Microalgae Growth and Morphology of *Skeletonema costatum* On Physiological Stress Nutrient Silicon (Si)

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Abstract. One type of microalga that can be cultivated as biodiesel raw material candidate is *Skeletonema costatum*. This study aims to determine the effect of nutrition physiological stress on the growth and 1,150,000 cells / ml at 72 hours and the lowest cell density in the treatment of stress that without the provision of Si of 550,000 cells / ml. Based on Dunnet test there was no significant difference between the growth of S. costatum the treatment of stress control treatment with 25% Si and 50% Si stress, but there was a significant difference in growth between the control and the treatment of stress Si 75% and 100%. *S. costatum* microalgae morphology can be seen that with the increasing stress of nutrient Si indicates cell lysis much experience.

1. Introduction

Biodiesel is a renewable alternative energy where it functions the same as diesel oil. The main ingredient or biodiesel precursors are triacylglycerols (TAGs). TAGs are the main components of vegetable oil which can be converted into fatty acid methyl esters by utilizing the catalysts in the esterification process [1]. In Indonesia, microalgae have potential as a biodiesel feedstock candidates are very promising.

One type of microalga that can be cultivated as biodiesel raw material candidates is diatoms. The research that has been done by Abdulgani et al. [2] showed that *S. costatum* is one of the diatoms that can be recommended as biodiesel feedstock with a lipid content of 7.42%. In addition, the advantages of microalgae *S. costatum* is growing rapidly (doubling time / time generation of 0.340 days) by 1,625 days of harvest time and the relative growth rate of 3.2764 and can be grown in bulk. As the biodiesel feedstock candidate lipid synthesis plays an important role. Lipids in microalgae can be driven one through physiological stress techniques nutrients Si. This is due to the drought stress, an organism would limit the nutrients for its growth but rather is directed to defense. One form of defense is the increase of lipids to maintain osmotic potential inside the cell [3]. Also reported by Moll et al. [4] and Jeffryes et al. [5] that the lack of elements Si at diatoms RGD-1 and deficiencies in Cyclotella sp Si
elements can increase the lipid content (Triacylglycerols / TAGs). Microalgae in its growth requires a wide range of inorganic elements, both macronutrients and micronutrients. Elements of macronutrients consisting of N, P, K, C, Si, S, and Ca as well as elements of micronutrients consisting of Fe, Zn, Cu, Mg, Mo, Co, B, and others [6].

2. Experimental Details
Preparation of Media Growing Microalgae S. costatum. The growing medium used is sea water that has a salinity of 34 ppt. The sea water media is sterilized by boiling [7]. Media were sterilized allowed to cool at room temperature. The next set of fertilizer used in the culture of S. costatum. Fertilizer use is fertilizer diatoms obtained from the Laboratory BPBAP (Balai Perikanan Budidaya Air Payau) Situbondo. Physiological Stress Treatment Nutrient Silicon (Si). The nutrient physiological stress treatment on Skeletonema costatum culture medium in the form of a decrease in nutrient concentration Na2SiO3 Si in the form of:
- Control (K): Addition of Na2SiO3 as much as 30 g/ L (100%)
- Treatment 1 (K1): Without Addition of Na2SiO3 (0%)
- Treatment 2 (K2): Addition of Na2SiO3 of 7.5 g/ L (75%)
- Treatment 3 (K3): Added Na2SiO3 of 15 g/ L (50%)
- Treatment 4 (K4): Added Na2SiO3 of 22.5 g/ L (25%)

Five treatments are applied to the culture medium S. costatum conducted in 100 L scale culture vessels (aquarium 100 L) filled combination of sea water and fertilizer diatoms accordance with the treatment. Furthermore S. costatum inoculated seed as much as 10% of the volume of media. This study uses a completely randomized design (CRD). The study used one factor in the form of nutrient concentration of silicon (Si) that differ in culture media S. costatum. The parameters observed in this study include the growth curve, growth and morphology of microalgae S. costatum. Data obtained from the research results were statistically analyzed using the methods of analysis of variance (ANOVA). If there is impact, then followed by Dunnett test at 95% confidence level (α = 0.05) to determine the significance of differences in each treatment with control.

3. Results and Discussion

![Figure 1](image)

**Figure 1.** Growth curve Microalgae S. costatum at each Treatment: K (100%) with the addition of Na2SiO3 as much as 30 g/L; K1 (0%) without Na2SiO3; K2 (75%) with addition of Na2SiO3 of 7.5 g/L; K3 (50%) with Na2SiO3 addition of 15 g/L, K4 (25%): with the addition of Na2SiO3 of 22.5 g/L

The growth curve of *S. costatum* in each treatment medium shows different growth phases as presented in Figure 1. Based on the growth curve in Figure 1 can be seen that in the control treatment (K), S. costatum experiencing the peak phase (late exponential) on hour-72 with a cell density of 1.15
The growth curve in Figure 1 shows that the control treatment (K) has a longer growth than the other treatment. The results of the study on growth show that the higher the stress Si is given, the growth phase of S. costatum progresses faster than the control. This is due to the control treatment, S. costatum is not gripped by the treatment medium so it can grow well. In the control treatment (K) and 25% Si (K4), S. costatum was able to live up to 120 hours (5 days), while at 100% stress, S. costatum was only able to live up to 72 hours (3 days). After entering a phase of adaptation, S. costatum nutrient demands quite a lot to do to reach the exponential phase of growth to high cell densities. Si is the main material forming the cell wall Bacillariophyceae (diatoms) so that Si becomes vital needs [8]. When Si deficiency occurs, diatoms can produce only a few cellular walls in silicon, but can still regulate their growth rate so that cell density follows these stress conditions [9]. Microalgae growth is influenced by internal factors and external factors. One of the external factors that influence the nutrition. Their nutritional stress will affect the growth of microalgae. Besides the stress will provide a response to microalga to persist in maintaining osmotic cell. The following shows the growth of Microalga S. costatum On Silicon Recovery Treatment Media (Si). Microalgae growth is influenced by internal factors and external factors. One of the external factors that influence the nutrition. Their nutritional stress will affect the growth of microalgae. Besides the stress will provide a response to microalga to persist in maintaining osmotic cell. The following shows the growth of Microalga S. costatum On Silicon Recovery Treatment Media (Si).

### Table 1. Growth of Skeletonema costatum In Media Treatment of Stress Silicon (Si)

| Physiological Stress Treatment Nutrient Si | Microalgae growth of S. costatum |
|------------------------------------------|---------------------------------|
| Control (K): Addition of Na₂SiO₃ as much as 30 g / L (100%) | 623.452 a |
| Treatment 1 (K1): Without Addition of Na₂SiO₃ (0%) | 215.327 |
| Treatment 2 (K2): Addition of Na₂SiO₃ of 7.5 g / L (75%) | 333.417 |
| Treatment 3 (K3): Added Na₂SiO₃ of 15 g / L (50%) | 451.471 a |
| Treatment 4 (K4): Added Na₂SiO₃ of 22.5 g / L (25%) | 573.738 |

Description: The same letters indicate significantly different results based Dunnett test with a level of 95%.

Dunnett test results, it can be seen that there is no significant difference between the growth of S. costatum in the control treatment (K), stress Si 25% (K4), and stress Si 50% (K3). Differences significant growth when compared to the control shown in the treatment of stress Si 75% (K2) and 100% (K1). The growth parameter is seen from cell density measured every 6 hours. Treatment K1 and K2 have a significant impact on the growth of S. costatum Si due to deficiency of 75% and 100% is a high stress for S. costatum because Si supply provided is less than required. In an environment that gripped the growth of S. costatum disorder where the level of cell damage is high. This is due to a supply of Si obtained for the formation of cell walls. With the high level of damage to cells, the cell division is also taking place more quickly. This is also supported by a statement Jeffryes et al. [5] which states that the algae that has gripped the growth phase is shorter than when grown in a normal environment. From the results of the study on the growth of the S. costatum microalgae shown in Table 1, it can be seen that the highest cell density is found in the control treatment (K), while the lowest is in the 100% stress (K1) stress treatment during the peak phase. The higher the Si stress, the more rapid the growth of S. costatum but the lower cell density. This is in accordance with the research that has been done by Moll et al. [4] which shows that by decreasing Si concentration, it can decrease cell density, but the growth phase is faster. When cells are deficient Si, can only produce a
little diatom cell walls are siliceous, but still be able to set the rate of growth so that the cell density following the stress state of the [9]. Such conditions will ultimately affect cell morphology. The morphology of the cells exposed to stress such Si can be shown in Figure 2.

![Figure 2. Skeletonema costatum morphology with 100 times magnification on various treatments of Si: K (100%) with 30 g/L Na$_2$SiO$_3$ addition; K1 (0%) without Na$_2$SiO$_3$; K2 (75%) with addition of Na$_2$SiO$_3$ of 7.5 g/L; K3 (50%) with Na$_2$SiO$_3$ addition of 15 g/L, K4 (25%): with the addition of Na$_2$SiO$_3$ of 22.5 g/L.](image)

Based on Figure 2, it can be seen that the growth of *S. costatum* at K treatment and K4 if the terms of its morphology, growth is normal. This can be seen from the form of *S. costatum* cells that form long colonies with good protoplast conditions and no visible lysis. The morphology of *S. costatum* in the treatment of K3 Figure 2 (d) shows that the *S. costatum* cell grows by forming a not too long colony and some cells are lysed, while Fig. 2 (c) shows that *S. costatum* cells with K2 treatment grow by forming a little colony but the cell size is longer than *S. costatum* by treatment of K, K3, and K4.

In addition, the cells in the K2 treatment have an imperfect protoplast. Many cells that do not have protoplasts and some are protoplasnya not meet the cell. The *S. costatum* cell with the K1 treatment shown in Fig. 2 (b) shows that the cell is experiencing imperfect growth. Many cells are damaged and have lysis, even colonies that are very short but long cells, such as cells in the treatment of K2. According to Sumeru and Anna [10] normal *S. costatum* cells are shaped like boxes with cytoplasm that fill cell contents. The research that has been done by Balzano et al. [11] suggest that stress causes morphological changes in *S. costatum*, one of which is indicated by changes in cell size.

4. Conclusion
Microalgae growth of *S. costatum* decreased with increasing stress concentration Si. The concentration of stress (deficiency) Si which produces the highest cell density were 0% (control), while the 100% concentration Si deficiency is stress that causes the most low cell density. However, the faster the growth phase with increasing stress. Given the increasing stress that many are experiencing cell lysis

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