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The Influences of Physiological Stress from Silicon (Si) Nutrient toward Total Lipid Content at *Skeletonema costatum*

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ABSTRACT

Biodiesel was both kind of renewable and alternative energy where played like diesel oil. Microalgae were placed as attractive biodiesel raw material candidates. One of them was *Skeletonema costatum*. This research obtained to know the impacts of Silicon (Si) nutrient physiological stress toward total lipid content for microalgae Skeletonema costatum. Various Silicon (Si) deficiency concentrations applied at growth culture medium of *Skeletonema costatum* consisted of 0%, 25%, 50%, 75%, and 100%. Silicon (Si) within Na_2SiO_3 compound form used in test. The result gave inform that silicon (Si) deficiency concentration at 75 % utilized the highest total lipid content amount 3.46%, whereas *Skeletonema costatum* under 100% silicon (Si) concentration produced the lowest total lipid content which was 2,25%.
KEYWORDS —total lipid, physiological stress , silicon (Si), *Skeletonema costatum*.

INTRODUCTION

National energy sector often challenged by the increasing of consumption of fossil fuel and it price. Imbalance of the supply increased volume of imported fossil fuel [1]. Alternative energy was required especially for renewable energy to overcome the dependence of petroleum. Biodiesel was an alternative energy which played like diesel oil. The main ingredient or precursor of biodiesel was triglyceride/triacylglycerols [2]. The potential candidate which abundant numbers in Indonesia for biodiesel ingredient were microalgae [3].

Diatom was majority microalgae who cultivated for biodiesel utilization. *Skeletonema costatum* had 7.42% lipid content and belong to a diatom groups. It also had rapidly growth (doubling time at 0.340 days and cropping time at 1.625 days, relative growth rate around 3.2764) and mass cultivated easily [4].

Microalgae growth required certain nutrient growth inorganic, micronutrient and macronutrient. Macronutrient element composed as N, P, K, C, Si, S, and Ca where micronutrient element consisted of Fe, Zn, Cu, Mg, Mo, Co, B, and etc [5]. Lipid synthesize placed in an important phase to scale mass up. Lipid increasing for microalgae might be imposed by Silicon (Si) physiological stress. Organisms under stress condition limited their growth nutrition production and prefer protected themselves. Increasing lipid number was one of defensive way to keep potential osmotic pressure within cell [6]. Silicon (Si) rolled in cell wall producing in diatom [7]. This research obtained to see Silicon (Si) physiological stress toward total lipid for *Skeletonema costatum*. This research was a proceeding from biodiesel production.

MATERIALS AND METHODS

Sterilization

Culture bottles, aquarium 100 L, measuring beaker glass, aerator slang, water hose, beaker glass, volume pipette, droppers pipette, stirrer, Petri disk, Erlenmeyer clean washed and soaked by HCl 0.2% as long as 24 hours and watered using distilled water. Heat-resistant equipments sterilized using autoclave 121°C with 1.5 atm pressure at 20 minutes. Chlorines 40 ppm used to sterilize all non heat-resistant equipments [8].
Sea water used in at this research with 34 ppt salinity. It boiled to sterile and kept cooling at room temperature [9].

Fertilizer

Diatom fertilizer collected from Marine Feed for Aquaculture Laboratory (Laboratorium Pakan Alami Balai Budidaya Air Payau (BBAP)) Situbondo.

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Table 1. Composition of diatom fertilizer [10]

Ingredient	Number (g/L)
KNO_3	75
PO_4	5
EDTA	5
Fe	3,15
Na_2SiO_3	30

Growth curve

Seeds of *Skeletonema costatum* isolates as much of 50 ml inoculated into 450 ml culture medium kultur (mixture of sea water and fertilizer). Haemocytometer improved Neubauer used to measure cell density in every 6 hours till death phase and made their growth curve by data which collected.

Treatment

Decreasing Silicon concentration in Na_2SiO_3 compound used as research treatment,

Control (K) : 0% (+ Na_2SiO_3 30 g/L)
Concentration 1 (K1) : 100% (without Na_2SiO_3)
Concentration 2 (K2) : 75% (+ Na_2SiO_3 7,5 g/L)
Concentration 3 (K3) : 50% (+ Na_2SiO_3 15 g/L)
Concentration 4 (K4) : 25% (= Na_2SiO_3 22,5 g/L)

All five treatments applied at *Skeletonema costatum* culture medium in 100 L volume. Amount 10% (10L) culture taken and grown at culture medium.

Cropping

Skeletonema costatum cropped at late exponential phase (top peak). Sateen textile used to filter and cropped microalgae [10].

Total lipid content analysis

Soxhlet method used to analyze total lipid content. Two grams of dried microalgae wrapped in a paper with catoon base seal. It was heat not more than 80°C in 1 hour. It implanted to Soxhlet with connected to lipid tubes with boiled stone and known it weight. It extracted using solvent mixture (hexane 39 ml + ethanol 13 ml) in 6 hours. It evaporated using oven at 105°C then weighed after reached room temperature [11].

$$total\ lipid\ content(\%) = \frac{total\ weight\ of\ lipid\ (g)}{weight\ of\ samples(g)} \times 100\%$$

Research design

Complete random sampling with single factor that composed various silicon nutrient concentrations at *Skeletonema costatum* culture medium. There were 5 treatments with two kind of repetition.

Data analysis

Data analyzed statistically using ANOVA. If any differs impacts followed by Dunnet test at 95% (α=0.05) confidence level to obtain how significant different in all treatments.

RESULTS AND DISCUSSION

Growth curve made to determine top peak of late exponential phase. It used decide cropping time.

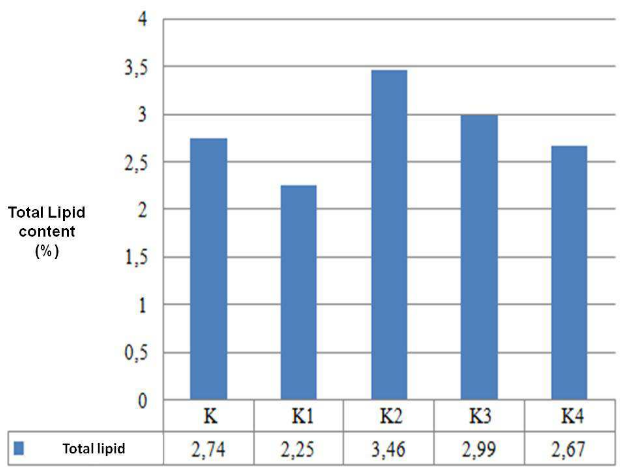

Figure 1. Total lipid content of *Skeletonema costatum* in all treatment

According to total lipid content test of *S. costatum* like in Figure 1., K2 treatment gave the highest total lipid content which was 3.46%. Even the cell density was at the opposite to number of total lipid that gained. The cell density was in small number than control (K), it fortunately produced much more total lipid content. Si deficiency at 75% was the optimum stress given for *S. costatum* in producing high total lipid content. At stress level 100% concentration, *S. costatum* produced the lowest total lipid content compared to others. Si deficiency at 100% to diatom decreased photosynthetic activity compared to diatom that got enough concentrations [12]. It affected to lipid synthesize especially triglyseride [13]. Due to triglyceride synthesize initiated by acetyl Ko-A from photosynthesis [14]. If only photosynthesis hampered thus both acetyl Ko-A and lipid synthesize also hampered and decreased (triglyceride).

ANOVA result informed that $P<0,05$ occurred. It was mean any influences from physiological stress for Silicon nutrient toward total lipid content in *Skeletonema costatum*. Dunnett test also confirmed any different for total lipid content in control and treatment (K1 and K2).

Table 2. Dunnett test for total lipid content of *Skeletonema costatum*

Si tress	Treatment	Total lipid content
K (0%)	+ Na_2SiO_3 30 g/L	2,74 a
K1 (100%)	Without Na_2SiO_3	2,25
K2 (75%)	+ Na_2SiO_3 7,5 g/L	3,46
K3 (50%)	+ Na_2SiO_3 15 g/L	2,99 a
K4 (25%)	+ Na_2SiO_3 22,5 g/L	2,67 a

Description: Number which followed by the same letter at of the same column figured no significant differ based on Dunnett test at 95% (α=0,05%) of confidence level.

Silicon (Si) was essential element in lipid synthesize which produced various kind of lipids included triglyceride (biodiesel precursor). Manipulating Si metabolism might be conducted by added Si deficiency toward *S. costatum*. It was able to increase total lipid production. Si deficiency became a trigger for producing triglycerides [15].

CONCLUSION

Skeletonema costatum which stressed using 75% silicon (Si) produced the highest total lipid content amount of 3.46%. Silicon (Si) at 75% concentration was optimum stress level to obtain high lipid content and fast for growth phase at *Skeletonema costatum* which played as biodiesel production raw material candidate.

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