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The Influence of Differences in Silicate Concentration on the Growth of Microalgae *Thalassiosira* sp. at the Laboratory Scale

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Abstract

Thalassiosira sp. is a planktonic microalga from the class Bacillariophyceae (diatom) used as natural feed in the cultivation of fresh and marine organisms. Some diatom species, including *Thalassiosira* sp., require silicate for the formation of their cell walls. This research aims to investigate the influence of different silicate concentrations on the growth of *Thalassiosira* sp. and determine the optimal silicate concentration. The method used is a laboratory experiment, A complete randomized design was conducted with six different silicate concentration treatments, specifically: 0ppm, 5ppm, 10ppm, 15ppm, 20ppm, and 25ppm, replicated three times. The research results show that different silicate concentrations significantly affect ($p<0.05$) the growth (cell density) of *Thalassiosira* sp. The optimal cell density of *Thalassiosira* sp. is observed at silicate concentrations of 15 ppm and 20 ppm, with peak densities of $70x10^5$cells/mL and 75.7 $x10^5$ cells/mL on the 6th day, respectively. The lowest cell density is recorded at a silicate concentration of 0ppm, reaching a peak density of $42x10^5$ cells/mL on the 6th day. Based on these results, it can be concluded that different silicate concentrations can be applied to enhance microalgal density. Throughout the study, water quality measurements were conducted, revealing temperature ranging between 22-25°C, salinity between 35-37ppt, and pH within the range of 8-8.5. The water quality used in the study remains optimal for the growth of *Thalassiosira* sp. Based on these results, it can be concluded that different silicate concentrations can be applied to enhance the density of the *Thalassiosira* sp. microalgae. Silicate concentrations of 15ppm and 20ppm can have the greatest impact on the density of *Thalassiosira* sp. Silicate is essential for *Thalassiosira* sp., in certain dose.

1. Introduction

Indonesia is known as a mega-biodiverse country due to its vast biodiversity, including diatomae. According to Sanjaya and Danakusuma (2018), approximately 20 to 25% of the earth's primary biomass productivity comes from diatom activities, highlighting the significant role of diatoms in ecosystems. Microalgae, tiny algae found in both freshwater and marine environments, include single-celled marine species capable of independent photosynthesis to meet their energy needs (Purbani *et al*., 2019).

Thalassiosira sp. is a planktonic microalga belonging to the Bacillariophyceae class (diatom) commonly used as natural feed for shrimp larvae. This microalga is easily cultivated as natural feed due to its single-celled structure and lack of chain formation, making it easily digestible (Devianti *et al*., 2022). Another advantage is its ease of cultivation. *Thalassiosira* sp. has a relatively large size, ranging from 4-32 µm, compared to *Chaetoceros* sp. with a size of 3–5 µm, making it easy to capture in later larval stages.

Shrimp feed containing *Thalassiosira* sp. has proven to increase survival rates, as its nutritious content is easily digestible for shrimp larvae (Fadila *et al.*, 2021). The high nutrient content in *Thalassiosira* sp., including carbohydrates, lipids, and proteins, is a key factor in its selection as natural feed (Iglesias *et al*., 2022). *Thalassiosira* sp. contains bioactive compounds such as exopolysaccharides, carotenoids, fatty acids, and amino acids, and its cell wall is composed of silica. Therefore, in its growth process, it requires macronutrients such as silica, nitrogen, and phosphorus (Anggraeni *et al.*, 2019). Different diatom species exhibit varying silica requirements to support their growth. Silica is a crucial element for diatoms, especially in the formation of their cell walls. For instance,

Skeletonema costantum requires 15 ppm of silicate (Fitriani *et al.*, 2017), while *Thalassiosira* sp. needs a range between 15 and 20 ppm for its growth (Vella *et al.*, 2019). Variability in silica requirements contributes to variations in dissolved silica content in the water, influencing diatom succession. Silicate fertilizers are commonly used by *Thalassiosira* sp. cultivators. However, the optimal dosage of silica (SiO_2) for the cultivation process remains unknown. Therefore, this study focuses on selecting the appropriate dosage of silicate fertilizer needed to achieve optimal *Thalassiosira* sp. development.

This research builds upon previous information, specifically the difference in silicate concentrations as a basis for identifying the effects on *Thalassiosira* sp. diatom growth treated with additional silicate in the media as a nutrient. The objective of this study is to determine the influence of different silicate concentrations on *Thalassiosira* sp. growth and identify the most optimal silicate concentration

2. Material and methods

2.1 Materials

This research utilized *Thalassiosira sp.* microalgae obtained from the Center for Brackish Water Aquaculture Fisheries (BBPBAP) in Jepara, KW21 fertilizer, and silicate.

2.2 Methods

The research method is the laboratory experimental method. The research design utilized a Completely Randomized Design with 6 treatments (C (without Silicate addition), Si5 (5 ppm), Si10 (10 ppm), Si15 (15 ppm), Si20 (20 ppm), Si 25 (25 ppm). All treatment were administered in 3 replications (Fitriani *et al.*, 2017).

Algae cultures were placed on two different shelves with each bottle spaced 5cm apart. The light source for this study consisted of two 35-watt lamps (TL) on each shelf, positioned 15 cm above the cultures. Each glass bottle was loosely covered with cotton between the aeration hose, with a steady flow of air. The distance between the aeration hose and the bottom of the glass bottle was approximately 1 cm in each medium.

2.2.1 Equipment Sterilization

The sterilization of equipment involves the preparation of containers and aeration equipment. Eighteen 250 mL Erlenmeyer flasks are used as containers. The flasks are washed, dried, and sprayed with 70% alcohol (Arguelles *et al*., 2021). All the equipment was cleansed with detergent and air-drying it. Aeration hoses and aeration stones, once dried, are immersed in a calcium hypochlorite solution for 10-15 minutes (Latif *et al.*, 2022). Afterward, the hoses and stones are rinsed with distilled water (aquades) and assembled onto the aerator.

2.2.2 Sterilization of Seawater and Freshwater as Culture Media

The seawater used in the study was obtained from a commercial supplier and had an initial salinity of approximately 40 ppt. The sterilization process for seawater and freshwater involved adding chlorine at a concentration of 60 ppm for 30 minutes to reduce contamination from other microorganisms that may be present. Subsequently, sodium thiosulfate with a concentration of 30 ppm was used to neutralize the chlorine in the water (Supriyantini, 2013).

2.2.3 Seed and Culture Media Preparation

Isansetyo and Kurniastuty (1995) stated that seed selection can be done through visual observation, where the seeds exhibit characteristics such as not settling at the bottom of the container and having a brown color. Microscopic observations were also conducted to ensure high cell density, intact cells, large cell size, freedom from contaminants such as bacteria or microorganisms, and achieving optimal density levels. Subsequently, based on the 1-liter culture stock, the seed was prepared according to the conditions to be used in the study. The steps in preparing culture media include pouring sterilized seawater into Erlenmeyer flasks, adding KW21 fertilizer and silicate fertilizer solution according to the measurements, ensuring the temperature, salinity, and pH of the culture media are suitable for diatom growth, and finally, introducing *Thalassiosira* sp. into the prepared culture container.

3. Results

3.1 Growth of Microalga *Thalassiosira* sp. at Different Silicate Concentrations

The growth of microalga *Thalassiosira* sp. cultured with varying silicate concentrations for 12 days can be seen in Table 1. The results obtained show a change in the color of the culture media. The color changing to a dark and intense brown indicates an increase in cell density in the microalga *Thalassiosira* sp. Figure 1.

Table 1. *Thalassiosira* sp. Density (x10^5 cells/mL ± SD) during the 12-day culture period with Different Silicate Concentrations

Day of Culture	Cell density (x10^5sel/ml)					
	0ppm (control)	5ppm	10ppm	15ppm	20ppm	25ppm
1	5±0.00	5±0.00	5±0.00	5±0.00	5±0.00	5±0.00
2	14.3±0.47	15.7±1.47	16.7±1.70	19.3±2.87	22.7±2.87	28.3±1.25
3	22±0.82	22.7±1.08	23±0.82	29±4.97	30.3±5.44	37.3±2.05
4	31±1.41	45±1.41	47.7±2.05	56.7±3.68	59.7±2.87	61.7±2.05
5	34.7±2.87	46.7±1.78	49±2.16	61.3±3.40	61.7±1.70	66.3±2.49
6	42±1.63	55±1.08	56.7±1.25	70±1.63	75.7±1.25*	35.3±5.91
7	20.7±2.45	41±1.87	42±1.41	46±1.63	51±1.63	39.3±3.30
8	10±0.47	34±1.41	37.3±1.70	40±1.63	44±2.45	20±2.83
9	7±1.63	15.3±1.63	18.5±0.82	20.3±1.25	30.3±1.25	12±4.32
10	4.3±2.05	10±0.71	15±0.82	18.7±1.25	27±1.63	2.7±1.70
11	1±1.41	5.7±1.08	8.7±0.94	9.3±1.25	22.3±2.49	0±0.00
12	0±0.00	1±0.18	2.7±1.64	4.7±1.70	17.3±2.49	0±0.00

Explanation: (*) indicates the peak highest density.

Figure 1. *Thalassiosira* sp., cultured in different silicate concentration

Based on the observation results, it can be noted that a specific growth pattern occurred in the culture of microalga *Thalassiosira* sp. over the 12-day period (Figure 2). This is indicated by the dynamic fluctuations of cells during the culture period. Based on its growth, the results show that the 20ppm silicate concentration has the highest cell density, reaching $75.7x10^5$ cells/mL on the 6th day of the culture period. The lowest peak cell density was obtained at the 0ppm (Control) silicate concentration, which is $42x10^5$ cells/mL on the 6th day of the culture period.

The above figure indicates that the microalga *Thalassiosira* sp. grows in all provided media. The results of this study show that the 20ppm silicate concentration has the highest peak cell density on the 6th day, with a density of $75.7x10^5$ cells/mL. Based on this, it can be concluded that higher silicate concentrations result in varied cell densities of microalga *Thalassiosira* sp. The relationship between the influence of different silicate concentration treatments on the growth of microalga *Thalassiosira* sp. is determined through regression analysis, as presented in Figure 3.

The results of the regression analysis, specifically the coefficient of determination (R square), indicate that different silicate concentration treatments have a 72% influence on the growth of *Thalassiosira* sp., while 28% is attributed to other factors. The correlation coefficient (r) or multiple R shows a value close to one, namely 0.849, meaning there is a strong relationship between different silicate concentration treatments and the growth of microalga *Thalassiosira* sp. The regression coefficient shows a positive result, meaning that different silicate concentrations have a positive or direct relationship with the growth of microalga *Thalassiosira* sp.

3.2 Water Quality Data

The measured water quality parameters in this study include temperature, salinity, and pH. Water quality parameters were measured daily at 09:00 AM. Based on the water quality measurements, the culture media conditions are considered normal and suitable for microalga life (Prihardianto *et al.*, 2023). The monitored temperature and salinity of the culture ranged from 22-25°C and 35-37 ppt, respectively, while the pH was recorded between 8-8.5.

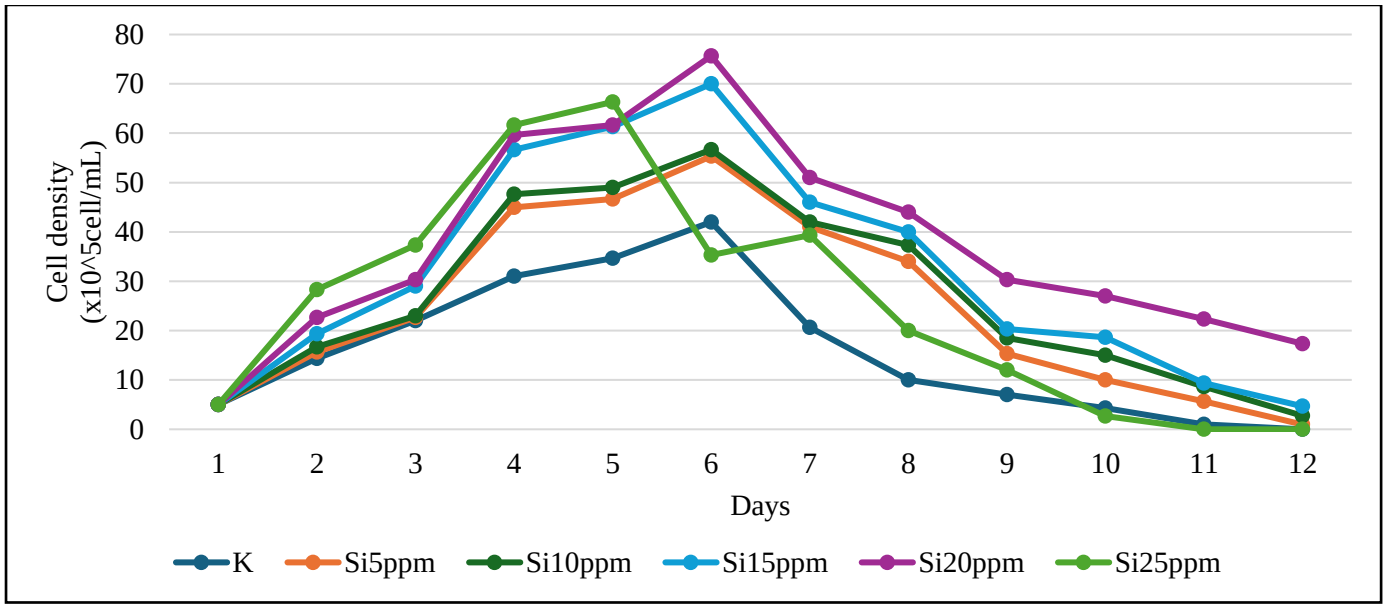

Figure 2. Growth Pattern of *Thalassiosira* sp. during the 12-day culture period with different silicate concentrations.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.849[a]	.720	.703	6.323

Predictors: (Constant), Treatment

Figure 3. Results of Regression Analysis Using SPSS Application

4. Discussion

The growth of microalga *Thalassiosira* sp. cultured at different silicate concentrations can be assessed qualitatively and quantitatively. Qualitatively, it can be observed visually by monitoring the color change of the culture media from clear to golden brown. Quantitatively, it involves calculating the cell density of microalga *Thalassiosira* sp.

The results obtained show a change in the color of the culture media. The color changing to a dark and intense brown indicates an increase in cell density in the microalga *Thalassiosira* sp. The color of the culture media is golden brown because microalga *Thalassiosira* sp. dominated carotenoid pigments in its composition. This is supported by Prasetyo *et al.* (2022), explaining that the golden-brown color of the culture media is due to the dominance of carotenoid pigments in microalga *Thalassiosira sp.*

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Microalga growth can also be assessed through cell density, with the calculation of cell numbers conducted daily during the culture period. The cell density at different silicate concentrations varies during the culture process, and statistical tests show significant differences between treatments ($p<0.05$). The research results indicate that microalga *Thalassiosira sp.* cultured for 12 days at different silicate concentrations resulted in the highest cell density at the 20ppm silicate concentration, which is $75.7x10^5$ cells/mL. The cell density of microalga *Thalassiosira sp*. at 0ppm, 5ppm, 10ppm, 15ppm, and 25ppm silicate concentrations, respectively, were $42x10^5$ cells/mL, $55x10^5$cells/mL, $56.7x10^5$cells/mL, $70x10^5$cells/mL, and $66.3x10^5$cells/mL. The variation in cell density for each treatment is presumed to be due to the different silicate concentration treatments (Umiatun *et al.*, 2017).

The lowest peak cell density in this study was obtained at the 0ppm silicate concentration, which is $42x10^5$cells/mL on the 6th day of the culture period. This is likely due to the absence of silicate in its growth process. This is supported by Umiatun *et al.* (2017), explaining that without silicate fertilizer, diatoms cannot form frustules properly, negatively impacting cell integrity and the cell's ability to withstand osmotic pressure, predation, or other environmental factors. The cell density of microalga at the 25ppm treatment did not experience optimal growth. This is caused by an excess of silicate, as stated by Sanjaya and Danakusuma (2018), explaining that silicate content exceeding the tolerance of microalga will disturb microalga growth. This condition is also accompanied by a decline and death of microalga *Thalassiosira* sp. This is reinforced by Wahyuni *et al.* (2021), explaining that a too high silicate concentration can interfere with the absorption of other nutrients by diatoms, disrupting the balance of nutrients required for healthy growth and reproduction. At 25ppm, silicate is not entirely absorbed for growth. This is further supported by Erlangga *et al.* (2021), stating that high cell density obstructs light penetration due to self-shading.

One characteristic feature of diatoms is the specific sculpting on the cell wall, which consists of silicate (Umiatun *et al*., 2017). Silicate influences microalga *Thalassiosira* sp. to have high resistance to environmental pressure (Sanjaya and Danakusumah, 2018). Silicate is an essential element for diatoms, especially for the formation of their cell walls. Various diatom species require different amounts of silicate. The difference in silicate content is one of the factors causing diatom succession. The cell wall of diatoms that protects structural units inside the cell is composed of silicate polymers (Annekov *et al.* 2020). Calcium also plays a role in aligning and regulating protoplasm activity and pH content within the cell.

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5. Conclusions

The silicate concentration significantly influences the growth of microalga *Thalassiosira* sp., with optimal concentrations (15ppm and 20ppm) supporting the highest cell density. Excessively high silicate concentration (25ppm) can disrupt growth, while low concentration (0ppm) also has negative impacts. The optimum silicate concentration for the growth of *Thalassiosira* sp. is 15ppm and 20ppm. The growth peaks on the 6th day with cell densities of $70x10^5$ cells/mL and $75.7x10^5$cells/mL, respectively. Different silicate concentrations can be applied to enhance the density of the *Thalassiosira* sp. microalgae. Silicate concentrations of 15ppm and 20ppm can have the greatest impact on the density of *Thalassiosira* sp. Silicate is highly crucial for *Thalassiosira* sp. and this is in a dose-dependent manner

Ethics approval

No need permit to *Thalassiosira sp.*

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Credit authorship contribution statement

SA is doing research ideas, data generation, sample image collection, and data analysis, HE is supervising, and writing the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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