Photoinhibition of algal photobioreactor by intense light

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Abstract. Algal photobioreactor is a translucent reactor equipped with media supply installation and gas emissions to cultivate microalgae with CO\(_2\). Alga as an autotroph organism, growth mechanism following photosynthesis system which use light exposure to develop and growth the algae cells. This study analyze the performance of *Chlorella sp.* within intense light intensity (6, 12, 18, and 24 hour) variable with using UV fluorescence. The environment condition and growth rate was analyze of microalgae in the photobioreactor. Algal growth influenced by the intense of light intensity (hours exposure), and likely will effect to nutrient concentration in the photobioreactor.

Keyword: algal growth, light intensity, photobioreactor

1. Introduction

Factors that affect the growth of microalgae, there are abiotic factors (sunlight, temperature, nutrition, O\(_2\), CO\(_2\), pH, salinity), and others biotic factors (bacteria, fungi, viruses, and competition with other microalgae), and technical factors (how to harvest, etc). Furthermore, it is believed that the nutrient is important\([1]\)[2][3].

According to previous study explain the growth is increasing the number of cell in a certain period. In further process, followed by increasing the size or the number of cells. Recently, cell density is used for the growth of microalgae in the cultivation of natural feed\([4]\).

*Chlorella sp.* is an autotrophic (protest) habitat because this habitat is capable of making its own food (autotroph). This organism has chlorophyll pigments so that it can carry out photosynthesis. This type of microalgae is the worldwide existence group of green algae, 90% of Chlorella live in fresh water, while the other 10% live in seawater.

Characteristics of chlorella sp. generally have chlorophyll, to store food reserves in food bags or pyrenoid. Some environmental factors that can increase the growth of the population of chlorella sp. There are temperature, light intensity, pH, dissolved oxygen, nutrient, and carbon dioxide. Chlorella sp. requires a high enough temperature for growth\([5]\). Meanwhile, in recent years *Chlorella sp.* has capability to improve CO\(_2\) uptake within in climate change issues.

Light is the main energy source and an important parameter for microalgae for cultivation. Microalgae convert light into energy through photosynthesis\([6]\). The wavelength range of light that can be used by microalgae between 400 nm - 700 nm is in accordance with visible light\([7]\). The intensity of light that is too intense can cause the inhibition of photosynthesis process because there is
an effect of saturation of light. The excess of this light changes to heat so that the culture temperature rises [8].

Microalgae is autotrophs which synthesize food directly. Carbon dioxide and air are used by microalgae to produce sugar and oxygen used as food. Algal growth rates need light intensities in the range densities (4, 8, 15, 30, 85, and 110 μmol photons m−2 s−1, light/dark cycle of 16:8 hL:D) [9]. were recorded at 20°C by increase of chlorophyll a. Simple photosynthesis with the following reaction equation 1:

$$6\text{H}_2\text{O} + 6\text{CO}_2 \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 \text{(Glucose)} + 6\text{O}_2 \quad (1)$$

Glucose is used to form other organic compounds such as cellulose. The process takes place through cellular respiration. In cellular respiration, sugar (glucose) and other compounds will react with oxygen to produce carbon dioxide, water, and chemical energy. Furthermore, in this study will analyze the Chlorella sp. performance in photobioreactors within the light variance dependence.

2. Materials and method

2.1. Research location

This research was conducted on a laboratory scale at the Research Laboratory, Department of Environmental Engineering, Faculty of Engineering, University of National Development “Veteran” East Java located on Jalan Raya Rungkut Madya, Gunung Anyar, Surabaya City, East Java Province.

2.2. Algae culture preparation

Early cultivation was used to grow microalgae to be ready for use in reactor. The initial seed of 300 mL was bred in 1 L beaker glass with the addition of fertilizer and aeration.

2.3. Seeding and acclimatization of microalgae

Cultivation is conducted to maximize microalgae growth that it is prepared to be used for the operation of the reactor by adding a certain amount of nutrients. Furthermore, this condition is to certain microalgae from getting nutritional needs. Light and nutrition factors need to be considered to have microalgae with high biomass. The certain chlorophyll a microalgae concentration indicates that the microalgae is ready for use.

While acclimatization aims to condition the microalgae with a reactor that will be used so that the microalgae perform optimally. The acclimatization process is carried out for 6-10 days. The acclimatization process is an advanced process of cultivation so that microalgae can adapt to their environment. The acclimatization process carried out by adding NPK fertilizer will produce optimum chlorophyll a.

Culture and acclimatization were carried out to obtain microalgae that were ready to be used during the running process. Mineral water is used as a medium in the process of microalgae culture and is given the addition of nitrate 20 mg / L and phosphate 5 mg / L. Microalgae culture was carried out for 2 weeks until the abundance of culture reached $6 \times 10^6$ cells / ml media and at the density level the microalgae was ready to be transferred to the reactor for the running process [2].

2.4. Experimental setup

The intensity used in the closed photobioreactor is 1000 lux. This is based on the optimal range of microalgae growth values in the range of 1000 lux - 5000 lux. The maximum resistance value can damage photosynthetic receptor systems. Because light absorbed not used in photosynthesis will turn into heat which will inhibit microalgae growth [10].

Research carried out over a period of ± 2 months with two research variables, namely control (without light) and four variations (6, 12, 18, and 24 hour) of the duration of irradiation using UV fluorescence T4 lamps. The reactor was added of pure CO₂ gas. As long as the research takes place, the optimal temperature is measured between 28°C - 30°C. In this study also carried out testing to determine the growth rate of microalgae as a control carried out by laboratory testing in the sample Chlorella sp. CO₂ supply is carried out to the reactor with variations in nutrient composition and long irradiation variations for 8 hours with a level of 0.5 L / minute. CO₂ gas is supplied to the reactor using
a diffuser pump. The reactor design used consisted of a closed plastic jar with a diameter of 12 cm and a height of 18 cm with a volume of 2 liters, the reactor was then installed on a 5-tiered rack with 1 stacking rack for the control and variations long irradiation. The running process carried out in support of the reactor has been prepared and carried out based on the microalgae life cycle.

2.5. Analytical procedure
Research conducted the main and additional parameters is carried out every day during the microalgae life cycle. Algal biomass was analyze by chlorophyll. Phosphate was analyze as a nutrient concentration. Light intensity, temperature, and pH conducted in order to determine the environment condition. Table 1 is procedures which conducted in this research.

| No. | Analytical Procedure/Equipment |
|-----|--------------------------------|
| 1   | Chlorophyll-a ADAC 942.04.2005 (chromatography) |
| 2   | Phosphate Indonesia National Standart, 06-6989.31-2005 (spectrofotometer) |
| 3   | pH pH meter |
| 4   | Temperature Termometer |
| 5   | Light Intensity Lux meter |

3. Results and discussion

3.1. pH condition
pH condition of Chlorella sp. in this study it was in the range 7 - 8.5, with the highest pH of 8.5 and the lowest pH of 7.2. The low pH in this study does not significantly affect the life of microalgae because the pH of the media is still within the permissible range of 6.6 - 8.5. Figure 1.a shown a pH in our photobioreactor. In each treatment, pH tends to fluctuate. Increased pH is influenced by increased metabolism of microalgae and photosynthesis [11]. It has been well established that environmental conditions can influence the cellular chemical composition of phytoplankton.

3.2. Dissolved Oxygen
Dissolved oxygen on Chlorella sp. shown in Figure 1.b. The range of DO values is 5.45 - 8.00 mg / L which is properly for the life processes of aquatic biota. Increased DO is caused by increased photosynthesis and there is a large supply of photosynthesis and aeration, while a decrease in DO is caused by photosynthesis. In this study by five variations on the duration of irradiation showed a range of appropriate DO values so that the alga process in the culture of Chlorella sp. goes properly.

3.3. Light intensity
Based on Figure 2, it can be seen that in the 9 days of the study, low or higher light levels were carried out indicating that Chlorella sp. can still survive even at the lowest light intensity conditions of 23 lux and the highest light intensity of 1917 lux.

**Figure 2. Light intensity of algal photobioreactor**

3.4. Algal growth (chlorophyll)

Figure 3 has shown the algal concentration in the photobioreactor. In the beginning of the reactor can be seen that low or higher algal concentration dependent with light intensity in the 9 days of the study. The content of chlorophyll-a in the results of the above analysis ranges from 0.100 - 1.607 µg / L. The lowest chlorophyll-a content was found in the provision of control photobioreactor (no light intense). Furthermore, in intense of 6, 12, and 18 hour will improve the algal concentration. After 24 hour of light intensity, biomass decreasing slightly in 6-9 days.

**Figure 3. Algal biomass concentration (left), and algal correlation between algal biomass and light intensity (right)**

In the water, presence of sunlight, CO2, and nutrient, Chlorella cells will growth faster. Previous research was conducted with algal bioreactor with light intensities (i.e. 400, 800, 1200, 1600, 2000, and 2400 µmol m-2 s-1) was observed under the wavelength of red light. Light intensity 400 was too low to maintain the growth of microalgae Chlorella vulgaris, whereas 2400 µmol m-2 s-light intensity was too high to avoid photo inhibition [10]. This is similar with our research while research conducted in range 20-2000 Lux while low light intensity will not improve the algal growth (Section E). In this research, light exposure to the photobioreactors was not directly induce algal growth. The
maximum light intensity in the reactor ±2000 lux. Meanwhile, along in 9 days, biomass will inherence of the light induction [12]. Light/dark cycle (6, 12, 18 and 24 hour intensity) will affect the biomass performance [11].

### 3.5. Correlation between algal biomass and light intensity

Previous study found that growth algal biomass dependence on light intensity on the first/beginning phase. This phase believed during which irradiation was limiting. A second phase in which light had an inhibitory effect. As light may be limiting for growth. Otherwise, if the light in excess, leads to oxidative stress. The flashing light could also decrease the production of microalgae, when the dark/light cycle or the frequency of the flashes is not optimized [13]. In the beginning, the highest intensity tested maybe slight similar cultures showed growth similar to that at low intensity, indicating that cells can protect themselves from such strong light excess and yet maintain significant biomass accumulation [14][15]. Figure 3b explain the correlation between algal biomass and light intensity. Control photobioreactor has low algal growth affected with low light intensity.

### 3.6. Correlation between phosphate concentration and light intensity

Nutrient limitation will generally cause a reduction in intracellular nutrients while they are important elements for cell composition. In other hand, nutrient such as nitrogen and phosphorus limitation is likely to affect normal cellular functions (e.g. photosynthesis, respiration and some enzyme activities in the case of algae) [11].

![Figure 4](image.png)

**Figure 4. Correlation between phosphate concentration and light intensity**

Varying the light intensities, biomass growth resulted in significantly different trends, whereas varying the nutrients loads. According to operating conditions tested, nutrient removal rate mainly depended on nutrients availability rather than light intensities. Moreover, it could be further analysis in order of dependence of phosphate concentration with light intensity. It was observed in previous study that biomass production performed better when light intensity increased in the range 20-100 μmol s⁻¹ m⁻², whereas performed poor when nutrients concentrations increased [16]. The other explanation that algae could adsorb nutrient when algae in the lack of energy (nutrient removal) [17]. In Figure 4 was shown that nutrient in reactor control have reducing performance to remove phosphate concentration. Nevertheless, is not clearly explain the direct connection between nutrient and light intensity.

### 4. Conclusion

This study was stated according to operating conditions tested, nutrient removal rate mainly depended on nutrients availability rather than light intensities. Light intensities in this research is not directly effect to algal biomass production, while the light intensities might be low to enhance the biomass. Furthermore increasing period of the exposure by time, the results was not clearly improve within biomass and nutrient removal indeed.
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