Tomini Journal of Aquatic Science, Vol 3(1): 1–7, May 2022
Received: 2021-02-18; Accepted: 2022-04-15

e-ISSN: 2722-3787
Tomini Journal of Aquatic Science
Homepage: http://ejurnal.ung.ac.id/index.php/tjas

The growth of *Chlorella* sp. cultivated in walne media with different intensities of light

Arafik Lamadi [1*], Mulis [1], Agatha Elda Kristanto [1]

[1]Faculty of Fisheries and Marine Science, Universitas Negeri Gorontalo, Indonesia
*Corresponding author: arafik_lamadi@ung.ac.id

ARTICLE INFO

Keywords:
Chlorella sp.; Light Intensity; Growth; Walne

How to cite:
Lamadi, A., Mulis, M., & Kristanto, A. E. (2022). The growth of Chlorella sp. cultivated in walne media with different intensities of light. *Tomini Journal of Aquatic Science*, *1*(1), 1–7

ABSTRACT

This research was composed to identify the effect of different light intensities on the growth of Chlorella sp, as well as the optimal amount of light to improve said growth. This experimental research employed a completely randomized design, with 5 treatments of different light intensities; A (3400 lux), B (4400 lux), C (5400 lux), D(6400 lux), and E (7400 lux) with 4 replications for each treatment. Based on the ANOVA, the findings revealed the different effects ofthe treatments ($p < 0.05$), in which treatment D (6400 lux) was considered the best treatment with an average density of 11,483,333 cell/ml of cultivation peak.

INTRODUCTION

One of the determining factors for the success of fish farming, namely in the hatchery stage, (Tampubolon et al., 2016) explained that the main problem found at the hatchery stage is the low percentage of survival because at this time fish larvae do not have a perfect digestive system. Qualitatively, natural food cannot be replaced by artificial feed. So that the availability of sufficient natural food is needed both in terms of quantity and quality to be able to support the success of fish farming (Chilmawati, 2008). One of the microalgae species that is widely cultivated is Chlorella sp. which is used in hatcheries as feed. Chlorella sp., Is also often used as feed for bivalve mollusks and crustacean larvae.

According to (Utami et al., 2012) Chlorella sp., Is autotrophic and has a high reproductive rate. But to meet the needs of natural feedstocks for fish or zooplankton, the culture of Chlorella sp. under normal light intensity (2500-3500 lux) still cannot meet the required target. This makes light a limiting factor in the growth of Chlorella sp. Light is used as an energy source to synthesize carbohydrates, lipids, proteins, and other organic materials to be converted into food. Based on the above background, this research was conducted to determine the density of Chlorella sp. That is high by using different light intensities for the laboratory scalable Chlorella sp. Culture.

MATERIAL AND METHODS

***Research Tools*.** The tools used in the research are: glass jars, culture racks, blowers, aeration hoses, pipettes, test tubes, measuring cups, microscopes, object-glass, haemocytometers, hand counters, refractometers, thermometers, pH meters, DO meters, lux meters, 40 watts TL lamp and 14 watts LED lamp.

***Research Materials*.** The materials used in the research were: Chlorella sp. Seeds, walne fertilizer, sterile seawater, distilled water, and 4% formalin.

***Research design*.** The method used was a completely randomized design (CRD) experiment with 4 treatments and 4 ulangan, the treatment used is the difference in light intensity. Treatment A 3400 lux, treatment B 4400 lux, treatment C 5400 lux, treatment D 6400 lux, and treatment E 7400 lux. The duration of irradiation is 24 hours bright and 0 hours dark. The data collected is in the form of Chlorella sp growth data which is observed every 24 hours and water quality data.

Procedure

Seeds and fertilizing. The seeds of Chlorella sp were obtained from the Jepara BBPBAP seed culture laboratory and were cultivated with 1 ml / L walne fertilizer.

Table 1. Composition of Walne Fertilizer

No.	Chemical Components	Dose
1.	NH4NO3	100 ppm
2.	NaH2PO4	20 ppm
3.	H3BO3	33.6 ppm
4.	Na EDTA	45 ppm
5.	FeCl3	1.3 ppm
6.	MnCl2	0.36 ppm
7.	Vitamin B12	0.001 ppm

1. Culture of Chlorella sp. As much as 2 liters of seawater media used for the culture of Chlorella sp, was taken from the waters of Jepara BBPBAP which was then sterilized and given 1ml / L walne fertilizer. Seedlings of Chlorella sp are stocked at a density of 1,000,000 cells/ml, to calculate the volume of seeds that will be stocked the formula (Mudjiman, 2004):

$V_1 = \frac{N_2 \, x \, V_2}{N_1}$ in liters or ml

Information:
V1 = volume of inoculum required
V2 = volume of water medium used
N1 = inoculum density (cells / ml)
N2 = density of chilled inoculum (cells / ml)

Light Source Preparation. The light source used is light from a 40 watt TL lamp and a 14 watt LED lamp, with each treatment receiving the light intensity according to what has beendetermined, namely treatment A 3400 lux, treatment B 4400 lux, treatment C 5400 lux, treatment D 6400 lux, and treatment E 7400 luxby adjusting the distance from the lamp to the jar and measured using a lux meter. The following is the distance used to get the light intensity according to the treatment.

Table 2. Distance the Culture Container from the Light Source

Treatment	Type of Lamp		
	LED TL lamp	LED Light Bulb	Black Plastic Cover
A	1 cm	-	-
B	1 cm	-	√
C	3 cm	15 cm	√
D	2 cm	17 cm	√
E	5 cm	6 cm	√

RESULTS AND DISCUSSION

***The density of Chlorella* sp.** The results of the analysis of variance (ANOVA) showed that different light intensity treatments had a significant effect on the growth of Chlorella sp. ($p < 0.05$). Based on the data that has been obtained, the average density calculation results for Chlorella sp. cultured with different light intensities can be seen in Figure 1.

From the first day until the second day, it can be seen that Chlorella sp. still adapting to the new environment, especially to relatively high light exposure. On day 2 entering day 3 to day 5(peak growth) growth of Chlorella sp. starts to enter the exponential phase i.e. happens increase in cell density due to the fast and constant division of microalgae. Then from day 5 to day 6, the graph culture shows that there is a decrease in cell density or begins to enter the declination phase or there is a slowdown in cell division due to reduced availability of nutrients.Furthermore, the growth stationary phase of Chlorella sp. started to be shown on the 6th to 8th day of culture, in this phase there was a slowdown in cell division due to the reduced availability of nutrients in the media and storing their products in the form of lipids, at stationary the number of living cells was the same as the number of dead cells so that growth was relatively constant (Norbawa & Yudiati, 2013).

Enter the death phase on days 9 and 10. In the death phase, it is also possible in addition to reducing the nutrients in the media, the shading effect which is a phenomenon when cells in a population do not get light because they are blocked by other cells. This indirectly affects the ability of cells to carry out photosynthesis (Muchammad et al., 2013). From the results above, it can be seen that the best growth pattern occurs at treatment D (6400 lux) with the highest average density of 11,483,333 cells/ml because on day 5 it reached the highest density and in the stationary phase and entered the death phase of Chlorella sp. Shows a steady growth pattern. Whereas in treatment E (7400 lux) the growth reached a peak faster but there was a drastic decrease on the 5th day until the last day of maintenance. This is thought to have happened because in treatment E (7400 lux) more light absorption and accelerated growth rate. (Rasyid, 2009) states that the photosynthetic process can take place quickly or slowly because it is influenced by light, when the photosynthesis process is fast it will produce more energy, and some of it is stored by microalgae in the form of food reserves.

However, this also causes cell growth to decline faster because in the stationary phase the availability of nutrients in the culture media of Chlorella sp. is reduced due to being absorbed more at the exponential phase to compensate.

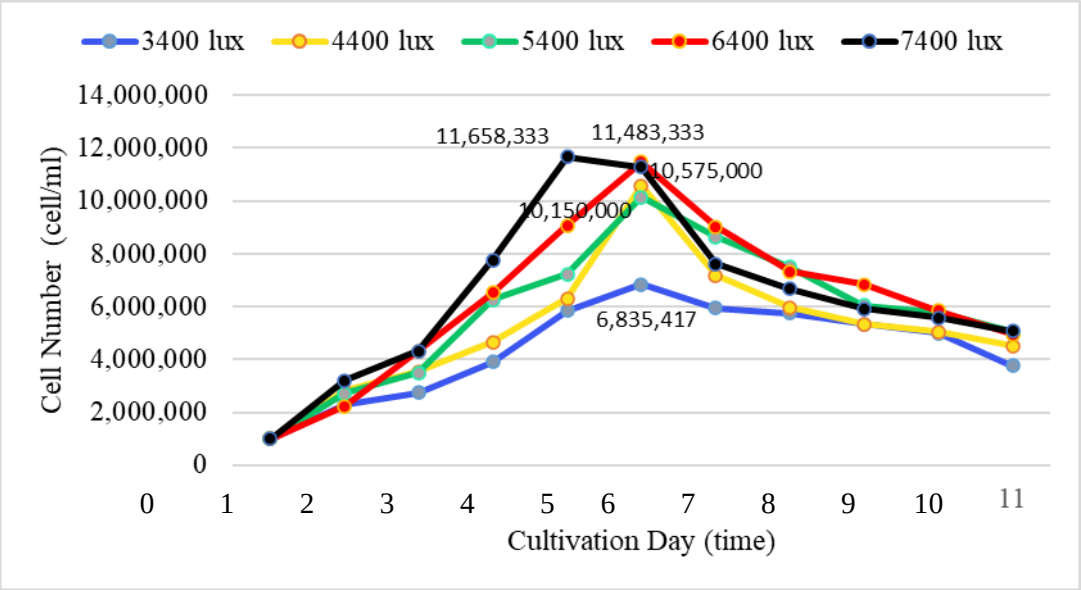

Figure 1. Growth curves of *Chlorella* sp. (cells/ml) under different light intensities

For the absorption of energy from light, and because too high light exposure allows photosynthesis inhibition and even damage the death phase in the 7400 lux treatment occurs more quickly due to cell damage. This is supported by (Lehmuskero et al., 2018) who state that when the light intensity is too high, photo-inhibition will occur in the photosystem II process and will produce radicals resulting in photo-oxidative damage, and in some cases, this can be a contributing factor. death in microalgae.

(Kim et al., 2019) also stated that light intensity can influence the effectiveness of photosynthesis if it is used at the optimal intensity for microalgae, but if exposed to too high light the microalgae will make adjustments by reducing the chlorophyll content or limiting light absorption to minimize light absorption and prevent photo-damage from happening.

The inhibiting factors that were found were The presence of contaminants in the form of protozoa that are thought to come from the aerator pipe, but the population of protozoa in the culture does not grow so much that it does not have the potential to inhibit the growth of Chlorella sp., this also depends on the space of the culture media and available nutrients. (Susanti et al., 2013) stated that when microorganisms (bacteria/protozoa) enter the microalgae culture they can potentially become competitors, this can cause a decrease in the growth rate of the microalgae due to limited nutrients in the culture media while the more microorganisms in it, thereby reducing the speed at which microalgae can divide.

***Water quality*.** One of the parameters that have a direct relationship with light intensity is temperature, there is low-temperature light intensity is the limiting factor for photosynthesis. The results of the water quality analysis during the study are presented in table 3.
The temperature range for this measurement is from 20-24℃, this is normal for the growth of Chlorella sp. This is supported by (Choi & Lee, 2011) explaining that temperature has a relationship with a light intensity which can affect the metabolic rate of microalgae. Microalgae do not have an internal temperature control function which affects the biomass components of the microalgae, metabolism, and metabolic reaction speed. The more the temperature increases, the photosynthetic rate will also increase, but at high-temperature conditions, it canslow down the rate of photosynthesis rapidly. This shows that the photosynthesis process is influenced by enzymes. This temperature range is included in the optimal range of 20-24 ℃,

according to (Nurhayati & Hermanto, 2013), namely the optimal growth of Chlorella sp. 23-30 ℃.

The range of pH values during the maintenance period for all treatments was between 7.6 - 8.8. The pH value increased along with the increasing growth of Chlorella sp., During the maintenance period, it was seen that the increase in pH began to appear on day 2, b ffffffut the increase was relatively small for all treatments. The range of pH values in this study wasstill within the normal pH range for maintenance of Chlorella sp. However, at a higher light intensity of 7400 lux, the pH tends to increase higher than other treatments during the maintenance period. This is by (Gong et al., 2014) who showed that at a light intensity of 7920lux and 11920 lux the pH of water increased to 10.8.

In this study, the DO concentration range was 6.9-7.2 mg/L. The concentration does not experience an excessive decrease or increase due to the direct supply of a controlled aerator. However, it can be seen that the highest concentration change occurred in treatment E (7400 lux), which was the highest at 8.84 mg/L, this is presumably because at the highest light intensity there was a higher photosynthetic rate than other treatments and produced more O_2 as a product of photosynthesis.

Table 3. Results of Water Quality Measurements

Treatment	Range of Water Quality Measurement Results					
	Salinity (g/L)	Temperature (℃)	pH	DO (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
A	30-32	19.2-24.2	7.68–8.03	7.0–7.2	1.139	1.681
B	30-32	22.5-24.1	8.02–8.63	7.0–7.1	0.899	1.066
C	30-32	21.3-23.3	8.08–8.61	7.0–7.1	0.894	0.944
D	30-32	22.2-23.8	7.64–8.51	6.9–7.2	0.759	0.708
E	30-32	22.8-24.4	8.31–8.84	7.0–7.1	1.035	0.482

Dissolved oxygen in the waters is obtained from the photosynthesis of plants that have chlorophyll. The higher the density in the treatment, the higher the dissolved oxygen content in the culture media of Chlorella sp in the treatment (Susanti et al., 2013).

In controlled conditions the DO concentration supplied from the aerator must be adjusted, if the aeration is too fast it will result in more water evaporation and have a bad impact on microalgae. (Kazbar et al., 2019) stated that at high light intensity large DO concentrations can cause photochemical damage to the photosynthetic apparatus and result in a decrease in the concentration of pigments in microalgae cells.

From the observation of salinity values, it was obtained a range from 30-32 ppt, the increase in salinity was thought to be due to evaporation in the culture of Chlorella sp. presumably due to strong aeration and temperature changes. However, this salinity range is still within normal limits for the growth of Chlorella sp. namely 15-35 g/L (Aprilliyanti et al., 2016).

Nitrogen and phosphorus are the fundamental factors in microalgae cells. Nitrogen present in cells is used to synthesize enzymes and cell structures. Meanwhile, phosphorus contributes to various metabolic processes such as energy production and photosynthesis. However, if ammonium is too high (reaching a certain level) it can cause ammonium poisoning in cells because the ammonium produced by the assimilation process in cells cannot be quickly transferred to amino acid synthesis and will affect growth (Feng et al., 2020). From the measurement results, the concentration of nitrate and phosphorus is0.759-1.139 mg/L and 0.482-1.681 mg/L, these concentrations are classified as optimal for the growth of Chlorella sp., (Aprilliyanti et al., 2016) stated that Chlorella sp. requires a nitrate content of 0.9-3.5

mg/L and phosphate 0.27-5.51 mg/L. Nitrate content at levels below 0.1 ppm or above 45 ppm can be a limiting factor for fertility.

CONCLUSION

Light intensity 3400 lux, 4400 lux, 5400 lux, 6400 lux, and 7400 lux have a significant effect on the growth of Chlorella sp. cultured on wine media and Light with an intensity of 6400 lux can produce the best growth in Chlorella sp. with an average density of 11,483,333 cells/ml.

REFERENCES

Aprilliyanti, S., Soeprobowati, T. R., & Yulianto, B. (2016). Hubungan kemelimpahan *Chlorella* sp dengan kualitas lingkungan perairan pada skala semi masal di bbbpbap jepara. *Jurnal ilmu lingkungan*, *14*(2), 77. https://doi.org/10.14710/jil.14.2.77-81

Chilmawati, D. (2008). PENGGUNAAN MEDIA KULTUR YANG BERBEDA TERHADAP PERTUMBUHAN Chlorella sp. *Jurnal Saintek Perikanan*, *4*(1), 42-49–49. https://doi.org/10.14710/ijfst.4.1.42-49

Choi, H.-J., & Lee, S.-M. (2011). Effect of Temperature, Light Intensity, and pH on the Growth Rate of Chlorella Vulgaris. *Journal of Korean Society of Environmental Engineers*, *33*(7), 511–515. https://doi.org/10.4491/ksee.2011.33.7.511

Feng, P., Xu, Z., Qin, L., Asraful Alam, M., Wang, Z., & Zhu, S. (2020). Effects of different nitrogen sources and light paths of flat plate photobioreactors on the growth and lipid accumulation of Chlorella sp. GN1 outdoors. *Bioresource Technology*, *301*(January), 122762. https://doi.org/10.1016/j.biortech.2020.122762

Gong, Q., Feng, Y., Kang, L., Luo, M., & Yang, J. (2014). Effects of light and pH on cell density of Chlorella vulgaris. *Energy Procedia*, *61*, 2012–2015. https://doi.org/10.1016/j.egypro.2014.12.064

Kazbar, A., Cogne, G., Urbain, B., Marec, H., Le-Gouic, B., Tallec, J., Takache, H., Ismail, A., & Pruvost, J. (2019). Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Research*, *39*(June 2018), 101432. https://doi.org/10.1016/j.algal.2019.101432

Kim, D. W., Shin, W. S., Sung, M. G., Lee, B., & Chang, Y. K. (2019). Light intensity control as a strategy to improve lipid productivity in Chlorella sp. HS2 for biodiesel production. *Biomass and Bioenergy*, *126*(January), 211–219. https://doi.org/10.1016/j.biombioe.2019.05.014

Lehmuskero, A., Skogen Chauton, M., & Boström, T. (2018). Light and photosynthetic microalgae: A review of cellular- and molecular-scale optical processes. *Progress in Oceanography*, *168*, 43–56. https://doi.org/10.1016/j.pocean.2018.09.002

Muchammad, A., Kardena, E., & Rinanti, A. (2013). Pengaruh intensitas cahaya terhadap penyerapan gas karbondioksida oleh mikroalga tropis *Ankistrodesmus* sp. Dalam fotobioreaktor. *Jurnal Tehnik Lingkungan*, *19*(2), 103–111. https://doi.org/10.5614/jtl.2013.19.2.1

Mudjiman, A. 2004. Makanan Ikan. Jakarta. Penebar Swadaya.

Norbawa, P., & Yudiati, E. (2013). Pengaruh perbedaan periode aerasi karbondioksida terhadap laju pertumbuhan dan kadar total lipid pada kultur *Nannochloropsis oculata*. *Journal of Marine Research*, *2*, 6–14.

Nurhayati, T., & Hermanto, M. B. (2013). *Penggunaan fotobioreaktor sistem batch tersirkulasi terhadap tingkat pertumbuhan mikroalga chlorella vulgaris, Clorella sp. dan nannochloropsis oculata the recirculation batch system of photobioreactor against growth rate of chlorella vulgaris microalg*. *1*(3), 249–257.

Rasyid, A. (2009). Distribusi klorofil-a pada musim peralihan barat - timur di perairan Spermonde Propinsi Sulawesi Selatan. *Jurnal Sains Dan Teknologi*, *9*(2), 125–132.

Susanti, T. I., Lutfi, M., & Nugroho, W. A. (2013). Pengaruh penambahan plant-growthpromoting bacteria the influence of addition plant-growth promoting bacteria (*Azospirillum* sp .) For growth rate of microalgae (*Chlorella* sp.) In the synthetic waste

water of tofu. *Jurnal Keteknikan Pertanian Tropis Dan Biosistem*, *1*(3), 239–248.

Tampubolon, T. P., Raharjo, E. I., Fakultas, A., Kelautan, I., Pontianak, U. M., Perikanan, F., & Pontianak, U. M. (2016). *Pengaruh beberapa jenis pakan alami terhadap pertumbuhan dan kelangsungan hidup larva ikan koi (cyprinus carpio) effect of some types of natural feed on the growth and survival rate of fish koi larvae (cyprinus carpio)*. *4*(1), 28–33.

Utami Nofi Puji, Yuniarti MS, K. H. (2012). Chlorella sp . *Pertumbuhan Chlorella sp. yang dikultur pada perioditas cahaya yang berbeda*, *3*(2), 159–164.