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# EFFECT OF RED LIGHT-BLUE AND NITROGEN STARVATION ON GROWTH OF MICROALGAE *Tetraselmis* sp.

Thoriq Teja Samudra, Hilda Wati Nasution, Wiga Pratama, Chandra Pradhitaningrum, Rezika Alyza, and Eko Agus Suyono

Biotechnology Laboratory, Faculty of Biology,
Gadjah Mada University, Yogyakarta
Corresponding author: Eko Agus Suyono
Email: eko_suyono@ugm.ac.id

## ABSTRACT

Microalgae has many benefits for human being. Microalgae is potential source for biodiesel and bioethanol, but the biomass produced from the culture is relatively low. So that, the research has to be done to improve the productivity of the microalgae. Red light and blue light spectrum are known as effective spectrums for the photosynthesis process. Then nitrogen starvation medium is usually performed in order to improve the lipid content. However, nitrogen starvation treatment will decrease microalgae biomass. Therefore, this research aims to study the effect of red and blue light and nitrogen starvation on the growth of *Tetraselmis* sp. The parameters measured were the cells number, dry weight and chlorophyll a and b. In this study, microalgae *Tetraselmis* sp. was treated using red and blue light for 7 days. Then, followed by nitrogen starvation treatment with concentration 100%, 50%, and 0% of the f/2 normal medium until day 14. The number of the cells was counted every day for 14 days using a haemocytometer and dry weights were counted at day 0, 1, 5, 7 and 14. Chlorophyll contents were calculated by Jeffrey and Humphrey's Trichromatic Equations method at absorbance 664 nm, 647 nm, 630 nm, and 750 nm. The results indicated that the red light increased the number of cells to twice as normal, while the blue light increased the number of cells to 1,5 times as normal. Furthermore, the 50% of nitrogen treatment under the red and blue light increased the dry weight to 25% and 60%.

Key words: Microalgae, *Tetraselmis* sp., Nitrogen Starvation, Blue and Red light

## INTRODUCTION

Nowadays, energy for human needs is increase. According Nuryadhyn (2012), in Indonesia, the energy consumption by the public is still focused on fossil fuel. Fossil fuel consumption is currently reaches 1.3 million barrels per day. Meanwhile, oil production by the contractor only reached 900,000 barrels per day. Therefore, it is necessary to develop alternative energy sources.

Alternative energy sources have actually been developed, they can be either bioethanol or biodiesel. Bioethanol and biodiesel using feedstock are derived from food crops such as oil palm, castor, sugarcane, corn, and soybeans.

One of the potential bio-based fuel sources is microalgae. Microalgae can produce lipids that can be used as a substrate for biodiesel. Compared with corn and palm oil, productivity of microalgae is much higher, where the productivity of biodiesel from microalgae can reach 58 700 L / ha, while palm oil only 5,950 L / ha (Chisti, 2007). One of microalgae that potential to develop as a biofuel is *Tetraselmis* sp. which has a relatively high lipid content, which may reach 15-23% of the dry weight (Chisti, 2007).

The productivity of microalgae needs to be increased. It is done by optimizing its photosynthetic. Blue light and red light are known to be effectively used for photosynthesis reac-


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tion, because the best light absorption of chlorophyll is under blue light and red light. (Taiz & Zeiger, 2002). In general, the blue light on microalgae increased photosynthetic activity. Furthermore, blue light also regulates metabolism in microalgae. In one study, blue light increased lipid productivity followed by increased productivity and decreased carbohydrate protein in microalgae (Marchetti *et al.*, 2012). To increase the lipid content in microalgae culture stressing methods can be used to reduce the levels of N in the growth medium. This is because in these conditions the carbon metabolic pathway is directed to the synthesis of lipids (Allsul & Omar, 2012; Hu, 2004).

## MATERIALS AND METHODS

### Materials

Materials used in this study are *Tetraselmis* sp. ancol isolates, sea water, distilled water, alcohol, and chemicals to make the f/2 medium, The tools used in this study are thermometers, culture bottles, aerator, aerator hose, erlenmeyer, refractometer, pH indicators, autoclave, microscope, and haemacytometer.

### *Tetraselmis* sp. culture

*Tetraselmis* sp. cultured in medium f/2 with a bottle. A total of 50 mL isolate *Tetraselmis* sp. Inoculat µ…ñed in 150 mL of medium f/2, incubated for one week with continuous aeration and illumination. The cultures were incubated with a different light (blue, red, and white as a control). Lighting for 18 hours each day. After 1 week of microalgae cultures that had been treated with a different lighting transferred into a f/2 medium with a nitrogen content 100%, 50% and 0% of the prescription

### Growth Parameters

The growth rate of *Tetraselmis* sp. was determined by measuring the number of cells, dry weight and the content of chlorophyll a and b in all treatments. Culture samples were taken every 24 hours during the incubation period. Number of cells was counted under a light microscope using a haemocytometer. Biomass productivity *Tetraselmis* sp. was measured by calculating the dry weight at the beginning and end of each treatment and control. Calculation of chlorophyll a and b was performed on days 0,1,3,5,7, and 14 by using the Jeffrey and Humphrey's Tricrhomatic Equation method.

## RESULT AND DISCUSSION

Table 1 showed that the growth of cultured microalgae *Tetraselmis* sp. with the highest number of cells was under the red light treatment with 50% N medium (4.8 million cells.ml$^{-1}$). The highest dry weight was produced by culture of *Tetraselmis* sp.was under red light with 100% nitrogen f/2 medium. Highest chlorophyll content was obtained in cultures of microalgae *Tetraselmis* sp. treated with blue light.

The results showed that the red light was effective in increasing the number and dry weight of cells. This could be occurred because red light has a wavelength that could be used by *Tetraselmis* sp. to carry out the photosynthesis process effectively. So *Tetraselmis* sp. grown faster, it could be seen from the acquisition of cell number and dry weight of red

light treatment. While blue light also had the optimal wavelengths for photosynthesis, but the blue light had more energy than red light (Marchetti *et al.,* 2012). The blue light treatment effected on chlorophyll synthesize, because the blue light provided more energy for photosynthesis. The *Tetraselmis* sp adapted it by producing more chlorophyll.

Table 1. Number of cells, the amount of chlorophyll, and dry weight of *Tetraselmis* sp. on all treatments

| Treatments | Red light | Control | Blue light |
|---|---|---|---|
| Medium N 0% | Σ Cell : 2,5 million cell.ml$^{-1}$<br>Σ Chlorophyll : 6 ng.ml$^{-1}$<br>Dry weight : 300 µg.ml$^{-1}$ | Σ Cell : 2,3 million cell.ml$^{-1}$<br>Σ Chlorophyll : 7 ng.ml$^{-1}$<br>Dry weight : 310 µg.ml$^{-1}$ | Σ Sel : 2,3 million cell.ml$^{-1}$<br>Σ Chlorophyll: **7,5 ng**.ml$^{-1}$<br>Dry weight : 250 µg.ml$^{-1}$ |
| Medium N 50% | Σ Cell: 4,8 million cell.ml$^{-1}$<br>Σ Chlorophyll: 6 ng.ml$^{-1}$<br>Dry weight : 400 µg.ml$^{-1}$ | Σ Cell: 1,5 million cell.ml$^{-1}$<br>Σ Chlorophyll : 7 ng.ml$^{-1}$<br>Dry weight: 325 µg.ml$^{-1}$ | Σ Cell : 3,2 million cell.ml$^{-1}$<br>Σ Chlorophyll : 7,5 ng.ml$^{-1}$<br>Dry weight: 310 µg.ml$^{-1}$ |
| Medium N 100% | Σ Cell : 3 million cell.ml$^{-1}$<br>Σ Chlorophyll: 6 ng.ml$^{-1}$<br>Dry weight : 500 µg.ml$^{-1}$ | Σ Cell: 1,5 million cell.ml$^{-1}$<br>Σ Chlorophyll : 7 ng.ml$^{-1}$<br>Dry weight : 320 µg.ml$^{-1}$ | Σ Cell : 2,1 million cell.ml$^{-1}$<br>Σ Chlorophyll : 7,5 ng.ml$^{-1}$<br>Dry weight : 350 µg.ml$^{-1}$ |


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