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Lipid accumulation of *Skletonema costatum* under silica, Nitrogen and aeration stress

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ABSTRACT

Microalgae are photosynthetic microorganisms that could potentially be a source of raw material for biodiesel because it contains triglycerides. *Skeletonema costatum* was a microalgae that produces lipids up to 7.42%. Nutrients granted for microalgae growth. Aeration created water circulation so that cells obtained nutrients in the medium cultivation. The purpose of this study was to determine the effect of aeration and nutrient stress (N and Si) on growth and lipid content of *S. costatum* microalgae. Method starting from preliminary test of S. costatum culture with different fertilizer concentration and 0 hour, 24 hours, and 48 hours aeration. Cell density calculations used to figure growth curves out. Biomass measurement obtained from 8 liter culture result. Factorial randomized design followed by ANOVA test. Lipid accumulation analyzed using fluorescence microscope. N1Si1, N2Si1, and N3Si1 were the treatment that produced high cell density. Growth of N1Si1 in 24-hours aeration was the highest cell density among the three aeration treatments. Yellow luminescence was present in the treatment of N2Si2 (KNO3 37.5 g/L and Na2SiO3 15 g/L) with 48 hour of aeration, N2Si3 (KNO3 37.5 and Na2SiO3 0 g/L) and N3Si3 (KNO3 0 g/L and Na2SiO3 0 g/L) with 24 Hours Aeration.

Key words: *Skletonema costatum, Aeration, Nitrogen, Silica, Lipid*

Introduction

Biodisesel as produced from various fatty acids, that could be produced by microalgaes. Triacylglyceride was the main lipid component that utilized from them especially diatom group. Their abundance vary from 20.000 up to 200.000 species either on lakes or ocean, so that it was potential as biodiesel feedstock (Guiry, 2012). Aeration and nutrients were the factors that influence the growth of microalgae. Aeration plays role to keep microalgae in suspension form, provide carbon sources for growth, control pH, release O_2 from culture media, and prevent saturation (Priyadharsani, 2014). Cells had obtained nutrients in the cultivation medium evenly because of the circulation of water in the culture container through aeration (Amini, 2010). Nutrients played to support the growth of microalgae. Silica (Si) was an element that necessary to form cell walls for diatoms while Nitrogen (N) required for protein synthesis (Umiatun, 2017). When the cell division rate in culture limited by nutrients, cells changed their metabolism and converted energy to produce backup substances. Protein and chlorophyll synthesis decreased caused by limited nutrient frequencies, however lipid concentrations was

increase (Costard, 2012). Under a variety of stress conditions (aeration and physiological stress) photosynthetic membranes of algae encountered rapid degradation and the accumulation of TAGs (Triacylgliserols) lipid bodies. The effects of aeration and nutrient stress (N and Si) were and researched on *S. costatum* lipid content.

Materials and Methods

Modification media is used for algae culture**.** Semi-mass culture conducted at the Situbondo Brackish Water Aquaculture Center (BPBAP). The cultivation presented in sea water enriched-Conway medium at 10 L. Furthermore, the growth medium of microalgae was sterile sea water, reducing the composition of elements of nitrogen and silica by 25%, 50% and 75% respectively and given aeration stress 0 hours, 12 hours and 48 hours. Microalgae harvested at 7 days in the exponential phase (growth phase).

Harvesting and Drying Techniques

S. costatum harvested in the exponential final growth phase (peak phase) using satin fabric. It was because the near stationary phase, algae culture had a high ability of lipid accumulation. Microalgae biomass dried directly under the sun. In addition, it also can be dried with a drysprayer.

Fig. 1. *Skletonema costatum* harvesting on artificial sea water medium that enriched by Conway fertilizer on various treatment

Observation

Supporting research parameters are temperature, pH, and salinity. The temperature is measured using a thermometer, pH was measured using pH indicator paper and salinity was measured using a refractometer. Measurement of supporting parameters is done every day to support the main parameters, namely growth and total lipid content of microalgae *Skeletonema costatum*.

Nile Red Staining

Qualitatively lipid content of microalgae cells was observed using Nile Red dye (Duong, 2012). Nile Red stock solution prepared by adding 1 mg of Nile Red to 10 mL of acetone. Furthermore, the solution stored in a dark and closed room. Amount 1 mL of microalgae sample put into the microtube and added 0.2 mL of Nile Red stock solution. The sample was shake to be homogeneous. The microalgae cells in the microtube were then rinsed using aquadest in a ratio of 1: 1 (i.e. entering 50 µL of microalgae samples that were stained with 50 µL of distilled water into another microtube). It centrifuged to separate microalgae cells with water. Supernatant (30 µL) from the centrifugation then dripped into the slide and viewed intracellular lipid luminescence using a fluorescence microscope with a wavelength of 450-495 nm. Seen in color on microalgae, if it was shiny yellow it was mean that the microalgae contains lipids (Duong, 201). The least amount of lipid content qualitatively determined using the color index as presented in Fig. 2.

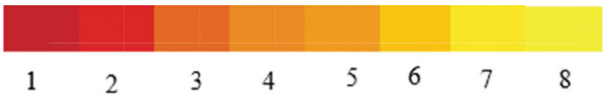

Fig. 2. Lipid Qualitative Test Color Index
Description of lipid content:1: +; 2: ++ ;
3: +++; 4: ++++; 5: +++++; 6: ++++++; 7: +++++++;
8: ++++++++
*more signs (+) was mean that more lipid content in the treatment

Statistical analysis

The results of this study explained by describing the effect of aeration and nutrient stress (N and Si) on the growth and lipid content of *S. costatum*. The data obtained displayed in the form of tables and interpreted in the form of descriptions.

Results

Lipid Qualitative Test

The results of lipid qualitative test of *Skeletonema costatum*, based on observations under fluoressnce microscopy are shown in Table 1.

Discussion

Based on Table 1, the most luminesce yellow color

Table 1. Lipid content of *S. Costatum* through aeration and physiological (N and Si) stress

Nutrient	0 hour	24 hours	48 hours
N1Si1	+	+	+++++++
N1Si2	++	++++++	+++
N1Si3	+++++	++++++	+++++++
N2Si1	+++++	++++++	+++++++
N2Si2	++++++	+++++++	++++++++
N2Si3	++++	++++++++	+
N3Si1	++	++	+++++++
N3Si2	++++++	++++++	++++++
N3Si3	+++++	++++++++	+

Description: (N1Si1: 75 g/L KNO_3 and 30 g/L Na_2SiO_3; N1Si2: 75 g/L KNO_3 and 15 g/L; Na_2SiO_3; N1Si3: 75 g/L KNO_3 and 0 g/L Na_2SiO_3; N2Si1: 37,5 g/L KNO_3 and 30/ L Na_2SiO_3; N2Si2 : 37.5 g/L KNO_3 and 15 g/L Na_2SiO_3; N2Si3: 37,5 g/L KNO_3 and 0 g/L Na2SiO_3; N3Si1: 0 g/L KNO_3 and 30 g/L Na_2SiO_3; N3Si2: 0 g/L KNO_3 and 15 g/ L Na2SiO3; N3Si3: 0 g/L KNO_3 and 0 g/L Na_2SiO_3)

was *S. costatum* with N2Si2 treatment (Nitrogen 37.5 g/L and Silica 15 g/L) 48 aeration every hours, N2Si3 (Nitrogen 37.5 g/L and Silica 0 g/L) and N3Si3 (Nitrogen 0 g/L and Silica 0 g/L) 24 aeration every hours. Treatment of N2Si2 with aeration for 48 hours obtained 3.2 grams of biomass. N2Si3 has 1.18 gram biomass and 0.715 g produced by N3Si3. The treatment of N3Si3 by giving 24 hours of aeration has the lowest biomass compared to other treatments on 24 hours aeration. The whole treatment that has the highest lipid accumulation is not the treatment with the highest biomass. In this treatment, biomass is inversely proportional to the lipid content in which low biomass but high lipids are produced.*S. costatum* containing low lipid consists of N1Si1 (Nitrogen 75 g/L and Silica 30 g/L) without aeration, N1Si3 (Nitrogen 75 g/L and Silica 0 g /L) aerated in a day, N2Si3 (Nitrogen 37, 5 g/L and Silica 0 g/L) with 2 day aeration, and N3Si3 (Nitrogen 0 g/L and Silica 0 g/L) with 2 day aeration as indicated by red light. The treatment of N1S1 was the control (no aeration) that had the highest biomass on 1.15 grams. The treatment of N1Si3 has a 1.585 g biomass. The treatment of N3Si3 has a 0.715 g biomass. Both N1Si3 and N3Si3 treatments were given 24 hours of aeration. The 48-hour N2Si3 treatment has 1.1 g of biomass.

The treatment allegedly accumulated high lipids in the administration of Nitrogen 37.5 g/L and 0 g/ l (N2Si2 48 aerated hours, N2Si3 and 24 aerated hours. According to (El-sheekh, 2013) in conditions

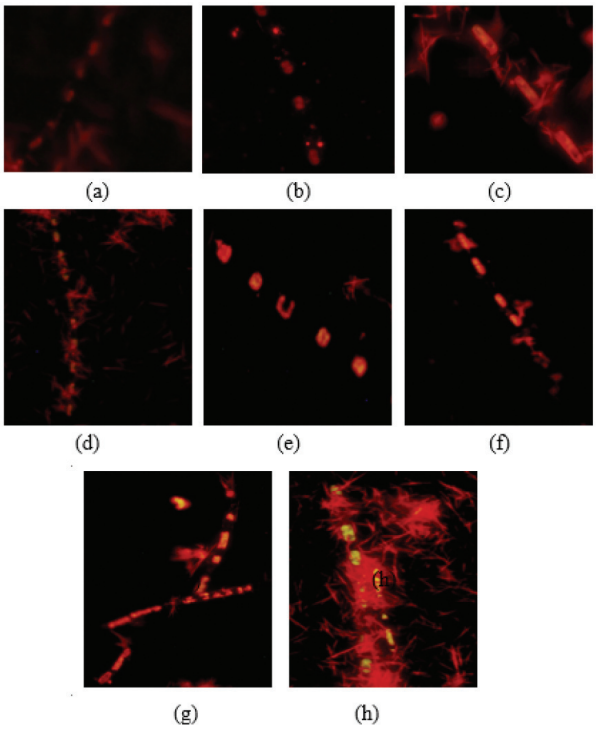

Fig. 3. Cellular-luminescence Morphology of *Skletonema costatum* at stress medium: Description: (a) N1Si1: 75 g/L KNO_3 and 30 g/L Na_2SiO3 + 0 hour of aeration; (b) N3Si1 : 0 g/L KNO_3 and 30 g/L Na_2SiO_3 + 24 hours of aeration; (c) N1Si2 : 75 g/L KNO_3 and 15 g/L Na_2SiO_3 + 48 hours of aeration; (d) N2Si3 : 37,5 g/L KNO_3 and 0 g/L Na_2SiO_3 + 0 hour of aeration; (e) N1Si3 : 75 g/L KNO_3 and 0 g/ L Na_2SiO_3 + 0 hour of aeration; (f) N1Si2 : 75 g/L KNO_3 and 15 g/L Na_2SiO_3 + 24 hours of aeration; (g) N2Si1 : 37,5 g/L KNO_3 and 30 g/l Na_2SiO3 + 48 hours of aeration; (h) N3Si3 : 0 g/L KNO_3 and 0 g/L Na2SiO_3 + 24 hours of aeration.

of nitrogen limited algae cells tend to collect carbon metabolism in the form of lipids. In the nitrogen treatment media, 37.5 g/L and 0 g/L increase in lipid content when compared with nitrogen treatment 75 g/L, but the cell density tends to be low. Nitrogen was a macronutrient that affected the growth and lipid metabolism in microalgae. It also impacted the functional process and cell structure (Ahlgren, 2003). The absence of nitrogen in the culture medium inhibits the proliferation of microlaga (Sharma, 2012). Nitrogen starvation also caused an increase in free fatty acids that function as cell defences (Belloti, 2013). Optimization of lipid production was influenced by many factors, which was nutrient limitation or nutrient starvation in growth

and cellular components (Khozin-Goldberg, 2002). (Ip & Chen, 2005). In the end of the explanation that treatment without Si given to diatoms causes a decrease in photosynthetic activity when compared to diatoms that obtain Si supply. In Silica's abundant environment, *S. costatum* used its energy to survive rather than for cell division. In Silica's deficient condition, the formation of the cell wall which is made from Silica becomes abnormal because Silica's supply is reduced. So that the formation of cell/frustula walls was imperfect. This affects the turgidity of the cell. When the environment was hypertonic while hypotonic cells can harm cells. When the hypertonic environment, fluid penetrated the cell that had a hypotonic state continuously so that the cell broken. For self-defense, It required lipids for osmoprotection. This was in accordance with (Moll, 2014) stating that in gripping conditions, an organism will limit itself to growth but is more directed to defense, one of which increases lipids to maintain osmotic potential in cells, so that *S. costatum* can maintain its turgidity in environment that lacks Silica.

Aeration treatment was also affect microalgae lipid content beside N and Si nutrient factors. *S. costatum* with the highest lipid accumulation was N2Si2 treatment (Nitrogen 37.5 g/L and Silica 15 g/L) 48 aerated hours, N2Si3 (Nitrogen 37.5 g/L and Silica 0 g/L) and N3Si3 (Nitrogen 0 g/L and Silica 0 g/L) 24 aerated hours. It suspected to contain low lipid in the treatment of N1Si1 (Nitrogen 75 g/L and Silica 15 g/L 0 aerated hours, N1Si3 (Nitrogen 75 g /L and Silica 0 g/L) 24 aerated hours, N2Si3 (Nitrogen 37, 5 g/L and Silica 0 g/L) 48 aerated hours, and N3Si3 (Nitrogen 0 g/L and Silica 0 g/L) 48 aerated hours. Excessive aeration was lead excess oxygen supply and higher pressure (Affifah, 2013), so microalgae was try to defend themselves by accumulating lipids.

Lipids produced by microalgae divided into polar lipids and non-polar lipids. Non polar lipids in TAG-shaped (Triacylglycerides) consisting of saturated and unsaturated fatty acids. It processed into biodiesel through transesterification. Phospholipids were polar lipids in the form of sterols which acted as constituents of cell membranes and organelles.

Microalgae utilized TAG to change energy metabolic pathways so that cells defend themselves in conditions that were not optimal or stressed. Lipid synthesized especially TAG during photosynthesis (bright reactions). It stored in the cytosol whereas non-polar lipid generated into polar in a dark reaction (Thomson, 1996). TAG synthesis was consists of 3 steps. The first step was the formation of acetyl CoA that occurr through photosynthesis. Chain elongation of fatty acids was the second step. The last was the formation of triglycerides (Jeffreys, 2013). Acetyl CoA converted to malonyl CoA catalyzed by Acetyl CoA Carboxylase enzyme (ACCase). ACCase was trigger triglyceride synthesis from the glycerol 3 phosphate pathway. The rise of TAG impacted to fatty acid and lipid content accumulation at the end (Katilli, 2012). In normal conditions the results of microalgae lipid metabolism were relatively low at 5-20% of dry weight. On the contrary when microalgae stressed they changed the lipid biosynthesis pathway to the formation and accumulation of non-polar lipids (20-50%) from dry weight, especially in the form of TAG. TAG is a precursor of biodiesel raw materials (Hu, 2008).

Positive results indicated by the presence of yellow luminescence in the cell when exposed under a fluorescence lamp, while the red luminescence gave negative results (Mata, 2010). Microalgae containing lipids was tend to show a shiny yellow color. According to (Gunawan, 2010), the red color was occur because Nile Red react to microalgae cell lipids by changing the ligand to yellow. Luminescence results of the color of the *S. costatum* cell based on the qualitative test color index are seen in Figure 3.

Conclusion

Nitrogen, silica and aeration stress effected on lipid accumulation for cell. It informed that the highest total lipid reached at N2Si2 treatment (KNO_3 37.5 g/L and Na_2SiO_3 15 g/L) with 48 aeration hours, N2Si3 (KNO_3 37.5 g/L and Na_2SiO_3 0 g/L) and N3Si3 (KNO_3 0 g/L and Na_2SiO_3 0 g/L) 24 aeration hours which indicated by yellow luminescence

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