Biosaintifika 16 (3) (2024): 498-507 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Optimizing Light Intensity for *Navicula* sp. Cultivation: Impacts on Growth, Biomass, and Biochemical Productivity

Siti Mudrikah[1], Renata Adaranyssa Egistha Putri[2], Dedy Kurnianto[3], Abdurrahman Muhammad Fikri Rasdi[4], Tia Erfianti[1], Eko Agus Suyono[1]*

[1]Faculty of Biology, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia 55281 Telephone/Fax: +62 (274) 580839
[2]Study Program of Biotechnology, Graduate School of Universitas Gadjah Mada,Yogyakarta, Indonesia 55281 Telephone/Fax: +62(274) 564305
[3]Research Centre for Food Technology and Processing, National Research and Innovation Agency, Yogyakarta, Indonesia 55861
[4]College of Medicine and Veterinary Medicine, Edinburgh Medical School, The University of Edinburgh, United Kingdom

*Corresponding Author: eko_suyono@ugm.ac.id

Submitted: 2024-07-07. Revised: 2024-10-01. Accepted: 2024-12-05.

Abstract. Global energy demand is steadily rising, necessitating the exploration of sustainable alternatives. Microalgae, such as *Navicula* sp., offer a promising solution. This study investigated the impact of light intensity on the growth, and biochemical composition (carbohydrates, lipids, proteins, and carotenoids) of *Navicula* sp. Cultures were grown in f/2 medium under four light intensity treatments: 2100 lux (control), 3500 lux, 4500 lux, and 5500 lux. Cell growth was measured through optical density analysis. Carbohydrate, lipid, protein, and carotenoid assays were performed using phenol-sulfuric acid, Bligh and Dyer, Bradford, and spectrophotometric methods. Maximum growth was observed on the eighth day of cultivation at 4500 lux light intensity. The best specific growth rate was achieved at 5500 lux. The highest biomass, carbohydrate, lipid, and protein productivity were attained at 5500 lux (24.65 mg/mL, 0.374 mg/mL/day, 0.399±0.204 mg/mL/day, and 0.025±0.018 mg/mL/day). Conversely, the highest carotenoid productivity was recorded at 2100 lux, 0.278±0.085 mg/mL/day. By those means, the increase in light intensity was positively correlated with microalgal cell growth, biomass, carbohydrate content, and carbohydrate, lipid, and protein productivity. However, a negative correlation was found with carotenoid pigment productivity. From this research, it is evident that a light intensity of 5500 lux offers a feasible option for the industrial-scale cultivation of *Navicula* sp. microalgae for biomass, carbohydrate, and protein production, while 2100 lux is more suitable for carotenoid production. This research compares the findings to previous studies, emphasizing the unique way *Navicula* sp. responds to different light levels.

Keywords: Biomass; Diatom; Metabolic productivity, *Navicula* sp., Specific growth rate

How to cite : Mudrikah, S., Putri, R. A. E., Kurnianto, D., Rasdi, A. M. F., Erfianti, T., & Suyono, E. A. (2024). Effect of Light Intensity on Growth and Primary Metabolites Content of *Navicula* sp. . *Biosaintifika: Journal of Biology & Biology Education*, *16(3)*, 498-507.

DOI: http://dx.doi.org/10.15294/biosaintifika.v16i3.13899

INTRODUCTION

The dwindling reserves of fossil fuels, accompanied by massive exploitation, have led to an accumulation of CO_2 in the atmosphere, adversely impacting climate conditions. It is indispensable to develop alternative energy sources that are cleaner and more widely utilized (Raha et al., 2018). Various biomass forms, comprising chemical compounds contained in different organisms, have been proven to serve as raw materials for producing many biofuels, such as biodiesel, bioethanol, biohydrogen, and biogas (Ferrero et al., 2021). One of the organisms that can be utilized as a raw material for biofuels is microalgae. Microalgae are microorganisms capable of producing high amounts of lipids, with some species yielding more than 70% lipids, significantly higher than traditional oily crops (Udayan et al., 2023). Another advantage of using microalgae is that their cultivation requires relatively little land, and their productivity is high due to their rapid growth rates (Costa et al., 2019). Additionally, during the growth phase, microalgae require only water, nutrients, CO_2, and sunlight, which are generally available at a low cost (Gris et al., 2014).

The benthic diatom *Navicula* sp. is a

prominent phytoplankton member and relatively easy to cultivate. However, the biochemical content of diatoms can change depending on their growth rate and environmental conditions, including temperature, nutrition, pH, irradiation, and light intensity during this life cycle phase. *Navicula* sp. has significant potential for polysaccharide production due to the mucilage that contains high levels of extracellular polymeric substances (EPS), including lipids, proteins, nucleic acids, and carbohydrates (Telussa et al., 2019). Generally, carbohydrate storage in diatoms occurs in the form of chrysolaminarin, which can accumulate up to 30-60% of their dry weight. Under optimal growth conditions, diatoms can also produce lipids constituting 1-6% of their dry weight (Marella et al., 2021; Stonik & Stonik, 2015). Despite their potential, *Navicula* sp. is rarely used for commercial applications due to low biomass and metabolite production, making its productivity not yet industrially feasible (Dhali et al., 2022).

Modification of the culture media has a direct impact on reproduction, growth, and metabolic pathways (González-Vega et al., 2021). Light intensity is one of the crucial factors affecting the growth of microalgae in nature, as it impacts their photosynthetic efficiency and biomass production (Bialevich et al., 2022; Maltsev et al., 2021). Prins et al. (2020) has examined the effect of light intensity on diatom physiology with high light intensity treatments, above 10,000 lux. Previous research by Fimbres-Olivarría et al. (2015) showed that higher light intensity can increase the growth of *Navicula* sp. Although these studies used the same range of light intensity, they used different wavelengths of light and only investigated the content of carbohydrates, lipids, and proteins. In addition, a study by Zhao et al. (2014) used the same range of light-intensity treatments but only measured growth, without metabolite content. Thus, this study needs to be carried out with some updates, both in light intensity treatment (2100 lux, 3500 lux, 4500 lux, and 5500 lux) and the parameters measured, including growth, biomass, metabolite content of carbohydrates, lipids, proteins, and carotenoid pigments in diatom species *Navicula* sp. These light intensities may yield better results than those reported in earlier studies. The effects of these various light intensities on the lipid, carbohydrate, protein, and pigment production of *Navicula* sp. have yet to be explored. Assessing the impact of different light intensities will provide more information on the most effective conditions for industrial purposes for enhancing specific metabolites from *Navicula* sp. Not only that, a larger volume of culture is used in this study (500 mL), compared to the previous study by Zhao et al. (2014) that use 250 mL culture volume. As it is well known that different size of culture volume might provide different results. Different metabolites may require distinct light intensities to achieve optimal productivity. Therefore, this research is necessary to determine the effects of light intensity on cell growth and biomass, as well as the content of lipids, carbohydrates, proteins, and carotenoid pigments in *Navicula* sp. Thus, this research aims to discover a more feasible method to increase the metabolite production of *Navicula* sp. by manipulating the light intensity received by the culture. *Navicula* sp. can be considered a valuable source of metabolites for industrial applications.

METHODS

Navicula sp. culture was obtained from the Biotechnology Laboratory of the Faculty of Biology UGM. Seawater was collected from Porok Beach, Yogyakarta, and sterilized before use. The medical-grade cotton, f/2 medium components, 70% alcohol, distilled water, 10% SDS, chloroform, methanol, 5% phenol, H_2SO_4, Bradford reagent, LED lamp, and Bovine Serum Albumin by Merck were also used in this research.

Culture Medium Preparation

The medium used for cultivating *Navicula* sp. is medium f/2, according to (Guillard, 1975). The sterilized seawater (4975 mL) with a salinity level of 26 or 27 was added into a 15 L water tank, including 1 mL of 75 g/L $NaNO_3$, 1 mL of 5 g/L^{-1} $NaH_2PO_4 \cdot H_2O$, 1 mL of 30 g/L^{-1} $Na_2SiO_3.9H_2O$, vitamin B_1 (2.96 x 10^{-7}), vitamin B_{12} (3.69×10^{-10}), and 1 mL of trace metals solution. The trace metal solution composed of 1.17×10^{-5} M $FeCl_2 \cdot 6H_2O$, 1.17×10^{-5} M $Na_2EDTA \cdot 2H_2O$, 9.10×10^{-7} M $MnCl_2 \cdot 6H_2O$, 7.65×10^{-8} M $ZnSO_4 \cdot 7H_2O$, 4.20×10^{-8} M $CoCl_2 \cdot 6H_2O$, 3.93×10^{-8} M $CuSO_4 \cdot 5H_2O$, and 2.60×10^{-8} M $Na_2MoO_4 \cdot 2H_2O$. The medium was then homogenized using a magnetic stirrer before being distributed into 500 mL bottles according to the experimental design. After distribution, the medium and other instruments were autoclaved.

Navicula sp. Cultivation

Navicula sp. was cultivated in 500 mL culture bottles with a 2:3 ratio of culture and medium. Cultivation was carried out at room temperature,

whereas homogenization was carried out with aeration, light shaking twice daily, and light intensity treatment variation. The treatment consisted of a control treatment with a light intensity of 2100 lux and three variations of light intensity treatment, including 3500 lux, 4500 lux, and 5500 lux, with an LED lamp in triplicate.

Measurement of Growth and Biomass

The cell growth was measured by using a spectrophotometer (Thermo Scientific Spectrophotometer Genesys 50 UV-Vis) with a wavelength of 680 nm and 860 nm (Zhao et al., 2014). *Navicula* sp. biomass was measured using the dry weight method. The grown culture was taken for 2 mL and put into the previously weighed microtube. It was then centrifuged at 1200 rpm for 10 minutes, and the supernatant was discarded while the pellets were dried overnight in an oven at 37℃. The next day, the microtubes were weighed again, and the biomass data was obtained from the difference in the final weight minus the initial weight.

Carbohydrate Test

The carbohydrate content test was carried out using the phenol-sulphuric acid method (Nielsen, 2010), with some modifications according to Erfianti et al. (2024) and Nurafifah et al., (2023). Five mL of the sample was centrifuged at 4000 rpm for 10 minutes, and the supernatant was discarded. The pellet was added with 0.5 mL 5% phenol and 1 mL H_2SO_4. Samples were allowed to stand for 30 minutes and homogenized. A total of 2 mL of sample was put into a glass cuvette. After that, the absorbance was calculated using a 490 nm wavelength in a spectrophotometer (Thermo Scientific Spectrophotometer Genesys 50 UV-Vis). The blank solution was used as a comparison using 0.5 mL of distilled water plus 0.5 mL of 5% phenol and 1 mL of H_2SO_4. Next, a standard curve was made by measuring the absorbance of glucose compounds with various concentrations of 500, 1000, 1500, 2000, 2500, 3500, 4000, 6000, and 8000 mg/mL. The results of carbohydrate absorbance measurements were calculated based on the linear standard curve equation of the glucose solution.

Lipid Test

The lipid content test was carried out using the Bligh and Dyer method (Breil et al., 2017). Empty petri dishes are weighed on an analytical balance. Five mL of microalgae samples were put into the conical tube and then centrifugated at 4000 rpm for 10 minutes. The supernatant was discarded, then the pellet was added to 2 mL of methanol and 1 mL of chloroform. The mixture was homogenized with a vortex. A total of 1 mL of chloroform and 1 mL of distilled water were added, then homogenized again. After that, it was centrifuged at 4000 rpm for 10 minutes. Centrifugation results in three layers, then the yellow lipid at the bottom is taken, placed in a petri dish, and then incubated. The petri dish was weighed, and the lipid content was calculated by dividing the difference between the final and initial weight of the petri dish by the sample volume.

Protein Test

The protein content test was carried out using the Bradford method (Kielkopf et al., 2020). Two mL of sample in a microtube was centrifuged at 12000 rpm for 5 minutes. The supernatant was discarded, and the pellet was incubated until dry. Pellets were added 1 mL SDS 10% and heated at 95℃ for 5 minutes. Next, the samples were incubated at 4℃ for 5 minutes. After that, 8 µL of the sample was taken and put into the micro-well plate, and 200 µL of the Bradford reagent was added. Readings were made with an ELISA reader BioTEk ELX800 with a wavelength of 595 nm. A standard curve was made by measuring the absorbance of Bovine Serum Albumin (BSA) with various concentrations of 0, 50, 100, 200, 400, and 800 ppm. The reading results of the ELISA reader are calculated based on the linear standard curve equation of the BSA.

Carotenoid Test

The method followed the protocol proposed by (Scott, 2001), with some modifications according to Erfianti et al. (2024) and Thrane et al. (2015). Two mL of sample was put into a microtube and centrifuged at 12000 rpm for 5 minutes, then the supernatant was discarded, and the pellet was added to 2 mL of 100% methanol. Then, the microtubes were wrapped in aluminum foil and incubated at 4℃ for 24 hours. Samples were centrifuged at 12000 rpm for 5 minutes. Next, the absorbance of the supernatant was measured with Thermo Scientific Spectrophotometer Genesys 50 UV-Vis at a wavelength of 480 nm.

Data Analysis

The data on growth parameters, biomass, levels of carbohydrates, lipids, proteins, and carotenoid pigments were tested for normality

using the Shapiro-Wilk test. A non-parametric test was performed using the one-sample Kolmogorov-Smirnov test. Then, a homogeneity test was performed. In addition, all treatments were also analyzed using a One-Way Analysis of Variance (ANOVA) ($p < 0.05$) and Tukey's posthoc multiple comparison test with IBM SPSS Statistics 25. A p-value less than 0.05 is considered the treatment statistically significant.

RESULTS AND DISCUSSION

Navicula sp. cell growth was indicated by the optical density data or the turbidity of the culture. Ideally, microalgae growth consists of four main phases: lag, log, stationary, and death (Chen et al., 2013). The control showed maximum growth on the 12th day, while the culture with a light intensity of 3500 and 5500 lux had maximum growth on the 10th day. However, the maximum growth of *Navicula* sp. on 4500 lux occurred on the eighth day (Figure 1). *Navicula* sp. specific growth rate based on the results of statistical tests showed no significant difference between the treatments given light intensity (Figure 2). Based on the dry weight data (Figure 3), *Navicula* sp. produces maximum biomass on the 5500 lux treatment of 24.65 mg/mL, significantly different from all light-intensity treatments. The lowest biomass production of *Navicula* sp., as shown by the culture with 2100 lux light intensity control treatment of 3.475 mg/mL. The increase of biomass reach around eight times compared to control in 5500 lux treatment. It is higher increase than the treatment of microalgae *Botryococcus* sp. that underwent biomass increase up to three times of control in the treatment of 10 Gy Gamma ^{60}Co radiation (Ermavitalini et al., 2017).

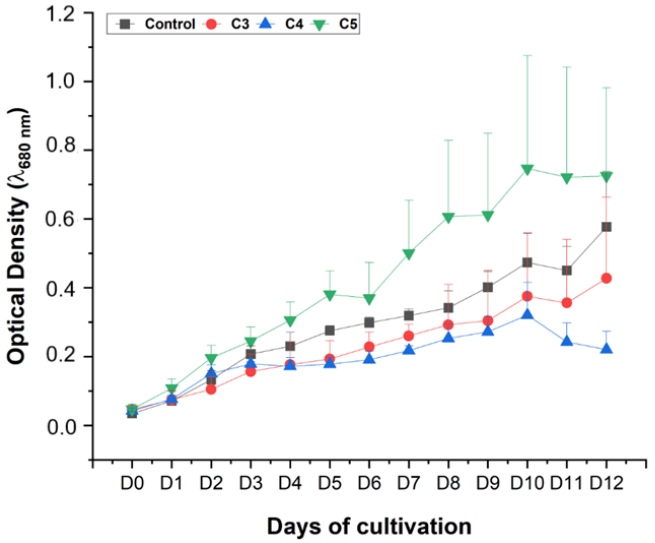

Figure 1. Growth of *Navicula* sp. under variations of light intensity

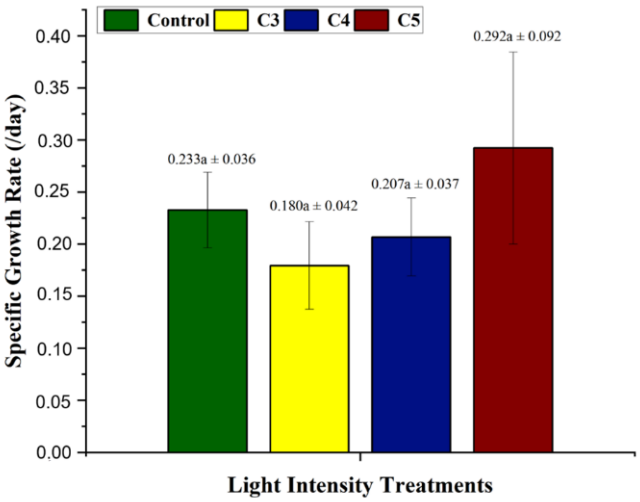

Figure 2. The specific growth rate of *Navicula* sp. under variations of light intensity

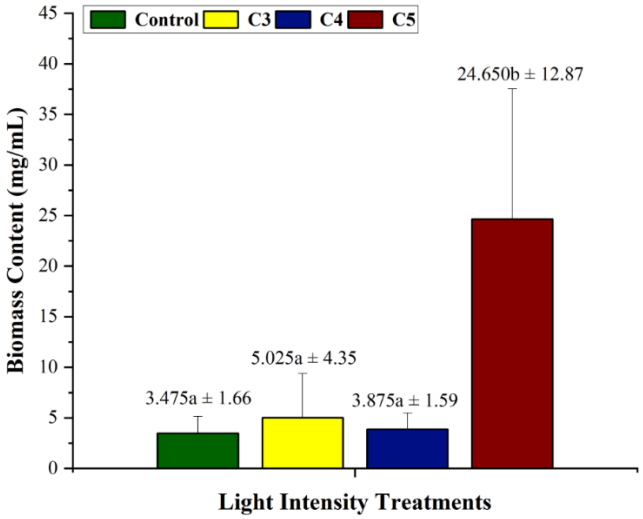

Figure 3. Biomass content of *Navicula* sp. under variation of light intensity treatments

Light is one of the leading environmental factors involved in the growth and development of microalgae. The quality and quantity of light affect the increase or decrease in growth rate to achieve optimal biomass productivity (Maltsev et al., 2021). Generally, adequate culture conditions for microalgae growth have a light intensity range of 1000-10,000 lux (Kumar et al., 2019). Based on this study, the most optimal growth occurs in a light-intensity treatment of 5500 lux compared to the control and other treatments. The cultivation result of *Navicula* sp. in a light-intensity treatment of 50 µmol/m^2/second or equivalent to ±3500 lux and 100 µmol/m^2/second or equivalent to ±7000 lux indicates a relatively low concentration of cells (Prins et al., 2020). Therefore, the treatment of light intensity is directly proportional to the growth of *Navicula* sp. culture; the higher the light intensity, the higher the concentration of microalgae cells will be.

The quantity of light microalgae cells receive is directly related to the carbon fixation capacity. When exposed to high light intensity, the cells accumulate carbohydrates and triacylglycerols, leading to higher biomass productivity and cell growth rate. However, if the light intensity is extremely high, the cells will be damaged due to photoinhibition. Under these conditions, the cell's specific growth rate becomes inhibited, leading to cell death (Havurinne & Tyystjärvi, 2017). Previous research also reported that high-light-intensity exposure helped increase the microalgae biomass content. Research on *Thalassiosira pseudonana* doubled biomass production at 120 µmol/m^2/second (8400 lux) light-intensity (Palanisamy et al., 2022). Likewise, current research shows that the highest biomass productivity was achieved in *Navicula* sp. with the highest light intensity, namely 5500 lux.

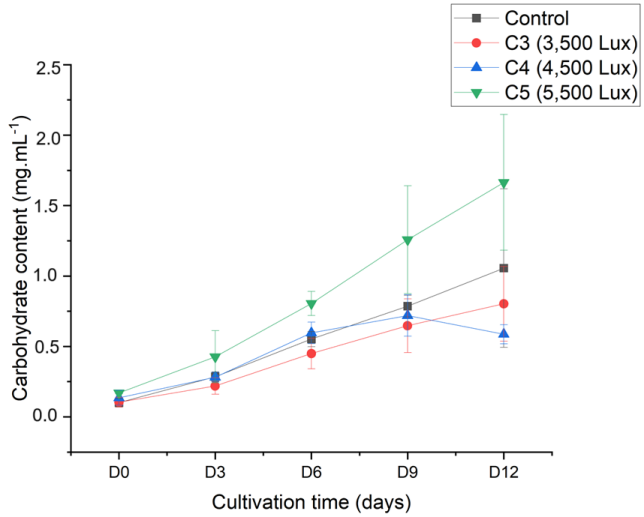

Figure 4. Carbohydrate contents of *Navicula* sp. under variations of light-intensity treatments

Based on Figure 4, the carbohydrate content produced by *Navicula* sp. with the highest value was in the treatment of 5500 lux light intensity (C5) at 1.664 mg/mL. This quantity showed results that exceeded the carbohydrate content in the control treatment. Meanwhile, the 3500 lux (C3) and 4500 lux (C4) light intensity treatments showed results below the control treatment. Microalgae cultured under environmental stress use carbon to produce energy storage products, including carbohydrates. The carbohydrates are then used for consumption in unfavorable cultural ecological conditions. The goal is for the microalgae to survive under environmental stress, such as low light intensity. Low light intensity conditions trigger microalgae to allocate energy for its growth. Increased light intensity causes microalgae to supply energy for synthesizing food ingredients like carbohydrates. Previous research found that the microalgae *Staurosira* sp. accumulates carbohydrates at the highest light-intensity conditions (Cointet et al., 2019). In line with this research, current research also shows that the highest carbohydrate content of *Navicula* sp. was achieved in cultures with the highest light-intensity treatment of 5500 lux. In this study, the most accumulation of carbohydrates occurred on the 12th day after *Navicula* sp. was cultured.

The productivity of carbohydrates, lipids, proteins, and carotenoid pigments is presented in Figure 5. The highest productivity of carbohydrates was shown by *Navicula* sp. with a light-intensity treatment of 5500 lux of 0.374 mg/mL daily. This amount was 42% higher than the control treatment with 2100 lux, whereas when compared to the 3500 and 4500 lux light intensity treatments, the 5500 lux light intensity treatment was twice as high. The productivity of carbohydrates cultivated at various light intensities was not statistically significantly different at $p > 0.05$, although each treatment showed a difference in yields of carbohydrate productivity (Figure 5A).

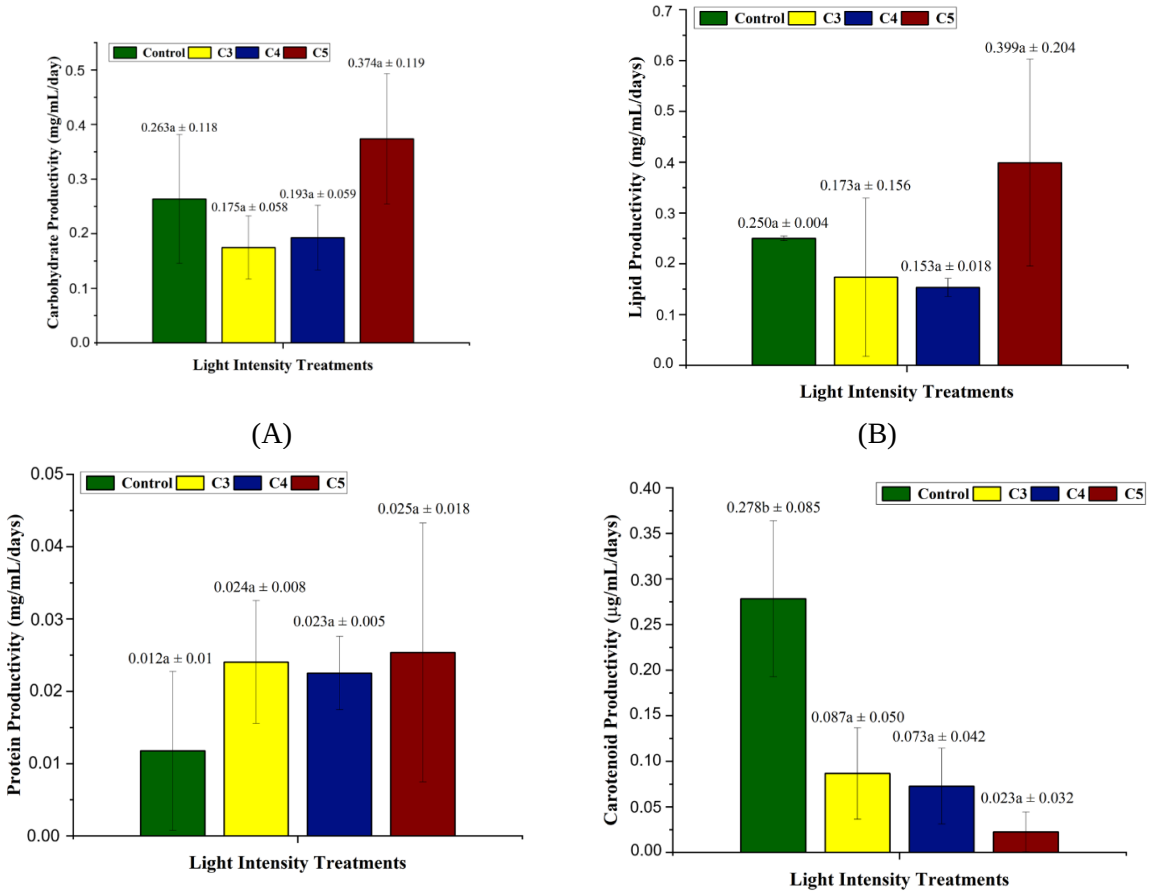

Figure 5. Productivity per day of Navicula sp. under variation of light intensity treatments. (A) Carbohydrate, (B) Lipid, (C) Protein, and (D) Carotenoid

Lipid productivity was the same between the 3500 and 4500 lux light-intensity treatments, 0.2 mg/mL daily. Lipids produced by *Navicula* sp. cultured for 12 days showed no significant difference ($p > 0.05$) between each light-intensity treatment. Based on the data in Figure 5B, the control treatment with a light intensity of 2100 lux showed a maximum lipid productivity of 0.6 mg/mL per day. The light intensity treatment of 5500 lux showed lipid productivity of 33% of the lipid productivity in the control treatment, which was 0.4 mg/mL per day. It is higher than previous finding in which *Navicula* sp. able to produce around 18% of lipid when cultivated in tapioca liquid waste media added with 25% NaCl concentration (Insan et al., 2018).

The lipid productivity of *Navicula* sp. culture was optimally achieved in the treatment of light intensity of 2100 lux. Gammanpila et al. (2015) stated that giving low light intensity causes a decrease in lipid productivity. This is due to the limited energy required for a series of microalgae photosynthetic reactions. Although this report does not support the results of the present study, the results indicate that lipid productivity is higher at a light intensity of 5500 lux when compared to lipid productivity at a light intensity of 3500 and 4500 lux. These results indicate that the potential for lipid productivity is relatively higher at higher light intensities. Previous studies on the effect of light intensity on the lipid content of *Chlorella vulgaris* microalgae showed appropriate results. A higher percentage of *C. vulgaris* lipid content was produced at a light intensity of 5000 lux than at a lower light intensity (Gammanpila et al., 2015).

The productivity of *Navicula* sp. culture protein has the same value in the light intensity treatment of 3500 and 4500 lux, which is 0.02 mg/mL per day. Based on statistical tests on the results of protein productivity in *Navicula* sp. No significant difference ($p > 0.05$) between the control light intensity treatment and the three light intensities given (Figure 5C). Nonetheless, a protein produced in *Navicula* sp. with the light intensity treatment of 3500, 4500, and 5500 lux was higher than the protein produced in the control treatment. *Navicula* sp. showed the highest protein productivity value with a light-intensity treatment of 5500 lux, equal to 0.025 mg/mL daily. The protein productivity reached three times the protein produced in a culture treated with control light intensity or 2100 lux.

In addition to carbohydrates and lipids, light intensity also affects protein productivity. Sui & Harvey (2021) reported that the protein content in microalgae decreased as the light intensity increased. The protein content of *Dunaliella salina* is relative to ash-free dry weight, which decreases with increasing light intensity. The provision of white light showed a protein content of 74% AFDW at a light intensity of 100 µmol/m^2/second (7000 lux), decreased to 52% AFDW at a light intensity of 600 µmol/m^2/second (42000 lux). This is because the light intensity of 600 µmol/m^2/second is too high, causing microalgae to experience photoinhibition. However, the decrease was insignificant; the protein content was still relatively high because it was more than 50%. These results are in line with research conducted by Seyfabadi et al. (2011) that the highest protein concentration was achieved at a light intensity of 100 µmol/m^2/second, and the minimum protein concentration was achieved at a light intensity of 37.5 µmol/m^2/second (2625 lux). However, the study concluded that an increase in light intensity also increased the protein productivity of microalgae. In this study, the maximum protein productivity of *Navicula* sp. was achieved in cultures with 5500 lux light intensity (0.03 mg/mL/day). Protein productivity at this light intensity was not much different from protein productivity at 3500 lux light intensity.

Figure 5D shows that the highest productivity of carotenoid pigments in *Navicula* sp. was in the control light intensity treatment or 2100 lux, which was 0.28 µg/mL per day. These results differed significantly ($p < 0.05$) with the three light intensity treatments, 3500, 4500, and 5500 lux. The second highest carotenoid productivity was shown by the light intensity treatment of 3500 lux, which was 0.09 µg/mL per day. This productivity value, when compared with the light intensity treatment of 4500 and 5500 lux, was 1.3 times and 4.5 times higher, respectively, even though it was not statistically significantly different ($p > 0.05$) (Figure 5D).

Carotenoids are pigments or groups of bioactive compounds that photosynthetic or non-photosynthetic organisms can synthesize. Carotenoids usually play a protective role for microalgae under stressful environmental conditions. The group of carotenoid compounds can react with lipid peroxidation products to stop the chain reaction and inactivate the excited chlorophyll. The aim is to scavenge the accumulation of ROS in chloroplasts. The carotenoid concentration of *Navicula* sp. in cultures treated with the light intensity of 4000 lux can reach 20 mg/L after 11 days of cultivation (Ding et al., 2017). After 12 days of cultivation,

the maximum carotenoid productivity of *Navicula* sp. microalgae was achieved at 2100 lux light intensity (0.28 µg/mL/day) and decreased with increasing light intensity. These results occur because specific light intensities in diatoms affect photosynthetic capacity, so it can provide mechanisms and strategies to optimize photosynthetic rates. Irradiation treatment with high light intensity causes inhibition of photosynthetic pigment synthesis. This can occur because when light conditions are photocatalytic stress, the chloroplast structure loses the ability to absorb excess excitation. Previous research reported that the diatom *Phaeodactylum tricornutum* experienced increased carotenoid synthesis at lower light intensities. Carotenoid productivity of the species reached a maximum in the light intensity treatment of 150 µmol/m^2/second compared to the high light intensity of 750 µmol/m^2/second (Conceição et al., 2020).

Our results indicated that the increase in light intensity was positively correlated with cell growth along with biomass and carbohydrate content. The productivity of carbohydrates, lipids, and protein increases together with biomass. However, the increase in light intensity is negatively correlated with carotenoid pigment productivity. This study illustrates that proper selection of light intensity should be considered for growth and biomass enhancement, whether carbohydrates, lipids, proteins, or carotenoid pigments. The fastest growth of *Navicula* sp. in reaching the maximum number is on the eighth day after culture by the light intensity of 4500 lux. Light intensity of 5500 lux had the best specific growth rate, although the result did not show significant differences ($p > 0.05$). Thus, it concluded that light intensity of 5500 lux and 2100 lux could be a feasible environmental parameter for the *Navicula* sp. microalgae industrial culture. This research provides a different light intensity from that of the previous research. This confirms the previous research by (Fimbres-Olivarría et al., 2015), which shows that the increase in light intensity will increase the biomass and carbohydrates. However, the previous research did not measure the amount of pigments from the culture. This research provides new insight into light intensity's effect on the amount of pigments produced. The number of carotenoids pigment decreases along with the increase in light intensity.

CONCLUSION

The light intensity in the cultivation of *Navicula* sp. plays an essential role in cell growth and metabolite production. The light intensity of 5500 lux gave the best result regarding cell growth, biomass, carbohydrate, and lipid. The carotenoid production reaches the optimum value at a light intensity of 2100 lux. Thus, the light intensity used in the cultivation depends on the targeted metabolites produced. In future research, it is recommended that another range of light intensity be conducted to fully grasp the relation between light intensity and the increase of growth and metabolite production in *Navicula* sp. culture. An in-depth analysis of the constitution of metabolites and a transcriptomic analysis need to be examined to study the metabolic pathway or genetic expression caused by the difference in light intensity. Mixing various environmental parameters besides light intensity might produce different results compared to only light intensity treatments.

ACKNOWLEDGMENT

This manuscript is supported by the Universitas Gadjah Mada as part of "Program Asistensi Riset 2024" with grant number 10604/UN1.P4/PT.01.03/2024.

REFERENCES

Bialevich, V., Zachleder, V., & Bišová, K. (2022). The Effect of Variable Light Source and Light Intensity on the Growth of Three Algal Species. *Cells*, *11*(8), 1293. https://doi.org/10.3390/cells11081293

Breil, C., Abert Vian, M., Zemb, T., Kunz, W., & Chemat, F. (2017). "Bligh and Dyer" and Folch Methods for Solid–Liquid–Liquid Extraction of Lipids from Microorganisms. Comprehension of Solvatation Mechanisms and towards Substitution with Alternative Solvents. *International Journal of Molecular Sciences*, *18*(4), 708. https://doi.org/10.3390/ijms18040708

Chen, L.-F., Chen, J.-J., Chung, C.-Y., Liao, S.-W., Gau, H.-S., & Lai, W.-L. (2013). Specific Fluorescent Signatures for Microalgae and Their Released Organic Matter. *Journal of Biobased Materials and Bioenergy*, *7*(2), 240–246. https://doi.org/10.1166/jbmb.2013.1316

Cointet, E., Wielgosz-Collin, G., Bougaran, G.,

Rabesaotra, V., Gonçalves, O., & Méléder, V. (2019). Effects of light and nitrogen availability on photosynthetic efficiency and fatty acid content of three original benthic diatom strains. *PLOS ONE*, *14*(11), e0224701. https://doi.org/10.1371/journal.pone.0224701

Conceição, D., Lopes, R. G., Derner, R. B., Cella, H., Do Carmo, A. P. B., Montes D'Oca, M. G., Petersen, R., Passos, M. F., Vargas, J. V. C., Galli-Terasawa, L. V., & Kava, V. (2020). The effect of light intensity on the production and accumulation of pigments and fatty acids in Phaeodactylum tricornutum. *Journal of Applied Phycology*, *32*(2), 1017–1025. https://doi.org/10.1007/s10811-019-02001-6

Costa, J. A. V., Freitas, B. C. B. D., Lisboa, C. R., Santos, T. D., Brusch, L. R. D. F., & De Morais, M. G. (2019). Microalgal biorefinery from CO2 and the effects under the Blue Economy. *Renewable and Sustainable Energy Reviews*, *99*, 58–65. https://doi.org/10.1016/j.rser.2018.08.009

Dhali, S., Jain, R., Malik, A., Sharma, S., & Raliya, R. (2022). Cultivation of Navicula sp. On rice straw hydrolysate for the production of biogenic silica. *Bioresource Technology*, *360*, 127577. https://doi.org/10.1016/j.biortech.2022.127577

Ding, T., Yang, M., Zhang, J., Yang, B., Lin, K., Li, J., & Gan, J. (2017). Toxicity, degradation and metabolic fate of ibuprofen on freshwater diatom Navicula sp. *Journal of Hazardous Materials*, *330*, 127–134. https://doi.org/10.1016/j.jhazmat.2017.02.004

Erfianti, T., Fakhruddin Yusuf, A., Handayani, S., Ryan Sadewo, B., Setiadi Daryono, B., Budiman, A., & Agus Suyono, E. (2024). Enhancing growth and metabolite profiles in indigenous Euglena gracilis through explorative light spectrum effect. *Egyptian Journal of Aquatic Research*, *50*(3), 318–331. https://doi.org/10.1016/j.ejar.2024.09.003

Ermavitalini, D., Yuliansari, N., Prasetyo, E. N., & Saputro, T. B. (2017). Efect of Gamma 60Co Irradiation on The Growth, Lipid Content and Fatty Acid Composition of Botryococcus sp. Microalgae. *Biosaintifika: Journal of Biology & Biology Education*, *9*(1), 58. https://doi.org/10.15294/biosaintifika.v9i1.6783

Ferrero, G. O., Faba, E. M. S., & Eimer, G. A. (2021). Biodiesel production from alternative raw materials using a heterogeneous low ordered biosilicified enzyme as biocatalyst. *Biotechnology for Biofuels*, *14*(1), 67. https://doi.org/10.1186/s13068-021-01917-x

Fimbres-Olivarría, D., López-Elías, J. A., Martínez-Córdova, L. R., Carvajal-Millán, E., Enríquez-Ocaña, F., Valdéz-Holguín, E., & Miranda-Baeza, A. (2015). Growth and Biochemical Composition of Navicula sp. Cultivated at Two Light Intensities and Three Wavelengths. *Israeli Journal of Aquaculture - Bamidgeh*, *67*. https://doi.org/10.46989/001c.20715

Gammanpila, A. M., Rupasinghe, C. P., & Subasinghe, S. (2015). Light Intensity and Photo Period Effect on Growth and Lipid Accumulation of Microalgae *Chlorella vulgaris* And *Nannochloropsis* sp. for Biodiesel Production. Proceedings of 12th ISERD International Conference, Tokyo, Japan, 26th Sept. 2015, ISBN: 978-93-85832-00-0

González-Vega, R. I., Cárdenas-López, J. L., López-Elías, J. A., Ruiz-Cruz, S., Reyes-Díaz, A., Perez-Perez, L. M., Cinco-Moroyoqui, F. J., Robles-Zepeda, R. E., Borboa-Flores, J., & Del-Toro-Sánchez, C. L. (2021). Optimization of growing conditions for pigments production from microalga Navicula incerta using response surface methodology and its antioxidant capacity. *Saudi Journal of Biological Sciences*, *28*(2), 1401–1416. https://doi.org/10.1016/j.sjbs.2020.11.076

Gris, B., Morosinotto, T., Giacometti, G. M., Bertucco, A., & Sforza, E. (2014). Cultivation of Scenedesmus obliquus in Photobioreactors: Effects of Light Intensities and Light–Dark Cycles on Growth, Productivity, and Biochemical Composition. *Applied Biochemistry and Biotechnology*, *172*(5), 2377–2389. https://doi.org/10.1007/s12010-013-0679-z

Guillard, R. R. L. (1975). Culture of Phytoplankton for Feeding Marine Invertebrates. In W. L. Smith & M. H. Chanley (Eds.), *Culture of Marine Invertebrate Animals* (pp. 29–60). Springer US. https://doi.org/10.1007/978-1-4615-8714-9_3

Havurinne, V., & Tyystjärvi, E. (2017). Action Spectrum of Photoinhibition in the Diatom Phaeodactylum tricornutum. *Plant and Cell Physiology*, *58*(12), 2217–2225. https://doi.org/10.1093/pcp/pcx156

Insan, A. I., Christiani, C., Hidayah, H. A., & Widyartini, D. S. (2018). The Lipid Content of The Culture Microalgae Using Media of Tapioca Liquid Waste. *Biosaintifika: Journal of Biology & Biology Education*, *10*(2), 440–448. https://doi.org/10.15294/biosaintifika.v10i2.12381

Kielkopf, C. L., Bauer, W., & Urbatsch, I. L. (2020). Bradford Assay for Determining Protein

Concentration. *Cold Spring Harbor Protocols*, *2020*(4), pdb.prot102269. https://doi.org/10.1101/pdb.prot102269

Kumar, G., Nguyen, D. D., Huy, M., Sivagurunathan, P., Bakonyi, P., Zhen, G., Kobayashi, T., Xu, K. Q., Nemestóthy, N., & Chang, S. W. (2019). Effects of light intensity on biomass, carbohydrate and fatty acid compositions of three different mixed consortia from natural ecological water bodies. *Journal of Environmental Management*, *230*, 293–300. https://doi.org/10.1016/j.jenvman.2018.09.026

Maltsev, Y., Maltseva, K., Kulikovskiy, M., & Maltseva, S. (2021). Influence of Light Conditions on Microalgae Growth and Content of Lipids, Carotenoids, and Fatty Acid Composition. *Biology*, *10*(10), 1060. https://doi.org/10.3390/biology10101060

Marella, T. K., Bhattacharjya, R., & Tiwari, A. (2021). Impact of organic carbon acquisition on growth and functional biomolecule production in diatoms. *Microbial Cell Factories*, *20*(1), 135. https://doi.org/10.1186/s12934-021-01627-x

Nielsen, S. S. (2010). Phenol-Sulfuric Acid Method for Total Carbohydrates. In S. S. Nielsen (Ed.), *Food Analysis Laboratory Manual* (pp. 47–53). Springer US. https://doi.org/10.1007/978-1-4419-1463-7_6

NurAfifah, I., Hardianto, M. A., Erfianti, T., Amelia, R., Maghfiroh, K. Q., Kurnianto, D., siswanti, D. U., Sadewo, B. R., Putri, R. A. E., & Suyono, E. A. (2023). The Effect of Acidic pH on Growth Kinetics, Biomass Productivity, and Prima-ry Metabolite Contents of Euglena sp. *Makara Journal of Science*, *27*(2). https://doi.org/10.7454/mss.v27i2.1506

Palanisamy, K. M., Rahim, M. H. Ab., Govindan, N., Ramaraj, R., Kuppusamy, P., & Maniam, G. P. (2022). Effect of blue light intensity and photoperiods on the growth of diatom Thalassiosira pseudonana. *Bioresource Technology Reports*, *19*, 101152. https://doi.org/10.1016/j.biteb.2022.101152

Prins, A., Deleris, P., Hubas, C., & Jesus, B. (2020). Effect of Light Intensity and Light Quality on Diatom Behavioral and Physiological Photoprotection. *Frontiers in Marine Science*, *7*, 203. https://doi.org/10.3389/fmars.2020.00203

Raha, H. E., Shafii, M. B., & Roshandel, R. (2018). Energy efficient cultivation of microalgae using phosphorescence materials and mirrors. *Sustainable Cities and Society*, *41*, 449–454. https://doi.org/10.1016/j.scs.2018.06.002

Scott, K. J. (2001). Detection and Measurement of Carotenoids by UV / VIS Spectrophotometry. *Current Protocols in Food Analytical Chemistry*, *00*(1). https://doi.org/10.1002/0471142913.faf0202s00

Stonik, V., & Stonik, I. (2015). Low-Molecular-Weight Metabolites from Diatoms: Structures, Biological Roles and Biosynthesis. *Marine Drugs*, *13*(6), 3672–3709. https://doi.org/10.3390/md13063672

Sui, Y., & Harvey, P. J. (2021). Effect of Light Intensity and Wavelength on Biomass Growth and Protein and Amino Acid Composition of Dunaliella salina. *Foods*, *10*(5), 1018. https://doi.org/10.3390/foods10051018

Telussa, I., Rusnadi, & Zeily Nurachman. (2019). Dynamics of β-carotene and fucoxanthin of tropical marine Navicula sp. As a response to light stress conditions. *Algal Research*, *41*, 101530. https://doi.org/10.1016/j.algal.2019.101530

Thrane, J.-E., Kyle, M., Striebel, M., Haande, S., Grung, M., Rohrlack, T., & Andersen, T. (2015). Spectrophotometric Analysis of Pigments: A Critical Assessment of a High-Throughput Method for Analysis of Algal Pigment Mixtures by Spectral Deconvolution. *PLOS ONE*, *10*(9), e0137645. https://doi.org/10.1371/journal.pone.0137645

Udayan, A., Pandey, A. K., Sirohi, R., Sreekumar, N., Sang, B.-I., Sim, S. J., Kim, S. H., & Pandey, A. (2023). Production of microalgae with high lipid content and their potential as sources of nutraceuticals. *Phytochemistry Reviews*, *22*(4), 833–860. https://doi.org/10.1007/s11101-021-09784-y

Zhao, X., Pang, S., Liu, F., Shan, T., & Li, J. (2014). Biological identification and determination of optimum growth conditions for four species of Navicula. *Acta Oceanologica Sinica*, *33*(8), 111–118. https://doi.org/10.1007/s13131-014-0465-y