV irradiation for 2 hours (Dianita, Hasibuan and Syafriadiman, 2020).

Cultivation

Cultivation was carried out at a ratio of 1:2 (*C. vulgaris*: sterilized seawater) at a volume of 3000 mL. Walne fertilizer was given at a dose of 3 mL (1 mL/L) (Lasmarito *et al*., 2022). Media parameters observed included: pH, salinity, temperature, and dissolved oxygen. The media parameters were also measured once every 24 hours during cultivation. In *C. vulgaris* cultivation, aeration was also given to distribute nutrients well, preventing temperature stratification and fertilizer deposition (Prasetyo *et al*., 2022). *C. vulgaris* cell density was calculated every 24 hours for 8 days by taking 1 mL for each *C. vulgaris* cultivation sample treatment and placing it in a sample bottle. *C. vulgaris* cell density was calculated using a Haemocytometer Neubauer Improved Assistant Germany under a binocular microscope with a magnification of 25x and counted with a hand counter. The equation used in calculating the cell density of *C. vulgaris* refers to Elystia *et al*., (2021) as follows:

$$N = n\ x\ 10^4$$

Note: N = abundance of cells (cells/mL); n = number of counted cells; 10^4 = Volume of the hemocytometer box

Biomass Harvesting

Biomass harvesting was carried out during the growth phase of *C. vulgaris* in the stationary phase. *C. vulgaris* biomass was harvested using centrifugation to obtain the wet biomass at 3000 rpm for 10 minutes. The biomass as a paste is then weighed using an analytical scale to determine the wet weight.

Spectrophotometry Analysis for Chlorophyll Content of *C. vulgaris*

The *C. vulgaris* biomass was extracted using PA acetone solvent by taking a ± 1 gram sample for each treatment, pulverizing it with a mortar, and adding 10 mL of PA acetone. Samples were incubated for 12 hours at room temperature, and the supernatant was centrifuged at 3000 rpm for 10 minutes (Lasmarito *et al.,* 2022). The supernatant obtained was analyzed with spectrophotometry at the absorbance of 663 and 645 nm (Rizzi *et al.*, 2021).

Chlorophyll Content Estimation

The content of chlorophyll-a and chlorophyll-b with acetone solvent is calculated according to the equations referred to Sumiati (2021) as follows:

$$Chlorophyll - a \left(\frac{mg}{l}\right) = (12.7\ x\ OD\ 663) - (2.69\ x\ OD\ 645)$$

$$Chlorophyll - b \left(\frac{mg}{l}\right) = (22.9\ x\ OD\ 645) - (4.68\ x\ OD\ 663)$$

The total chlorophyll content with acetone solvent is calculated using the equation referred to Rajalakshmi & Banu (2015) as follows:

$$Total\ Chlorophyll = (20.2\ x\ OD\ 645) + (8.02\ x\ OD\ 663)$$

Statistical Analysis

Research data were analyzed using SPSS 22 and Microsoft Excel. Statistical analysis was performed using prerequisite, normality, and homogeneity tests to analyze variance (ANOVA) (Khadse *et al*., 2020). The parametric test conducted to determine the significant effect of light intensity on chlorophyll content in *C. vulgaris* in *C. vulgaris* is One-way ANOVA.

RESULT AND DISCUSSION

Different Light Intensities on C. vulgaris Cell Density

The growth rate of *C. vulgaris* is presented in Figure 1, the result of light intensity on the density of *C. vulgaris*. The density of *C. vulgaris* will increase with the increase of light intensity given. The highest cell density was obtained in treatment C (4500 lux) with an average cell density of 6569.7 cell/ml of eight days counting, followed by treatment B (3000 lux) of 5804.7 cell/ml, and the lowest density was obtained in treatment A (1500 lux) of 4601.7 cell/ml.

The high cell density in treatment C is due to the high light intensity, which can lead to high photosynthetic activity, so that algae growth will increase as the light intensity increases. Light will affect algae cultivation, and optimal light exposure is needed for microalgae to achieve maximum productivity because algae will absorb light and store this light in the form of ATP *(Gatamaneni et al.,* 2018). In treatment A, it was known that *C. vulgaris* reached the peak of the logarithmic phase faster on the fifth day and started its stationary phase on the sixth day, which is faster compared to treatment B and C. *C. vulgaris* reached the stationary phase faster due to photo limitation, a condition where there is not enough light is obtained by the microalgae to achieve the maximum rate of photosynthesis (Chen *et al.*, 2011). Statistical analysis was conducted using

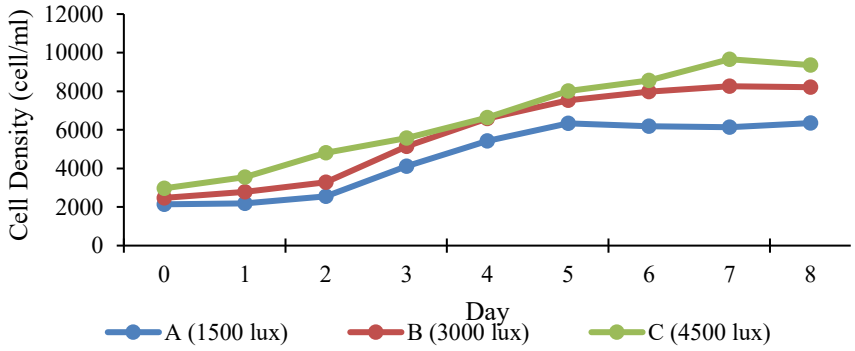

Figure 1. Different Light Intensity Treatments on Cell Density of *C. Vulgaris*

Table 1. Analysis of *One-way ANOVA* of Light Intensity on Cell Density of *C. vulgaris*

Source of Variation	Sum of Squares	df	Mean Square	F	*P-value*	F crit
Between Groups	19684184	2	9842092	2.134761	0.137808	3.354131
Within Groups	1.24E+08	27	4610396			
Total	1.44E+08	29				

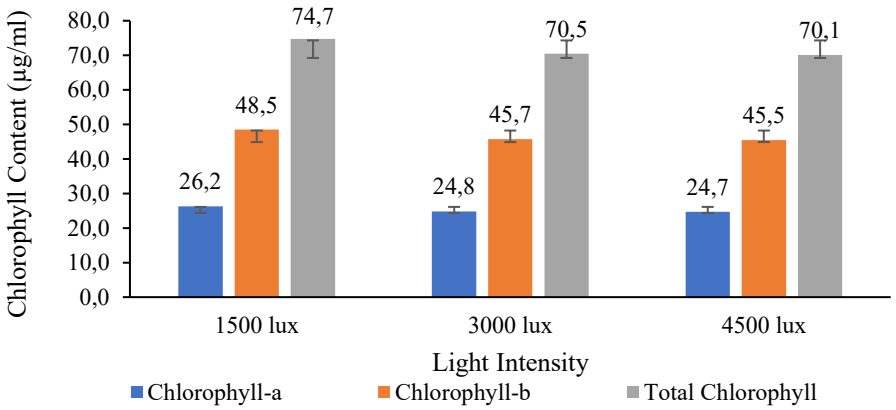

Figure 2. Chlorophyll Content of *C.vulgaris* on Different Light Intensity

Table 2. Analyses of *One-way ANOVA* of Light Intensity on Chlorophyll Content of *C. vulgaris*

Source of Variation	Sum Squares	df	Mean Squares	F	*P-value*	F crit
Between Groups	17.20972	2	8.604862	0.01585	0.984316	5.143253
Within Groups	3257.264	6	542.8774			
Total	688.0478	8				

the One-way ANOVA test presented in Table 1. No significant effect ($p>0.05$) was observed between light intensity and *C. vulgaris* cell density.

There was no significant effect of light intensity on *C. vulgaris* cell density due to the range of light intensity is still considered in the optimal light range for *C. vulgaris* to grow (1500, 3000, and 4500 lux). According to Febrieni *et al*. (2020), *C. vulgaris* can grow and photosynthesize optimally if the need for light is sufficient, which is in the range of 500 – 5000 lux; therefore, the range of light intensity used in this study is still classified as sufficient for the growth of *C. vulgaris,* so there is no significant effect of light intensity on *C. vulgaris* cell density. Similar research was also conducted by Indrastuti *et al*. (2014) regarding the effect of light on the growth of *Spirulina platensis* with treatments of 16 watts (450 – 750 lux), 23 watts (1000 – 1300 lux), and 45 watts (8000 – 9500 lux) showed that light intensity did not have a significant effect on *S. platensis* growth.

Different Light Intensities on Chlorophyll Content of C. vulgaris

Chlorophyll-a and chlorophyll-b are the main pigment components found in *C. vulgaris* (Serratos *et al.*, 2021). The content of chlorophyll-a, chlorophyll-b, and total chlorophyll in *C. vulgaris* is presented in Figure 2. It was found that the most considerable chlorophyll content was found in treatment A (1500 lux) with chlorophyll-a, chlorophyll-b, and total chlorophyll content of 26.2, 48.5, and 74.7 µg/ml, respectively.

According to Figure 2. presented above, the chlorophyll content of *C. vulgaris* will decrease as the light intensity increases. According to Kirk (2011), when microalgae are cultivated in conditions that require high pigments, such as conditions of low light intensity and high nutrient concentrations in the medium, the chlorophyll levels contained in microalgae tend to be higher than those found in natural conditions. Microalgae will carry out light-shade adaptations in which the photosynthetic activity carried out by microalgae will be reduced, resulting in less energy. This energy will produce a more efficient light-catching system which causes the chloroplasts in the cells to expand more. The result of light-shade adaptation will involve an increase in chlorophyll. In contrast, cells at higher light intensities will spend less energy on chlorophyll synthesis and focus more on enzymes related to enzyme fixation, such as carboxylase synthesis. Therefore *C. vulgaris* in treatment A has higher chlorophyll content *et al.*, 2015).

The results of the One-way ANOVA statistical analysis on light intensity on *C. vulgaris* chlorophyll contents are presented in Table 2. It

shows no significant effect ($p>0.05$) on light intensity on chlorophyll-a, chlorophyll-b, and total chlorophyll levels in *C. vulgaris*.

There is no effect of light intensity on chlorophyll content in *C. vulgaris* because microalgae make adjustments by reducing chlorophyll content or limiting light absorption to minimize light absorption to prevent damage to the photosynthetic apparatus when exposed to higher light intensities which are stated by Kim *et al*. (2019). According to Marzetz *et al*. (2020), the proportion of chlorophyll tends to decrease at high light intensities because chlorophyll decomposition increases; at high light intensities, microalgae reduce their chlorophyll contents to protect the photosynthetic system from the formation of oxygen radicals.

CONCLUSION

The results showed that the highest *C. vulgaris* cell density was obtained in treatment C (4500 lux) with a density of 6569.67 cells/ml. The highest chlorophyll content of *C. vulgaris* was obtained in treatment A (1500 lux) with chlorophyll-a, chlorophyll-b, and total chlorophyll content of 26.2, 48.5, and 74.7 µg/ml, respectively. Light intensity on cell density and chlorophyll content in *C. vulgaris* showed no significant effect ($p>0.05$).

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