Light Intensity Promote Pigment Contents, Biomass Production, Total Lipid and Specific Fatty Acid Profile on *Nannochloropsis* sp. Culture

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Abstract

Nannochloropsis sp is marine microalga and widely cultured for its benefits. Pigments, lipid, and fatty acid compounds of Nannochloropsis sp are essential elements in the industry. This research aimed to determine the best light intensity on the growth rate, cell density and size, biomass, pigments (chlorophyll a, b, carotenoids), total lipid and fatty acid profile. Nannochloropsis sp. culture was carried out with three light intensity treatments (100, 155, and 180 µmol), with two replications. Periodicity was set up (16:8) with the ratio of dark (8h) to light (16h). The highest cell density and total pigment content of 180 µmol were significantly different ($p<0.05$) with 155 and 100 µmol. The highest weight of chlorophyll a, b, and carotenoids were found from the intensity of 180 treatment ($p < 0.05$), followed by 155 and 100 µmol as the smallest one. The bigger cell size was reached from 180 and 155 treatments compared to 100 µmol treatment. The higher wet weight was gained from 155 (564 grams) and followed by 180 µmol (549 grams). The 100 µmol light intensity produced the lowest wet weight (490 gr) ($p<0.05$). The highest total lipid content was obtained from 155 µmol treatment (0.14 g ww). The microalgae contain SFA/Saturated Fatty Acids (Palmitic, Stearic Acid) and UFA/Unsaturated Fatty Acid (Oleic Acid). The microalgae from 180 µmol produced Eicosanoic acid (Omega-6). The production of certain compounds has differed in light intensity. In the future, the light intensity can be adapted as the alternative solution for producing microalgae for industrial approach, whether for pigments or biodiesel production.

Keywords: *Fatty acid; Lipid; Nannochloropsis sp.; Pigment content*

Introduction

Microalgae, including *Nannochloropsis* sp., is a profitable marine organism rich in nutrients. It has rigorous cell cellulose polymer characteristics (Bernaerts *et al*., 2020) with unicellular cells and cell sizes deviating between 2-6 µm (Borowitzka, 2013). Many studies show that *Nannochloropsis gaditana* is commercially used as a skin protector (Letsiou *et al*., 2017), biofuel raw material (Teo *et al*., 2014), and health supplement (Kent *et al*., 2015).

Nannochloropsis sp. retains pigment and lipids on its cells (Hossain *et al*., 2019). Chlorophyll a, chlorophyll b, and carotenoids are the ubiquitous pigments in these microalgae cells (Hokmalipour *et al*., 2011). Lubián *et al*. (2000) research shows that *Nannochloropsis* sp. have chlorophyll a, violaxanthin and vaucheriaxanthin. Chlorophyll recreates a role in its oxygenic photosynthesis (Jubert *et al*., 2009) and entraps light energy while emitting high-energy electrons (Jalal *et al*., 2014). Moreover, *Nannochloropsis* sp also has chlorophyll b as an accessory pigment in photosynthesis (Alam *et al*., 2018). On light-harvesting, chlorophyll b helps chlorophyll a to increase the efficiency of the process (Koh *et al*., 2019). In addition, *Nannochloropsis* sp also contains carotenoid pigments, including beta carotene (Faé-Neto *et al*., 2018). The carotenoids in microalgae also act as light traps (Solovchenko *et al*., 2008) and play a role in restoring the resilience of the complex structure and function in the photosynthesis process (Guedes *et al*., 2011).

Pigment (Dufossé *et al*., 2005) and fatty acids (Deshmukh *et al*., 2019) are fundamental elements in industrial sectors. Chlorophyll has been used as a nutraceutical agent with antioxidant, anti-

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inflammatory, antimutagenic, and antimicrobial possessions (da Silva and Sant'Anna, 2017). Examination by Lauritano *et al*. (2020) shows that chlorophyll derived from the diatom *Cylindrotheca colostrum* has anti-inflammatory activity. Carotenoid pigments have health applications and their use in the food, feed, nutraceutical, pharmaceutical, and cosmetic industries (Ambati *et al*., 2019). Lipids produced by microalgae have many functions in various fields, such as medical and renewable energy sources (Zewdie and Ali, 2020). Lipid content in microalgae is a vital provision that determines the feasibility of microalgae for commercial production, such as biodiesel needs (Deshmukh *et al*., 2019). Fatty acid in microalgae can take 5-50% ratios from cells' dry weight (Breuer *et al*., 2012). Fatty acids production from microalgae can be used for high-lipid feedstock (Hu *et al*., 2008). It has been prominent that *Nannochloropsis* sp. has high lipid content, a rapid growth rate, and a high carbon fixation rate (Raj *et al*., 2019). Thus, this research attempted to confine the best light intensity on the growth rate, cell density, cell size, biomass production, pigments (chlorophyll a, b, carotenoids), total lipid content, and fatty acid profile of *Nannochloropsis* sp.

Materials and Methods

Strain and culture preparation

A single culture of six-liter pure *Nannochloropsis* sp was obtained from Lampung Marine Aquaculture Center, Lampung District. Soon as the pure culture arrived, it was then acclimated and aerated. The *Nannochloropsis* sp. culture was performed by up scaling from 1 L to 5 L, then to 15 L and 30 L for each treatment.

Experimental design

This research was carried out under sterile conditions, both on instruments and materials. Appliances such as aerator hoses and stones, tub receptacles were soaked in 20-30 ppm chlorine for 24 hours. Seawater was sterilized in an autoclave for 15 minutes, screened using cotton blanketed with a plankton net, and UV for 2 h (Sari and Manan, 2012). Three treatments of light intensities were applied, referred from Wagenen *et al*. (2012), namely 100, 155, and 180 µmol. Every *Nannochloropsis* sp. culture media (30 L) treatment were administered out in six containers containing 22.5 L of 35 ppt seawater and 7.5 L of algae inoculum (3:1). Treatments were replicated two times. The initial algae density was $1.745 \times 10^{11} \pm 42.9$ cells ml^{-1}. The experiment was carried out in a closed dark room; the only lighting sources came from the growth lamp operated to cultivate algae. The fertilizer used was Conwy PA at a rate of 1 $ml.L^{-1}$ seawater. It contains NaEDTA, H_2BO_3, $NaNO_3$, $FeCL_3$ $6H_2O$, $NaHPO_4$, Aquadest, Trace Metal, and vitamins (Yarti *et al*., 2014).

Cell density, cell size, and biomass

The density of *Nannochloropsis* sp. was counted on a hemocytometer and observed every day until the stationary phase was attained (6 d). The cell area of algae was analyzed according to Baroni *et al*. (2019) method, using ImageJ software connected to a microscope. The length and width of the cells were measured according to the hemocytometer scale and observed at the 7th day before being harvested.

Biomass was harvested on the 7th day by the method referred to Sales and Abreu (2015) and Chua *et al*. (2019) using NaOH and chitosan. The NaOH solution was mixed at 10 $g.L^{-1}$, and the chitosan was dissolved up to 22 ppm. The biomass was centrifuged for 15 min at 5000 rpm and the pellet was then collected.

Pigment content

Biomass analysis is carried out for pigment content namely chlorophyll a, chlorophyll b, and carotenoids. The extraction, calculation of chlorophyll a, b and carotenoids were done basically by Fakhri *et al*. (2017) methods. Biomass was dissolved in 10 ml of 90% acetone and stored at 4°C for 12-14 h. The biomass solution was centrifuged at 5000 rpm for 2 x 5 min until the solution was performed. The decanatant was then collected. After centrifugation, the solution was then analyzed using a spectrophotometer with wavelengths of 665, 652, and 480 nm.

Lipid content

Lipid extraction was carried out according to the Melanie and Fithriani (2019) method. Microalgae were macerated using n-hexane as a solvent for 24 hours. After maceration, the solvent and microalgae are separated by centrifugation at 10000 rpm for 5 minutes. The remaining solvent was evaporated to obtain microalgae oil.

Fatty acid profile

Fatty acid profile was assessed using GC and GC-MS. GC analysis was run to Hewlett-Packard 6890 chromatography. The instrument was operated with split injector; 250°C and flame ionization detector at 300°C, colom fused silica (Omegawax 250; 30m x 0.25 nm internal diameter with 0.25 film thickness). It was used as gas carrier. The oven temperature programme was set at 70-280°C with velocity at $5^{o}C.min^{-1}$ and held in 10 mins. The MS condition was full scanned (50-600$m.z^{-1}$), 0.65 cycle time and electron ionization at 70eV.

Data analysis

Most data in this research used three treatments with two repetitions each. Due to the restriction on getting adequate materials (biomass for total lipid contents), we combined the materials from each treatment. Statistical analysis using One-way ANOVA with R Studio software, with a significant value of 0.05, and ensured that the data were typically normal distributed and homogeneous.

Result and Discussion

The growth rate in microalgae is influenced by environmental parameters, and exceptionally light intensity (Biswal *et al*., 2012). Light intensity plays an important role in microalgae conditions such as growth rate and photoautotrophic reproduction (Rocha *et al*., 2003). The growth rate and cell density of *Nannochloropsis* sp. in three treatments (100, 155, 180 µmol) of different light intensities are presented in Figure 1.

ANOVA results showed that there was a significant difference ($p<0.05$) on the growth rate and cell density of *Nannochloropsis* sp. among three treatments. The highest growth rate and cell density were reached from 180 µmol, while the smallest growth and lowest cell density derived from 100 µmol light intensity. D0 to D1 shows the lag phase where the microalgae adapt to the culture medium. D1 to D4 shows the performance of a log/exponential phase. The highest density was obtained from 180 µmol, followed by 155 µmol and 100 µmol was the lowest one. The pattern was similar throughout the culture period (day). When entering the exponential phase, the cells were still actively divided. This may due to the low competition for light and nutrients so that the microalgae population density grows optimally (Cotteau, 1996). D4 to D5 indicate the stationary phase, while the D5 to 6 show the mortality phase. The 180, 155, and 100 µmol decreased from 5.222×10^{11} to 4.125×10^{11} (180 µmol), 3.120×10^{11} to 4.675×10^{11} (155 µmol), and 4.125×10^{11} to 2.373×10^{11} cell.ml^{-1} (100 µmol), respectively. Cell density decreased as competition for light and nutrients increased. Research by Burson *et al. (*2019) shows that the denser the population of *Nannochloropsis* sp., the higher the need for nutrients and light becomes.

The cell size of microalgae in three treatments (100, 155, and 180 µmol) of different light intensities are presented in Figure 2. Treatments 180 and 155 µmol were significantly higher compared to 100 µmol ($p<0.05$). Cell size of *Nannochloropsis* sp. in 180, 155, 100 µmol treatments were 7.83 ± 0.53, 7.5 ±0.14, and 6.15±0.22 µm^2, respectively. Research from Baroni *et al*. (2019) stated that the culture conditions influence the cell size of *Nannochloropsis* sp.; one of the parameters is light intensity. The results of this study were in agreement with research by Trinh *et al*. (2019), which states that cell area and sizes are increased according to light intensity.

The 180 µmol light intensity treatment is optimal for obtaining the highest population density. The high intensity of the given light triggers the rate of photosynthesis and more cell division. As Becker (1994) denoted, the greater the rate of photosynthesis, the greater the density of the microalgae population. The rate of photosynthesis affects the biomass of microalgae. The wet weight of microalgae is present in Figure 3. The light intensity treatment of 155 µmol resulted in the slightly highest wet weight of microalgae (564 g), followed by 180 µmol (549 g), and 100 µmol, with the lowest weight of (490 g). According to research from Nzayisenga *et al*. (2020), a high level of biomass in microalgae is found in high light intensity.

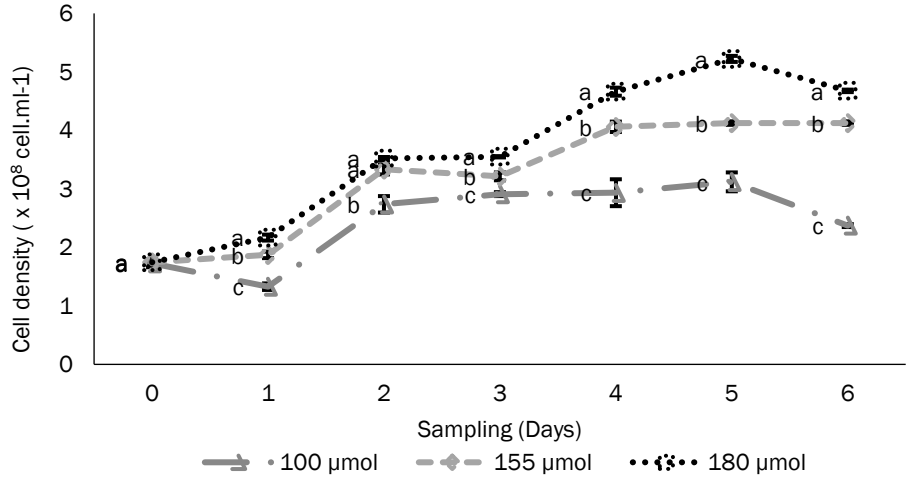

Figure 1. *Nannochloropsis* sp. growth and cell density with different light intensity in 6 days of culture. Data at the same sampling time with different letters indicate significant differences ($p<0.05$)

Pigment content

Photosynthesis in microalgae is an essential source of the number of biomasses contained in the algae (Vadiveloo *et al*., 2016). Chlorophyll is the primary pigment in the photosynthesis of *Nannochloropsis* sp. microalgae (Sakhtivel and Devi, 2014). The pigment content in this microalga from the three treatments are shown in Figure 4. Based on Fig 4, the ANOVA results show that chlorophyll a, chlorophyll b, and carotenoids are significantly different ($p<0.05$). The highest chlorophyll a pigment contents were reached at 180 µmol light intensity (90±0.04 g.mL^{-1}) followed by 155 µmol 0.62±0.06 g.mL^{-1} and 100 µmol (0.60±0.03 g.mL^{-1}), respectively. Research from Metsoviti *et al*. (2019) strengthened our data and reported that light intensity affects the levels of microalgae pigments.

The chemical structure of chlorophyll is unique with free electrons structure from nitrogen atoms (Nakano *et al*., 2022) and the crucial role of pigments is to neutralize and combat the free radicals and the reactive oxygen species accumulation (Luis *et al*., 2018). The oxygenic photosynthetic chlorophyll b is an accessory pigment that helps chlorophyll a to capture light. Figure 4 shows that the best chlorophyll b content was reached from 180 µmol light intensity ($p<0.05$). The 180 µmol produced 1.54±0.08 g.mL^{-1}, then 155 µmol produced 1.43±0.01 g.mL^{-1}, and 100 µmol produced the lowest chlorophyll b (1.23± 0.101 g.mL^{-1}). According to research from Xu *et al*. (2018), the carbon assimilation rate will increase at high light intensity so that chlorophyll production will also increase. Nutrients from fertilizers also affect the chlorophyll content produced by microalgae (Yudiati *et al*., 2021a) as well as macroalgae *Gracilaria* sp. (Yudiati *et al*., 2020; Yudiati *et al*., 2021b).

The carotenoid content was significantly different ($p<0.05$) among 180, 155, and 100 µmol treatments. The highest carotene content was found in the 180 µmol light intensity (0.5±0.161 µg.mL^{-1}) and the lowest from 100 µmol treatment (0.1±0.02 g.mL^{-1}). The high carotenoids content in higher light treatment is due to the high chlorophyll produced by *Nannochloropsis* sp. for photosynthesis. Carotenoids

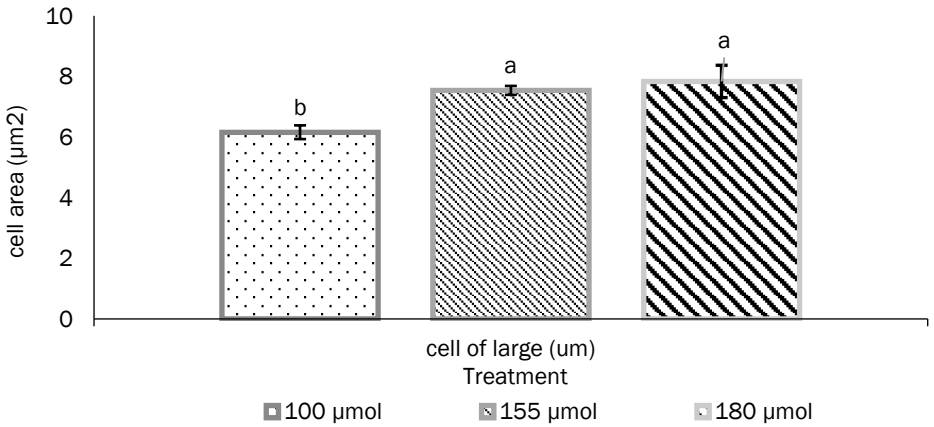

Figure 2. *Nannochloropsis* sp. cell size with different light intensity. Data with different letters indicate significant differences ($p<0.05$)

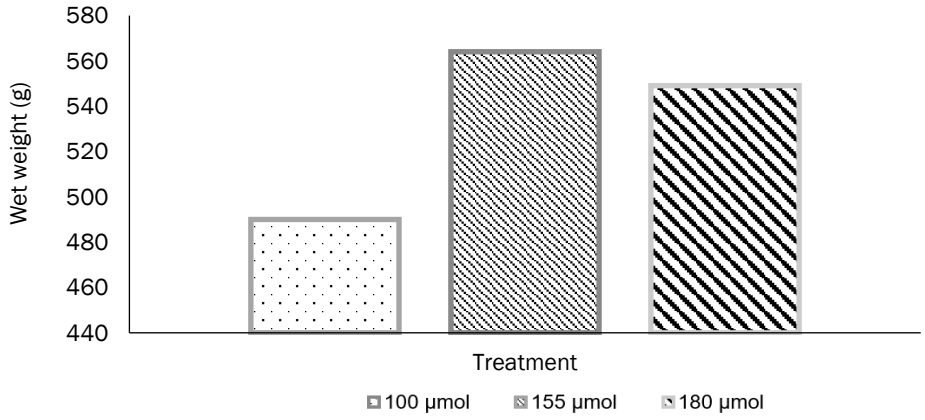

Figure 3. *Nannochloropsis* sp. wet weight, cultured in different light intensity at the end of experiment. Data with different letters indicate significant differences ($p<0.05$)

the tetraterpene pigments that display yellow, red, purple, and orange colours are essential pigments in photosynthetic organs beside chlorophylls (Maoka, 2019). Carotenoids supports chlorophyll for taking in light energy. The 180 µmol light intensity treatment was optimal for obtaining high pigment levels.

Total lipid content

The extraction of lipids in this *Nannochloropsis sp.* biomass was done by gravimetry methods, based on wet weight. The lipid content in *Nannochloropsis* sp. from the three treatments is shown in Figure 5. The highest lipid weight was obtained from 155 µmol treatment (0.14 g ww), follows by 180 µmol (0.10 g ww), and the lowest from 100 µmol (0.03 g ww). This result is higher than Maynardo *et al*. (2015), which reports that their lipid weights were 0.005 g and 0.01 g, respectively. In addition, Chavoshi and Shariati. (2019) was also noted, when microalgae are stressed, the lipids contained in the microalgae can also accumulate more under high salinity pressure and high light intensity. Moreover, Gong and Jiang. (2011), also reported that high lipid content in microalga shows that this organism shows an auspicious potential in the biotechnology industry, for example, biodiesel production.

Fatty acid profile

The chromatogram of GC-MS analysis shows the fatty acid content of different Nannochloropsis sp. which cultures in different light intensities (Table 1.).

Based on Table 1, all the microalgae from different light intensity produces saturated and unsaturated fatty acid (Oleic acid). The microalgae from 180 µmol light intensity gets more diverse fatty acid type than the two others. The most commonly found fatty acids type are Stearic acid, Palmitic acid, and Oleic acid. This shows the same result as the research of Bartley *et al*. (2013), Bondioli *et al.* 2012, and Valdez *et al.* (2011); the fatty acid profiles found were C16:0 (Palmitic acid), C18:1 (Oleic acid), and

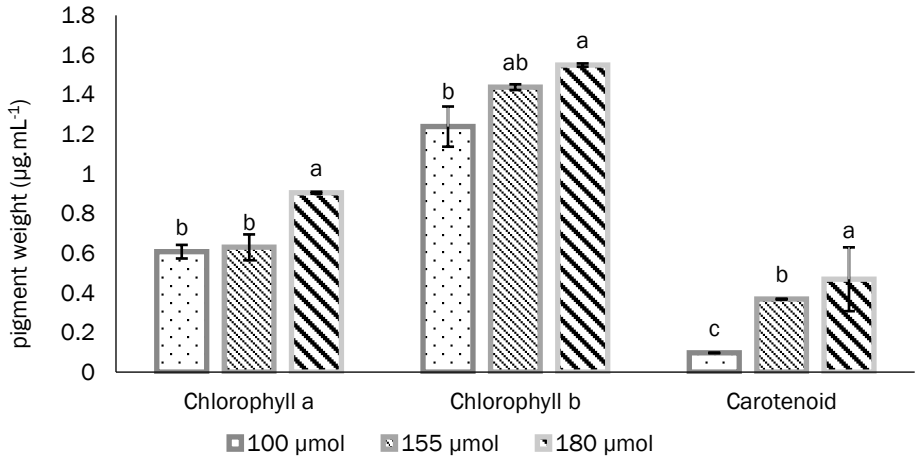

Figure 4. *Nannochloropsis* sp. pigment contents with different light intensities at the end of the experiment. Data at the same pigment type with different letters indicate significant differences ($p<0.05$)

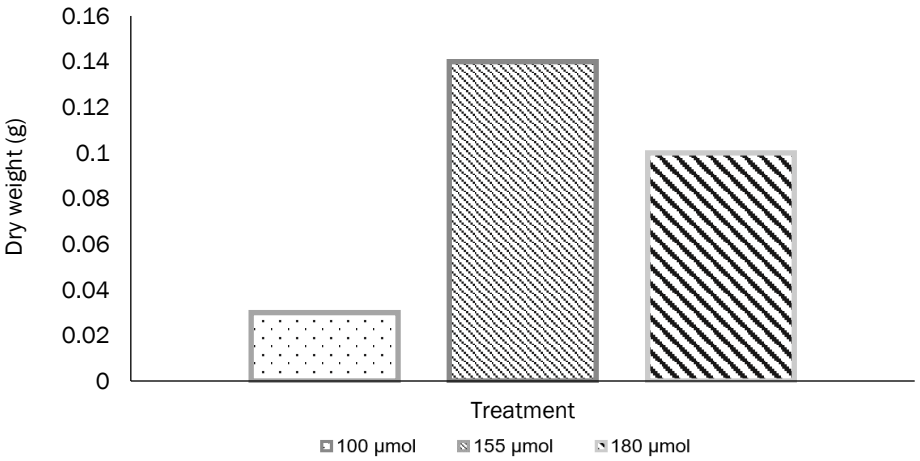

Figure 5. The dry weight of Lipid content of *Nannochloropsis sp.* under different light intensity regime

Table 1. Fatty Acid Profile of *Nannochloropsis sp.* under different light intensity regime

Light Intensity	Fatty acid
100 µmol	Palmitic Acid (saturated fatty acids) (C16:0)
	Oleic Acid (unsaturated fatty acids) (C18:1)
155 µmol	Stearic Acid (saturated fatty acids) (C18:0)
	Oleic Acid (unsaturated fatty acids) (C18:1)
180 µmol	Palmitic Acid (C16:0)
	Eicosanoic acid (unsaturated fatty acids, Omega-6 fatty acids) (C20:0)
	Oleic Acid (unsaturated fatty acids) (C18:1)

C18:0 (Stearic acid). The same type of fatty acid was also found in other species such as *Chaetoceros* sp and *Tetraselmis suecica* (Chaisutyakorn *et al*., 2017). Moreover, research by Seyfabadi *et al.* (2011) mentioned that total saturated fatty acids increased, while monounsaturated and polyunsaturated fatty acids decreased with increasing irradiance and light duration.

Biodiesel is an alternative to fossil fuels. Biodiesel is environmentally friendly because it is made from materials obtained from plants or nature. Some of the fatty acids contained in plants can be used as ingredients in biodiesel manufacture. The fatty acid component in biodiesel production, according to Sharmin *et al*. (2016), are components of palmitic acid (C16:0), Lysergic acid (C14:0), Stearic acid (C18:0), and Oleic acid (C18:1). Making biodiesel requires a high percentage of fatty acids in several components. Moazami *et al*. (2012) noted that oleic acid, which has a high percentage of fatty acids, will produce good quality biodiesel. In addition, the fatty acids in *Nannochloropsis* sp. can also be developed in the cosmetic industry. Palmitic acid contained in *Nannochloropsis* sp. is one of the raw materials in the manufacture of soap and other cosmetics (Kelm and Gary, 2017).

Conclusion

The application of three regimes of light intensity treatment significantly affected cell population density, biomass, cell size, pigment content of chlorophyll a, b, carotenoids, lipid and fatty acid profile of microalgae *Nannochloropsis* sp. Different light intensities resulted in different target compounds. The 180 µmol light intensity produced more pigment contents, and 155 µmol produced slightly higher biomass as well as total lipid content. The 180 µmol light intensity produces more diverse fatty acids type. Therefore the production of particular compounds was in light intensity dependent manner. The light intensity can be adapted as the alternative solution for producing microalgae for industrial approaches, in the future.

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