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Microalgae *Dunaliella salina* (Teodoresco, 1905) Growth using the LED Light (Light Limiting Dioda) and Different Media

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Abstract

The purpose of this research was to analyze the growth of *Dunaliella salina* (Teodoresco, 1905) with different media types and based light source of LED as well as obtaining the optimum media and light source that generate the highest growh of *D. salina*. The data was analyzed using ANOVA. The material used was *D. salina* which cultured on 3 L bottles, with three treatments and three replications. The treatments given were respectively Walne Jepara, Walne Lampung, and Za added with NPK, which were then illuminated by using red LED and blue LED. The density of *D. salina* cells were determined using a light microscope with a *Neubauer chamber*. The highets result of *D. salina* density is 8.504×10^4 cell · mL^{-1} which produced by the red LED treatment in the media of Walne Jepara. The results of blue LED treatment showed that *D. salina* density was 5.768×10^4 cell · mL^{-1} with Walne Jepara.

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Keywords: Culture growth; *Dunaliella salina* (Teodoresco, 1905); LED; Walne Pro–analyze; Walne technic

1. Introduction

Dunaliella salina (Teodoresco, 1905) is a microalgae and their cell was highly responsive to osmotic changes permitting arapid changes in the cell shape. Reproduction of *D. salina* is asexual flagellated cells that may produce

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thick walled cysts, which allow survivorship under an environmental stress. The accumulation of carotenoids in *D. salina* allowed the intensity of light as the main stimulus for *β*-carotene references by Lamers et al. (2010). Utilization of *D. salina* was quite diverse ranging from health food as it has been marketed in developed countries (Chang et.al., 2011). *D. salina* in response to stressful conditions such as high light intensity, temperature and salt concentration causes the buildup of various secondary metabolites such as lycopene, β-carotene, lutein and zeaxanthin (Lamers et.al., 2012). Microalgae are expensive to produce, although many efforts are under way addressed to achieve cost–efficient modes for mass cultivation of these organisms. Different systems have been designed for the growth and handling of microalgae on a large scale references by Zainuri et.al., (2008). The objective of this research was to analyze the growth of *D. Salina* at different media types based on light and source of LED (light–emitting dioda), as well as obtaining the optimum media and light source that generate the highest growth of *D. salina*.

Medium Walne is a basic medium that is often used in the cultivation of *D. salina*. However, this basic medium has the shortcomings include the nutrient content that is still able to accelerate the growth of *D. salina*. Pro-analyze walne Jepara of fertilizer is very expensive so if they are used for mass scale by the public will be detrimental to farmers (Andersen, 2005). Therefore, the aim of the research was to looking for an alternative nutrient thus saving fertilizer in cultivation of *D. salina*. The first treatment that is done to meet the nutrient requirement is the provision of ZA fertilizer containing ammonium sulfate or $(NH4)_2SO_4$ and non–technical walne.

The microalgae cultivation facilities typically use sea water enriched with nutrients, especially carbon, nitrate and phosphate (Fu et.al., 2012). *D. salina* need a complete nutrient composition can affect the concentration of biomass production and nutrient content of microalgae. The research to increase the growth of microalgae is to control the content of both macro and micro nutrients in the cultivation environment (Harisson, 2005).

2. Material and methods

The material used was *D. salina* from the brackish water aquaculture in Jepara in Central Java, Indonesia and then it cultured in 3 L bottles, with three treatments and three replications. The method used was Completely Randomized Design (CRD) in time. The purpose of the Completely Randomized Design in time was to determine the effect of treatment on the provision in improving water quality (Creswell, 2010).

The treatment given were as follows: type media the first was Walne pro-analyze from brackish water from Jepara, Central Java, Indonesia, second Walne techniques from backish water from Lampung in North Sumatra Indonesia and the last was media ZA (ammonium sulfate) added NPK (Nitrogen Phosphorous Potassium). The factor two was were then illuminated by using red LED and blue LED.

Cells were determined by direct counting, using a light microscope (magnification 40×) with a Neubauer chamber basic Hemocytometer Usage. The Neubauer chamber was a thick crystal slide with the size of a glass slide (30 mm × 70 mm and 4 mm thickness).

Culture of *D. salina* can be done when it reaches the peak of the population. Population peaks can be seen from the color change in the culture medium and the total population based on growth patterns. According to some sources, harvesting was done at the time of *D. salina* was located at the end of the exponential phase, approximately on 7 d. The culture has reached a population peak was precipitated first by lethal aeration. Then the solids obtained by using a centrifuge, and then weighed to determine the biomass produced. *D. salina* then has weighed, dried at cool room temperature, around 21 °C for about 4 d, from each sample.

The research was carried out in the Laboratory of Marine Biology, University of Diponegoro, Semarang, Indonesia. The data were analyzed using SPSS factorial analysis of variance (ANOVA two ways).

3. Result and discussions

The results showed that on difference media types and based light source given different real influence on the pattern of growth of *D. salina*. The result of growth of *D. salina* were highest of 850.4×10^{-4} cell · mL^{-1}, in using a media Walne Jepara and red LED. The highest results of blue LED was of 576.8×10^{-4} cell · mL^{-1}, Walne Jepara.

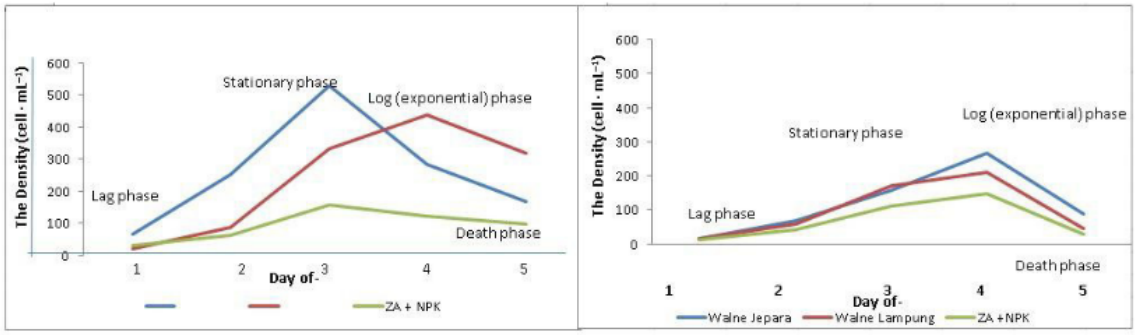
Test statistics on cell density data *D. salina* showed that the normal kinds of data spread, homogenous and are additive. Data source of light and the media is very significant or significant to the growth of *D. salina*. The highest

growth results by using red LED was Walne Jepara Figure 1 (a). The content is $FeCl_3.6H_20$, $FeCl_2.4H_20$, H_3BO_3, EDTA, $NaH_2PO_4.2H_2O$, $NaNO_3$, and trace metal solution. This makes the content of the growth of grow with perfect shapes cell wall. A red LED makes growth lasts for 3 d with the highest density.

Nevertheless Walne Lampung growth results for 5 d until the stationary phase and the density was almost equivalent to Walne Jepara. ZA added with NPK has the lowest density growth and selected mineral ingredients contained in ZA added with NPK not good enough for the growth of *D. salina*. The use of red LED give very real effect on the rapid growth of *D. salina*, it was suggested that the red color beams of LED triggered more rapid the reproduction of *D. salina*. The growth phase of cultivation *D. salina* with blue LED Figure 1 (b) give the result that it was the highest cell density compared with the Walne Lampung and ZA added with NPK. However, the length of time it takes of *D. salina* to grow longer than the red LED. This was suggested that the length of blue light to perform photosynthesis and reproduction does not trigger the stress on *D. salina*. The research has analyzed the growth *D. salina* in different media types and on based light source.

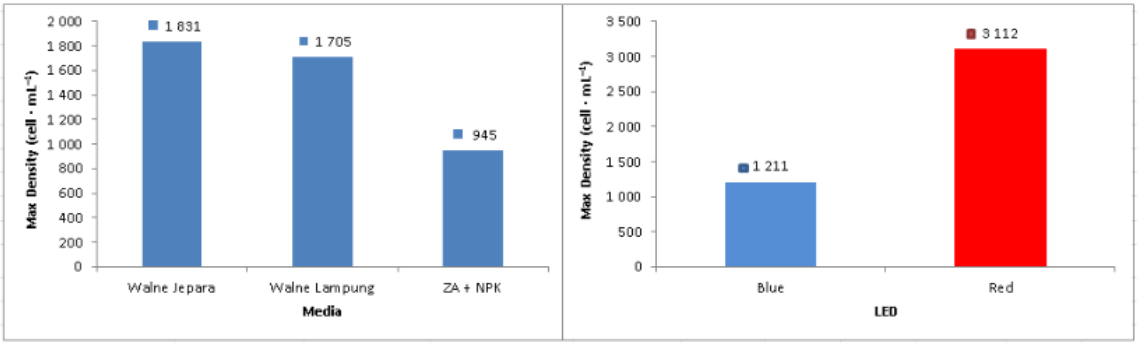
Table 1 Maximum cell density and specific growth rate of *Dunaliella salina*

Light LED	Walne Pro–analysed	Walne Technical	ZA added with NPK
	Maximum cell density (cell · mL^{-1})	Maximum cell density (cell · mL^{-1})	Maximum cell density (cell · mL^{-1})
RED	8.504×10^4	5.242×10^4	4.897×10^4
BLUE	5.768×10^4	5.725×10^4	5.317×10^4



(a) (b)

Figure 1a. The density of *D. salina* treated with red LED (b); the density of *D. salina* treated with blue LED



(a) (b)

Figure 2a. Media effect on the growth *D. salina* (b); the LED effect on the growth *D. salina*.

D. salina growth in this study includes five phases, namely the phase of adaptation, specific or exponential growth phase, stationary phase, the phase of maximum density or peak and death phase. The results of the study Figure 2 (a) and Figure 2 (b) showed the results of a real influence on the growth of media and sources giving different LEDs.

These results were confirmed by the processing of ANOVA using SPSS 16, which explains that the effect the media and the LED shown to have significantly different conclusions with a value of 0.000, which means significant, as well as significant value on the LED has given 0.000 results. As for effect media and the LED has a fixed value of 0.031, which means significantly to the growth of *D. salina*.

Differences in the duration of adaptation allegedly because of differences in nutrient content of fertilizers applied to different light sources. *D. salina* cell growth patterns in the adaptation phase or phase lag all observed within the time duration for 4 hours in less than one day later accompanied by the growth rate.

D. salina has a generation time is very fast, therefore, in a relatively short time, the multiplication of the cells will occur very quickly, especially if the available light as an energy source, although in a minimal amount. At the optimum culture conditions, the growth rate in this phase can achieve growth with the maximum. The regression analysis shows that the growth of medium and light sources significantly affected the adaptation phase.

Photosynthesis Occurs in two stages. In the first stage, the light reaction or light reaction absorbs light energy and use it to generate energy storage molecule ATP and NADPH. The second stage, the dark reactions using this product to absorb and reduce carbon dioxide. When the cells were exposed to light *D. salina* they start producing carotenoids. Green cells dominated by chloroplast Began to change orange. Chloroplasts shrinks, decreasing the size of the chloroplast membrane and carotenoid- lipid-containing granules.

4. Conclusion

The growth of *D. salina* in different media types and based light source of LED: the highest result of *D. salina* density was 8.504×10^{-4} cell · mL^{-1} which produce by the red LED treatment in the media of Walne Jepara. While the results of a blue LED showed a *D. salina* density of 5.768×10^{-4} cell · mL^{-1} in Walne Jepara medium. The results by Duncan test results and states that the value of 6:41 > 1.08, which means the media and the LED significantly affect the growth of *D. salina*.

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