AL-KAUNIYAH: Jurnal Biologi, 18(1), 2025, 1-13
Website: http://journal.uinjkt.ac.id/index.php/kauniyah
P-ISSN: 1978-3736, E-ISSN: 2502-6720

# SILICA'S MEDIUM IN VARIOUS CONCENTRATION EFFECT ON *Navicula* sp. METABOLISM

## PENGARUH VARIASI KONSENTRASI SILIKA DALAM MEDIUM TERHADAP METABOLISME *Navicula* sp.

**Khalid Erlangga Karillanata[1], Dwi Hardianti Luthfiana[1], Khusnul Qonita Maghfiroh[1], Dedy Kurnianto[2], Ria Amelia[1], Tia Erfianti[1], dan Eko Agus Suyono[1]***

[1]*Universitas Gadjah Mada, Teknika Selatan Street, Daerah Istimewa Yogyakarta*
[2]*Food Technology and Process Research Center, National Research and Innovation Agency (BRIN), Gunung Kidul, Daerah Istimewa Yogyakarta 55861*
**Corresponding author:* eko_suyono@ugm.ac.id

Submitted: 14 February 2023; Revised: 22 October 2023; Accepted:17 December 2023

**Abstract**

*Navicula* sp., an aquatic microalgae species, are numerous and diverse, with high metabolites so they hold great potential in biotechnology. Although it has many advantages, it is often not used in industry. In silica medium, *Navicula* sp. produces various metabolites depending on their concentration. This research aims to discover how adding silica to the medium affects the growth and production of *Navicula* sp. metabolites so that cultivation can be carried out at low cost and with maximum results. For 21 days, this experiment was carried out with three concentrations of silica (1; 1.5; and 2 mL/L) and one control (0 mL/L) grown each in a 500 mL culture of *Navicula* sp. Repetition was done thrice for each measurement parameter; growth speed, biomass production, lipids, carbohydrates, and protein. Medium silica 1.5 mL/L was the optimal concentration for growth speed, biomass production, and carbohydrate production for *Navicula* sp. (0.083; 0.54; and 0.075 mg/day, respectively). Meanwhile, the optimal silica concentration for lipid and protein production for *Navicula* sp. were 1 mL/L medium and control medium (0.517 and $0.8 \times 10^{-2}$ mg/day, respectively). Overall, this research can be used to grow *Navicula* sp. in producing specific metabolites optimally.

**Keywords:** Culture; Metabolites; Microalgae; *Navicula* sp.; Silica

***Abstrak***

*Navicula sp., spesies mikroalga akuatik, sangat banyak dan beragam dengan metabolit yang tinggi, sehingga memiliki potensi yang besar dalam bioteknologi. Meskipun memiliki banyak keunggulan, spesies ini seringkali tidak digunakan dalam industri. Dalam medium silika, Navicula sp. menghasilkan berbagai metabolit tergantung pada konsentrasinya. Tujuan dari penelitan ini adalah untuk mengetahui bagaimana penambahan silika dalam medium memengaruhi pertumbuhan dan produksi metabolit Navicula sp., sehingga kultivasi dapat dilakukan dengan biaya yang rendah dan hasil yang maksimal. Selama 21 hari, eksperimen ini dilakukan dengan tiga konsentrasi silika (1; 1,5; dan 2 mL/L) dan satu kontrol (0 mL/L) yang ditumbuhkan di dalam 500 mL kultur Navicula sp. Pengulangan dilakukan tiga kali untuk setiap parameter pengukuran, yaitu kecepatan pertumbuhan, produksi biomassa, lipid, karbohidrat, dan protein. Medium silika 1,5 mL/L merupakan konsentrasi yang optimal untuk kecepatan pertumbuhan, produksi biomassa, dan produksi karbohidrat bagi Navicula sp. (0.083; 0.54; dan 0.075 mg/hari, secara berurutan). Sementara itu, konsentrasi silika yang optimal untuk produksi lipid dan protein bagi Navicula sp., secara berurutan, adalah medium 1 mL/L dan medium kontrol (0.517 dan $0.8 \times 10^{-2}$ mg/hari). Secara keseluruhan, penelitian ini dapat dijadikan sebagai solusi untuk menumbuhkan Navicula sp. dalam memproduksi metabolit tertentu secara optimal.*

***Kata Kunci**: Kultur; Metabolit; Mikroalga; Navicula sp.; Silika*

**Permalink/DOI: http://dx.doi.org/10.15408/kauniyah.v18i1.31078**

© 2025 The Author(s). This is an open article under CC-BY-SA license (http://creativecommons.org/licenses/by-sa/4.0/)

## INTRODUCTION

Diatoms are the largest group of single-celled eukaryotic microalgae in waters. Diatoms dominate the phytoplankton group in the sea with a contribution of 20% of the total biological primary production on earth. One of the unique characteristics of diatom cells is a cell wall composed of silica, or frustule, which has a thickness of ten to hundreds of micrometers. This frustule consists of two parts, the upper epitheca, and the lower hypotheca, resembling a petri dish attached to a girdle; the protoplast is enclosed in this frustule where the pore array extends along the structure (Kröger & Wetherbee, 2000). Diatoms have a high metabolite content, such as lipids, that might be utilized in biotechnology (Li et al., 2017a). Despite having an attractive attribute, the commercial production of diatoms is still in its infancy due to low biomass output and expensive artificial media. Culturing diatoms on a large scale for biomass generation is contingent on several parameters. The most important are nutrient composition, temperature, and light (Li et al., 2017a). These factors control the diatom's physiological and metabolic pathway, impacting its productivity (Dhali et al., 2022).

The nutrient availability ensures the synthesis of primary nutrients, which eventually contribute to cellular structure and activity. Carbohydrates, proteins, and lipids are examples of primary metabolites that participate in a wide range of physiological and metabolic processes that significantly alter species growth, development, and reproduction (Dhali et al., 2022). One of the important compounds for diatom growth that results from the polymerization of silicic acid, which is composed of a chain of $SiO_4$ tetrahedral units with the general formula $SiO_2$, called silica. In nature, silica compounds are found in several natural materials, such as sand, quartz, glass, and many other sources (Sulastri & Kristianingrum, 2010). Silica has an important role in the formation of diatom cell walls, hence changing its quantities has been shown to have a huge effect on the growth and physiology of diatoms cultivated under intense conditions (Singh et al., 2022). Cell walls are formed in diatoms as resistance to environmental stress. Diatoms without a cell wall have no defense structures from their external environment, so they cannot survive. Different types of microalgae require different amounts of silicas. Therefore, the more optimal the application of silicas to diatoms, the better it is for forming their cell walls, and these diatoms only require a small amount of silica (Jati, 2012).

*Navicula* sp. is a benthic diatom that has a propensity to build biofilms (Lawijaya et al., 2023). *Navicula* sp. is one of the diatoms considered the best microalgae in biodiesel production compared to other biodiesel raw materials because it does not compete with terrestrial plants (Christi, 2007). *Navicula* sp. can be used as a raw material for biodiesel because it can produce high-energy compounds, such as triglycerol and starch, in high quantities compared to other phytoplankton (Christi, 2007) with Fatty Acid Methyl Ester (FAME) production of 185 mg/L/week based on research by Matsumoto (2010). The advantages of using *Navicula* sp. include a fast growth rate, not requiring large areas of land, high environmental tolerance, and no cultural season restrictions (Christi, 2007). It has been claimed that several synthetic media, the most popular one is f/2-Si (Guillard's medium), may be used to cultivate diatoms in the laboratory; however, these media are costly and unsuitable for large-scale culture (Dhali et al., 2022). Therefore, this study aims to determine the impact of silica on the production of carbohydrates, lipids, and proteins and the growth of *Navicula* sp. so the cultivate can achieve a better yield with minimum cost. It is hoped that this experiment can become a huge step for the advancement of the world's biotechnology and industry.

## MATERIALS AND METHODS

This research was conducted from September to October 2022. The culture used was *Navicula* sp., which is available at the Biotechnology Laboratory of Gadjah Mada University and is the site for analysis of the composition of *Navicula* sp.

### Guillard f/2 Medium

Guillard's medium f/2 formulation (Guillard, 1975) was made according to the guidelines of da Coêlho (2013). Before making the medium, a stock was made in sterile distilled water with a dilution concentration of 100 times. Each stock solution (Table 1) (macronutrient stock, EDTA base solution, iron stock, and micronutrient stock) was sterilized by autoclaving at 122 °C for 20 minutes to prevent contamination. Vitamin stock is sterilized using a millipore filter. The medium used for diatom

culture was seawater enriched with Guillard medium f/2 + Si in different concentrations, namely 1; 1.5; and 2 mL/L. The stock medium was dissolved in sterile seawater, filtered through filter paper, and autoclaved at 121 °C at 1 atm pressure for 15 minutes. The final medium was prepared by adding the following stock components, macronutrients are $NaNO_3$ 1 mL, $NaH_2PO_4.2H_2O$ 1 mL; trace metals are $FeCl.6H_2O$ + $Na_2.EDTA.2H_2O$ 1 mL; micronutrients are $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.4H_2O$ 100 µL, $MnCl_2.2H_2O$ 100 µL and $SiO_2$ 30 ppm 0.25 mL, 0.5 mL, and 1 mL; Vitamins are 1 mL of cyanocobalamin/vitamin B12, 1 mL of thiamin HCl/vitamin B1, and 1 mL of Biotin in every liter of seawater.

**Table 1.** Guillard f/2 medium composition for diatom 1 L

| Solution | Reagent | Stock solution (g 1.000 $mL^{-1}$) | Culture medium (mL) |
|---|---|---|---|
| 1 | $NaNo_3$ | 75.0 | 1.0 |
| 3 | | | |
| 4 | | | |
| 2 | $NaH_2PO_4.H_2O$ | 5.0 | 1.0 |
| | $Na_2O(SiO_2)2$ | 12.0 | |
| 3 | $CuSO_4$ | 9.8 | 1.0 |
| | $ZnSO_4$ | 22.0 | |
| | $MgCl_2$ | 10.0 | |
| 4 | $Na_2MoO_4.2H_2O$ | 6.3 | |
| | $FeCl_2$ | 3.0 | 1.0 |
| | $Na_2EDTA.2H_2O$ | 4.6 | |
| | Thiamine hydrochloride | 2.0 | |
| 5 | Pyridoxine hydrochloride | 2.0 | 0.5 |
| | Cyanocobalamin | 0.1 | |
| 6 | Distilled water | | 1,000 |

## Experimental Design

This experiment was carried out with three concentrations of silica (1; 1.5; and 2 mL/L) and one control (0 mL/L) grown each in 500 mL culture of *Navicula* sp in 21 days. Repetition was done thrice for each measurement parameter: growth speed, biomass production, lipids, carbohydrates, and protein. The experimental design used was a Completely Randomized Block Design, so determining number of repetitions by using specific formula was unnecessary.

## Culture Stock

The prepared medium was then divided into 500 mL bottles containing 350 mL medium and 100 mL *Navicula* sp., which was previously cultured for 1 week. Cultivation using medium f/2 + silica in liquid form was taken 1; 1.5, and 2 mL/L liquid silica solution into different mediums. The medium was prepared with silica as a test and without silica as a control medium. Each treatment was given three repetitions with a total of 12 culture bottles, as shown in Table 2. Cultivation was carried out for 21 days in a controlled environment. Constant aeration was given through a diaphragm pump with an airflow of 3 L per minute. The room temperature and the light provided were maintained at 25 ± 1 °C and 200 µ/s.$cm^2$, respectively, using a fluorescent lamp and were given continuously. This experiment was carried out for 21 days.

**Table 2.** Treatment of variations in silica concentration in *Navicula* sp medium

| Silica concentration | Repetition | | |
|---|---|---|---|
| | First | Second | Third |
| Control treatment (PK) | $PK_1$ | $PK_2$ | $PK_3$ |
| 1 mL/L (P1) | $P1_1$ | $P1_2$ | $P1_3$ |
| 1,5 mL/L (P2) | $P2_1$ | $P2_2$ | $P2_3$ |
| 2 mL/L (P3) | $P3_1$ | $P3_2$ | $P3_3$ |

**Parameters**

**1. Culture Density**

Density measurements were carried out from day 0 to day 21. The sample was taken from 12 culture bottles with the amount of 2 mL each into a cuvette and was done every 24 hours. The cuvettes were run through a spectrophotometer (Thermo Scientific GENESYS™ 10UV Scanning) with a wavelength of 680 nm.

**2. Biomass**

Samples were collected on days 0, 2, 4, 6, 8, and 12 with as much as 2 mL in a 2 mL microtube that had been dried and weighed before. The sample was centrifuged at 3,300 rpm for 10 minutes, and the supernatant was discarded. The sample's pellet was then dried in an incubator at 40 °C with the tube cap opened until the liquid was removed entirely and the weight stabilized. The dry weight that has been obtained is subtracted from the previous microtube weight to get the actual dry weight of the microalgae.

**3. Neutral Lipid Content**

Lipid level analysis was carried out using the Bligh and Dyers (1959) method. First, 5 mL of the sample was centrifuged for 10 minutes at 4,000 rpm. The supernatant was then removed, and 1 mL of chloroform and 2 mL of methanol were added to the sample in order. Next, the mixture was homogenized by vortexing for 30 seconds at room temperature. The mixture was then added with 1 mL of chloroform and 1 mL of distilled water, and then centrifuged for 10 minutes at 4,000 rpm to form two layers. The yellow layer was formed and transferred to a petri dish using a pipette, which was previously weighed on an analytical balance. The petri dish was placed in the incubator for 24 hours, and the sample mass was measured.

**4. Carbohydrate Content**

The Phenol-sulfuric acid method was utilized for carbohydrate level analysis. 5 mL samples were taken on days 0, 7, 14, and 21, then put in a 15 mL conical tube and centrifuged at 4,000 rpm for approximately 15 minutes. The supernatant was removed from the pellet. The pellet was added with 0.5 mL 5% phenol and 1 mL $H_2SO_4$ and then incubated for 30 minutes. Samples were taken as much as 2 mL and then transferred into the cuvette. The cuvette was inserted into the spectrophotometer (Thermo Scientific GENESYS™ 10UV Scanning), and the absorbance and concentration were calculated at a wavelength of 490 nm following the guidelines from Nielsen (2010). Preparation of a blank solution, namely 0.5 mL of distilled water + 0.5 mL of 5% phenol + 1 mL of $H_2SO_4$. Standard glucose solutions were prepared at concentrations of 500; 1,000; 1,500; 2,000; 2,500; 3,500; 4,000; 6,000; and 8,000 µg/mL.

**5. Protein Content**

The method used to measure protein levels is Bovine Serum Albumin. In the initial stage, 2 mL of the sample was centrifuged at 4,000 rpm for 10 minutes. Then the pellet is separated from the supernatant. Then, 1 mL of 10% SDS solution was added to the pellet. The mixture was heated at 95 °C using a water bath for 5 minutes, then incubated at 4 °C for 5 minutes. Next, 800 µL of the sample was taken and put into the microwell plate. Afterward, the sample was added with 200 µL of Bradford's solution and homogenized with an orbital shaker for 5 minutes. The microwell plate is then run using Elisa Reader with a wavelength of 595 nm. The blank used was 200 µL of Bradford's solution plus 800 µL of distilled water.

**6. Data Analyses**

Calculating culture density, biomass, lipid, carbohydrate, and protein content with a standard deviation was performed on Microsoft Excel software and made into a graph. Then, an Analysis of Variance (ANOVA) and Duncan's multiple range test (DMRT) were performed using SPSS. The formulas used in this experiment.

Metabolite productivity is a major part of this experiment, which was formulated using $P_0 = \frac{Amax}{A0}$, where $P_0$ is metabolite productivity (mg), $A_{max}$ and $A_0$ as the maximum absorbance density and first-day absorbance density of the specific metabolites, respectively. Next formula is doubling time which is formulated using Doubling Time (Dt), $Dt = \frac{\ln(2)}{P0}$ (Korzyńska & Zychowicz, 2008). Another formula used in this experiment is specific growth rate is defined as an increase in cell density/unit time (Bhatia, 2015), and formulated as $SGR = \frac{\ln(\frac{Wt}{W0})}{t}$ (Kang et al., 2011). Where SGR is the Specific Growth Rate (μ), Wt is the absorbance on the last day, and $W_0$ first day of the growth period (*t*). The growth curve of each sample is made by using the triplicate mean value. The next formula is the carbohydrate quantification formula, is obtained by processing the carbohydrate calibration curve against the spectrometry results curve which is based on Hewitt's (1958) work. The following formula is obtained $X = \frac{\ln(\frac{Y}{0.58})}{0{,}0003}$, where, X is the carbohydrate quantification (µg/mL) and Y is carbohydrate absorbance.

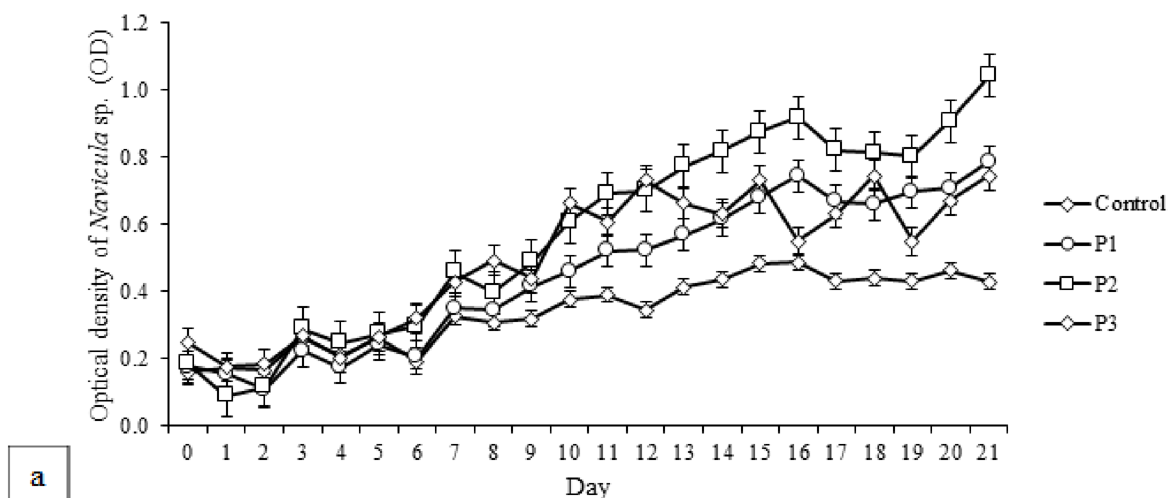
# RESULTS

## Growth Rate

The culture growth of the examined microalgal species differed in each treatment and was increasing until the final test day (Figure 1a). The addition of silica at a concentration of 1.5 mL/L appears to have a more efficient growth rate than growth at other concentrations (Figure 1a). The control treatment has a minor increase in optical density. The impact of this silica application on the specific growth rate of each silica concentration during the 21-day experiment is presented in Figure 1b. The specific growth rate of *Navicula* sp. in treatment P2 or 1.5 mL/L was generally higher (μ= 0.083 $day^{-1}$) than other treatments (control: μ= 0.047 $day^{-1}$, P1 or 1 mL/L: μ= 0.072 $day^{-1}$, P3 or 2 mL/L: μ= 0.053 $day^{-1}$). The use of silica above 1.5 mL/L seems to cause a decrease in the specific growth rate of *Navicula* sp.

## Biomass

The growth of *Navicula* sp. biomass showed different variations in each treatment of silica concentration (Figure 2a). The control treatment seemed to fluctuate from day 0 to the last day. With treatment P1 or 1 mL/L, biomass increased until the 10th day (3.6 mg) and decreased afterward. In general, the P2 treatment, or 1.5 mL/L, produced higher biomass compared to the other treatments, with a peak on the $8^{th}$ day (7.9 mg). Treatment P3 or 2 mL/L increased for 10 days, (6.8 mg, not higher than treatment P2) and decreased the next day. We observed biomass productivity (Figure 2b) in treatment P2 is the highest (0.54 mg/day) among other treatments (Control: 0.27 mg/day, P1: 0.18 mg/day, P3: 0.49 mg/day). However, the highest doubling time (Figure 2c) is in P1, 3.8097 cells/day, almost double the amount of the treatment P2 and P3 (1.2929 cells/day and 1.4259 cells/day, respectively).



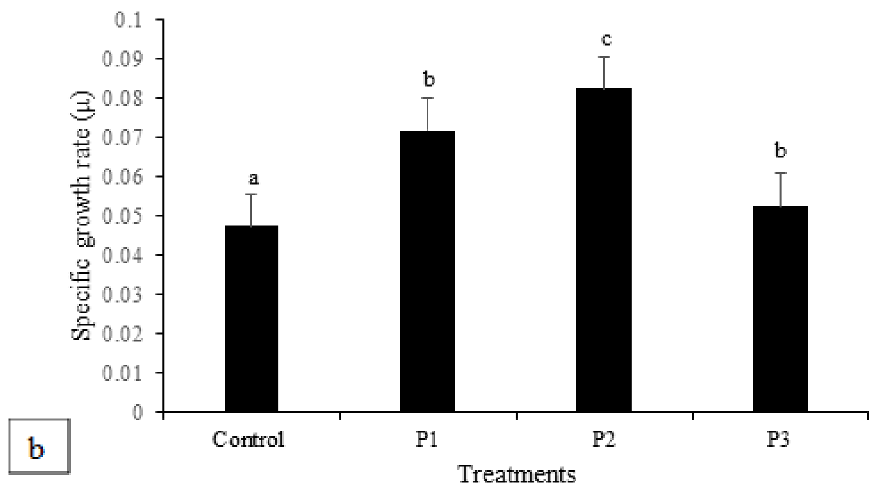
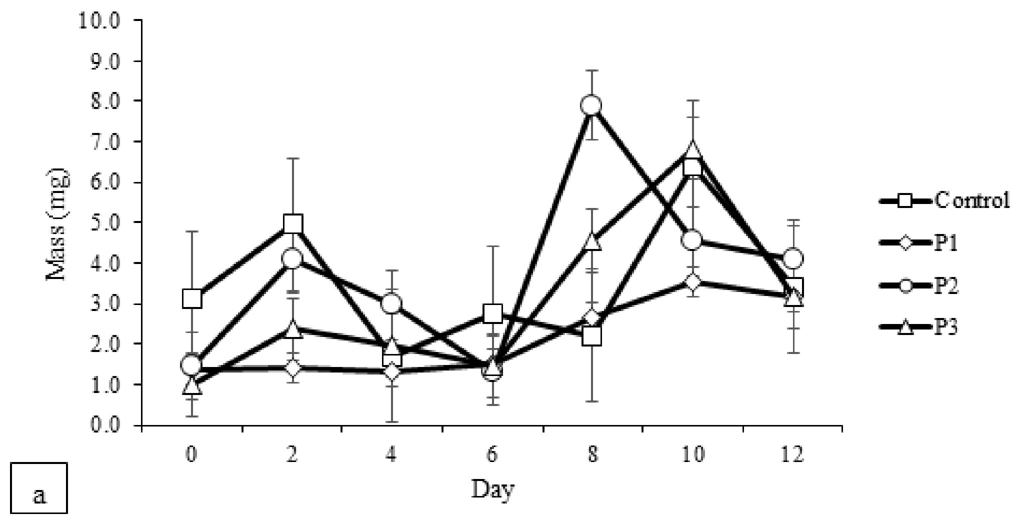
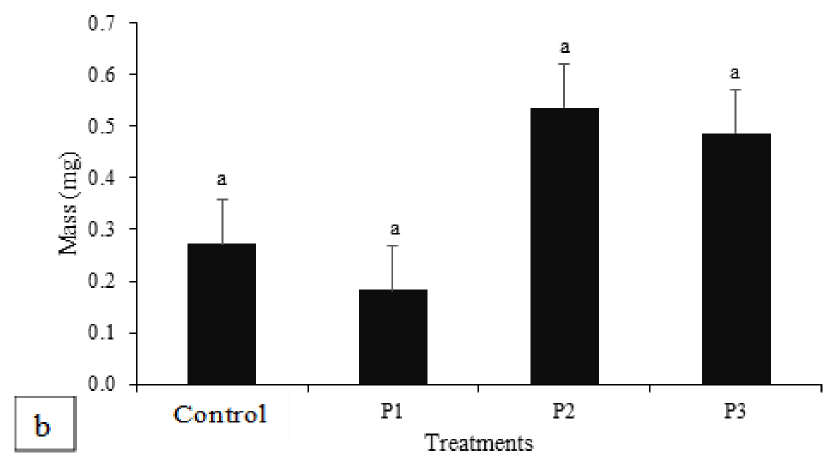



Figure 1. Optical density (OD) (a) and specific growth rate (b) of *Navicula* sp. in one culture period. Different letters in Figure 1b indicated significant differences between treatments and were calculated through one-way ANOVA followed by Duncan Multiple Range Test (DMRT) ($P < 0.05$)



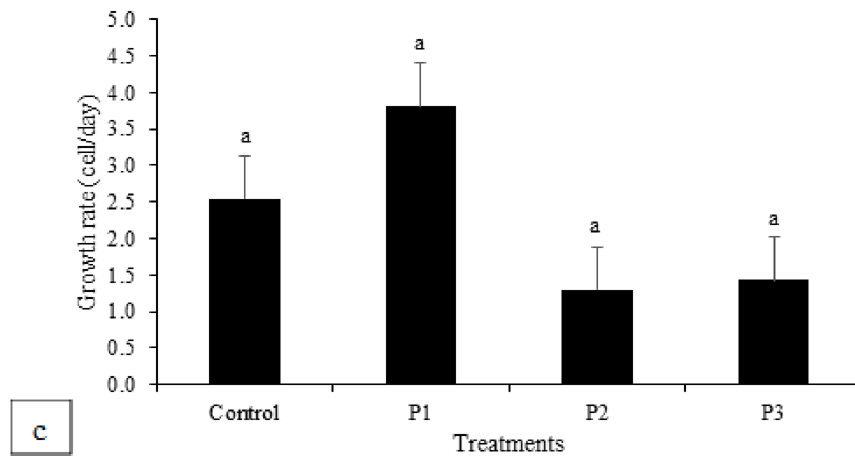



**Figure 2.** *Navicula* sp. biomass in 12 days (a), biomass productivity (b), and doubling time (c). The same letter (a) indicated insignificant differences among groups and was calculated through one-way ANOVA followed by Duncan Multiple Range Test (DMRT) (P >0.05)

**Lipid Content**

The lipid content produced by Navicula sp. fluctuated for 20 days in each treatment (Figure 3). The highest lipid content (Figure 3a) was found in treatment P1 on day 12, and the lowest was in the same treatment on day 0. Lipid productivity (Figure 3b) differs between control, P1, P2, and P3 treatments. The highest productivity was found in treatment P1, which is 0.517 mg/day. However, the lipid productivity values of the control, P2, and P3 treatments differed slightly from P1 (0.385; 0.435; and 0.472 mg/day, respectively).

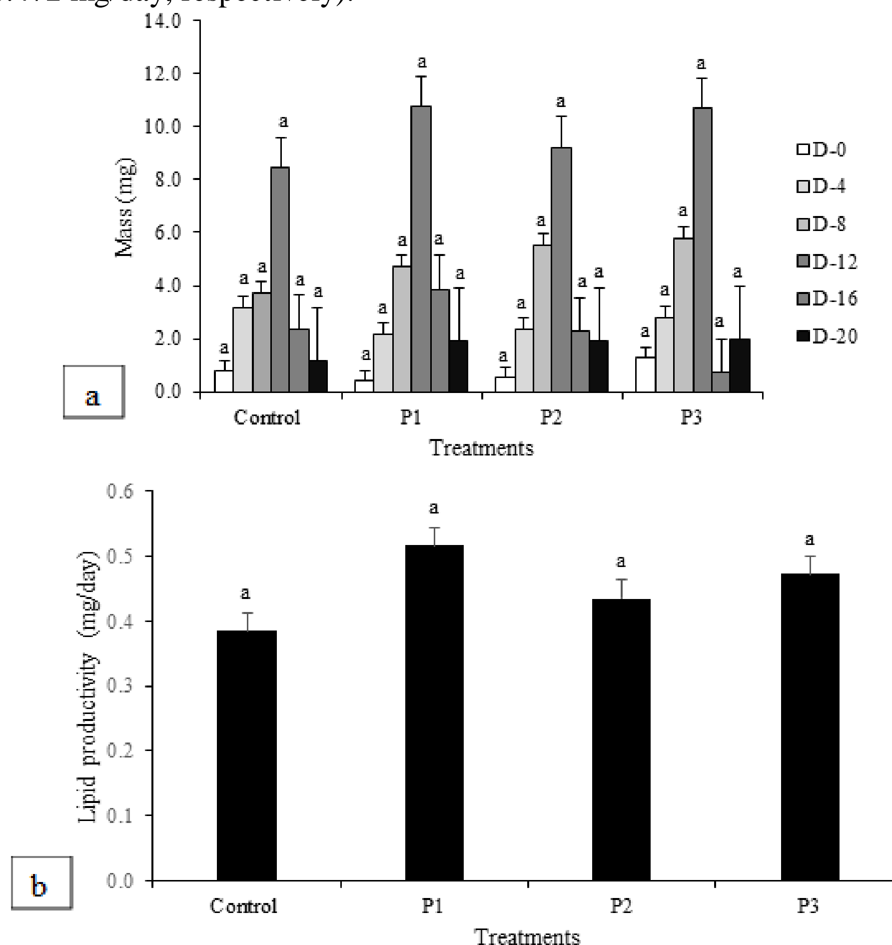



**Figure 3.** Lipid content (a) and lipid productivity (b) of *Navicula* sp. during a culture period of 20 days. The same letter (a) indicated insignificant differences among groups and was calculated through one-way ANOVA followed by Duncan Multiple Range Test (DMRT) (P >0.05)

## Carbohydrate Content

The highest carbohydrate content (Figure 4a) was found in the P2 treatment, reaching 5.4 mg/mL on day 14, while the lowest result was in the control and P3 treatment, with the same value of 1.2 mg/mL on day 0 and day 7 respectively. In Figure 4b, the highest carbohydrate productivity that we observed was found in the P2 treatment, which was 0.075 mg/day, and the lowest was in the control treatment, which was 0.010 mg/day.

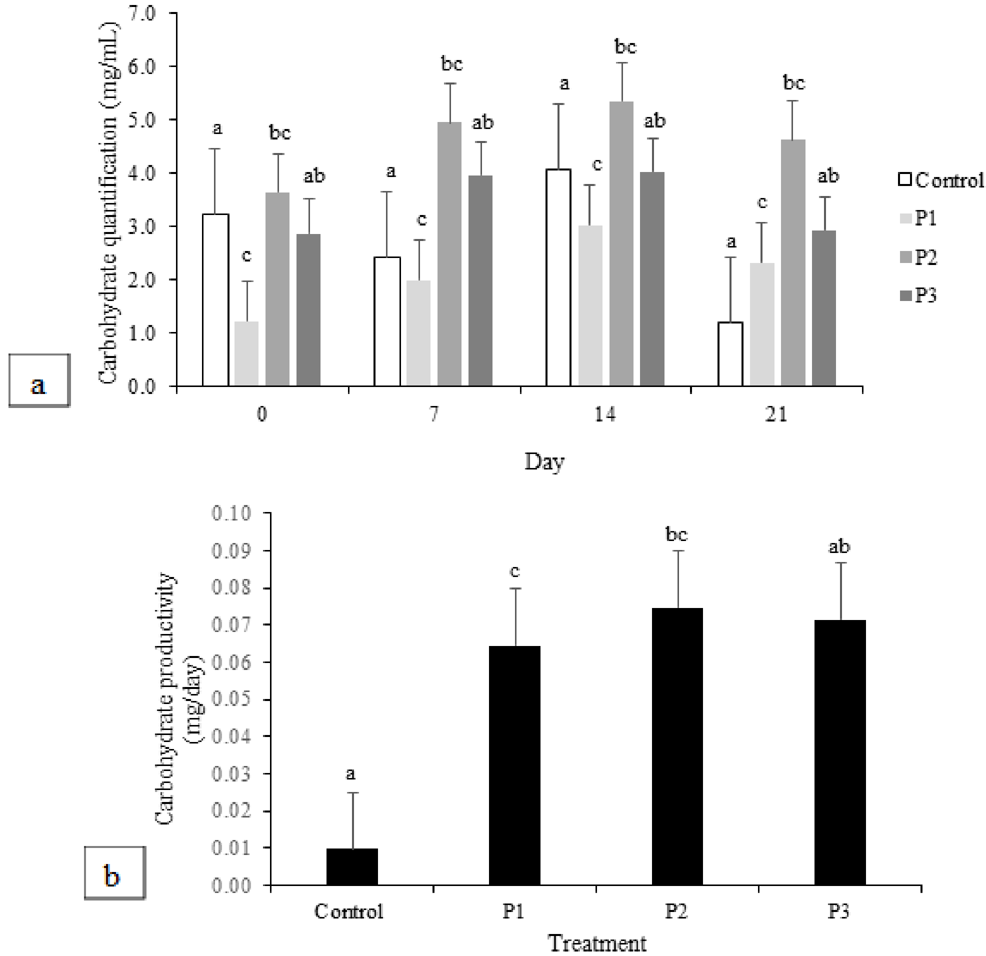



**Figure 4.** Carbohydrate content of *Navicula* sp. (a) and carbohydrate productivity (b). Different letters indicated significant differences between treatments and were calculated through one-way ANOVA followed by Duncan Multiple Range Test (DMRT) (P <0.05)

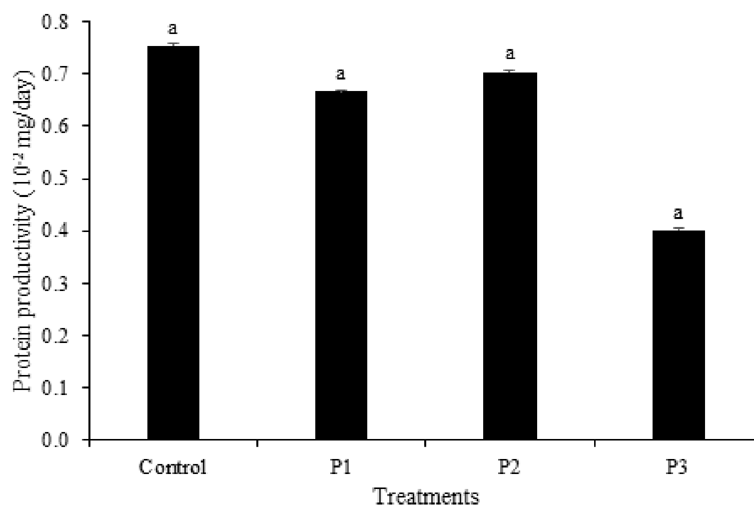



**Figure 5.** Protein productivity of *Navicula* sp. during the culture period. Letter (a) indicated insignificant differences among groups and were calculated through one-way ANOVA followed by Duncan Multiple Range Test (DMRT) (P >0.05)

**Protein Content**

Protein productivity between the control and the test treatment did not show a significant difference from each other (Figure 5). The highest protein productivity that we observed is on the control treatment, which is $0.8 \times 10^{-2}$ mg/day. However, the protein productivity value between P1, P2, and P3 treatments ($0.7 \times 10^{-2}$; $0.7 x 10^{-2}$; and $0.4 x 10^{-2}$ mg/day, respectively).

## DISCUSSION

**Growth Rate**

Measurements using optical density (OD) show how much the density of *Navicula* sp. is present in a sample. This data can also be used to see the growth rate of *Navicula* sp. in one culture period. This curve is designed to determine the growth phase of cells, which is critical for increasing culture productivity (Suyono et al., 2016). Collection and measurement of OD samples of *Navicula* sp. performed under aseptic conditions (Figure 1a). The normality of the resulting data is above 0.05 (P >0.05) in all treatments, which means that the data is normal. However, the treatment with silica had a significantly different effect on the growth of *Navicula* sp because it had a significance value below 0.05 (P <0.05). *Navicula* sp. density was the highest in the 1.5 mL/L silica treatment on day 21, 1.041 L $mol^{-1}$ $cm^{-1}$, and the lowest density of *Navicula* sp, in general, was in the control treatment. Based on the graph in Figure 1a, the P2 treatment had a higher *Navicula* sp. density than the other treatments, as evidenced by post hoc analysis using the Duncan Multiple Range Test with a higher value than the other treatments (P <0.05). Figure 1b shows that the P2 treatment has a higher Specific Growth Rate (SGR), which is 0.0825 $day^{-1}$. Various factors, such as nutrients, temperature, and pH, seriously impact phytoplankton growth (Li et al., 2017b). The level of acidity regulates the solubility and accessibility of ionized minerals, which influences how quickly nutrients are absorbed (Nurafifah et al., 2023). de Viçose et al. (2012) said that limited cell size and cell division are microalgae strategies for overcoming limited resource conditions, one of which is silica used to form cell walls.

Previous studies showed that silica stress has an indirect correlation with growth rate because it may be focused on increasing its survivability by enhancing adaptation responses, hence lowering the growth rate in the late development phase (Singh et al., 2022; Dhali et al., 2022). However, in this experiment, the growth rate of the control, P1, P2, and P3 tests kept increasing until the last day of the growth period. This result might be due to the overall measurement of cell density using a spectrophotometer, without being able to distinguish between living and dead cells, so that residual dead cells are still counted. The highest SGR obtained in this experiment is much lower than in the previous experiments. For example, Yang's (2014) experiment used higher concentrations than the amount of silica concentration used in this experiment, which obtained a higher SGR value using the same genus. The highest SGR results in this experiment (1.5 mL/L: $\mu$= 0.0825 $day^{-1}$) were lower than the average SGR control treatment results found in Yang's (2014) experiment (0 mL/L: $\mu$= 0.0942 $day^{-1}$). This result might be caused by the silica concentration used in this experiment being lower than the nutrient required for *Navicula* sp. to grow optimally. Furthermore, the algal species strain used in the experiment might significantly impact the results obtained on growth rate results or other metabolites, even though they are from the same genus because different species respond differently (Li et al., 2017b).

**Biomass**

The impact of providing silica in different concentrations on the biomass of *Navicula* sp. is shown in Figure 2. The ANOVA test resulting from the data obtained was not significantly different between the control and test treatments (P >0.05). In general, the biomass content in the control treatment was lower than in the treatment with silica administration. Biomass productivity (Figure 2b) shows that the P2 treatment has a higher value than the other treatments, 0.536 mg/day. In addition, *Navicula* sp., the P2 treatment, also had a shorter doubling time (Figure 2c) than the other treatments (1.293 cells/day).

As the experiment approached the last day, it was seen that the growth and productivity of the biomass from *Navicula* sp. decreased (Figure 2a). This can be caused by the silica content in the medium has begun to run out, resulting in silica starvation. The limiting of silica likely hindered

further cell division, resulting in a poor growth rate during the late development phase and a low biomass production overall. In addition, this silica deficiency lowered the efficiency with which other nutrients were absorbed after day seven when the cell density was inadequate (Dhali et al., 2022).

Biomass should be directly proportional to the growth rate because both contain the density of *Navicula* sp. in a sample. Nutrients are a crucial factor for algal growth and development, therefore impacting biomass productivity (Li et al., 2020). Previous literature of *Navicula* sp. biomass yield varies between 21.1 and 139 mg/L (Malibari et al., 2018; Marella et al., 2018; Gonz'alez-Vega et al., 2021; Hogan et al., 2021; Saxena et al., 2022). However, the biomass obtained from this research increases and decreases each day, which is not normal, as shown in Figure 2a. and is much lower compared to the reported literature. This data ambiguity can be caused by inaccuracies when weighing the biomass samples. For example, the sample may not be so dry that it may still contain liquid from the medium which could affect the mass. In addition, factors in inconsistent sampling also cause errors in measuring biomass content.

**Lipid Content**

Lipid concentration in *Navicula* sp. to the administration of silica in different concentrations can be seen in Figure 3. The ANOVA test resulting from the data obtained showed no significant difference ($P > 0.05$). The obtained result (Figure 3) is different from Encinas-Arzale et al. (2020) in their research, which said that the lipid production of *Navicula* sp. is in contrast to the silica concentration in the medium. Algae synthesize lipids as potent molecules for storage in response to harsh environmental conditions (Rangkuti et al., 2023). Therefore, limiting the concentration of silica causes an increase in the lipid content in the sample. Under favorable conditions, oily microalgae, such as diatoms, produce small amounts of neutral lipids, especially TAGs, and then accumulate large amounts of lipid droplets when given stressful conditions, such as limited resources (Sabu, 2019). In other words, the limitation of silica in the *Navicula* sp. growth medium can cause an increase in lipid production (Dhali et al., 2022). Furthermore, in several studies, the limitation of specific resources, such as nitrogen and silica, led to an increase in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Encinas-Arzale et al., 2020). In other words, *Navicula* sp. lipid metabolism reacts antagonistically to silica addition in the medium.

**Carbohydrate Content**

Carbohydrates obtained during one culture period are presented in Figure 4. The resulting data is normal and homogeneous ($P > 0.05$). The ANOVA test showed that the control treatment and the treatment with silica in various concentrations showed a significant difference ($P < 0.05$). This result means that adding silica affects the carbohydrate production in the *Navicula* sp. microalgae. Post hoc analysis using the Duncan Multiple Range Test showed that the P2 treatment had a more significant value than the other treatments ($P < 0.05$), which was also characterized by higher productivity of the P2 treatment (Figure 4b). Carbohydrate quantification showed an increase in carbohydrate production on day 14 with a peak in treatment P2 of 5353.70 μg/m. The availability of nutrients is essential in the production of crucial nutrients in cellular structure and cell activity. Primary metabolites, such as carbohydrates, are involved in various physiological and metabolic pathways that regulate certain species' growth, development, and reproduction. In the case of diatoms, nitrogen, phosphorus, and silicas are important nutrients that regulate their cellular composition (Dhali et al., 2022).

However, the results obtained in this test and previous research are different. The productivity of carbohydrates (Figure 4b) is directly proportional to the production of lipids, which depends on the silica concentration stress. The less silica, the higher the production of carbohydrates in *Navicula* sp. cells. Singh et al. (2022) study showed that carbohydrates produced reach a maximum when diatoms experience silica limitations because this species increases the activity of proteins related to lipids and carbohydrates to survive unfavorable conditions.

**Protein Content**

The protein obtained during one culture period is presented in Figure 5. The resulting data is normal and homogeneous ($P > 0.05$). The ANOVA test showed no significant difference between the

control treatment and the administration of silica in various concentrations (P >0.05). The highest protein content was found in the control medium, which doesn't have any silica additions. This result indicates that this species might have increased its protein level under silica stress to survive. The results obtained were by the research of Singh et al. (2022), which said that the protein metabolism of each diatom was influenced by the presence of silica in the medium. In Singh's experiment, he used different species of diatoms, *Thalassiosira* sp., *Skeletonema* sp., and *Chaetoceros* sp., and they showed a different number of protein contents in each species. Silica addition might have caused a decrease in the protein level contained in Navicula sp. because the cells are focused on the production of carbohydrates and cellular division.

## CONCLUSION

This research focused on the production of lipids, carbohydrates, and proteins and the diatom species *Navicula* sp. growth for various silica concentrations. The results indicated that the growth and biomass of *Navicula* sp. were affected by the optimal administration of silica at a concentration of 1.5 mL/L which increased the growth rate and some metabolites. In contrast, silica did affect growth rate also the production of metabolites, such as lipids, carbohydrates, and proteins. Further studies are needed regarding the optimal concentration of silica to increase the production of lipids, carbohydrates, and proteins without affecting the growth of *Navicula* sp.

## ACKNOWLEDGMENTS

The author's thanks are addressed to the 2022 Kedaireka Project as well as the Merdeka Belajar - Kampus Merdeka Mikroalga UGM, who facilitated and funded this research. Khalid Erlangga wants to thank, in particular, the uncountable support received from Dinda Ayuningtyas since the author entered the college. I hope we stay together.

## REFERENCES

Bhatia, S., Sharma, K., Dahiya, R., & Bera, T. (2015). Plant tissue culture. *Modern applications of plant biotechnology in pharmaceutical sciences*, 31-107.

Bligh, E. G., & Dyer W. J. (1959). A lipid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911-917.

Christi, Y., (2007). Biodiesel from microalgae. *Biotechnology advances*, *25*(3), 294-306.

Coêlho, A. A Da-C., Barros, M. U. G., Bezerra, J. H. C., da Silva, J. W. A., Moreira, R. L., & Farias, W. R. L. (2013). Growth of the microalgae *Tetraselmis tetrathele* and nitrate depletion in culture medium Guillard f/2 and Conway. *Acta Scientiarum. Biological Sciences*, *35*(2), 163-168.

de Viçose, G. C., Porta, A., Viera, M. P., Fernández, P. H., & Izquierdo, M.S. (2012). Effects of density on growth rates of four benthic diatoms and variations in biochemical composition associated with the growth phase. *Journal of Applied Phycology, 24*, 1427-1437.

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.

Encinas-Arzate, J. J., Marquez-Ríos, E., López-Elías, J. A., Torres-Areola, W., Huerta-Ocampo, J. Á., & Ramírez-Suárez, J. C., (2020). Effect of the deficiency of nitrate and silica on the growth and composition of the benthic diatom *Navicula* incerta. *Latin American journal of aquatic research*, *48*(2), 280-286.

González-Vega, R. I., Cárdenas-López, J. L., López-Elías, J. A., Ruiz-Cruz, S., Reyes-Díaz, A., Perez-Perez, L. M., ... 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.

Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates. In W. L. Smith, & M. H. Chanley (Eds.), *Culture of marine invertebrate animal* (pp. 29-60). New York, USA: Plenum Publishing.

Hewitt, B. R. (1958). Spectrophotometric determination of total carbohydrate. *Nature*, *182*(4630), 246-247.

Hogan, P., Otero, P., Murray, P., & Saha, S. K. (2021). Effect of biomass pre-treatment on supercritical $CO_2$ extraction of lipids from marine diatom *Amphora* sp. and its biomass evaluation as bioethanol feedstock. *Heliyon*, *7*(1), e05995.

Jati, F., Hutabarat, J., & Herawati, V. E., (2012). Pengaruh penggunaan dua jenis media kultur teknis yang berbeda terhadap pola pertumbuhan, kandungan protein dan asam lemak omega 3 EPA (*Chaetoceros gracilis*). *Journal of Aquaculture Management and Technology*, *1*(1), 221-235.

Kang, N. S., Jeong, H. J., Yoo, Y. D., Yoon, E. Y., Lee, K. H., Lee, K., & Kim, G. (2011). Mixotrophy in the newly described phototrophic dinoflagellate *Woloszynskia cincta* from Western Korean waters: Feeding mechanism, prey species and effect of prey concentration. *Journal of Eukaryotic Microbiology*, *58*(2), 152-170.

Korzyńska, A., & Zychowicz, M., (2008). A method of estimation of the cell doubling time-based on the cell culture monitoring data. *Biocybernetics and Biomedical Engineering*, *28*(4), 75-82.

Kröger, N., & Wetherbee, R. (2000). Pleuralins are involved in theca differentiation in the diatom *Cylindrotheca fusiformis. Protist, 151*(3), 263-273.

Lawijaya, E., Siswanti, D. U., & Suyono, E.A. (2023). Optimization of bioflocculation using *Anabaena* sp. and *Navicula* sp. for harvesting of Glagah microalgae consortium. *Tropical Agricultural Science, 46*(4), 1-11.

Li, X. L., Marella, T. K., Tao, L., Li, R., Tiwari, A., & Li, G. (2017a). Optimization of growth conditions and fatty acid analysis for three freshwater diatom isolates. *Phycological Research*, *65*(3), 177-187.

Li, X. L., Marella, T. K., Tao, L., Peng, L., Song, C. F., Dai, L. L., … Li, G. (2017b). A novel growth method for diatom algae in aquaculture wastewater for natural food development and nutrient removal. *Water Science and Technology*, *75*(12), 2777-2783.

Li, S., Ji, L., Chen, C., Zhao, S., Sun, M., Gao, Z., … Fan, J. (2020). Efficient accumulation of high-value bioactive substances by carbon to nitrogen ratio regulation in marine microalgae *Porphyridium purpureum*. *Bioresource Technology*, *123362*, 1-9.

Malibari, R., Sayegh, F., Elazzazy, A. M., Baeshen, M. N., Dourou, M., & Aggelis, G. (2018). Reuse of shrimp farm wastewater as a growth medium for marine microalgae isolated from Red Sea–Jeddah. *Journal of Cleaner Production*, *198*, 160-169.

Marella, T. K., Parine, N. R., & Tiwari, A. (2018). The potential of the diatom consortium developed by nutrient enrichment for biodiesel production and simultaneous nutrient removal from wastewater. *Saudi Journal of Biological Sciences*, *25*(4), 704-709.

Matsumoto, M., Sugiyama, H., Maeda, Y., Sato, R., Tanaka, T., & Matsunaga, T. (2010). Marine diatom, *Navicula* sp. strain JPCC DA0580, and marine green algae, *Chlorella* sp. strain NKG400014 as potential sources for biodiesel production. *Applied Biochemistry and Biotechnology*, *161*(1), 483-490.

Nielsen, S. S. (2010). Phenol-sulfuric acid method for total carbohydrates. In *Food analysis laboratory manual* (pp. 47-53). Boston, MA: Springer.

Nurafifah, I., Hardianto, M. A., Erfianti, T., Amelia, R., Maghfiroh, K. Q., Kurnianto, D., … Suyono, E. A. (2023). The effect of acidic pH on gr ect of acidic pH on growth kinetics, biomass pr growth kinetics, biomass productivity, and primary metabolite contents of *Euglena* sp. *Makara Journal of Science*, *27*(2), 97-105.

Rangkuti, P. M., Siswanti, D. U., & Suyono, E. A. (2023). Salinity treatment as bacterial control and its impact on growth and nutritional value of *Spirulina* (*Arthrospira platensis*) culture in open pond system. *Journal of Fisheries and Environment*, *47*(1), 63-74.

Sabu, S., Singh, I. S. B., & Joseph, V. (2019). Improved lipid production in oleaginous brackish diatom *Navicula phyllepta* MACC8 using two-stage cultivation approach. *3 Biotech*, *9*(12), 1-15.

Saxena, A., Singh, P. K., Bhatnagar, A., & Tiwari, A. (2022). Growth of marine diatoms on aquaculture wastewater supplemented with nano silica. *Bioresource Technology, 344*, 126210.

Singh, P. K., Bhattacharjya, R., Marella, T. K., Saxena, A., Mishra, B., Savio, S., … Tiwari, A. (2022). Production of lipids and proteins from marine diatoms under changing pH and

silica. *Bioresource Technology*, *362*, 127766.

Sulastri, S., & Kristianingrum, S., (2010). Berbagai macam senyawa silika: Sintesis, karakterisasi dan pemanfaatan. *Prosiding Seminar Nasional Penelitian, Pendidikan dan Penerapan MIPA, Fakultas MIPA, Universitas Negeri Yogyakarta, 15*, 1755-1315.

Suyono, E. A., Nopitasari, S., Zusron, M., Khoirunnisa, P., Islami, D. A., & Prabeswara, C. B. (2016). Effect of silica on the carbohydrate content of mixed culture *Phaedactylum* sp. and *Chlorella* sp. *Biosciences Biotechnology Research Asia*, *13*(1), 109-114.

Yang, M., Zhao, W., & Xie, X. (2014). Effects of nitrogen, phosphorus, iron, and silicon on growth of five species of marine benthic diatoms. *Acta Ecologica Sinica*, *34*(6), 311-319.