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# Effect of Photoperiod and Stress Physiological Nutrients (N and Si) on the Growth and Lipid Content of Microalgae *Skeletonema costatum*

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ABSTRACT

*Skeletonema costatum* is a microalgae that can be used as raw material for biodiesel. *S. costatum* has a relatively rapid doubling time, and a high lipid content is 7.42% dry weight. Microalgae lipid accumulation increased in drought stress. This study aimed to determine the effect of photoperiod and physiological stress nutrients N and Si on growth and lipid content *S. costatum*. The method used is a culture of *S. costatum* in physiological media stress nutrient N and Si with variations of photoperiod. Cell density measurements every 6 hours using Haemocytometer Improved Neubauer. Harvesting is done at the end of the exponential phase. The parameters measured were the growth curve, biomass and lipid levels. Data were analyzed using two-way ANOVA. The results showed that growth is highest in the treatment of A24 with a cell density of 132 500 cells / ml and 0.49 gram of biomass, while the highest lipid levels in the treatment of B24 by 14.42%.

***Keyword** : Physiological stresses, Photoperiod, lipids, Skeletonema costatum*

## Introduction

*Skeletonema costatum* has the ability to produce lipids (Pratiwi *et al.*, 2015). Lipids can be converted into biodiesel through transesterification. Growth and accumulation of microalgae lipid known to be dependent on growing conditions. Increased lipid accumulation in drought stress (Barqi, 2015). According to the study (Gammanpila *et al.*, 2015), photoperiod can affect the lipid content of microalgae. This is because light is an energy source for microalgae photosynthesis. Increased photosynthesis will be followed by an increase in productivity of microalgae. Microalgae requires periods of light on a photochemical phase to produce ATP and NADPH. While the dark period in the synthesis of molecules important role in growth. Thus, protein, carbohydrates and lipids in microalgae can vary with variations in photoperiod (Barqi, 2015). In a study (Ferdianto, 2018), has obtained the highest cell density in $KNO_3$ stress (stress nutrient N) by 100% and $Na_2SiO_3$ (nutrient stress Si) of 50%. As for the highest lipid accumulation results using $KNO_3$ stress by 50% and by 50% $Na_2SiO_3$. However, the studies were not given variations in photoperiod. Therefore, in this research, treatment variations of photoperiod and physiological stress nutrient N and Si using a concentration of research (Ferdianto, 2018).

### Methodology

#### Sterilization Equipment and Material Culture

Sea water sterilization carried out by boiling

(Prabowo, 2009). The sea water is maintained at 34 ppt salinity. Increased salinity of sea water, then add sterile distilled water to obtain a salinity of 34 ppt. Glassware sterilized using an autoclave at a temperature of 121 °C with a pressure of 1.5 atm for 15 minutes. Sterilization non heat-resistant glass that does not use the washing process with detergent and soaking in a solution of 40 ppm krolin. Further equipment is washed with running water (Triswanto, 2011).

**Fertilizer Diatoms**

Fertilizer diatoms obtained from the Laboratory of Pakan Alami Balai Budidaya Air payau (BBAP) Situbondo with corresponding composition in Table 1.

**Table 1.** Fertilizer composition Diatoms (Setyaningsih *et al.*, 2018).

| Ingredients | Number (gr/l) |
|---|---|
| $KNO_3$ | 75 |
| $PO_4$ | 5 |
| EDTA | 5 |
| Fe | 3.15 |
| $Na_2SiO_3$ | 30 |

**Growth Curve**

*S. costatum* growth curve was made to determine the final exponential phase as the basis for harvesting. 10% of the culture volume used for *S. costatum*. The culture was inoculated in sea water medium plus diatom fertilizer according to the research treatment. In 1 L of culture media, 1 ml of diatom fertilizer is needed. At this stage, 500 ml of microalgae culture was used for each treatment, 50 ml of *S. costatum* culture was needed and inoculated into 450 ml of culture media. Media contains *S. costatum* by photoperiod according to treatment with TL-40 lamp lighting watt as well as by aeration. Furthermore, the *S. costatum* cell density measurements every 6 hours using Haemocytometer Improved Neubauer. Cell density was calculated using the formula:

$$\text{Cell Density} = \frac{\Sigma \text{ cells within 4 blocks } x\ 10^4}{\Sigma \text{ blocks (4)}} \text{ cell /ml}$$

**Research Treatment**

The treatment in this study using a combination of stress concentrations (N and Si) and photoperiod different.

**Table 2.** Combination Research Treatment of Photoperiod Variation and Physiological Stress (N and Si)

| Fertilizer | Photoperiode 24 : 0 | 16 : 8 | 12 : 12 |
|---|---|---|---|
| A | A24 | A16 | A12 |
| B | B24 | B16 | B12 |
| C | C24 | C16 | C12 |

Description
A: Control (Concentration $KNO_3$ 75 gr / L and $Na_2SiO_3$ 30 g/l)

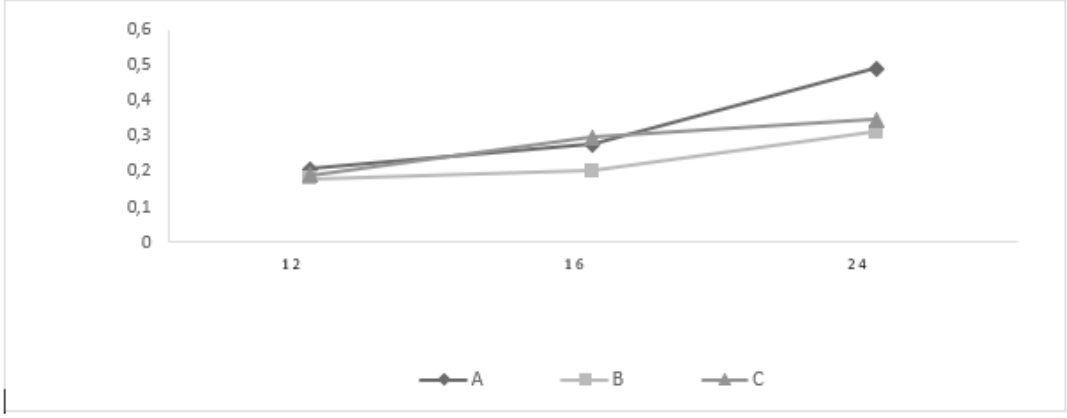


**Fig. 1.** Graph Biomass *S. costatum* Variation In Treatment photoperiod and Nutren Physiological Stress (N and Si)
Description:
A: Control (Concentration $KNO_3$ 75 g/l and $Na_2SiO_3$ 30 g/l)
B: 100% nutrient stress N and 50% Si (concentrations of $KNO_3$ 0 g/l and $Na_2SiO_3$ 15 g/l)
C: Nutrient stress N 50% and Si 50% ($KNO_3$ concentration 37.5 g/l and $Na_2SiO_3$ 15 g/l)

B: 100% nutrient stress N and 50% Si (concentrations of $KNO_3$ 0 g/l and $Na_2SiO_3$ 15 g/l)
C: Nutrient stress N 50% and Si 50% ($KNO_3$ concentration 37.5 g/l and $Na_2SiO_3$ 15 g/l)

### Harvesting of *S. costatum*

Harvesting *S. costatum* done at the end of the exponential phase. Harvesting of microalgae using satin fabric. The results measured filtration biomass and lipid levels in total. Biomass was measured using an analytical balance. While the total lipid levels were measured using Soxhlet method.

## Results

### Microalgae Biomass *S. costatum*

Microalgae biomass contains important components such as carbohydrates, proteins, fats and nucleic acids (Jeffryes *et al.,* 2013). Based on the two-way ANOVA test, nutrient treatment, photoperiod and their interactions had no effect on the culture biomass of *S. costatum*. This is indicated by the p-value which is more than the alpha value (0.05), namely 0.651 for nutrient factor, 0.183 for photoperiod factor and 0.927 for the interaction of the two. The results of *S. costatum* biomass is shown in Figure 1.

### Lipid levels Microalgae *S. costatum*

Analysis of the total lipid content quantitatively using Soxhlet method. The results of the total lipid content microalgae *S. costatum* of 9 treatments shown in Figure 2.

## Discussion

Based on Figure 1 can be seen that the treatment resulted in the highest biomass is treated with the photoperiod 24: 0, kind of nutrient treatment A (control), B (100% stress nutrients N and Si 50%) and C (stress nutrient N 50% and Si 50%). While the treatment resulted in the lowest biomass is treated with a 12:12 photoperiod, both in nutrient treatment A (control), B (100% stress nutrients N and Si 50%) and C (N nutrient stress 50% and 50% Si). However, based on two-way ANOVA test, nutrient stress and photoperiod treatments that do not give effect to biomass culture, it is suspected due to growth factors include aeration, salinity, pH and temperature suitable for the growth of *S. costatum*. *S. costatum* environmental conditions that support causes cell survival and regeneration, although the amount is less (Darsi *et al.,* 2012). In addition, each cultivated cells respond differently to a given treatment. By (Alqadi *et al.,* 2017), giving photoperiod 24: 0 and 16: 8 does not have a significant influence on the biomass from the culture *Nannochloropsis sp*. where the photoperiod 24: 0 biomass culture of 0.046 g / l while the photoperiod 16: 8 biomass culture of 0.027 g / l. In this study, biomass *S. costatum* culture in photoperiod 24: 0 by giving the control nutrient is 0.49 g, while photoperiod 16: 8 culture biomass of 0.31 grams and 12:12 photoperiod culture biomass of 0.345 g.

In addition to photoperiod, nutrient treatment is also given to obtain *S. costatum* with high lipid content. In this study, it was found that the treatment A24 (without stress nutrients to photoperiod 24: 0) is a treatment that results in the highest cell density (132 500 cells / ml) and the highest biomass (0.49 grams). This is consistent with the statement (Selvika *et al.,* 2016) that the more the number of

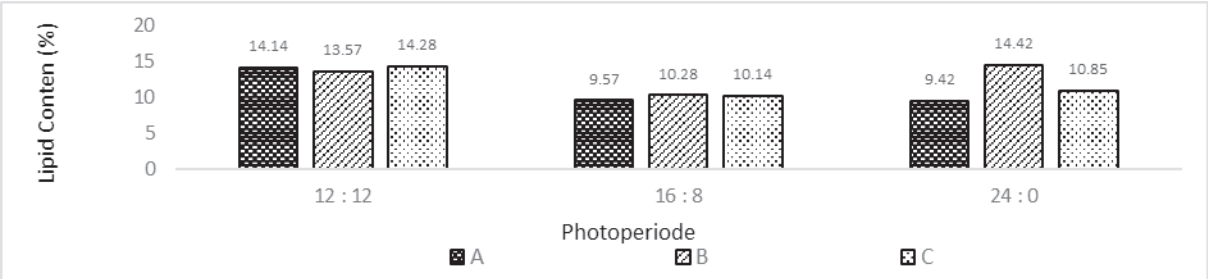


**Fig. 2.** Total Lipid Levels of *S. costatum* in the Treatment of Photoperiod Variations and Physiological Nutrient Stress (N and Si)

Description
A: Control (Concentration $KNO_3$ 75 g/l and $Na_2SiO_3$ 30 g/l)
B: 100% nutrient stress N and 50% Si (concentrations of $KNO_3$ 0 g/l and $Na_2SiO_3$ 15 g/l)
C: Nutrient stress N 50% and Si 50% ($KNO_3$ concentration 37.5 g/l and $Na_2SiO_3$ 15 g/l)

cells, then the higher the required nutrients. Limited nutrients causes competition among microalgae that cell density decreased. However, A24 treatment had the lowest lipid content, namely 9.42%. In contrast, treatment of B24 cells and biomass densities lower than A24, ie 118 750 cells / ml and 0.31 g, but has a higher lipid content compared with other treatments, ie 14.42%. This suggests that high growth does not necessarily result in a high lipid content. B24 treatment allegedly less than optimal to support the growth of *S. costatum*, because it is not given the addition of nutrients N, while nutrient Si is only given 50% of the dose should be. According to (Sheehan *et al.,* 1998) microalgae under drought stress limits its growth, but accumulate lipids for self-defense. According to (Endrawati *et al.,* 2012), stress makes microalgae tend to maintain viability than cells multiply. The adaptation process is done by the energy generated is used to survive, so that growth tends to slow and the energy stored in large quantities. Microalgae in nature accumulate lipids under certain conditions. In non-optimal conditions, microalgae continue to carry out photosynthesis and accumulate it in the form of carbohydrates and lipids. Microalgae total lipids accumulate in large quantities to find a good growing environment (Endrawati and Riniatsih, 2013). Thus, it can be assumed that the stress of silica 100% and nitrogen 50% by photoperiod 24: 0 to inhibit the growth and increase the lipid content of *S. costatum*. According to (Umiatun *et al.,* 2017), silica is the most important nutrient for diatom growth. Required by the diatom's silica cell wall formation. In silica deficiency conditions, diatoms can only produce a small amount of silica cell walls, but can still regulate their growth rate. The formation of the cell wall becomes abnormal because of the insufficient supply of Si, as a result the cell wall becomes imperfect. This affects the cell turgidity. When the environment is hypertonic while the cells are hypotonic, it can harm the cells, because the fluid will enter the cells continuously so that the cells burst. For self-defense, *S.costatum* require lipids to osmoproteksi. From the observations that have been made in this study, it was found that the cells undergo lysis image on silica stress treatment.

While nitrogen is an essential element required for the formation of cell proteins and nucleic acids (Brown *et al.,* 1993). According to (Hu *et al.,* 2008), microalgae cells in a nitrogen-limited state cause these cells to rapidly absorb inorganic nitrogen.


**Fig. 3.** The morphology of *Skeletonema costatum* at W Lounge Haemocytometer, (A). Treatment A24, (B). Treatment B24 (a: lysed cells, b: normal cells)

Meanwhile, carbohydrates in cells are used to meet the needs of cell physiological changes. Furthermore, the limited nitrogen can reduce carbon dioxide fixation, chlorophyll content and productivity. In addition, according to (Goncalves *et al.,* 2016), at the time of nitrogen deficiency, glikoksilat cycle and gluconeogenesis to be blocked and the availability of acetyl-CoA for the synthesis of fatty deposits increased.

At 12:12 photoperiod treatment, B12 treatment resulted in cell density of 90,000 cells / ml, higher than the treatment of A12 with a cell density of 64 062 cells / ml. However, biomass and lipid content A12 (0.205 g) is greater than the treatment of B12 (0.18 g). It also happens to photoperiod 16: 8, where the treatment of B16 (86 562 cells / ml) had a higher cell density than treatment A16 (64 750 cells / ml), but the biomass A16 (0.275 g) was higher than B16 (0.2 grams). This is because stress is given on the treatment of B12 and B16 causes the cells to accelerate growth. This is in accordance with (Balzano *et al.,* 2010) which states that the Skeletonema costatum can withstand drought stress by speeding up growth, when the bad quality of the resulting cells (cell lysis, small and unable to absorb a lot of carbon for cell metabolism). From the results of the study (Ferdianto, 2018) found that in an environment that gripped can cause damage to cells Skeletonema costatum. Damage to the cell causes rapid cell division. According to (Hu *et al.,* 2008) cells that grow and divide requires a sufficient supply of nitrogen. Should the supply of nitrogen in a limited environment, the cells tend to maximize the natural nitrogen supply of cells to divide.

## Conclusion

Photoperiod treatment, nutrients and their interaction no effect on the growth and lipid content S. costatum. Growth is highest in the treatment of A24

with a cell density of 132 500 cells / ml and 0.49 gram of biomass, while the highest lipid content contained in B24 treatment with lipid content of 14.42%.

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