

# Effect of difference of Photoperiod in culture chlorella sp. with the continuous photobioreactor system

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**Abstract**— Research on the effects of differences in cultures of *Chlorella sp* photoperiod. with a continuous photobioreactor system. This research was conducted in April 2019 at the laboratory of Fish Hatchery and Breeding University of Riau. The purpose of this study was to find out the right photoperiod in *Chlorella sp*. culture by using a blue LED light with a continuous photobioreactor system to population density and specific growth rates. The method used is the experimental method by using a completely randomized design with four treatments and 3 repetitions. The treatment used is P1 (24 hours bright - 0 hours without lighting), P2 (20 hours bright - 04 hours without lighting), P3 (16 hours bright - 08 hours without lighting), P4 (12 hours bright - 12 hours without lighting). The results of the study found that lighting 16 hours bright - 08 hours without lighting give the best results with a cell density of  $336.67 \times 10^4$  cells / ml and the specific growth rate of 0.341 / day d with peak growth occurring on day 8.

**Keywords**— photoperiod, photobioreactor, *Chlorella sp*.

## I. BACKGROUND

Natural feed is live food for fish larvae and seeds includes phytoplankton, zooplankton and benthos. Feed use in larval rearing dominant influence on fish growth because feed functions as an energy supplier to spur growth and maintain life (Melianawati, 2005). Small fish (larvae) need more feed with nutrient content especially higher protein content than large fish (Djarifah, 1995). Many high protein content found in natural food in the form of phytoplankton type *Chlorella sp*. This species is widely consumed by the larvae of fish like milkfish, tilapia and others. Although the availability of natural food can be replaced with artificial feed, high protein requirements for fish larvae cannot be fulfilled by artificial feed.

Optimizing the capabilities of microalgae can be obtained by designing a photobioreactor. Photobioreactors are places where conversion takes place involving certain organisms to be the desired result (Jordening and Winter, 2005). Besides being more efficient in using the place, culture with photobioreactor systems is also easy to do and control, smaller contamination and higher productivity because of the high agitation process (Nadya, 2017).

Photobioreactors are divided into two types, namely closed photobioreactor and open photobioreactor. Conditions on closed photobioreactor are easier to control and the possibility of contamination of microalgae is smaller compared to open photobioreactors. One type of closed photobioreactor is Tubular photobioreactors which have the highest photosynthetic efficiency compared to other closed photobioreactor types (Hadiyanto et al., 2012).

Giving light greatly affects the photosynthesis process of plants one of them is microalgae. Microalgae are photosynthetic organisms, microalgae absorb light in the form of photons. The energy of the photon will be used by chlorophyll to break hydrogen bonds in water which will later be with CO<sub>2</sub> in photosynthesis it will be used to produce O<sub>2</sub>. *Chlorella sp*. able to photosynthesize using artificial light sources. One light source that can be used is an LED light (light emitting diode), blue LED wavelengths range from 450 - 500 nm (Syafriyudin et al., 2015), while the light that can be absorbed in photosynthesis is light with a wavelength of 380nm to 700nm. But you need to know how long the radiation or photoperiod is right to produce optimal microalgae culture. Based on these considerations it is necessary to do research on how long the best photoperiod in *Chlorella sp*. culture by using blue

LED (light emitting diode) lights on a continuous bioreactor system.

## II. RESEARCH METHOD

This research was conducted in April 2019 in the Laboratory of Fish Spawning and Breeding, Faculty of Fisheries and Marine Sciences, University of Riau. The first step before starting the research is preparing tools and materials to be used. The material used in this study is *Chlorella* sp. which comes from the Laboratory of Microalgae, Faculty of Fisheries and Marine, University of Riau as material to be observed, fertilizer as a source of nutrients and nutrients for microalgae, and water. The tool used in this study is 4-liter glass containers totaling 12 pieces as a photobioreactor tool, LED lights are used as light sources, water pump used to drain *Chlorella* sp. so that the agitation process occurs. The rack is used as a photobioreactor, pH meter, DO meter and thermometer used to measure acidity, oxygen and temperature levels of cultured water, tissues and napkins as a glass cleaner and other equipment. The method used in this study is experimental method. The design used is a Completely Randomized Design (CRD) one factor with 4 treatments and 3 replications. In this study used LED (Light Emitting Diode) lamps with wavelengths ranging from 450 - 500 nm (Syafriyudin et al., 2015). With the intensity of light used is 1000 lux, According to previous research (Utami et al., 2012) the treatments used in this study are as follows.

P1: 24T-0G (for 24 hours lighting is given 24 hours and 0 hours without lighting)

P2: 20T-04G (for 24 hours lighting is done 20 hours and 04 hours without lighting)

P3: 16T-08G (for 24 hours lighting is done 16 hours and 08 hours without lighting)

P4: 12T-12G (for 24 hours lighting is done 12 hours and 12 hours without lighting)

After the container is cleaned then the container is filled with 3.5 liters of clean water and given 400 ml of liquid fertilizer (dahril solution) in each container as a

source of nutrients, circulation with a pump is given to stir the fertilizer to be evenly distributed, then add 100 ml of *Chlorella* sp. in each container (Djarjah, 1995), the calculation of cell numbers is first done using the haemocytometer which was observed under a microscope with the help of a hand counter. *Chlorella* sp. which is then further illuminated by LED lights according to each treatment. The process of adding *Chlorella* sp and fertilizer can be seen in Figure 1.

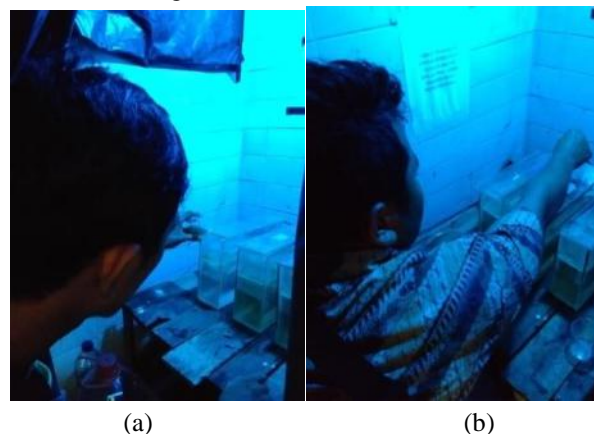


Fig.1. a. Addition of *Chlorella* sp. b. Addition of fertilizer

The parameters measured in this study are cell density / ml and specific growth rate (SGR), and parameters Water quality measured is temperature, dissolved oxygen, and pH.

## III. RESULTS AND DISCUSSION

### Cell Density / ml

Early in the culture, it was known that stock *Chlorella* sp. amounting to 700,000 / ml. Growth of *Chlorella* sp. can be seen from the increasing density of *Chlorella* sp. From the results of observations of *Chlorella* sp. in this study showed a growth pattern that is divided into several phases. Data density of cells / ml of culture observation *Chlorella* sp. during the research takes place can be seen in Figure 2 below.

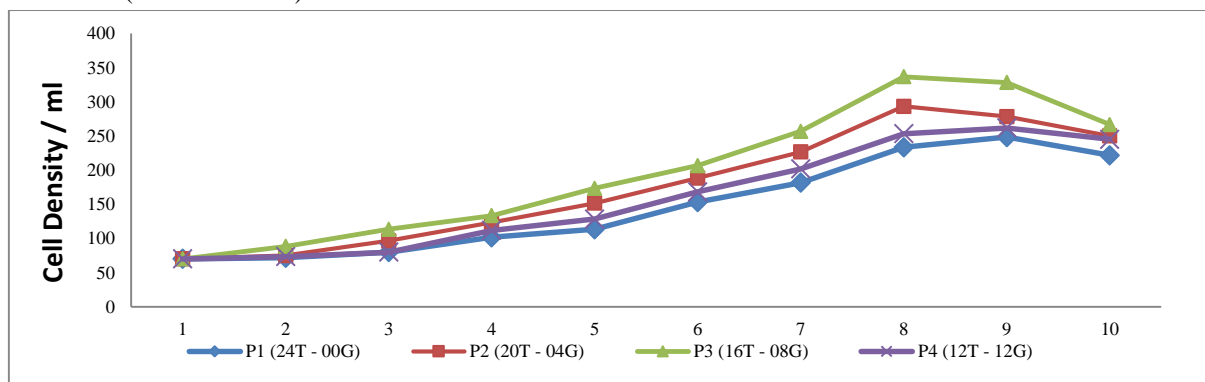


Fig.2. Average Cell Density / ml Graph

Note : Value of cell / ml density in  $10^4$

P1: 24 hour bright photoperiod treatment - 0 hours without lighting

P2: 20 hour bright photoperiod treatment - 4 hours without lighting

P3: 16 hour bright photoperiod treatment - 08 hours without lighting

P4: 12 hour bright photoperiod treatment - 16 hours without lighting

Based on Figure 2 it can be seen that in general, the population growth pattern of *Chlorella* sp. following the pattern of plankton growth in general, that is adaptation phase, exponential phase, the phase of the peak and decline phases of growth. In the adaptation phase, at P1, P4, it occurs on day 1 to day 2 while in the P2 and P3 treatment there is no adaptation process.

The results showed that the highest peak *Chlorella* sp. cell density in each treatment of different light periodicity occurred on different days. In figure 2 shows the highest cell density occurs on days 8 and 9, in treatment (16T-08G) and (20T: 04 G) population peak occurred on day 8 with cell density  $336.67 \times 10^4$  cells / ml and  $293.33 \times 10^4$  cells / ml. In the treatment (12T-12G) and (24T: 0 G) the population peak occurred on day 9 with cell density  $261.67 \times 10^4$  cells / ml, and  $248.33 \times 10^4$  cells / ml. The longer the radiation is given the higher the number of cells at the peak of the population until the irradiation time is 16 hours and when the duration of exposure increases, the number of cells eventually decreases, (Raymont 1963 in Andriyono 2001) stated that in photosynthesis there were 2 reactions namely light reaction and a dark reaction. In the light, cells will divide asexually, so that the child cells are smaller in size than the parent. While in the darkened state, cell development occurs to reach normal size.

The treatment (12T-12G) is the lowest treatment, too short lighting time affects the utilization of nutrients such as nitrate and phosphate, but microalgae need dark

conditions for their cell productivity (Meseck, et al., 2005). Giving light (16T - 8G) gives the best influence on the growth of *Chlorella* sp. because it can provide the highest cell / ml density. The statement above is supported by Ohi, et al. (2006) and Danesi, et al. (2004), that is Biomass production can decrease due to less optimal radiation or less duration of radiation while biomass can increase if the duration of optimal irradiation.

In the process of photosynthesis, light plays a very important role in both light intensity and long irradiation. Novianti (2015), states that the duration of irradiation can affect the synthesis of organic matter in photosynthesis because only with sufficient energy the process can proceed smoothly. Inthe (2012), states that, the reactions that occur in the process of photosynthesis are divided into 2 namely light reactions and dark reactions. Light reaction is very dependent on the availability of light. Light reactions are steps to convert light energy into chemical energy. Light absorbed by chlorophyll moves electron transport and hydrogen from water to the receiver (receptor) called NADP + which functions as an electron carrier in cellular respiration. Dark reactions are reactions to the formation of sugar from  $\text{CO}_2$  that occurs in the stroma. Unlike the light reaction, dark reactions or light-independent reactions can occur during day and night, however during the day the dark reaction rate is certainly lower than the light reaction rate (Inthe, 2012). In dark reactions, cyclic series reactions occur which form sugars from the basic ingredients of  $\text{CO}_2$  and energy (ATP and NADPH), which energy is used in the dark reaction is obtained from the light reaction (Novianti, 2015). Dark reaction aims to convert compounds containing carbon atoms into sugar molecules.

#### Specific Growth Rate (SGR)

Based on data obtained from observations of *Chlorella* sp. Culture. during the study, the specific growth rate can be seen in Figure 3 below by having different results in each treatment.

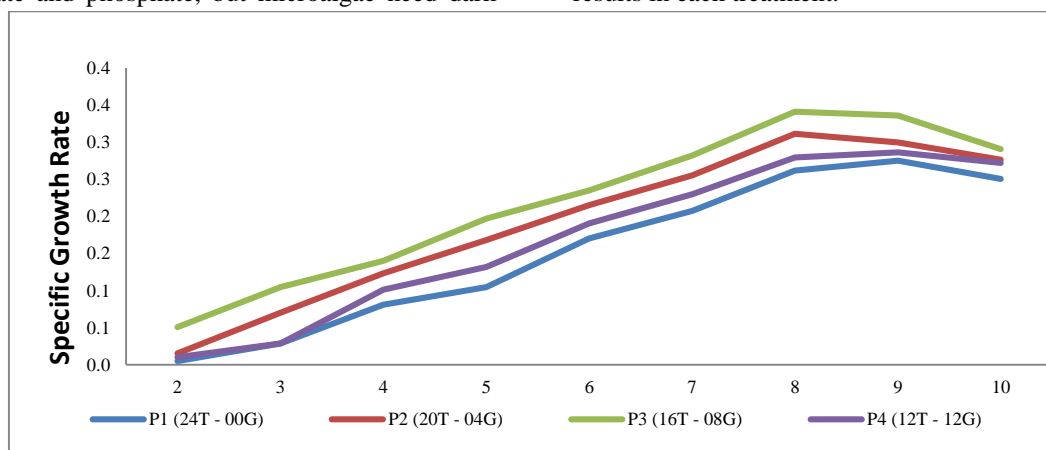


Fig.3. Graph of Specific Growth Rate

Specific growth rate is a parameter that describes the rate of growth of cells *Chlorella* sp. per unit time. Based on the results of observations on specific growth rates can also be known also the ideal time of harvesting cells *Chlorella* sp. The ideal harvest time is when the specific growth rate reaches the maximum value, because at that time the *Chlorella* sp. cell biomass reaches optimum concentration. According to Vonshak (1997), the optimum biomass concentration will correlate with the highest productivity.

Based on figure 3 the highest growth was obtained at treatment 16:08 (hours of light: dark) that is equal to 0.341 / day, then in the treatment (20G: 04 T) of 0.311 / day, then in the treatment (24G: 0T) of 0.286 / day, and in treatment (12G: 12T) of 0.275 / day. Suminto and Hirayama (1996), in their study stated that the greater SGR value means that the process of algae cell division is faster, so that cell growth per unit time will be greater than the increase in time itself. Although the maximum specific growth rate occurred at the same time, the growth rate in the treatment of 16T - 08G showed a higher value than the other treatments. This difference exists presumably due to the presence of different lighting times resulting in different cell growth rates. According to Fogg (1975) light is the energy source needed in photosynthesis, the amount of energy received depends on the quality, quantity and period of irradiation.

According to Lavens and Sorgeloos (1996) states that light irradiation must be appropriate in culturing phytoplankton, if light is too bright it will inhibit the process of photosynthesis. Long exposure very decisive in the process of synthesis organic material on photosynthesis because only enough energy the process can run smoothly. Long exposure can affect the biochemical composition were cultured besides the culture media, temperature, pH, light intensity, and stage time of harvest (Novianti, 2015).

#### WATER QUALITY

Water quality is an important factor for the growth of *Chlorella* sp. The results of measurements of water quality parameters during the study are presented in Table 1.

Table 1. Water Quality Measurement Results During Research

NO	Parameter	Value
1.	Temperature	29-33, °C
2.	pH	7-8,4
3	DO	7,3-8,5ppm

Growth of *Chlorella* sp. which is good besides being influenced by the nutritional content also influenced by environmental conditions in the media of maintenance.

Environmental factors that support the growth of *Chlorella* sp. is temperature, pH and dissolved oxygen. In this study, the range of water quality is still in good condition for the growth of *Chlorella* sp. The temperature at the time of the study reached a range of 29-32 °C. Isnansetyo and Kurniatuty (1995) state that the optimal culture of *Chlorella* sp. laboratory scale is 20-40 °C, according to Fachrullah (2011), changes in temperature affect the processes of chemistry, biology and physics, an increase in temperature can reduce the solubility of the material and can cause an increase in the speed of metabolism and microalgae respiration in the waters. The value of the degree of acidity (pH) of the media in this study is also still in a fairly good range for the growth of *Chlorella* sp., pH range obtained between 7 - 8.9. pH it is still in the optimal range for the growth of *Chlorella* sp. Ciferri (1983) in Winarti (2003) states that *Chlorella* sp. can grow well at pH 6-9. Measurement of dissolved oxygen during the study ranged from 8 ppm - 8.5 ppm and can still be tolerated by *Chlorella* sp. Oxygen Level (O<sub>2</sub>) is one of the factors that influence the growth of microalgae. Photosynthesis that works well will produce enough oxygen for the growth of microalgae Widyawatik (2018).

#### IV. CONCLUSIONS AND RECOMMENDATIONS

From the results of this study it can be concluded that giving 16-hour bright photoperiod treatment and 8 hours without lighting using blue LED lights affect cell density / ml and specific growth rate (SGR) in *Chlorella* sp. culture with a continuous photobioreactor system with cell / ml density obtained is  $336.67 \times 10^4$  cells / ml, with a specific growth rate (SGR) of 0.341 / day. It is recommended to culture *Chlorella* sp. with blue LED lights using a continuous photobioreactor system you should use a bright 16 hour photoperiod and 8 hours without lighting.

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