

Optimization of Photoperiod Treatment for Enhanced Biomass Production and Primary Metabolite Content in *Euglena* sp. Strain Isolated from Yogyakarta, Indonesia

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Abstract. Cultivating microalgae as an alternative energy resource requires the optimization of the cultivation process. *Euglena* is a biofuel microalga with proteins, essential amino acids, vitamins C and E, lipids, beta carotene, and beta-1,3-glucan (paramylon). Light intensity and photoperiod are crucial factors in determining microalgae biomass production because of their relationship to maximum photosynthesis efficiency. As a result, optimising growth conditions in microalgae cultivation is critical for maximising productivity. This study looked at how different photoperiod treatments (12:12, 14:10, 16:8, 18:6, and 24:0 h) affected the growth, biomass, lipid, carbohydrates, and protein content of *Euglena* sp. cultures in a modified CM (Cramer-Myers) medium. The 18:6 photoperiod treatment produced the highest specific growth rate, biomass, lipid carbohydrate, and protein content, according to the study's findings. The biomass concentration was 0.4190.184 g/L, and the specific growth rate was 5.960.66 (OD680/OD680/hx10⁻³). The lipid concentration was 0.2030.078 g/L, the carbohydrate concentration was 378.061252.135 mg/mL, and the protein concentration was 371.142107.126 g/L.

Keywords: *Euglena* sp.; photoperiod; isolation; optimization; cultivation.

1. Introduction

Due to the decreasing supply of fossil fuels and the limited number of agricultural lands, microalgae cultivation is becoming one of the subjects that deserves further attention. In addition, microalgae also have excellent capabilities in terms of waste treatment (domestic or agricultural waste) and free CO₂ binding (Douskova *et al.*, 2009). Until now, many research groups and microalgae-based industrial companies have conducted various microalgae studies due to their limitless

opportunity for being utilised in different sectors. One type of microalgae that is considered the potential for many uses is *Euglena* sp.

Euglena has a unique structure; its cells are surrounded by a membrane made of protein, that causes *Euglena* to be highly nutritional and absorb nutrients efficiently to support and restore cellular activity (Suzuki, 2017). Previous studies stated *Euglena* as one of the most promising microalgae species to be used as a raw material for biofuels (Toyama *et al.*,

2009). *Euglena* is widely used as raw material for food and feed, beauty products, and biofuels and is even commonly used in environmental management, such as for water treatment and CO₂ reduction. *Euglena* also contains 59 essential nutrients the human body needs for optimal growth. In addition, *Euglena* has a larger biomass and cell size when compared to other microalgae. It also contains various nutrients such as protein, essential amino acids, vitamins C and E, lipids, beta carotene and beta-1,3-glucan (paramylon) (Inui *et al.*, 1982; Calvayrac *et al.*, 1981; Takeyama *et al.*, 1997). Information and a study regarding the optimal cultivation process are currently needed to obtain a high growth rate and productivity, especially when cultivating local strains that are still highly dependent on environmental factors.

Similar to plants, microalgae also carry out photosynthesis. In the process of photosynthesis, light plays an important role, but the light required for each type of plant and algae to grow optimally is different (Lavens & Sorgeloos, 1996). Besides light intensity, photoperiod plays a vital role in supporting microalgae growth. Light intensity and photoperiod are essential in determining microalgae biomass production because both are related to the maximum efficiency of photosynthesis. The photoperiod can directly influence the photosynthetic mechanism, making it an essential factor for determining optimal growth conditions in microalgae cultivation (Kishore *et al.*, 2017). Light is also known to be a promoter of microalgae growth, but more prolonged exposure to a high light intensity could lead to photodamage in which the process reduces the photosynthetic efficiency (Parmar *et al.*, 2011; Wahidin *et al.*, 2013). Previous studies have shown that the photoperiod variation in microalgae cultivation shows significant changes in microalgae biomass (Richmond, 2004).

It is known that the laboratory-scale cultivation of microalgae is an essential component of basic information that can be used for large-scale cultivation processes. The main components of microalgae biomass are carbohydrates, lipids and proteins. These components' concentration depends on cultivation conditions, the growth medium, the intensity and duration of light, and also the supply of CO₂. Variations in the dark/light regime trigger changes in the cellular content of proteins, carbohydrates and lipids (Tzovenis *et al.*, 1997; Price *et al.*, 1998; Fabregas *et al.*, 2002). This study was conducted to determine the effect of photoperiod on growth, biomass, lipid, carbohydrates and protein content in the culture of the newly isolated *Euglena* sp. from Indonesia.

2. Materials and Methods

2.1 Sampling and Isolation

Samples were collected from ponds and rice fields around Yogyakarta (7,80565° S, 110,29830° E). 10 mL of samples were collected into a 15 mL conical tube, and 2.5 mL of 5 times modified CM (Cramer-Myers) medium was added into each sample for the selection process. The samples were left at room temperature for approximately one day. After that, 1 mL of each sample was moved into 24 well-plate (IWAKI), and 2 mL of 1 time-modified CM medium was added. The sample was incubated at room temperature with continuous light for approximately two weeks until the sample turned green. Perumal *et al.* (2015) conducted isolation according to the micromanipulation protocol. Micromanipulation is isolating an algal cell from a drop of enrichment sample. The cell is sucked up into a micropipette while being observed. The cell is placed on an agar plate in a drop of sterile medium (Pachiappan *et al.*, 2015). The cell is transferred to a drop of sterile medium and an agar plate. The cultures were examined

morphologically under the microscope for preliminary identification and were compared to an online algal database. Before performing all the experiments, isolated *Euglena* was pre-cultured in modified CM medium using 50 mL test tubes (IWAKI) until the optical density reached 0,4-0,6 (OD 680 nm)

2.2 Photoperiod Treatment and Experiment Design

Photoperiod (light: dark) treatments in this study were 12:12, 14:10, 16:8, 18:6 and 24:0 h (continuous light/control). All the cultures for each treatment were grown in 250 mL Erlenmeyer flasks containing 200 mL of modified CM (Cramer-Myers) medium. CM medium with the following composition (mg/L): (NH₄)₂HPO₄, 1000; KH₂PO₄, 1000; MgSO₄·7H₂O, 200; CaCl₂·2H₂O, 20; FeSO₄·7H₂O, 3; MnCl₂·4H₂O, 1.8; CoSO₄·7H₂O, 1.5; ZnSO₄·7H₂O, 0.4; Na₂MoO₄·2H₂O, 0.2; CuSO₄·5H₂O, 0.02; Vitamin B12, 0.0005; Thiamine HCl, 0.1. The temperature was set to 29°C; light intensity was 180 μmol m⁻² sec⁻¹, and aeration was set to 1 v/v/m (Suzuki, 2017). Each culture's initial optical density was around 0,1 (OD 680 nm). Each treatment was performed in triplicate.

2.3 Growth Rate Measurements and Biomass Production

Microalgae growth was monitored regularly at intervals of 2 days for 18 days. The concentration of *Euglena*'s cells in each culture was determined by monitoring the optical density (OD) of the culture using a spectrophotometer (UV-VIS Genesys) at an absorbance of 680 nm (Kishore *et al.*, 2017; Suzuki *et al.*, 2015), with distilled water as the blank. To determine the dry weight (DW), 5 mL of the cell culture sample was centrifuged at 4000 rpm for 10 min. The cell pellet was obtained and dried in the pre-weighed conical tube at 50 °C for 12 h. The pellet and conical tube were weighed using analytical scales

(AL204). The final DW was calculated using the following formula from Richmond (2004):

$$DW \text{ (m.g.mL}^{-1}\text{)} = \frac{\text{total weight} - \text{weight of conical tube}}{\text{volume of samples}} \quad (1)$$

The growth rate was observed from the optical density. Doubling time and specific growth rate were also calculated using the following formulas from Lee & Shen (2004):

$$T_d = \ln 2 \frac{t}{\ln \ln \frac{N_t}{N_0}} \quad (2)$$

$$\mu = 0.693/T_d \quad (3)$$

where: T_d is doubling time, μ is a specific growth rate, N_t is the optical density at the end of the exponential Phase, N_0 is the optical density at the beginning of the exponential phase, and t is the time interval (hour).

2.4 Lipid, Carbohydrate, and Protein Content Measurements

To measure the lipid content of the sample, 5 mL was taken and put into a 15 mL conical tube and then centrifuged at 4000 rpm for 10 minutes at 4°C. The supernatant is separated from the pellets. To the pellets, 2 mL of methanol and 1 mL of chloroform were added and then homogenised using a vortex. After homogeneity, the pellets were added with 1 mL chloroform, and 1 mL distilled water and then homogenised again. The sample was centrifuged at 4000 rpm for 10 minutes at 10 °C. Three layers are formed, and the bottom part is taken and placed on a petri dish that has been previously dried and weighed. The chloroform is then evaporated in an oven at 50°C until only neutral lipids are left in the petri dish. The lipid extraction was performed according to the Bligh & Dyer (1959) method and calculated using the following formula from Smedes & Thomasen (1996).

$$\text{Total lipid (g.mL}^{-1}\text{)} = \frac{\text{Total weight} - \text{weight of petri dish}}{\text{volume of samples}} \quad (4)$$

To measure the carbohydrate content of the sample, 5 mL of the sample was taken and put into a 15 mL conical tube, which was then centrifuged at 4000 rpm for 10 minutes at 4°C. The supernatant is separated from the pellets and discarded. Then add 1 mL of concentrated sulfuric acid and 0.5 mL of 5% phenol and incubate for 30 minutes at room temperature. The carbohydrate content was calculated spectrophotometrically at 490 nm according to the phenol-sulfuric acid method by Dubois *et al.* (1956). A Standard d-glucose solution was used for the standard curve. To determine the protein content of the sample, 2 mL of it was placed in 2 mL microtubes and centrifuged at 4000 rpm for 10 minutes. Following the formation of the pellets and supernatant, the pellets were taken and mixed with 1 mL of 10% SDS solution (Chemix, Yogyakarta, Indonesia). The samples were then placed in a 95°C oven for 5 minutes. After incubation, the sample was taken and placed in a refrigerator at 4°C for 5 minutes. The sample was then divided into eight L and placed in a 500 L microplate (IWAKI) with 200 L of Bradford reagent. [A1] Bradford's (1976) method calculated protein content spectrophotometrically at 595 nm. The standard curve was created using a solution of Bovine serum albumin (BSA).

2.5 Data Analysis

All the experiments were conducted in triplicate, and the data presented are the means. To evaluate the significance of photoperiod on the growth rate, biomass, protein, carbohydrate, and lipid contents of the culture, a one-way analysis of variance (ANOVA) was performed. Duncan Multiple Range Test (DMRT) was also performed if a significant difference was obtained. All analyses were performed using a confidence level of 95% ($\alpha=0,05$).

3. Result and Discussion

One of the alternative forms of energy is the utilisation of biomass or other products produced by microalgae. In this study, we

isolated and identified *Euglena* sp. as a potential microalga due to its benefits. The size of isolated *Euglena* sp. ranges from 20-50 μm (Fig. 1), similar to the previous study by Kishore *et al.* (2017). Some of the sampling locations included rice fields, fishponds, and sewage channels. These sampling sites were chosen because it is known that *Euglena* lives in freshwater and brackish water habitats due to its richness in organic matter (Suzuki, 2017). A previous study by Suzuki *et al.* (2015) shows that *Euglena gracilis* can grow at low pH in CM medium. Although in-depth research needs to be conducted, it is safe to assume that the isolated *Euglena* was *Euglena gracilis*. The cell shapes vary from round to oval to elongated. *Euglena* sp. does not have a cell wall; thus, the shape is diverse.

This study showed that 18:6 photoperiod treatment has the highest optical density while the 24:0 photoperiod treatment (control) has the lowest optical density during the cultivation process (Fig. 2(a)). Prolonged exposure to light (24:0 photoperiod) for this strain showed the lowest growth. It is assumed that continuous light beyond the saturation point can be a stressor due to photo-oxidation reactions that eventually will impact the growth rate. 18:6 photoperiod treatment showed the best result for growth of this isolated strain. This study showed similar results as the previous study conducted by Kishore *et al.* (2017). In a study conducted by Kishore *et al.* (2017), it is known that the *Euglena* strain isolated from the western Himalayas had the best growth in the 16:8 photoperiod treatment, followed by the 18:6 and 14:10 treatments, while the lowest results were obtained at the 12:12 and 24:0 treatments. Previous studies and our findings indicate that the lowest growth rate of *Euglena* is achieved at the lowest and most continuous photoperiod treatments.

The initial lag phase occurred within the first three days of cultivation. The log phase can

be observed from day four until day nine of cultivation. All the cultures went to the stationary phase after ten days of cultivation until the 14th days of cultivation. The optical density for all the photoperiod treatments decreased after 15 days of cultivation, which indicated that the culture was entering the death phase. The highest optical density from the 18:6 photoperiod treatment was also followed by its high specific growth rate (Fig. 2(b)). The specific growth rate was determined by calculating the doubling time (t_d) of each culture in each treatment according to the equation from Lee & Shen (2004). A low doubling time value corresponds to a high specific growth rate value, and vice versa (Liu *et al.*, 2011).

Microalgae strains with short doubling times and high specific growth rates are primarily developed for large-scale production. These two characteristics are beneficial because a shorter harvest time can be achieved. In general, by comparing the optical density from all photoperiod treatments during the cultivation, it is known that the photoperiod treatment has a significant effect, and there is a significant difference between the mean absorbance values of each photoperiod treatment within the 18 days of cultivation (Table 1).

In this study, the highest biomass in terms of dry cell weight was obtained from an 18:6 photoperiod treatment on the 15th day of cultivation (0.613 ± 0.094 g/L). All the treatments showed a similar trend for biomass production in which the biomass increased from the first day of cultivation and reached the maximum on the 15th day of cultivation (Fig. 3(a)). The lowest biomass average was obtained from the 24:0 photoperiod treatment (0.228 ± 0.104 g/L) (Table 2). The highest to the lowest biomass productivity was obtained from the 18:6, 14:10, 16:8, 12:12, and 24:0

photoperiod treatments, respectively (Fig. 3(b)).

In comparison to other studies, it is known that optimal growth of *Chlorella* sp., *Nannochloropsis salina*, and *Phaeodactylum tricornutum* is obtained in continuous (24 hours) photoperiod treatment (Sirisuk *et al.*, 2018), 12 hours regime per day results in better production efficiency in *Spirulina* sp. (Budiardi *et al.*, 2010), exposure to lighting for 18 hours per day provides maximum biomass in *Isochrysis galbana* culture (Sirisuk *et al.*, 2018), a 16:8 regime is also known to provide maximum biomass for *Chlorella vulgaris* (Khoeyi *et al.*, 2012).

Biomass production in most microalgae species increases along with the longer exposure time. This can occur because an extended exposure period is usually followed by an increase in reproduction to the highest saturation point (Danesi *et al.*, 2004). In recent studies, higher light intensity and more extended photoperiod (continuous light) can be a stressor that results in decreased growth or death due to photo-oxidation reactions in cells and excess light that cannot be absorbed into the photosynthetic apparatus (Richmond, 2004). This could be why the 24:0 photoperiod treatments gave the lowest dry cell weight and biomass productivity.

Generally, microalgae synthesise low lipid content when it is under enough nutrient conditions. The main lipid components are membrane lipids, such as phospholipids and glycolipids. However, under stressful conditions such as nitrogen deficiency, it is known that the total lipid content increases with TAGs (Triacylglycerols) as the dominant component (Hu, 2004). Other factors, such as CO₂ concentration and light intensity, also play an essential role in the mass culture and lipid content of microalgae (Lv *et al.*, 2010). Light is known to be one of the triggers for fatty acid

synthesis in microalgae (Thompson, 1996). During this study, the total lipid content increased over time in the 14:10, 16:8 and 18:6 photoperiod treatments (Fig. 4(a)). The highest lipid content obtained on the last day of cultivation is possible due to a gradual decrease in cell division, and cells begin to store their products in lipids, carbohydrates or proteins (Guzman *et al.*, 2010). Our findings indicated similar results. Several previous studies have shown that nitrogen and phosphate deficiency during the late stationary phase can increase the accumulation of lipid content in microalgae cells but reduce biomass productivity (Rodolfi *et al.*, 2009).

Figure 4 (a) also shows that in the 12:12 and 24:0 photoperiod treatments, the total lipid content tended to be constant and did not exhibit a significant increase. This static total lipid content resulted in low lipid productivity in cultures treated with the 12:12 and 24:0 photoperiod treatments. This result aligned with the low biomass production obtained from the 12:12 and 24:0 photoperiod treatments. The highest lipid productivity was produced in cultures treated with the 14:10 photoperiod, followed by 18:6 and 16:8 photoperiod treatment (Fig. 4(b)). This study also showed a significant difference between total lipid content from 18:6, 14:10, and 16:8 photoperiod treatments with the control (Table 3).

The carbohydrate content of microalgae varies between species and growth conditions (Templeton *et al.*, 2012). Previous studies have also reported that *Euglena* has no cell wall made of carbohydrates. However, the extraction results from the cell surface found that it contains other compounds such as glucose, mannose, mucosa, xylose, and rhamnose (Barras *et al.*, 1965). Overall, the total carbohydrate content in the culture has a similar trend to the biomass (Fig. 5(a)). This finding indicated that the increasing carbohydrate content also follows higher

biomass. The carbohydrate content was increased from the beginning of the cultivation and reached its maximum on the 15th day of cultivation. This study showed that the highest carbohydrate content and productivity were obtained from the 18:6 photoperiod treatment (Table 4) (Fig. 5(b)).

The production of carbohydrates is known to be associated with photosynthesis, which is greatly influenced by the intensity and duration of light exposure. Carbohydrates have an essential role in cell growth activity. Without photosynthetic products, microalgae cells will not be able to compose cell components, and no products can be stored as food reserves (Ho *et al.*, 2011). *Euglena* is widely known as a producer of paramylon (β -1,3-glucan), a form of carbohydrate. The total amount of paramylon content in *Euglena*'s cells tends to increase from the beginning to the end of the cultivation process. These results indicated that paramylon accumulation in *E. gracilis* does not depend on temperature but rather depends on the stage of cell growth (Wang *et al.*, 2018). Thus, the lower carbohydrate content on the 18th day in this study is related to the growth phase in which the culture was in the death phase.

Figure 6 (a) shows that the protein content was similar between all photoperiod treatments from the 9th day to the 15th day of cultivation. The highest protein content in all photoperiod treatments was obtained on the 18th day of cultivation, with the maximum amount found in the 18:6 photoperiod treatment. According to a previous study, protein content in cells (compared to dry cell weight) is highest when cell growth is in the exponential phase and tends to be constant in the stationary phase (Wang *et al.*, 2018). Kunne & de Groot (1996) stated that protein synthesis in *E. gracilis* depends on temperature and light, where high exposure and low temperature can trigger protein synthesis. Wang *et al.* (2018) stated that apart from the influence of light and

temperature, protein synthesis also depends on the growth stage of *E. gracilis* cells. In this study, it is known that the peak of the exponential phase of culture in all treatments occurred on the ninth day, and after that, the cells entered the stationary phase. Thus, according to Wang *et al.* (2018), the protein content started to increase until the 9th day, and after that, it was stagnant until the 18th day of cultivation.

The protein content in Table 5 shows no significant difference in numbers between all photoperiod treatments. This is supported by the protein productivity of *Euglena* sp. in all photoperiod treatments, whose numbers were also similar (Fig. 6 (b)). The highest protein productivity was found in the 18:6 photoperiod treatment, while the lowest was in the 24:0 photoperiod treatment.

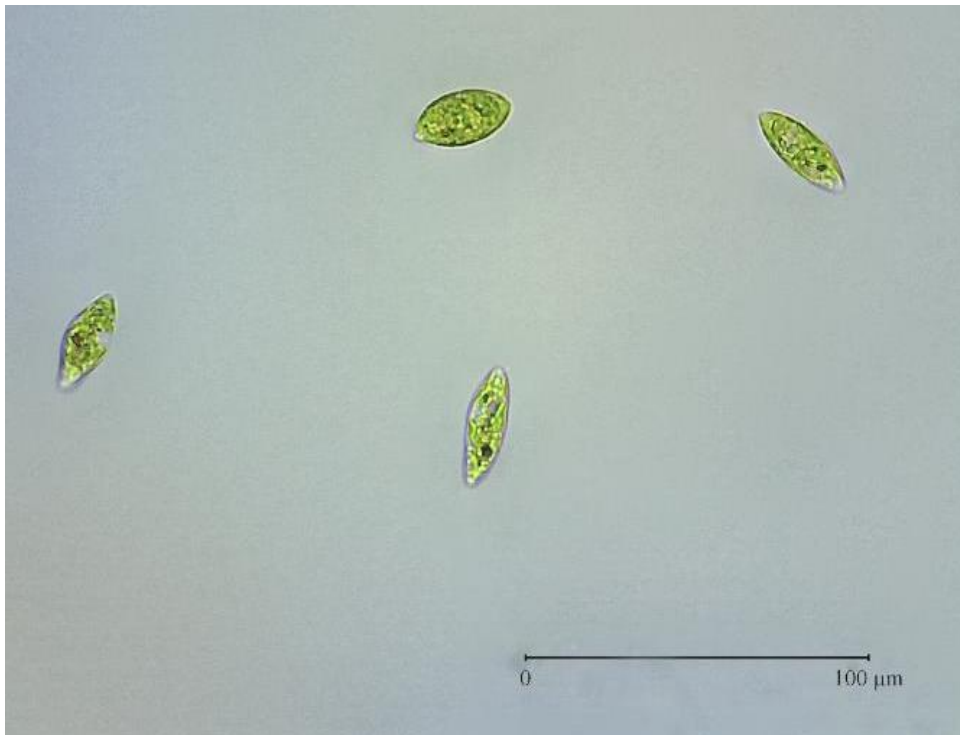


Fig. 1. Isolated and cultivated *Euglena* sp. (10x10 magnification) (Renaldy, 2020).

Table 1. Optical density of *Euglena* sp. culture during 18 days of cultivation in various photoperiod treatment (Renaldy, 2020).

Photoperiod treatment	Optical density (OD)
12:12	0.243 ^{ab} ±0.086
14:10	0.418 ^{bc} ±0.183
16:8	0.365 ^{abc} ±0.182
18:6	0.463 ^c ±0.230
24:0 (control)	0.201 ^a ±0.939

Data are expressed as mean ± standard deviation (n=18). Identical letters indicate no significant difference (p>0.05).

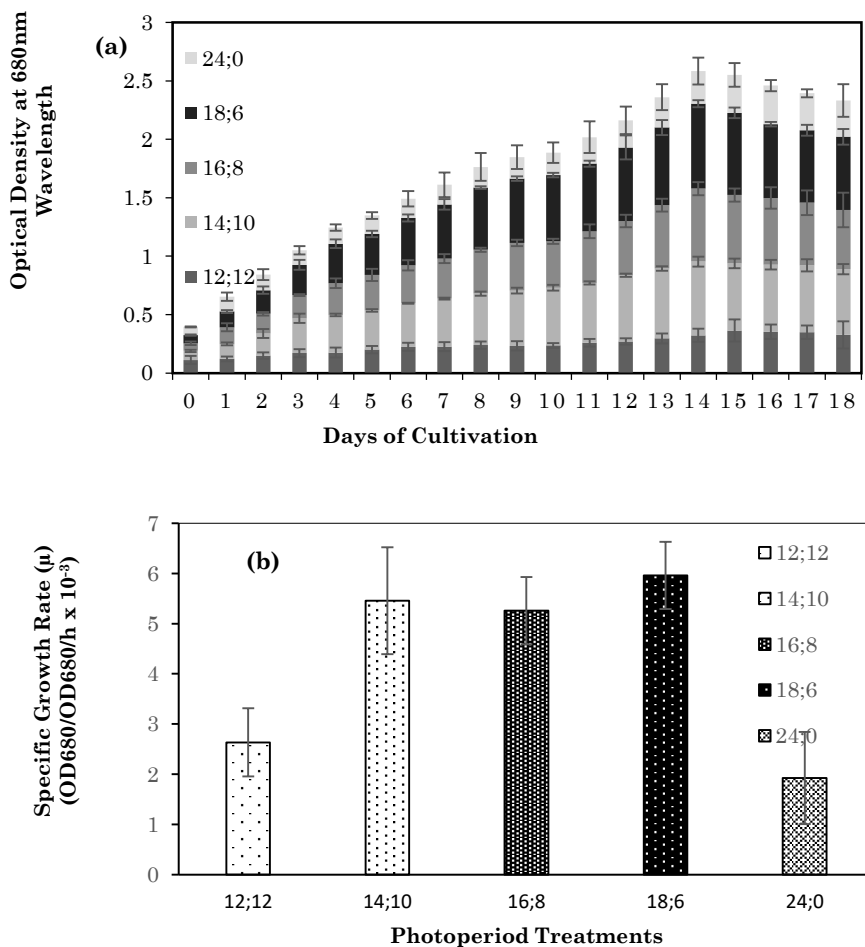


Fig. 2. (a) The growth of *Euglena* sp. (b) Specific growth rate of isolated *Euglena* sp. on various photoperiod treatment (Renaldy, 2020).

Table 2. Biomass of *Euglena* sp. culture during cultivation in various photoperiod treatment (Renaldy, 2020).

Photoperiod treatment	Dry cell weight (g/L)
12:12	0.263 ^{ab} ±0.106
14:10	0.367 ^{ab} ±0.170
16:8	0.352 ^{ab} ±0.163
18:6	0.419 ^b ±0.184
24:0 (control)	0.228 ^a ±0.104

Data are expressed as mean ± standard deviation (n=7). Identical letters indicate no significant difference (p>0.05).

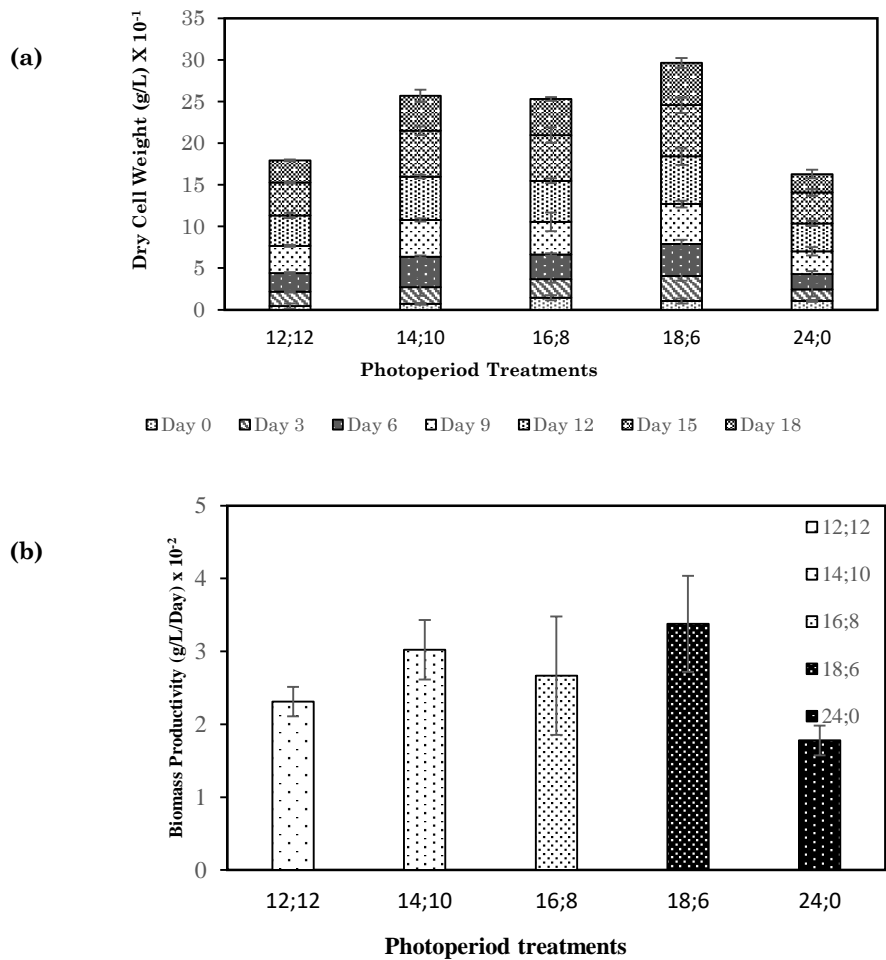


Fig. 3. (a) Dry cell weight of *Euglena* sp. (b) Biomass productivity based on dry cell weight of *Euglena* sp. on various photoperiod treatment (Renaldy 2020).

Table 3. Total lipid content of *Euglena* sp. culture during cultivation in various photoperiod treatment (Renaldy, 2020).

Photoperiod treatment	Total Lipid (g/L)
12:12	0.106 ^a ±0.015
14:10	0.186 ^b ±0.072
16:8	0.179 ^b ±0.047
18:6	0.203 ^b ±0.078
24:0 (control)	0.112 ^a ±0.016

Data are expressed as mean ± standard deviation (n=7). Identical letters indicate no significant difference (p>0.05).

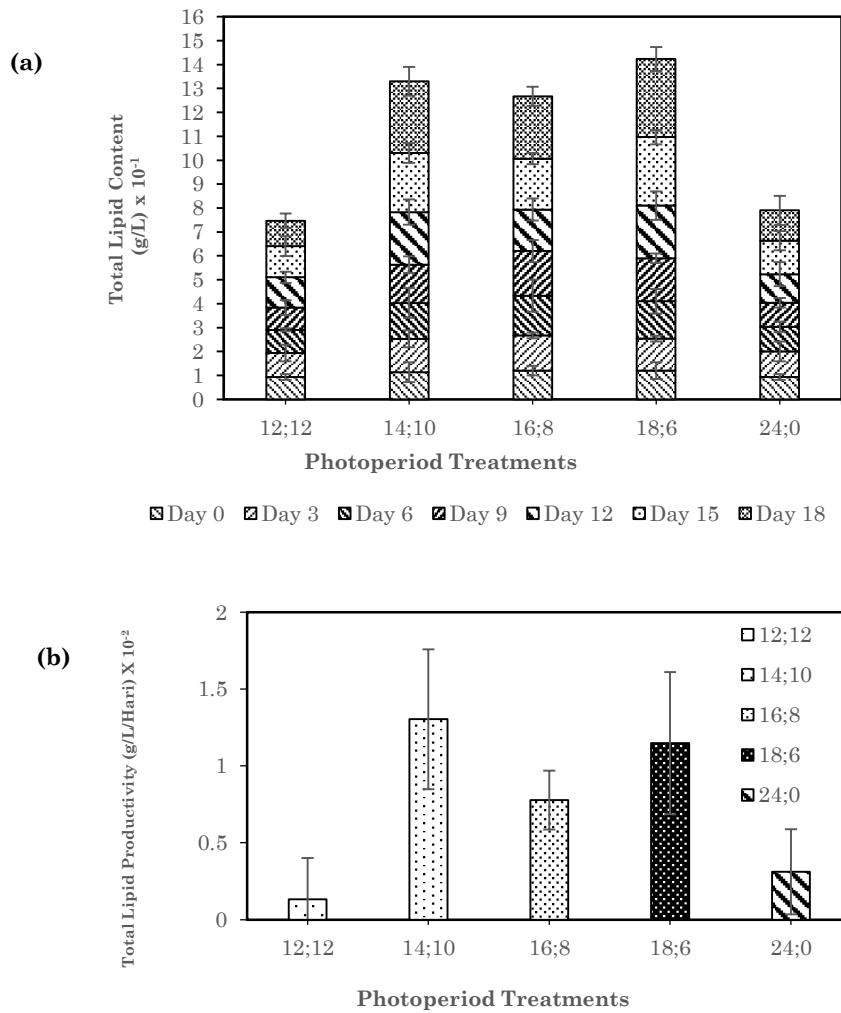


Fig. 4. (a) Total lipid content of *Euglena* sp. (b) Lipid productivity of *Euglena* sp. on various photoperiod treatment (Renaldy, 2020)

Table 4. Carbohydrate content of *Euglena* sp. culture during cultivation in various photoperiod treatment (Renaldy, 2020).

Photoperiod Treatment	Carbohydrate Content (mg/mL)
12:12	143.935 ^a ±67.032
14:10	314.958 ^{bc} ±155.488
16:8	264.662 ^{abc} ±147.888
18:6	378.061 ^c ±252.135
24:0 (control)	106.224 ^a ±76.063

Data are expressed as mean \pm standard deviation (n=7). Identical letters indicate no significant difference (p>0.05).

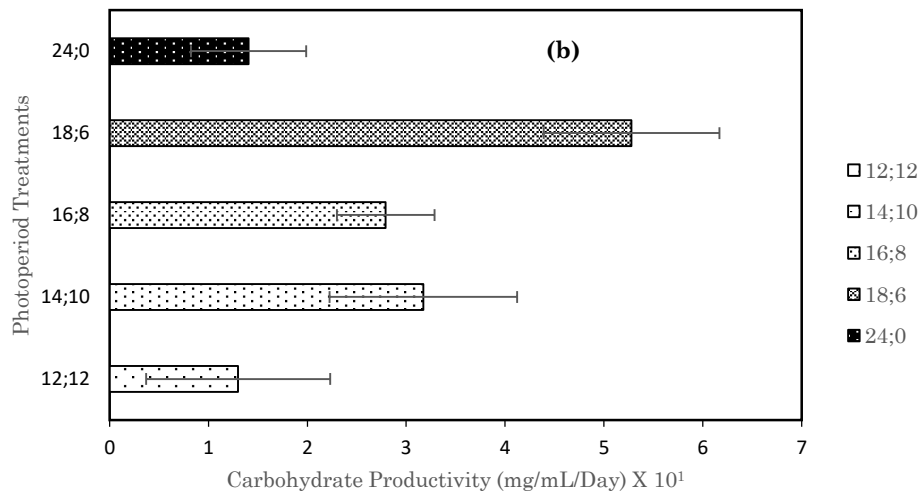
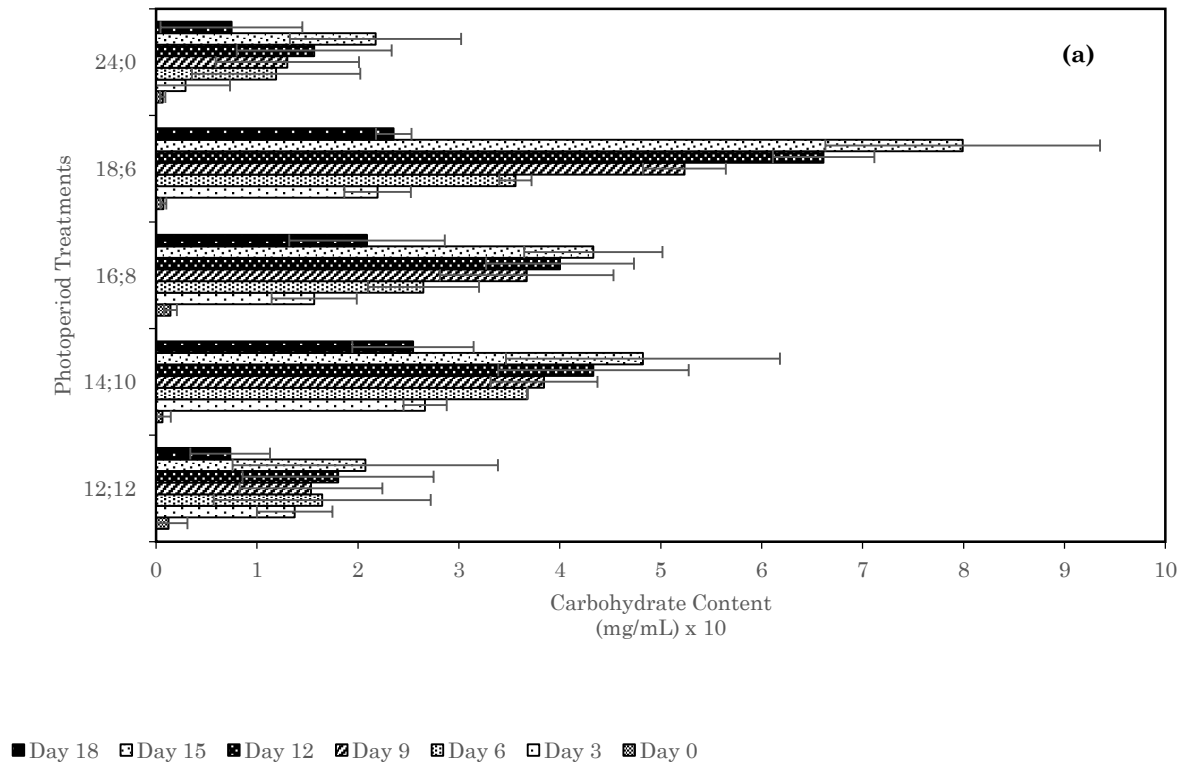


Fig. 5. (a) carbohydrate content of *Euglena sp.* (b) carbohydrate productivity of *Euglena sp.* on various photoperiod treatment (Renaldy, 2020).

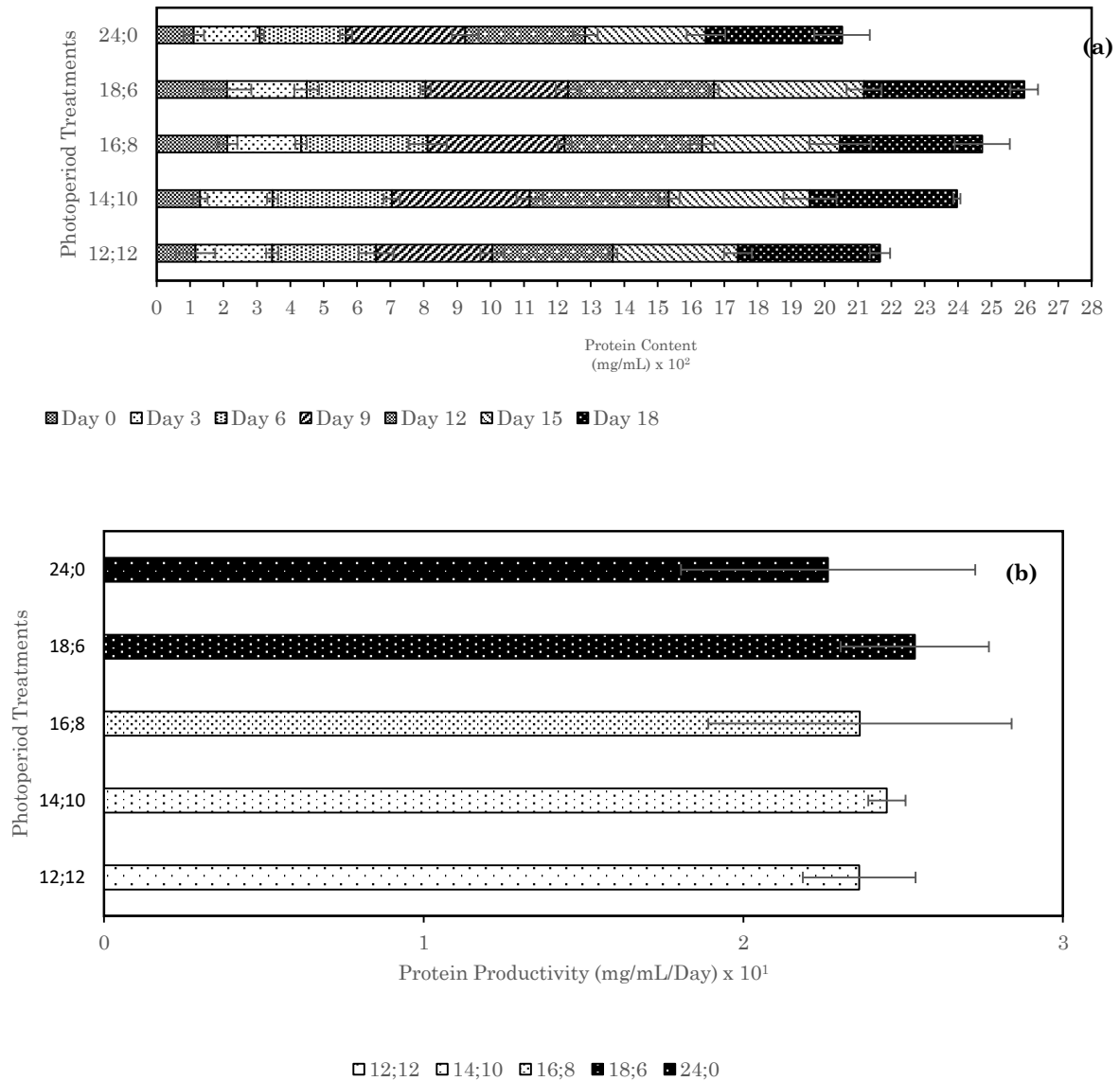


Fig. 6. (a) protein content of *Euglena* sp., (b) protein productivity of *Euglena* sp. on various photoperiod treatment (Renaldy, 2020)

Table 5. Protein content of *Euglena* sp. culture during cultivation in various photoperiod treatment (Renaldy, 2020).

Photoperiod treatment	Protein content ($\mu\text{g/ml}$)
12:12	308.573 ^a ±105.275
14:10	343.484 ^a ±118.768
16:8	353.181 ^a ±94.760
18:6	371.142 ^a ±107.126
24:0 (control)	273.333 ^a ±110.181

Data are expressed as mean \pm standard deviation (n=7). Identical letters indicate no significant difference ($p>0.05$).

4. Conclusions

Photoperiod is an essential factor in determining optimal growth conditions in microalgae cultivation in order to obtain the highest productivity. The results of this study indicated that the highest specific growth rate, biomass, lipid, carbohydrate, and protein content was obtained from the 18:6 photoperiod treatment. Therefore, 18:6 photoperiod treatment is the optimal light regime for the newly isolated *Euglena* sp. from Indonesia. The present study was performed in laboratory conditions in a small-scale condition. Further study needs to be explored especially in outdoor and large-scale conditions and take the cost of production into account.

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References

- Barras, D.R.** and **Stone, B.A.** (1965). Chemical composition of pellicle of *Euglena gracilis* var. *bacillaris*. *Biochemical Journal*, **97**, 14–15.
- Bligh, E.G.** and **Dyer, W.J.** (1959). A rapid method of total lipid extraction and purification. *Canadian Journal Biochemistry and Physiology*, **37**, 911-917.
- Bradford, M.** (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. *Analytical Biochemistry*, **72**, 248–254.
- Budiardi, T., Utomo, N.B.P.** and **Santosa, A.** (2010). The growth and nutrient content of *Spirulina* sp. in different photoperiod. *Indonesian Journal of Aquaculture*, **2**, 146-156.
- Calvayrac, R., Laval-Martin, D., Briand, J.** and **Farineau, J.** (1981). Paramylon synthesis by *Euglena gracilis* photoheterotrophically grown under low O₂ pressure. *Planta*, **53**, 6–13.
- Cramer, M.** and **Myers, J.** (1952). Growth and photosynthetic characteristics of *Euglena gracilis*. *Archiv Fur Mikrobiologie*, **7**, 1-4;384–402.
- Danesi, E.D.G., Rangel-Yagui, C.O.** and **Carvalho, J.C.M.** (2004). Effect of reducing the light intensity on the growth and production of chlorophyll by *Spirulina platensis*. *Biomass Bioenergy*, **26**, 29-335.
- Douskova, I., Doucha, J., Livansky, K., Machat, J., Novak, P., Umysova, D., Zachleder V.** and **Vitova M.** (2009). Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. *Applied Microbiology & Biotechnology*, **82**, 1, 179–185.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A.** and **Smith, F.** (1956). *Colorimetric Method for Determination of Sugars and Related Substances*, **28**, 3, 350-356.
- Fabregas, J., Maseda, A., Dominguez, A., Ferreira, M.** and **Otero, A.** (2002). Changes in the cell composition of the marine microalga, *Nannochloropsis gaditana*, during a light: Dark cycle. *Biotechnology Letters*, **2**, 4, 1699-1703.
- Guzman, H.M., de la Jara-Valido, A., Duarte, L.C.** and **Presmanes, K.F.** (2010). Estimate by means of flow cytometry of variation in composition of fatty acids from *Tetraselmis suecica* in response to culture conditions. *Aquaculture International*, **18**, 189–199.
- Ho, S.H., Chen, C.Y., Lee, D.J.** and **Chang, J.S.** (2011). Perspectives on microalgal CO₂ emission mitigation systems - a review. *Biotechnology Advances*, **9**, 189-198.
- Hu, Q.** (2004). Environmental effects on cell composition. in: *Handbook of microalgal culture*, (Ed.) Richmond, A. Oxford: Blackwell. 83-93.
- Inui, H., Miyatake, K., Nakano, Y.** and **Kitaoka, S.** (1982). Wax ester fermentation in *Euglena gracilis*. *FEBS Letters*, **150**, 89–93.
- Khoeyi, Z.A., Seyfabadi, J.** and **Ramezanzpour, Z.** (2012). Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, *Chlorella vulgaris*. *Aquaculture International*, **20**, 41–49.
- Kishore, G., Kadam, A.D., Daverey, A.** and **Arunachalam, K.** (2017). Isolation and evaluation of cultivation conditions of *Euglena* sp. from Western Himalaya for biofuel production. *Biofuels*, **9**, 2, 221-228.
- Kunne, A.** and **DeGroot, E.J.** (1996). Protein synthesis in *Euglena gracilis* is light- and temperature-dependent, oscillating in a circadian, temperature-compensated manner. *Botanica ACTA*, **109**, 57-63.
- Lavens, P.** and **Sorgeloos, P.** (1996). *Manual on the production and use of live food for aquaculture*. FAO Fisheries Technical Paper. No. 361. Rome: Food and Agriculture Organization of the United Nations.
- Lee, Y.K.** and **Shen, H.** (2004). Basic Culturing Techniques. In: Richmond, A. (ed.) *Handbook of Microalgal Culture: Biotechnology and applied Phycology*. Oxford: Blackwell Publishing Ltd.
- Lv, J.M., Cheng, L.H., Xu, X.H., Zhang, L.** and **Chen, H.L.** (2010). Enhanced Lipid Production of *Chlorella vulgaris* by

- Adjustment of Cultivation Conditions. *Bioresource Technology*, **101**, 17, 6797–6804.
- Parmar, A., Singh, N.K. and Pandey, A.** (2011). Cyanobacteria and microalgae: A positive prospect for biofuels. *Bioresource Technology*, **102**, 22, 10163–10172.
- Perumal, P., Prasath, B.B., Santhanam, P., Ananth, S., Devi, A.S. and Kumar, S.D.** (2005). *Isolation and Culture of Microalgae. Advances in Marine and Brackishwater Aquaculture*. New Delhi: Springer.
- Price, L.L., Yin, K. and Harrison, P.J.** (1998). Influence of continuous light and L:D cycles on the growth and chemical composition of Prymnesiophyceae including coccolithophores. *Journal of Experimental Marine Biology and Ecology*, **223**, 223–234.
- Renaldy, B.** (2020). Pengaruh Variasi Fotoperiode Terhadap Pertumbuhan, Biomassa, Kandungan Lipid, Karbohidrat dan Protein pada Kultur *Euglena* sp. *Undergraduate Thesis*.
- Richmond, A.** 2004. *Handbook of Microalgal Culture*. Oxford: Blackwell Science Ltd. 49.
- Rodolfi, L., Zittelli, G.Z., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M.J.** (2009). Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, **102**, 100–112.
- Sirisuk, P., Ra, C.H., Jeong, G.T. and Kim, S.K.** (2018). Effects of wavelength mixing ratio and photoperiod on microalgal biomass and lipid production in a two-phase culture system using LED illumination. *Bioresource Technology*, **253**, 175–181.
- Smedes, F. and Thomasen, T.K.** (1996). Evaluation of the Bligh & Dyer Lipid Determination Method. *Marine Pollution Bulletin*, **32**, 1, 681–688.
- Suzuki, K.** (2017). Large-scale cultivation of *Euglena*, In: Schwartzbach, S.D., Shigeoka, S. (Eds). *Euglena: Biochemistry, Cell and Molecular Biology*. Cham: Springer International Publishing. 285–293.
- Suzuki, K., Mitra, S., Itawa, O., Ishikawa, T., Kato, S. and Yamada K.** (2015). Selection and characterization of *Euglena anabaena* var. minor as a new candidate *Euglena* species for industrial application. *Bioscience, Biotechnology, and Biochemistry*, **79**, 10, 1730–1736.
- Takeyama, H., Kanamaru, A., Yoshino, Y., Kakuta, H., Kawamura, Y. and Matsunaga, T.** (1997). Production of antioxidant vitamins, β -carotene, vitamin C, and vitamin E, by two-step culture of *Euglena gracilis* Z. *Biotechnology and Bioengineering*, **53**, 185–190.
- Templeton, D. W., Quinn, M., Wychen, S.V., Hyman, D. and Laurens, L.M.L.** (2012). Separation and Quantification of Microalgal Carbohydrates. *Journal of Chromatography A*, **1270**, 225–234.
- Thompson, G.A.** (1996). Lipids and Membrane Function in Green Algae. *Biochimica et Biophysica Acta - Lipids and Lipid Metabolism*, **1302**, 1, 17–45.
- Toyama, T., Hanaoka, T., Yamada, K., Suzuki, K., Tanaka, Y., Morikawa, M. and Mori, K.** (2019). Enhanced production of biomass and lipids by *Euglena gracilis* via co-culturing with a microalga growth-promoting bacterium, *Emericella* sp. EG3. *Biotechnology for Biofuels*, **12**, 205.
- Tzovenis, I., Pauw, N.D. and Sorgeloos, P.** (1997). Effect of different light regimes on the docosahexaenoic acid (DHA) content of *Isochrysis* aff. galbana (clone T-ISO). *Aquaculture International*, **5**, 489–507.
- Wahidin, S., Idris, A. and Shaleh, S.R.M.** (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. *Bioresource Technology*, **129**, 7–11.
- Wang, Y., Seppänen-Laakso, T., Rischer, H. and Wiebe, M.G.** (2018). *Euglena gracilis* growth and cell composition under different temperature, light and trophic conditions. *PLoS ONE*, **13**, 4, 1–17.

تحسين المعالجة الضوئية لتعزيز إنتاج الكتلة الحيوية ومحتوى المستقلب الأولي في

Euglena sp. سلالة معزولة من يوجياكرتا، إندونيسيا

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^١ كلية الأحياء، جامعة غادجاه ماداء، إندونيسيا، و^٢ مركز أبحاث تكنولوجيا الأغذية وتصنيعها، الوكالة الوطنية للبحث والابتكار، يوجياكرتا ٥٥٨٦١، إندونيسيا، و^٣ باحث أول في معهد بحوث طاقة الطحالب، قسم دراسة جدوى إنتاج الكتلة الحيوية، تطوير تكنولوجيا الإنتاج، شركة يوجلينا المحدودة، اليابان

المستخلص. تتطلب زراعة الطحالب الدقيقة كمصدر بديل للطاقة تحسين عملية الزراعة. الحنذية عبارة عن طحالب دقيقة تعمل بالوقود الحيوي تحتوي على البروتينات والأحماض الأمينية الأساسية والفيتامينات C و E والدهون وبيتا كاروتين وبيتا ٣، ١ جلوكان (باراميلون). تعد شدة الضوء والفترة الضوئية من العوامل الحاسمة في تحديد إنتاج الكتلة الحيوية للطحالب الدقيقة بسبب علاقتها بأقصى كفاءة لعملية التمثيل الضوئي. ونتيجة لذلك، فإن تحسين ظروف النمو في زراعة الطحالب الدقيقة أمر بالغ الأهمية لزيادة الإنتاجية. نظرت هذه الدراسة في كيفية تأثير معالجات الفترة الضوئية المختلفة (١٢:١٢، ١٤:١٠، ١٦:٨، ١٨:٦، و ٢٤:٠ ساعة) على النمو والكتلة الحيوية والدهون والكربوهيدرات ومحتوى البروتين في *Euglena sp.* الثقافات في وسط CM (كريمير مايرز) المعدل. أنتجت المعالجة الضوئية ١٨:٦ أعلى معدل نمو محدد، والكتلة الحيوية، والكربوهيدرات الدهنية، ومحتوى البروتين، وفقاً لنتائج الدراسة. كان تركيز الكتلة الحيوية ١٨٤، ٤١٩٠، ٠، ٤١٩٠، ٠، ٧٨ وكان معدل النمو المحدد ٥، ٩٦٠، ٦٦ (OD680/OD680/hx10-3). كان تركيز الدهون ٠، ٢٠٣٠، ٠، ٧٨ و٣٧١، ١٤٢١٠، ٧، ١٢٦ جم/لتر. وتركيز الكربوهيدرات ٣٧٨، ٠، ٦١٢٥٢، ١٣٥ ملجم/مل، وتركيز البروتين ٣٧١، ١٤٢١٠، ٧، ١٢٦ جم/لتر.

الكلمات المفتاحية: يوجلينا س، الفترة الضوئية، عزلن تحسين، زراعة.

