Effect of medium type, light intensity, and photoperiod on the growth rate of microalgae Chlorococcum sp. local isolate

D S Putri¹, D A Sari¹, Marianah², S P Astuti², and I G A S Wangiyana³

¹Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Muhammadiyah Mataram, Mataram, Indonesia
²Department of Biology, Faculty of Mathematics and Natural Sciences, University of Mataram, Mataram, Indonesia
³Department of Forestry, Universitas Pendidikan Mandalika, Mataram, Indonesia

E-mail: dina.soes.putri@gmail.com

Abstract. Microalgae are microscopic organisms that are living in a watery environment, whether in fresh or seawater. As photosynthetic organisms, microalgae are the primary oxygen producers in the water. Furthermore, microalgae have various benefits for the sustainability of human beings. Chlorococcum sp. is green microalgae found in freshwater, seawater, brackish water, or even in wastewater. Publication data on this microalga are limited, but this alga is known for its high lipid content. Previously, Chlorococcum sp. was isolated from the Ampenan Estuary of Lombok Island and grown in a liquid medium using Walne's. The purpose of this study was to determine the optimum growth factors for the cultivation of Chlorococcum sp. The microalgae growth factors that were varied as treatments were the source of water media used (distilled water, seawater, and saline water), the light intensity (2000, 25000, 3000, and 3500 lux), and the photoperiod (16: 8; 20:4; and 24:0 hours). Based on the research data, it is known that the type of water media is very influential on the productivity of microalgae. Where the highest growth of Chlorococcum sp. was produced in a medium containing saline water. In addition, the number of cells in the initial culture also affects the life span of microalgae. The treatment with the lowest initial cells caused the cell death phase to be extended, starting from the 11th day of culture. In conclusion, the optimal growth of Chlorococcum sp. occurred on the 5th day with a cell density of 323×10⁴ cells/ml.

1. Introduction

Microalgae or commonly known as phytoplankton, are photosynthetic microorganisms that are easily found in aqueous environments. Microalgae are generally divided into two types: marine microalgae that live in seawater and freshwater microalgae [1]. In water, they acted as the bottom of the food chain and as the producer of oxygen for the other aquatic organisms [2]. As for humans, microalgae can contribute a variety of vital benefits for the survival of humankind in the future. For example, the sources of our food, feed, and energy.

Research related to microalgae has continued to develop globally for more than a decade because of its very varied applications in various fields of the commercial industry. For instance, in agriculture is used as feed and organic fertilizer [3], in the pharmaceutical field as daily food or supplements or drugs to prevent and or treat various diseases [3–5], in the field of renewable energy as biodiesel feedstock [6], biofuels, biohydrogen [3], and solar cells [7,8], in the field of cosmetics as a moisturizing agent, thickener, antioxidant, sunscreen, anti-aging, and colorant in lipstick and eye...
shadow [3,9], while in the food and beverage industry it can be used as an alternative food rich in nutrients, natural dyes, antioxidants, and others [3].

As an archipelagic country, Indonesia indeed collects a variety of marine microalgae that can be exported as much as possible. In addition, the sunlight needed by microalgae for photosynthesis is very abundant throughout the year. Therefore, as a tropical country, Indonesia has the potential to conduct research related to marine microalgae. Research related to marine microalgae is also suitable to be developed on the island of Lombok, which is one of the islands in Indonesia which has a relatively large area.

So far, many studies have identified the types of microalgae found in the waters of Lombok Island. Unfortunately, not many of the results of these studies are published in journals. Some of these publications including research on Mapak Beach (West Lombok), which has identified 18 species of microalgae [10], research on Cemara Beach (East Lombok) which has identified 35 species of microalgae [11], research in Pelangan Estuary (West Lombok) who have successfully identified 85 species of microalgae [12], and research in Ampenan Estuary (West Lombok) which has identified 48 species of microalgae [13].

One of the microalgae on the island of Lombok that has been successfully isolated and cultivated in the laboratory is the microalgae \textit{Chlorococcum sp.} [14]. \textit{Chlorococcum sp.} is one of the unicellular non-motile green microalgae with a spherical shape and commonly lives in solitaire. These algae can be found whether in aquatic or in terrestrial habitats [15]. In aquatic habitats, they can live in freshwater [16], seawater [17], brackish water [14], or even in wastewater [18].

\textit{Chlorococcum sp.} is one of the algae known for its lipid and starch content [15]. The lipid compounds can be applied as biodiesel, while the starch can be used to produce ethanol which is also a source of renewable green energy [3]. The green color of the cells indicated the abundance of chlorophyll $a$ and $b$. In addition, carotenoids, especially $\beta$-carotene, are always present as the accessory pigment. As the cells, the abundance of $\beta$-carotene will increase [15]. These three pigments are well known for their antioxidant and anticancer agents and widely used as safe coloring agents [15,19].

To get the maximum biomass of algae to be applied for commercial uses, optimum culturing is a must. At least six factors affect microalgae growth: nutrition, light, pH, salinity, aeration, and temperature [20]. This study aims to find the best culture conditions for the growth of microalgae \textit{Chlorococcum sp.} with variations in the type of culture medium, light intensity, and photoperiod.

2. Methodology

2.1. Nutrient preparation

The type of growth nutrition that was used in this research was Walne medium. Walne's medium recipe can be seen in Table 1 [2].
Table 1. Walne culture media composition

|                  | Quantity |
|------------------|----------|
| A solution (nutrient) |          |
| NaH$_2$PO$_4$.2H$_2$O | 20 gr/L  |
| NaNO$_3$         | 100 gr/L |
| Na$_2$EDTA       | 5 gr/L   |
| Na$_2$SiO$_3$    | 40 gr/L  |
| MnCl$_2$.H$_2$O  | 0.36 gr/L|
| FeCl$_3$         | 1.3 gr/L |
| H$_3$BO$_3$      | 10 gr/L  |
| Aquadest         | 1000 ml  |
| B solution (trace metal) |    |
| ZnCl$_2$         | 21 gr/L  |
| CoCl$_2$.6H$_2$O | 2 gr/L   |
| (NH$_4$)$_8$.Mo$_7$O$_{24}$.4H$_2$O | 0.9 gr/L |
| CuSO$_4$.7H$_2$O | 20 gr/L  |
| FeCl$_3$.6H$_2$O | 3.15 gr/L|
| Aquadest         | 100 ml   |
| C solution (vitamin) |       |
| B12              | 0.1 gr/L |
| Thiamine         | 20 gr/L  |
| Biotin           | 0.1 gr/L |

2.2. Tools preparation and sterilization

The equipment and growth medium used must be sterilized by autoclaving at a temperature of 121 °C and a pressure of 15 psi for 15 minutes. Walne medium (except vitamin solution) and heat-resistant (thermostable) equipment are sterilized in the same way. Meanwhile, for equipment that is not heat resistant and the work area is sterilized using 70% alcohol solution evenly [2].

2.3. Microalgae activation

Activation was carried out to adapt the microalgae stock to the new medium. Activation was carried out in a 1 L capacity bottle enriched with Walne's medium with a dose of 0.1% nutrient solution and 0.01% vitamin solution from the total growth medium. Activation is carried out for 1-3 days with culture conditions: aeration for 24 hours, room temperature, photoperiod 0:24 hours (dark: light), light intensity 2,000-2,500 lux, and sterile environmental conditions [8].

2.4. Microalgae cultivation in different medium type

Cultivation conditions in this treatment were the same as the activation stage. The difference is, the growth medium was varied (T1). The variation was using freshwater (aquadest), saline water (20ppm), and pure seawater. Cultivation is carried out for a week or more until the algae cells pass through their death phase. The death phase can be checked from the growth curve plotted from daily cell density data. Daily cell density was calculated using a hemocytometer Improved Neubauer under a microscope with 40× or 100× magnification. There is a counter space in the hemocytometer tool in the form of a square line in the middle with 25 spaces in every 1 mm square. Cell cultures for which density will be calculated were taken as much as 1 ml each per treatment with 3× repetitions. The formula for calculating cell density can be seen in Equation 1 [2].

\[ \text{Cell density} = \text{mean cells per space} \times 10^4 \text{ cells/ml} \]  

2.5. Cell density = mean cells per space \times 10^4 cells/ml .................(1)

2.6. Microalgae cultivation in different light intensity

Cultures were grown at several intensities (2000, 2500, 3000, and 35000 lux). The culture and technical conditions of implementation are the same as in step 4; the difference is that the medium type used is the best medium from stage 2.4, the saline water.
2.7. **Microalgae cultivation in different photoperiod**

In the photoperiod variation, cultures were grown at different light:dark durations (24:0; 20:4; and 18:6 hours). The culture conditions were the same as experiment 5 with the best light intensity from the experimental results, 3500 lux.

3. **Results and discussion**

This research aimed to determine the optimum growth factors for the cultivation of microalgae *Chlorococcum sp.* The optimum growth factor can be determined by analyzing the growth curve of microalgae obtained from the daily calculation of the number of microalgae cells in different treatment variations. The growth curve of microalgae generally consists of several phases: adaptation/lag, acceleration, exponential, retardation, stationary, and death phase [20]. The growth curve for the microalgae *Chlorococcum sp.* The variation of growth factors can be seen in Figures 1, 2, and 3. Meanwhile, the summary of the initial cell data and optimum cell microalgae culture can be seen in Table 2.

![Figure 1](image-url)

**Figure 1.** The growth rate of *Chlorococcum sp.* in various types of water sources: AQ (aquadest); SW (pure seawater); SAL (saline water 20 ppm). Microalgae growth in saline water showed the highest cell density at day 5.

Based on the growth curve in Figure 1, it shows that the growth medium using saline water (SAL) produced the highest biomass on day 5; faster and much more in quantity when compared to cultures using aquadest (AQ) or pure seawater (SW) medium. It means that microalgae *Chlorococcum sp.* will grow optimally in saline water than freshwater (aquadest) or seawater. In which the optimum biomass of saline culture was $323 \times 10^4$ cells/ml on day 5. Meanwhile, the optimum biomass of aquadest culture was $127 \times 10^4$ cells/ml on the 6th day, and the optimum biomass of seawater culture was $188 \times 10^4$ cells/ml on the 7th day.

In general, the growth of microalgae *Chlorococcum sp.* Different types of water medium used only go through four phases; acceleration, exponential, stationary, and death phase. In this culture, microalgae did not undergo the adaptation phase because they had previously undergone an activation process with the same culture conditions. The microalgae cells have adapted well.

The second treatment was the cultivation of microalgae at different light intensities. Based on the growth curve in Figure 2, it can be seen that the microalgae culture of *Chlorococcum sp.* generally increases in quantity when the intensity of light given to it is higher. Starting from the light intensity of 2500-3500 lux, all resulted in optimum microalgae cell density on day 5. Meanwhile, at the intensity of 2000 lux, the optimum cell density was obtained on the 6th day, slower than the other. From the four
variations of light intensity irradiated on microalgae culture, optimum growth occurred on day 5 with a light intensity of 3500 lux and produced cells up to $1.08 \times 10^4$ cells/ml.

There were differences seen in the growth stage passed by microalgae culture with different light intensities given. Under 2000 lux intensity, the culture was through four phases: lag, acceleration, exponential, and death phase. Under 2500 lux intensity, the culture was through five phases: lag, acceleration, exponential, stationary, and death phase. Meanwhile, cultures under light intensity between 3000-3500 lux only through acceleration, exponential, and death phases. It can be concluded that low light intensity can cause the process of physical adaptation of microalgae cells to the new environment to be longer. In addition, high light intensity could reduce the number of growth phase stages that microalgae will pass.

**Figure 2.** The growth rate of *Chlorococcum sp.* under various light intensity: 2000; 2500; 3000; and 3500 lux. The higher the light intensity used, the more microalgae growth.

The last treatment was microalgae cultivation in different photoperiods. Based on the growth curve in Figure 3, it can be seen that the longer the light hours given to the culture, the higher cell density produced by the microalgae *Chlorococcum sp.* A light duration of 24 hours and a dark duration of zero-hours resulted in optimum growth on day 9, faster than the culture given more dark hours duration. In other words, the growth of microalgae *Chlorococcum sp.* will be optimized when the light (source for photosynthesis) is given continuously.
Figure 3. The growth rate of Chlorococcum sp. under various photoperiods: 16 hours light:8 hours dark; 20 hours light:4 hours dark; and 24 hours light. The continuous light given to the culture made the cells grow optimally.

In general, the optimum growth in the photoperiod treatment was longer than the others. On treatment numbers 1 and 2, the mean optimal biomass occurred on the 5th day. While on treatment number 3 (photoperiod variation), the optimal growth occurred much slower on day 9 or 10. It is probably due to the high light intensity given, resulting in a rise in culture temperature. At certain high temperatures, the lag phase can be more extended, and the exponential phase increases further a few days [21]. That also explains why at treatment 3, the cells went through the lag phase while other treatments did not. Overall, the photoperiod variation did not significantly affect the growth productivity of the microalgae Chlorococcum sp. So, the most effective photoperiod at this experiment is at 16:8 (light:dark) hours because it can reduce the electricity used for the light source.

Table 2. Summary of Initial and Optimum Cells Density of Microalgae Chlorococcum sp. Culture on Various Treatment

| Parameters     | Treatments          | Initial Cells Density (×10^4 cells/ml) | Optimum Cells Density (×10^4 cells/ml) | Optimize on day |
|----------------|---------------------|---------------------------------------|----------------------------------------|----------------|
| Water source   | Aquadest            | 4.51±1.71                             | 126.64±19.18                           | 6              |
|                | Seawater            | 4.03±0.27                             | 188.04±9.46                            | 7              |
|                | Saline water        | 4.83±0.76                             | 322.92±17.00                           | 5              |
|                | 2000 lux            | 17.71±1.68                            | 85.65±5.74                             | 6              |
|                | 2500 lux            | 14.39±2.49                            | 70.13±2.52                             | 5              |
|                | 3000 lux            | 10.60±3.57                            | 85.76±5.39                             | 5              |
|                | 3500 lux            | 15.68±0.96                            | 108.27±9.79                            | 5              |
| Light intensity| 16:8 (light:dark)   | 1.67±0.25                             | 57.92±3.07                             | 10             |
| Photoperiod    | 20:4 (light:dark)   | 2.29±0.5                              | 58.11±1.08                             | 10             |
|                | 24:0 (light:dark)   | 2.13±0.5                              | 66.91±1.70                             | 9              |

The desired growth rate is the one that produces the most biomass (optimum) in a relatively short time so that production costs (cultivation) can be reduced. Based on the summary data from Table 2, it can be concluded that: 1) the type of water used as a growth medium greatly affects the productivity of microalgae Chlorococcum sp., while the variation of light intensity and photoperiod does not show a
significant difference in cells density, 2) the fastest optimum cell density production can be reached since the day 5 of cultivation, and 3) the number of initial cells can affect the lifespan of microalgae; the fewer initial cells were added, the longer the life span will be. In general, the microalgae *Chlorococcum sp.* will grow optimally in saline water growth medium with the light intensity of 3500 lux and duration of light:dark = 24:0 hours.

4. Conclusion

The optimum growth rate varies depending on the culture conditions and the initial cell density. Based on the result data, it can be concluded that the microalgae *Chlorococcum sp.* will grow optimally in a growth medium using saline water with a given light intensity of 3500 lux and duration of light:dark = 24:0 hours. In which the optimum growth of *Chlorococcum sp.* can occur since the 5th day of cultivation.

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