Optimization of light intensity on growth rate and total lipid content of Chlorella vulgaris

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Submission date: 15-May-2022 08:01PM (UTC+0700)
Submission ID: 1836616336
File name: Lamp._V_C-27.pdf (612.21K)
Word count: 3725
Character count: 19192

IOP Conference Series: Earth and Environmental Science

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Abstract. *Chlorella vulgaris* was a potential microalga as a source of biomass due to its high lipid content. This study aimed to determine the growth and total lipid content of *C. vulgaris* that cultured in different light intensity. There were four-level treatments of light intensity applied i.e.: 2,500; 5,000; 7,500 and 10,000 lux, and were replicated three times. Microalga *C. vulgaris* was cultured in the plastic container, 35-40 ppt salinity, moderately aerated, kept in 19-25^{0}C temperature, and pH 8-10 in the laboratory. The light intensity was given continuously for 24 hours. The culture media was enriched with Walne's fertilizer (1 ml/l media). The period of culture was 12 days and growth was measured as cell density. The harvested microalga was dried, and the total lipid was then determined. Total lipid content was determined by the gravimetric method. Results showed that there were significant differences ($p \leq 0.05$) in the growth among four-level treatments of different light intensity, as well as for the total lipid content ($p \leq 0.05$). The best total lipid content was reached from sample at 10,000 lux (17.40±0.52% dry weight), followed by 7,500 lux (15.06±0.25% dry weight), 5,000 lux (13.3±0.43% dry weight) and 2,500 lux (7.30±0.30% dry weight), respectively.

1. Introduction

Indonesia has diverse biodiversity of marine resources. In addition to diverse plants and animals, microalgae are also part of the abundance of diversity. There are approximately hundreds of types of microalgae that are dispersed throughout the waters. Microalgae can generally live in all waters (fresh and seawater) and have a high resistance to environmental changes. One of the microalgae that can survive in all these condition is *Chlorella vulgaris*. It is a single-celled green alga with a round shape measuring 3-8 microns [1]. *C.vulgaris* is a cosmopolitan microalga, therefore it can grow in humid spaces and can be lowered on the earth [2]. Microalgae are classified based on pigment colors such as Phaeophyceae (brown algae), Chlorophyceae (green algae), Chrysophyceae (golden yellow algae), Pyrrophyceae (dinoflagellate), and Rhodophyceae (red algae) [3]. *C. vulgaris* is classified in Chlorophyceae (green algae) [4].

Biomass of *C. vulgaris* contains important chemical compositions such as carbohydrates, proteins, chlorophyll, nucleic acids, and total lipid content. *C. vulgaris* have a high economic value, nutrient content such as lipid reaches 14-22%, and others are in the form of protein, carbohydrates, etc. [5], [6]. Lipid contained in this microalga, which is higher when, compared other materials. Fats/oils are usually called lipids and were composed of fatty acids. The special fatty acid content in *C. vulgaris*

microalga is in the form of omega-3, 6, and 9 [7]. This content is 900 times more than carrots. The omega-3 in the *C. vulgaris* microalga reaches between 50-60%. This makes *C. vulgaris* potentially to be utilized as nutritious foods (health supplements) as well as feeds (feed for larvae, fish, and shrimp).

One of the environmental factors affecting the lipid production of *C. vulgaris* is the light intensity. Research on light intensity on total lipid content [8], stated that the highest lipid content is produced by the light intensity of 10,000 lux within 7 days of cultivation time. If the cultivation period is extended, the lipid production will decrease. Furthermore, the stationary phase of the microalgae growth will finish and reach the death phase. Other studies [9] that developed from this stated that *C. vulgaris* microalga at a light intensity of 5,000 lux is able to produce a total lipid content of 13.9%. Another study on light intensity [10] revealed that with a light intensity of 2,390 lux, the *C. vulgaris* can grow optimally. Research on the effect of light intensity on total lipid content is very interesting. Stressing abiotic such as increased light intensity treatment to microalgae cultivation can be used to increase lipid production. Based on this reason, this research aims to determine the best light intensity to optimize the production of the total content of lipid *C. vulgaris*.

2. Research methods

2.1. Chlorella vulgaris and cultivation

This microalga was obtained from Brackish Water Aquaculture Development Center (BBPAP), Jepara. The *C. vulgaris* cultivation process was carried out on a laboratory scale with different light intensity conditions [11]. The light intensities (4 level) used to include 2,500; 5,000; 7,500 and 10,000 lux. This difference in light intensity was to investigate the effect of light intensity on the total lipid content of *C. vulgaris* microalga. The light intensity obtained from the 36 watt TL lamp was installed with four different light intensities at four different distances above the research container and justified using Lux meter. 2,500 lux at a distance of ± 15 cm; 5,000 lux at a distance of ± 12 cm; 7,500 lux at a distance of ± 8 cm; 10,000 lux with a distance of ± 5 cm. Lighting was given for 24 hours. *C. vulgaris* was cultured for 12 days of the growth period, while salinity at 35-40 ppt, the temperature at 16-21^{0}C with pH 8-10. All treatments were given the same treatment as a medium and aeration. The medium used for the cultivation of *C. vulgaris* microalga was Walne's fertilizer solution (1mL/L media). All containers were sterilized before cultivation. This was to avoid contaminants that could affect the growth of microalga. Then, each container was inoculated with microalga at an initial density of 25 x 10^7 cells/mL.

2.2. Total lipid content

The microalga was harvested using the flocculation method. The flocculation method used in harvesting microalga is with aluminum sulfate as the flocculant material [12]. The flocculation process was allowed to stand for 24 hours. The remaining microalga that has not precipitated was centrifuged at a speed of 3,000 rpm for 5 minutes. The wet microalga biomass was dried and weighed to be analyzed for water content. Total lipid was determined gravimetrically [13]. The biomass was macerated with methanol and partitioned by chloroform using a separating funnel. The chloroform fraction was dried with a rotary evaporator the extract weight was calculated as the total lipid content.

2.3. Statistical analysis

The research data was analyzed using the One Way ANOVA test ($\alpha = 0.05$), the ANOVA test results looked significantly different, then the follow-up test which was done with the Tukey HSD test. This test aims to determine the difference between each level of each light intensity treatment.

3. Result and discussions

3.1. Optimization of light intensity on growth rate

Microalga requires light for photosynthesis purposes. The process of photosynthesis produces organic compounds (carbohydrates, proteins, lipids) and energy. Some energy accumulates in the form of lipids. Based on the results of the study, all light intensity treatments reached the peak of growth occurring on the 11th day. Microalga was harvested after its growth passed through the peak and

entered the stationary phase, which is on the 12th day (Figure 1). Statistical tests indicate that light intensity has a significant effect between four-level treatments ($p \leq 0.05$). According to this research [14] which state that the light can increase the speed of photosynthesis of microalga. It increases the rate of growth of microalga. The intensity of medium-light such as 2,500 and 5,000 lux has increased quite rapidly; this is because the light is sufficient that the rate of photosynthesis and growth increases.

The statement of other research [15], C. vulgaris can grow by providing sufficient light intensity, which was in the range of 500 – 5,000 lux. This is also stated [16], that C. vulgaris need enough light 2,000 – 3,000 lux for photosynthesis and grow normally. Referring to those arguments can be said that at 2,500 and 5,000 lux light intensity provides good growth. However, this study different at 7,500 lux light intensity. The light intensity of 7,500 lux, its growth relatively decreased when compared to 5,000 lux. It is suspected that the received light is not optimal in absorbing photon energy by chlorophyll microalga, consequently occurs reducing the rate of photosynthesis. According to [17] also explained that the greater light intensity can increase in the rate of photosynthesis, but saturated and very high light can reduce the rate of photosynthesis, therefore it can reduce the growth. Meanwhile, the results of this study showed that C. Vulgaris had a different response to light intensity treatment 10,000 lux, the growth remained high. It was suspected because the microalga was already able to adapt to high-intensity light, therefore it was still can grow optimum. The harvesting of C. vulgaris microalga was carried out simultaneously on the 12th day; this was because on the 12th day the C. vulgaris microalga had a significant decrease since the 11th day. This stationary phase was chosen because it was to avoid the death of the microalga. Referring to [18], the stationary phase tends to be relatively short. During the stationary phase, the microalga suffers death and tends to try to adapt to survive with its stored energy reserves.

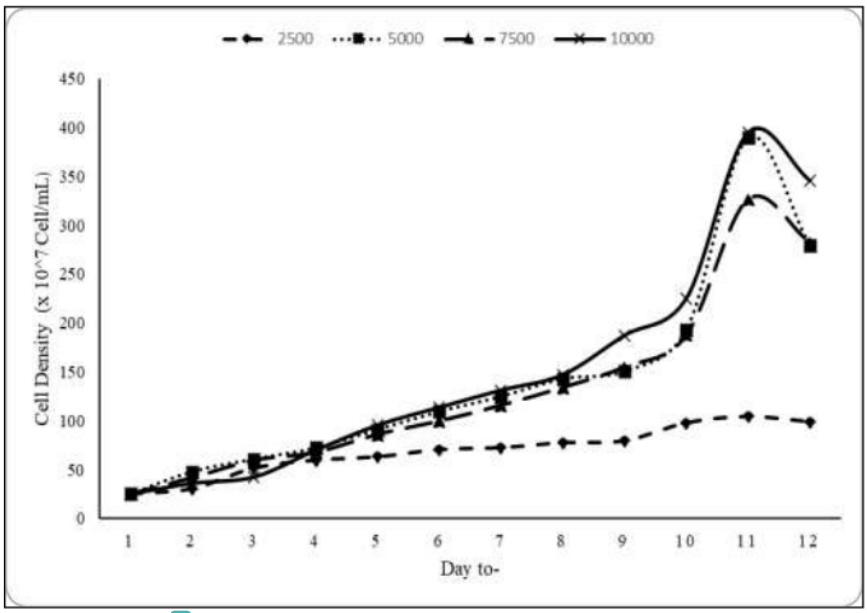

Figure 1. Growth curve of *C. vulgaris* under different light intensities

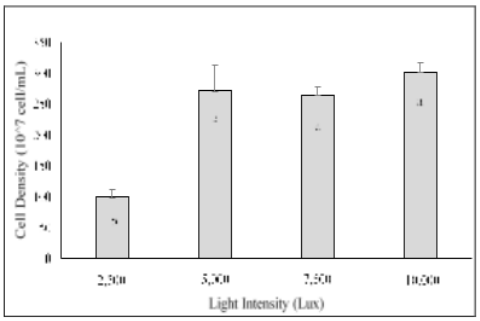

Figure 2. Cell density of *C. vulgaris* under different light intensity (note: different letters indicate the significant different value (p≤0.05))

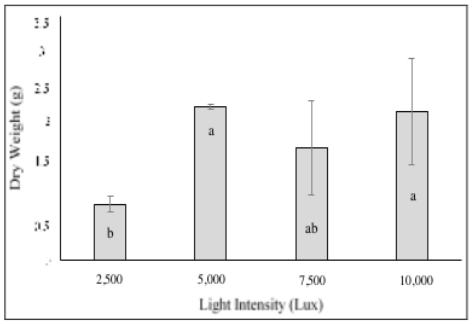

Figure 3. Dry weight of *C. vulgaris* under different light intensity (note: different letters indicate the significant different value (p≤0.05))

Microalga *C. vulgaris* was harvested in biomass form. Correlation of cell density to biomass weight depending on the content of the constituent components (carbohydrates, proteins, lipids). Based on Figures 2 and 3, the cell density profile when harvested was relatively the same as the biomass profile. Figure 2 showed a biomass graph after microalga was given the treatment a light intensity of 2,500-5,000 lux. The results of the statistical test of the study showed that light intensity had a significant effect (p ≤0.05) on dry biomass and total lipid content. The highest biomass obtained when *C. Vulgaris* was given light with an intensity of 5,000 lux and did not differ with the biomass from microalga cultivation using the 10,000 lux. Biomass production in microalga generally increases proportionally with the increase of light intensity. According to literature, optimal light intensity for biomass production and total lipid content varies in different species, depending on other factors such as temperature and nutrient in culture media [19]. Other studies [20] have reported that at higher light intensities, microalgae will degrade its biomass when it lacks the source of nitrogen in media culture. However, at high light intensities outside the saturation point, inhibition of photosynthesis is observed due to photosynthetic oxidation reactions that occur inside the cell [19]. This saturation point depends on the specific microalga species and the conditions of cultivation. In this study, it was found that light intensity effected biomass and the total lipid content of *C. vulgaris*. Effect of differences treatment in light intensity of 2,500, 5,000, 7,500, and 10,000 lux that was found in Figures 3 and 4, to be causing an increase in the biomass and the total lipid content. Faster growth rates for *C. vulgaris* microalga under higher light intensity has been reported by other researchers at light waves of 420 - 520 nm and 580-680 nm have an effect on the total lipid content of *C. vulgaris* but do not have an effect on biomass [21], [22]. This is because, at high light intensities, microalgae have decreased growth but accumulate energy reserves in the form of total lipid content. This indication shows that microalgae in stressful environmental conditions will tend to adapt by accumulating energy in the form of lipids [23].

In addition, the increased light intensity may decrease the function of chlorophyll. However, this constraint is addressed by the existence of carotenoids. These carotenoids will absorb photons during photosynthesis (as accessory pigment), it transfers part of the intensity of the light to be absorbed by chlorophyll [24]. It seems that an increase in microalgae growth will increase the weight of its biomass, as more light photons are available for photosynthesis. The results of this research proved that using light intensity until 10,000 lux can still be tolerated by *C. vulgaris*, accordingly to increase the production of biomass and lipid. However, referring to previous research, if the light intensity is too high to the saturation point will inhibit photosynthesis [25]. This causes the accumulation of

microalga biomass to be limited [26]. This is also experienced by other researchers [27] that the effect of light intensity of 110 µE /m^2/s (8,150 lux) has inhibited the rate of microalga growth and its biomass production.

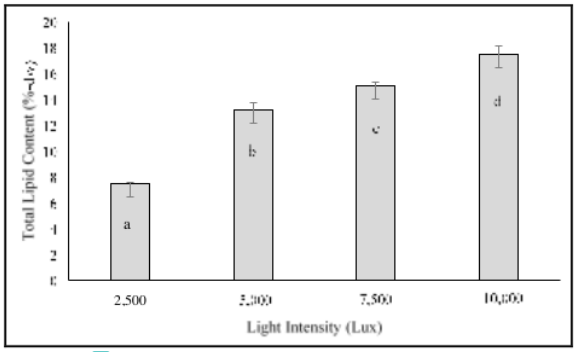

Figure 4. Total lipid content of *C. vulgaris* under different light intensity (note: different letters indicate the significant different value ($p \leq 0.05$))

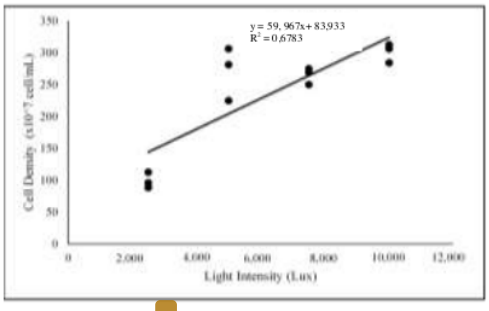

Figure 5. The effect of different light intensity on cell density of *C. vulgaris*

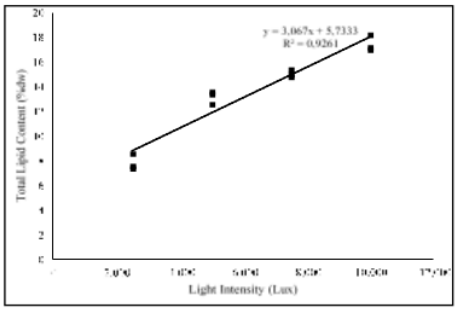

Figure 6. The effect of different light intensity on total lipid content of *C. vulgaris*

Light intensity affects microalgae in the production of biomass and lipids [28]. Figures 5 and 6 present a regression curve for the effect of light intensity treatment on biomass and total lipid content. The results of the regression analysis on the coefficient of determination (R^2) showed that the treatment of light intensity gave an effect of 92.6% with 7.4% caused by other factors. This means that the light intensity has a strong effect on the total lipid content. The lipid biosynthesis pathway competes with the carbon precursor pathway for growth. This makes the starch synthesis pathway blocked, thereby increasing lipid production [20], [29]. Microalgae that grow in high light often accumulate more lipids [28]. Another study, in the lipid production of *Scenedesmus obliquus*, it will increase at high light intensity (600 µE/m^2/s = 44,400 lux) [21]. Other studies also reported that *Chlorella* sp. could produce more lipids on high light intensity (600 µE/m^2/s) through intensity low-light treatment [30]. Lipid production increases several times when microalgae receive high-intensity light by converting excessive photon assimilation to lipids [31], [32].

Based on the results of the culture *C. vulgaris* using the light intensities of 2,500, 5,000, 7,500, and 10,000 lux for 12 days, the highest biomass produced was at a light intensity of 5,000 lux with a weight of 2.196 g, meanwhile, the highest lipid production at a light intensity of 10,000 lux was 17.4% (dry weight). The total lipid content in *C. vulgaris* microalga had increased below all light intensities but had decreased in biomass. It is suspected that fats and carbohydrates compete in the carbon precursor pathway [33], [34]. The formation of biomass by microalgae includes protein biosynthesis. When microalgae are given high-intensity treatment, there is an inhibition of protein production [19]. Inhibiting the process of protein synthesis will reduce the consumption of nitrogen and carbon for cell growth, therefore carbon can be used for fat biosynthesis (triacylglycerol) [19]. Other studies also show that total lipid levels at high light intensity are useful for storing excess photon assimilation that is then converted into chemical energy [35]. Lipid and triacylglycerol synthesis begins with the excess of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) through photosynthesis. ATP is a source of energy for lipid biosynthesis, while NADPH is a coenzyme that acts as a reducing agent in lipid synthesis [26].

C. vulgaris microalga contains protein, fat, unsaturated fatty acids, pigments, and vitamins [36]. The content of fats (lipids) and fatty acids (fatty acids) that are in microalga *C. vulgaris* are energy sources. This content is produced from photosynthesis with the help of the light intensity. It was found that *C. vulgaris* microalgae contain 3 classes of fatty acids, namely saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) C18: 2). The production of fatty acid i.e. : C16:0 (palmitic acid), C18:0 (Stearic acid), and C18:1 (oleic acid) at early stationary growth phase whilst C18:3 n-3 (α-linolenic acid) at later stationary growth phase [37]. The percentage of fatty acids in *C. vulgaris* is 73.3% unsaturated fatty acids and 26.3% saturated fatty acids [38]. *C. vulgaris* microalga in the presence of these unsaturated fatty acids can be used as food supplements, natural food, feed, etc. The results of this study proved that high light intensity treatment until 10,000 lux could increase the production of lipid *C. vulgaris*. Its method of cultivation is potentially developed to support industries requiring microalgae lipids.

Conclusion

There were significant differences ($p \leq 0.05$) in the growth among four-level treatments of different light intensity, as well as for the total lipid content ($p \leq 0.05$). The best total lipid content was reached from sample at 10,000 lux (17.40±0.52% dry weight), followed by 7,500 lux (15.06±0.25% dry weight), 5,000 lux (13.3±0.43% dry weight) and 2,500 lux (7.30±0.30% dry weight), respectively.

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Acknowledgement
The author would like to thank Ms. Sri Sedjati and Ms. Ervia Yudiati for providing support and motivation to complete this research.

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