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Effect of Aeration Time on *Chlorella vulgaris* Growth

Aliyah Devi Maysitha[1], Harmin Sulistiyaning Titah[2]

[1,2]Department of Environmental Engineering; Faculty of Civil, Planning, and Geo Engineering; Institut Teknologi Sepuluh Nopember (ITS), Surabaya 60111; Indonesia.

ABSTRACT: Microalgae is a raw material used in many different sectors and is helpful in absorbing carbon dioxide. *C. vulgaris* is one of best candidates as an additional material for biofuel. Biofuel is made in part from the fatty acids found in *C. vulgaris*. Thus, more research is required to identify the variables that can spur its growth. Aeration time is one of the most significant growth factors. In this research, experiments were carried out with differences in aeration time between 12 hours and 24 hours. Some of the fixed variables used are an air flow rate of 2 L/minute, 20 ppt of salinity, and a light intensity of 6,000 lux. Observations were made every day for 14 days. Measurements of the solution's pH, temperature, and carbon dioxide content in the reactor's air were made, along with the density of *C. vulgaris* cells. According to the observation data, *C. vulgaris* grew more effectively in the reactor that had 24-hours aeration. The peak day occurs on the tenth day, while the required adaptation phase lasts six days. Up until the peak day, the microalgae growth rate was 1.59 cells/mL.day.

KEYWORDS: aeration, cultivation, microalgae, photobioareactor, salinity

I. INTRODUCTION

Five groups of microalgae are recognized: *Phaeophyceae*, *Cyanophyceae*, *Phaeophyceae*, *Rhodophyceae*, and *Bacillariophyceae*. Prokaryotic and eukaryotic forms of microalgae are known to exist. Microalgae can develop in a variety of aquatic habitats, such as rivers, ocean, and brackish water. The size of microalgae are varies depending on its species [1]. A microalgae's life cycle lasts between ten and twelve days [2]. Microalgae use photosynthesis to lower CO_2 levels. The production of oxygen by this mechanism will raise the water's dissolved oxygen (DO) levels [3]. Microalgae has been used as material in several industries including biofuel, bioethanol, food, cosmetics, medicine, and fertilizer [4]. *Chlorella sp*. is one of mostly found microalgae in Indonesia [5].

The green microalgae *Chlorella sp*. belongs to the *Chlorophycea* family and reproduces asexually by producing autospores. Its fatty acid contents can be used as additional material of biofuel [6]. These microalgae reproduce best in salinities of 15-30 ppt [7]. Temperature and pH are two more variables that can affect *Chlorella sp*. reproduction in addition to salinity. The ideal pH range for culture is 6.8 to 9.4, and the usual temperature ranges from 20 to 42°C [8], [9]. Reference to [10], 6.400 lux is the best light intensity for its growth. The adaptation phase of *Chlorella sp*. lasts only around five days. Following that, growth persisted after the seventh day. The microalgae's adaptation phase, which lasts from days 2 to 6, is impacted by the addition of nutrients [11]. In addition, air and light are two other elements that need to be considered.

Microalgae growth is influenced by aeration and medium stirring. The microalgae shift from a middle position to the surface position and back again when stirred. In order to maintain the microalgae's viability, this is done to ensure that energy sources are distributed evenly and carbon dioxide sources are extracted [1]. An air flow rate of 0.7 air volume per culture volume per minute (vvm) is suitable for microalgae. Microalgae growth is also influenced by aeration duration; the longer the aeration period, the more microalgae growth is generated [12]. To stop the predatory bacteria *Vampirovibrio chlorellavarous* from growing, aeration must also be done during the dark hours. This will ensure that the quantity of microalgae will not decrease gradually [13].

To support its function as an additional material for biofuel, growth factors of *C. vulgaris* need to be identified. In this study one of the factor, aeration time, will be investigated. How does media aeration time affect the growth of *C. vulgaris* and which one is the best for cultivation.

II. MATERIALS AND METHODS

Research was done in batches. There was a duplicate sample utilized. Distilled water, *C. vulgaris* inoculum, sodium chloride (NaCl) made by Merck (Denmark), vitamins, trace metals, and Walne fertilizer were the materials employed in this study. A 50 mL beaker glass, a 100 mL measuring glass, a spray bottle, a spatula, and an analytical balance were the instruments utilized in this study. Bulb, aerator, rubber hose, and 1 L transparent plastic container are additional instruments required for facilitating the growth of microalgae. A bulb used in this research is a 4-Watt (420 lm) Philips brand with cool daylight type for each container. The aerator in use has a 2 L/minute air flow.

A. Media

The artificial brackish water utilized in this study had a salinity of 20 ppt. As a source of nourishment for *C. vulgaris*, distilled water combined with vitamins, trace metals, and Walne fertilizer. The first step in creating artificial brackish water was weighed 10 grams of NaCl for each container using an analytical balance. Three hundred fifty milliliters of distilled water were added to the plastic container after NaCl was added. Walne fertilizer, vitamins, and trace metals added 0.5 mL each. The addition of these three nutrients was conducted every week.

B. Inoculum of Microalgae

Inoculum of *C. vulgaris* is from Laboratorium Pakan Alam Situbondo. Inoculum added 150 mL into each plastic container filled with media.

C. Fixed Variables

Light and dark cycle done with 12:12 hours daily. The light intensity was arranged around 6.000 lux. There are 2 aeration time applied 12 hours and 24 hours. As for 12 hours variation applied when the lamp turned off. The airflow is set to 2 L/min.

D. Data Measurement

Temperature, pH, CO_2 concentration in the atmosphere, and the density of microalgae cells were the data observed in this study. For fourteen days, observations were done each day. Measuring instruments used were thermometer, pH meter, and CO_2 meter. Meanwhile, a microscope and hemocytometer were used to measure microalgae cell density. Hemocytometer measurement was made by choosing five squares based on the size of the microalgae. The volume of the square employed in this investigation was 4×10^6 mL because it was 0.2 mm in length and 0.1 mm thick. The average number of cells from five randomly chosen squares divided by the square's volume is the formula used to determine cell density [14].

$$\rho\ cell = \frac{\bar{x} \times fp}{V}$$

where:

ρ cell = cell density, cells/mL

$\bar{x}$ = average number of cells per square, cells

fp = dilution factor if any

V = volume of selected square, mL

III. RESULT AND DISCUSSION

This study used a media and inoculum ratio of 7:3. The media consisted of 20 ppt salinity; 0.5 mL Walne fertilizer; 0.5 mL vitamins; and 0.5 mL trace metal. *C. vulgaris* can grow well at salinities between 15 ppt – 30 ppt [8]. The results of physical observations as shown in Figure 1. The solution in the reactor with 24 hours aeration time was faster become dark green in colour compared to the 12 hours aeration treatment. Apart from that, the dark colour did not last long in a reactor with 12 hours of aeration. On the 10th day the concentration decreased which was then followed by the formation of a lot of precipitate on the 13th day.

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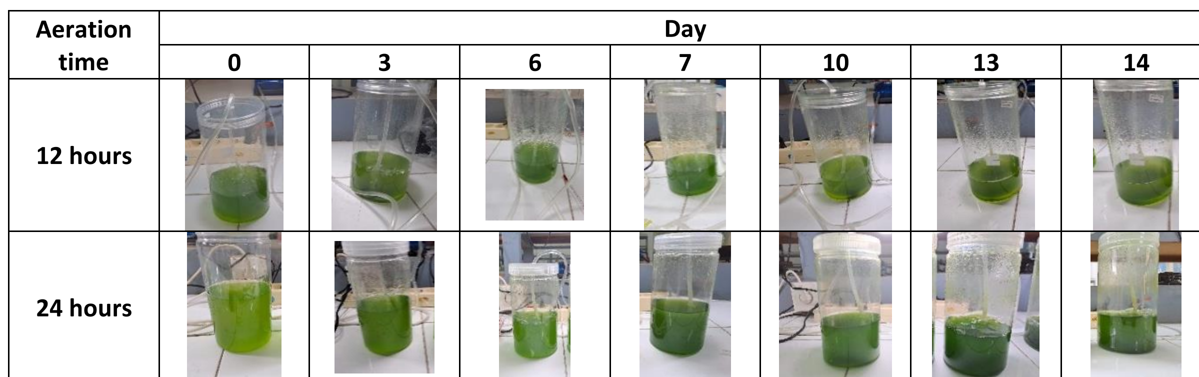

Aeration time	Day						
	0	3	6	7	10	13	14
12 hours							
24 hours							

Figure 1. Physical observation of the solution in the reactor

The results of quantitative observations on temperature parameters (Figure 2) show an increase in temperature with 24-hours aeration compared to 12-hours aeration. Continuous use of an aerator can affect the temperature of the air produced, thereby increasing the temperature of the solution. However, the range of temperature differences in both reactors were still within the optimum growth range for *C. vulgaris*, namely 20-42°C [8], [9]. The pH of both reactors was neutral, which also indicates that the solution is in optimum condition.

pH causes changes in the solubility of inorganic carbon in waters. Based on this graph, most of the available carbon is in the form of bicarbonate (HCO_3^-). Bicarbonate is a carbon nutrient used by microalgae for photosynthesis. The highest bicarbonate content is at pH 8 [1]. Reactors with 12-hours aeration experienced a decrease in pH (Figure 3). It can occur because when microalgae grow, more bicarbonate ions are used. Another reason might be because the CO_2 supply from the aerator is not always available, so it continues to decrease. In contrast to reactors with 24-hours aeration, there was a decrease at the beginning due to continuous aeration which increased CO_2 levels in the solution. During the exponential phase, the number of microalgae conducting photosynthesis increases so that the supply of bicarbonate in the solution can be balanced and the pH rises towards neutral.

CO_2 can keep the balance of its concentration in air and water [15]. CO_2 concentration in the air inside the 24-hours reactor is higher than 12-hour reactor (Figure 4). It happens because of two possibilities. Excessive dissolved CO_2 in 24-hours reactor is released to the air or CO_2 in 12-hours reactor is dispersed into the water that lack of dissolved CO_2. This also indicates that *C. vulgaris* inside the reactor were consumed the dissolved CO_2 daily. Photosynthesis happened and results on growing microalgae cell density.

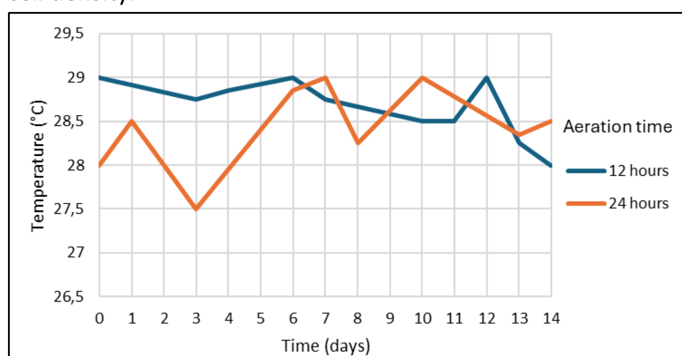

Figure 2. Temperature of the solution in the reactor

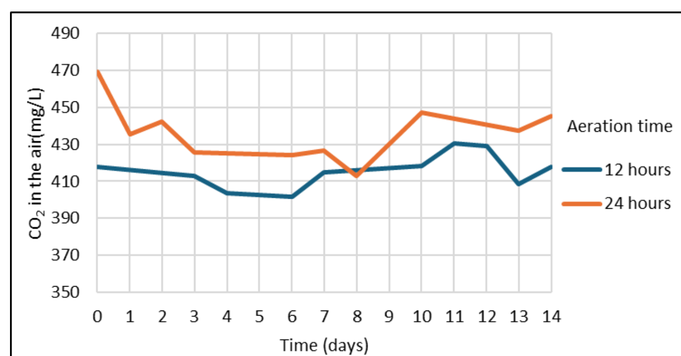

Figure 4. CO_2 concentration in the air inside the reactor

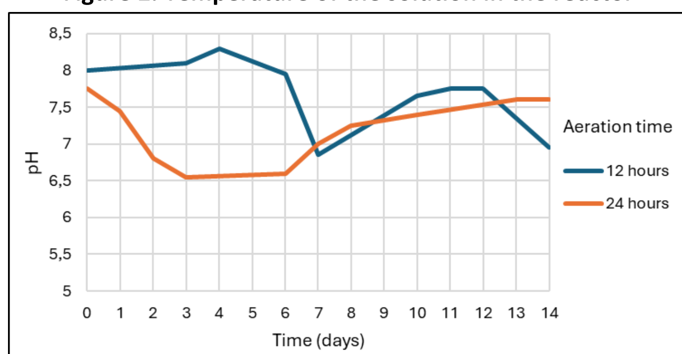

Figure 3. pH of the solution in the reactor

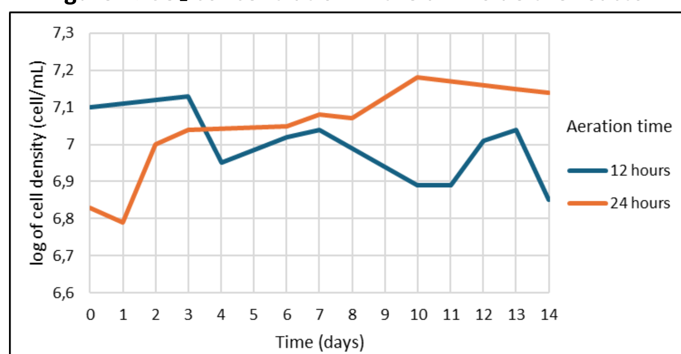

Figure 5. log of microalgae cell density in solution inside the reactor

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Microalgae cell density shows the difference between both reactor (Figure 5). Usually, microorganisms have their growth phases. It starts with adaptation phase (lag phase), exponential phase, and stationary phase. In a 24-hours reactor those phases can be seen as the number increases day by day. The first week is notably as an adaptation phase. After *C. vulgaris* adapt well, it continues to the exponential phase until reach the peak growth on 10th day. Followed by decreases in number slowly until the 14th day. In a 12-hours reactor these phases are not seen as the numbers keep decreasing day by day. It might be caused by lacks DO at night. DO is needed for respiration to recharge microalgae's electron acceptors on making ATP [3]. If the respiration process is disturbed, photosynthesis will be disturbed as well. It can cause low biomass production. From the data we can get specific growth number for 24-hours reactor by using this formula. The specific growth of 24-hours reactor from initial to peak day is 1,59 cells/mL/day.

$$\mu = \frac{ln\,(\rho_1 - \rho_0)}{t}$$

where:

μ = microalgae specific growth, cell/mL/day

$\rho 1$ = cell density on peak/final day, cell/mL

$\rho 0$ = cell density on initial day, cell/mL

t = time, day

IV. CONCLUSION

Continuous aeration for 24 hours is the optimal growing condition for *C. vulgaris*. The airflow used for 500 mL solution is 2 L/min. With a light : dark cycle duration of 12:12 and 6,000 lux for light intensity.

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