

The Effect of Nanosilica on the Growth of *Skeletonema costatum*

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Abstract. The marine diatom *Skeletonema costatum* is very important as natural feed for shrimp larva in the hatchery and brackish water pond. This diatom needs fertilizer like silica as limiting factor. The purpose of this research is to determine the most effective nanosilica to increase the growth of *S. costatum* cells. This research was conducted from March to April 2018 in the Laboratory of Invertebrates, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. In this study, different source of silica was used, namely the silica natrium fertilizer (control), nanosilica chemical reaction (amorf), and nanosilica of quartz sand as much as 10 mg / l with 3 replications each. The lag phase, exponential phase, stationary phase and death phase of *S. Costatum* was observed at 16, 34, 38 and 72 hours after inoculum spreading, respectively. The highest cell density was observed on the second day of experiment, at 02.00 am for all treatments. The highest density of the cells was achieved on the treatment of silica natrium fertilizer, with cell density of 2.72 x 106 cells / ml, followed by nanosilica chemical reaction and nanosilica fertilizer from quartz sand with density of 2.16 x 106 and 1.84 x 106 cells / ml.

Keywords: Growing, Nanosilica, *Skeletonema costatum*

1 Introduction

Skeletonema costatum is one of natural live feeds that are widely used in the aquaculture hatcheries. This marine diatom is very commonly used as feed for tiger shrimp larvae from nauplius to zoea stage. *S. costatum* is characterized by the areola on the cell wall which mainly formed by silica. Therefore, silica is essentially needed for the growth of *S. costatum* cell walls. Diatoms absorb silica in the form that is dissolved in water, namely as Si (OH)₄ (Bellinger and Sigeo 2010) [1]. The mechanism of absorption of silica by *S. costatum* is genetically controlled by proteins. These proteins have been known to regulate biosilification in vivo in the metabolic system under environmental conditions[2].

The current development of silica in the industrial field, allows the manufacture of silica in the form of nano, called nanosilica. Nanosilica can be defined as a silica made on a nanoscale with a size ranging from 10 to 1000 nm [32]. Little is known about the effect of nanosilica on diatoms, but the use of nanosilica as fertilizer on plants has been widely used, such as tomato and rice plants. Nanosilica can accelerate the absorption, thus it will improve photosynthesis, and quantitatively increase the plant growth. Additionally, the role of silica in plants is to stimulate photosynthesis and carbon dioxide translocation (Fitriani and Haryanti 2016)[34]. Unlike the case with nanosilica, sodium silica fertilizer in *S. Costatum* media has

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been widely used by natural food farmers, and natural feed cultivation center in Indonesia. Sodium silica compound is a compound made by reacting silica, oxygen, and sodium elements in a thermal process at high temperatures to produce pure compound[5].

Study on the use of sodium silica fertilizer in culture media has been widely carried out, and shows positive results. Indeed, *S. costatum* is able to absorb sodium silica in water, this is shown by the increased growth of cells when given sodium silica fertilizer. Therefore, it is necessary to conduct the study on which types of silica that can increase cell growth *Skeletonema costatum* effectively.

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2 Materials and Methods

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2.1 Microalgae Preparation

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In this study, *S. costatum* was obtained from the Development Center of South Brackish and Marine Culture (BPBAPLWS) Pangandaran. The inoculum that has been calculated for density was initially placed into a 2l jar with aeration and specific light intensity (100 $\mu\text{mol photons/m}^2/\text{s}$) and photoperiod (16:8 for light and dark period, respectively).

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2.2 Preparation of Culture Medium

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Sea water culture medium with salinity of 25 ppt was sterilized prior to algae cultivation. The flask was filled with sterile sea water medium as much as 1.8 l. Afterwards, a total of 40, 20, 1 and 10 mg/ l of NPK fertilizer, NaH_2PO_4 , FeCl_3 , and EDTA-2NA, respectively, was added into all treatments following method from [6]. Each cultivation flask was filled with silica according to treatment, which is 10 mg/ l for sodium silica, 10 mg/ l of nanosilica from quartz sand, and 10 mg/ l of nanosilic chemical reactions.

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The culture medium was aerated evenly and left for one day, then on the next day a total of 100,000 cells /ml of *S. costatum* inoculum was placed into the cultivation flask. The dilution formula was applied to calculate the volume of inoculum used for the distribution of the initial cell density of *S. costatum*:

$$V_1 = \frac{V_2 \times N_2}{N_1}$$

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Where :

V1: volume of inoculum needed (ml)

N1: inoculum cell density per ml (cell/ ml)

V2: volume of cultured water (ml)

N2: desired initial density (cell / ml)

The inoculation flask was maintained illuminated by a 40 watt TL lamp for 24 h with specific light intensity and photoperiod.

2.3 Calculation of Cell Counts

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Determination of cell density in *S. costatum* culture was conducted by sampling every 4 hours during the culture process, following the protocol from Natasamita et al. [7]. Calculation of

cell counts was performed by taking water samples using culture media pipette, then placed them into the counting chamber hemocytometer.

2.4 Data Analysis

Data collected were analyzed descriptively through observational analysis using supporting data and related literatures. Furthermore, data were analyzed using analysis of variance (F-test) at the confidence level of 95% to determine the effect of each treatment on the *Skeletonema costatum* growth patterns. When a significant difference between the treatments was found, the Duncan multiple distance test with $\alpha = 5\%$ was performed [8].

3 Results and Discussion

3.1 Cell density of *S. Costatum*

In the present study, the average cell density of *S. costatum* at the 4-day culture period were different for each treatment. The density of *S. costatum* cells in the treatment of sodium silica fertilizer at a dose of 10 mg/l results in higher density compared to other treatments. Cell density appeared to be constant at 100.00 cells/ml after 16 h of observation. The average of cell density of *S. costatum* with different types of nanosilica for 4 days of experiment is shown in Table 1, and the growth curve of *S. costatum* is presented in Figure. 1.

Table 1. Average Cell Density of *S. costatum* during the experiment

Date	Time	Average cell density of <i>S. costatum</i> (cell / ml)		
		Sodium Silica	Nanosilica by Chemical Reaction	Nanosilica from Quartz sand
Day 1	18.00-22.00	0.100 x 10 ⁶	0.100 x 10 ⁶	0.100 x 10 ⁶
	02.00	0.100 x 10 ⁶	0.100 x 10 ⁶	0.100 x 10 ⁶
Day 2	06.00	0.640 x 10 ⁶	0.453 x 10 ⁶	0.293 x 10 ⁶
	10.00	0.893 x 10 ⁶	0.827 x 10 ⁶	0.707 x 10 ⁶
	14.00	1.053 x 10 ⁶	0.907x 10 ⁶	0.760 x 10 ⁶
	18.00	1.453 x 10 ⁶	1.227 x 10 ⁶	0.960 x 10 ⁶
Day 3	22.00	2.320 x 10 ⁶	1.800 x 10 ⁶	1.347 x 10 ⁶
	02.00	2.720 x 10^{6(a)}	2.160 x 10^{6(b)}	1.840 x 10^{6(c)}
	06.00	2.587x 10 ⁶	2.160 x 10 ⁶	1.760 x 10 ⁶
	10.00	2.453x 10 ⁶	1.813x 10 ⁶	1.573x 10 ⁶
	14.00	1.973x 10 ⁶	1.440 x 10 ⁶	1.400 x 10 ⁶
	18.00	1.067x 10 ⁶	0.680 x 10 ⁶	0.587x 10 ⁶
Day 4	22.00	0.573x 10 ⁶	0.227 x 10 ⁶	0.187x 10 ⁶
	02.00	0.507x 10 ⁶	0.227x 10 ⁶	0.173x 10 ⁶
	06.00	0.400 x 10 ⁶	0.147x 10 ⁶	0.120 x 10 ⁶
	10.00	0.217x 10 ⁶	0.093x 10 ⁶	0.067x 10 ⁶
	14.00	0.080 x 10 ⁶	0.027x 10 ⁶	0.013x 10 ⁶
	18.00	0	0	0

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Note: the numbers followed by the lowercase letters indicate the difference between treatments at the 95% confidence level.

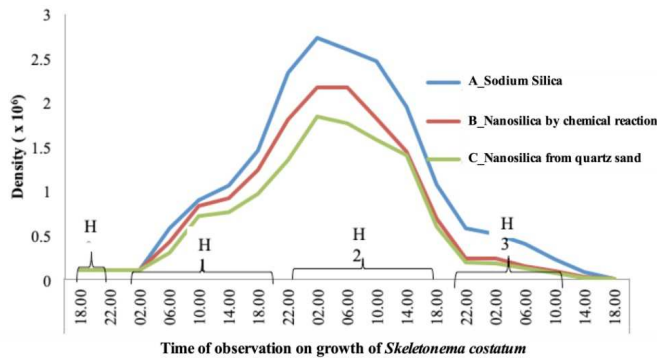


Fig. 1. *Skeletonema costatum* growth pattern

The peak population with the highest cell density was observed on day 3, at 02.00am for all treatments. The highest cell density of *S. costatum* was found in the treatment of sodium silica fertilizer, with cell density of 2.72×10^6 cells / ml, then followed by the treatment of nanosilica fertilizer from chemical reactions of 2.16×10^6 cells / ml, and the last is nanosilica fertilizer treatment from quartz sand with cell density of 1.84×10^6 cells / ml.

3.2 *S. costatum* cell growth

The growth of *S. costatum* cells are affected by environment and nutrient. Increasing temperatures and nutrient concentrations induced high growth rates and dominance by longer chains in a cultured *S. costatum* strain[9]. *S. costatum* abilities, in tolerating high nutrients level such as the ammonium[10] and nitrate[11], and in surviving the wide range of temperature and salinity[12]. The growth of *S. costatum* can be observed from their visual discoloration. The brown color formed during culture is caused by the pigment contained in *S. costatum* which points yellow pigments rather than green pigments. As previously demonstrated in [13] that carotenoids and diatomines are dominant pigments in *S. costatum*.

Based on statistical analysis revealed different pattern of growth of *S. costatum*. The highest growth of this algae appeared to be faound in the treatment of sodium silica fertilizer, followed by nanosilica fertilizer from chemical reactions and nanosilica from quartz sand. Additionally, Duncan test indicates that the sodium silica fertilizer 10 mg/l give significant effect on the growth of *S. costatum*.

Our findings demonstrate that the lag phase occurs for 16 hours after the inoculum added, which shown in the growth pattern curve of *S. costatum* that has not shown an increase

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in the number of cells in all treatments. While the exponential phase occurs quickly, this phase occurs on day 2 or 34 h after the inoculum was spread, and lasted for 2-3 hours. Cell density increases significantly in the exponential phase, suggesting that cells have successfully adapted and are optimal in nutrient absorption.

The exponential phase for the three types of fertilizer, the highest cell density of *S. costatum* was obtained at sodium silica with a density of 2.72×10^6 cells / ml, while the density for nanosilica from chemical reactions was 2.16×10^6 cells / ml, and nanosilica from quartz sand 1.84×10^6 cells/ml. These results are highest cells densities than *S. costatum* was cultivated at cement tank culture and recorded 1.23×10^6 cell/ml and 0.78×10^6 cells/ml in their respective f/4 medium and commercial fertilizer medium [14].

The stationary phase is characterized by the addition of a population that is balanced with the number of cell deaths. The stationary phase occurs 38 hours after the inoculum is added and lasts for 2-3 hours. Our results show that the number of cells in the stationary phase is not constant, but tends to decrease. This suggests that growth is beginning to be hampered, as also demonstrated by [15] who found that new cell growth will be hampered by the presence of dead cells and other limiting factors.

The death phase occurs on day 4 and is indicated by the condition that the culture media turns into a clear color, and biodeposition can be found at the bottom of the cultivation flask. Our result is congruent with previous study from [16] that the death phase was characterized by a decrease in *S. costatum* cell density that is caused by the decrease of nutrients availability in the culture medium. Therefore, the available nutrient could not meet the needs of *S. costatum* cells and there was competition among individuals in utilizing nutrients, space, light, and other supporting factors, such as turbidity that will inhibit photosynthesis. According to [17] *S. costatum* has rapid growth, with doubling time 8 hours, harvest time 39 h from the time of inoculum addition and relative growth rate of 3.3.

The use of sodium silica fertilizer is found to be the most effective in increasing *S. costatum* cell growth. This is because sodium silica dissolves in water and forms ions. [18] stated that diatoms absorb silica in the form dissolved in water, namely as $\text{Si}(\text{OH})_4$. [19] demonstrated that types of inorganic fertilizers, especially agricultural technical fertilizers such as urea, NPK and sodium silica, have small particle sizes and are easily soluble in water. The relative size of the particles facilitates or speeds up the process of absorption of nutrients by the cells of *S. costatum*.

The treatment of the three different types of silica fertilizers showed that the most ineffective was the use of silica from quartz sand, apart from its shape in nanoparticles when dissolved in water so it could not be absorbed by *S. costatum*. Moreover, the ineffectiveness of quartz sand is also due to many impurities as demonstrated by [20] that quartz sand has a high crystallinity and contains a lot of impurities which reduces its ability as an adsorbent. Quartz sand has a combined composition of SiO_2 , Al_2O_3 , CaO , Fe_2O_3 , TiO_2 , CaO , MgO , and K_2O , clear white or other colors depending on the impurity compound [21].

4 Fish Survival

Survival is one of the indicators of whether or not a cultivation system is feasible. Survival is closely related to whether or not food is sufficient as well as to fish health and good maintenance environment [14]. The protein content in feed can be used as a substance to form antibodies that function to fight against foreign substances that enter the fish [15]. The environmental conditions of the study site are influenced by the maintenance media hygiene factors. According to Sari [16], materials that are not useful and even detrimental to fish will be sedimented at the bottom of the maintenance container. In this container, a nitrogen cycle occurs due to the presence of decomposing bacteria and inorganic materials (from food waste and fish metabolic waste). Aquaponics system reduces this by absorbing the waste water using plant roots so that the remaining feed waste undergoes an oxidation process with the help of oxygen and bacteria [17].

Based on 30 days of maintenance, the survival rate of silver catfish maintained with an aquaponics system is very good, with a survival rate of 100%. This is probably caused by the ability of the maintenance media to properly meet the needs of the fish.

Increased Length of Stem in Lettuce and Water Spinach

The average stem length of water spinach at the end of the maintenance period was around 38.7 cm, while the length of lettuce was 20.7 cm which can be seen on Figure 2.

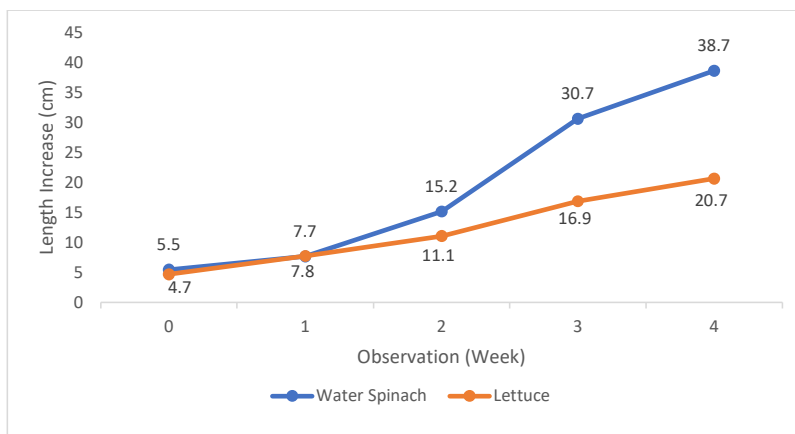


Figure 2. Increase in Plant Stem Length

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According to Wasonowati [18], plant growth is influenced by internal and external factors. The internal factors that influence plant growth are related to physiological processes while the external factors that affect plant growth include solar radiation, temperature, water, and nutrient supply. There are 3 important things that affect stem growth, namely the presence of light, growth regulators, and nutrients. The availability of water and nutrients affects the growth of segments, especially in terms of cell expansion. Plants that do not receive adequate light will present symptoms of etiolation, which is very fast plant growth in dark places but the condition of plant is weak and the stem is not sturdy [19]. The plant height and number of leaf increase are parallel to the increasing age of plants [20].

Addition in Number of Leaves in Lettuce and Water Spinach

The addition in the number of leaves during this study was around 16 leaves for water spinach and 17 leaves for lettuce as shown in Figure 3. At the beginning of planting, plants have an average of 4 leaves. But after maintenance, each treatment plant has a different number of leaves. The highest number of leaves was seen in treatment with water spinach and silver catfish of 17 leaves. The addition of the number of leaves occurs because of the availability of sufficient nutrients to be absorbed by plants.

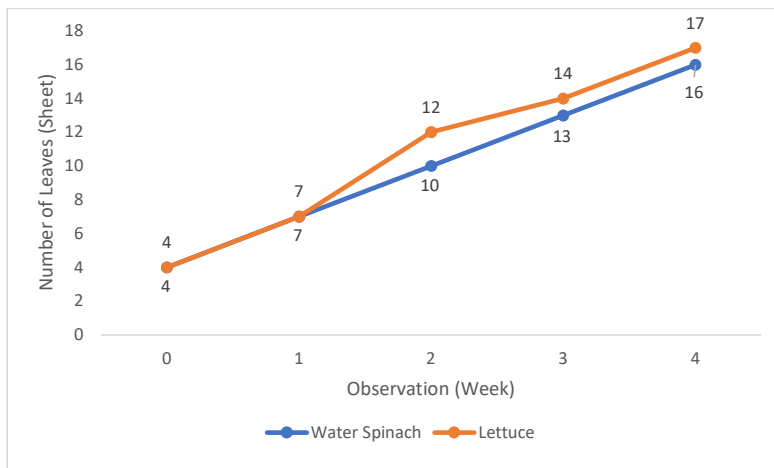


Figure 3. Number of Leaves

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Leaves are vegetative organs of plants and their number greatly affects plant growth because the leaves are the organ where photosynthesis occurs. The more leaves there are in plants, the greater the production will be. In addition, the number of leaves also determine the results of photosynthesis and affect plant growth and development [21]. Plants that do not get additional nitrogen will be stunted and the leaves formed will be smaller and thinner with a lower number while the plants that get enough nitrogen then the number of leaves formed will be higher and the leaves will be wider [22].

Conclusion

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The administration of 10 mg / l of sodium silica to the culture medium produced the highest cell density of *S.costatum* compared to the other two treatments. Sodium silica fertilizer is an effective fertilizer to increase *S.costatum* cell growth.

This study shows that the use of water spinach plant as an aquaponics system biofilter is more effective than lettuce. The combination of silver eafish and water spinach plants produces the highest productivity of the plant, i.e. stem length of 38.7 cm and the addition of 16 leaves. This

combination also produces the highest absolute growth of 7.79 grams/fish and a survival rate of 100%.

Acknowledgments

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