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Microalgae Cultivation and Its Potential for Phycoremediation Agent

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Abstract
Microalgae can be an ecological remediation agent known as phycoremediation. Understanding these factors is critical in producing the expected products efficiently. Several factors affect microalgae growth, including nutrients, temperature, pH, CO_2 concentration, lighting, microalgae photosynthesis processes, and hydrodynamic factors. Microalgae *Chlorella vulgaris* has the potential to survive in media contaminated with heavy metal and high salinity levels. It can reduce the concentration of heavy metals in water through several mechanisms. Further research must be conducted to determine the factors influencing microalgae growth. The research was conducted to observe the growth rate of microalgae in a controlled photobioreactor. The cultivation was carried out in a plastic photobioreactor equipped with aeration, lighting, and the addition of nutrients. The first stage of the research was observations on *C. vulgaris* growth with 30% inoculum and 20% inoculum. The *C. vulgaris* growth rate is 0.133 cells/mL/day and 0.927 cells/mL/day, respectively. The second stage of the research was observations on *C. vulgaris* growth with 20‰ salinity, and the variation used is the aeration, 3 L/min, and 4 L/min. The *C. vulgaris* growth rate is 0.263 cells/mL/day and 0.236 cells/mL/day, respectively.
Keywords: *aeration, Chlorella vulgaris, cultivation, photobioreactor, salinity.*

Abstrak
Mikroalga dapat menjadi salah satu agen remediasi lingkungan atau yang dikenal dengan fikoremediasi. Pemahaman terhadap faktor-faktor tersebut sangat penting dalam menghasilkan produk yang diharapkan secara efisien. Beberapa faktor yang mempengaruhi pertumbuhan mikroalga, antara lain nutrisi, suhu, pH, konsentrasi CO_2, pencahayaan, proses fotosintesis mikroalga, dan faktor hidrodinamik. Mikroalga *Chlorella vulgaris* berpotensi bertahan hidup pada media yang terkontaminasi logam berat dan tingkat salinitas yang tinggi. Mikroalga ini dapat menurunkan konsentrasi logam berat dalam air melalui beberapa mekanisme. Penelitian lebih lanjut perlu dilakukan untuk mengetahui faktor-faktor yang mempengaruhi pertumbuhan mikroalga. Penelitian ini dilakukan untuk mengamati laju pertumbuhan mikroalga dalam fotobioreaktor yang terkontrol. Kultur dilakukan dalam fotobioreaktor plastik yang dilengkapi dengan aerasi, pencahayaan, dan penambahan nutrisi. Tahap pertama penelitian adalah pengamatan pertumbuhan *C. vulgaris* dengan inokulum 30% dan inokulum 20%. Laju pertumbuhan *C. vulgaris* masing-masing adalah 0,133 sel/mL/hari dan 0,927 sel/mL/hari. Tahap kedua penelitian adalah pengamatan pertumbuhan *C. vulgaris* dengan salinitas 20‰, dan variasi yang digunakan adalah aerasi 3 L/menit, dan 4 L/menit. Laju pertumbuhan *C. vulgaris* masing-masing adalah 0,263 sel/mL/hari dan 0,236 sel/mL/hari.
Kata Kunci: *aerasi, Chlorella vulgaris, fotobioreaktor, kultivasi, salinitas*

1. Introduction

Water is the primary resource living organisms need and is widely used by individuals, groups, domestic activities, agriculture, and industrial scale. Population and industrial growth increase the need for clean water. All human activities utilizing water cannot be separated from producing residue or wastewater. Pollution can occur due to a lack of processing of the wastewater produced and the use of chemicals or other materials in large quantities, which become the primary source of water pollution. Heavy metals are among the pollutants that could become dangerous pollutants in the water ecosystem

Heavy metal pollution, Hg, Cr, and Pb, occurs in port waters and coastal areas [1], [2]. Many forms of wastewater treatment and environmental management are needed to reduce the impact of heavy metals on living organisms. In managing an environment contaminated with heavy metals, the focus of the treatment must be carried out on the polluted media. According to the sustainable environmental management point of view, seawater processing contaminated with heavy metals is the main idea that must be considered because it can disrupt the aquatic ecosystem and human health. The urgency of seawater management, processing polluted seawater, and maintaining aquatic ecosystems are among the main focuses in realizing the Sustainable Development Goals (SDG), which are currently being promoted. Indonesia is one country that supports and participates in recognizing the 17 SDGs. One of the SDGs goals related to the management of seawater pollution is SDGs number 14, Marine Ecosystems [3].

There are various methods for managing water contaminated with heavy metals. Remediation technology provides multiple methods to restore water contaminated with heavy metals. This management can be conducted physically, chemically, biologically, and through integration. Remediation methods have also been widely developed to utilize natural resources in treating polluted media. Several factors must be considered when selecting a remediation method, such as the extent of pollution, the contaminated area, environmental conditions, the specific remediation method, and the application of the selected method. Based on these considerations, remediation using living things is one option that can be used and offers more significant advantages. The bioremediation method using microalgae is one solution for treating water contaminated with heavy metals by combining sustainable natural resources.

Microalgae are unicellular organisms that can absorb energy from light through photosynthesis, which utilizes the CO_2 in the growth medium and converts it into chemical energy [4]. Microalgae play an essential role in environmental quality restoration. Several studies have been conducted to determine the ability of microalgae to restore polluted environments. The ability of microalgae to absorb heavy metals and the ability to survive in water contaminated with heavy metals with moderate concentrations make microalgae one of the remediation agents for water contaminated with heavy metals. Microalgae also have forms of intracellular and extracellular mechanisms that are used to protect themselves from heavy metal toxicity. Biosorption and bioaccumulation mechanisms are the main methods that occur in microalgae to remove heavy metals. In addition to being environmentally friendly, economically, and energy efficient, microalgae can also provide benefits such as biomass production that has good nutritional content and is a renewable material in technology [5]. Phycoremediation is a remediation technique using microalgae to remediate hazardous contaminants such as hydrocarbons, pesticides, and heavy metals. Research related to Phycoremediation is in great demand because of its advantages in remediating hazardous contaminants and its ability to produce valuable biomass, such as for fertilizers and biofuels [6], [7]. Phycoremediation has a great demand to be used as a pollution removal agent in soil, water, and air media. Microalgae offer various methods of pollutant removal from media, such as the ability to absorb pollutants in their biomass, their metabolic abilities, and the ability to degrade pollutants through the enzymes they produce. Based on these advantages, phycoremediation is one of the sustainable and eco-friendly options in sustainable environmental management [8].

Chlorella vulgaris as shown in **Figure 1** is a single-celled green microalgae containing chlorophyll, which it uses for photosynthesis. *C. vulgaris* can also accumulate high carbohydrate levels [9]. Culturing *C. vulgaris* can enhance lipid production through a metabolic process that utilizes various pollutants in its living medium. The cultivation process can simultaneously reduce COD (Chemical Oxygen Demand) and BOD (Biological Oxygen Demand) levels in wastewater through the oxidation process of microalgae.

The productivity and efficiency of microalgae in their utilization are influenced by several factors: energy, nutrients, and other parameters such as temperature, pH, CO_2 concentration, lighting cycle, microalgae photosynthesis process, hydrodynamic factors, and the design of photobioreactor [10]. The energy required by microalgae comes from lighting and CO_2 contained in the microalgae growth medium as a carbon source. The nutrients needed for microalgae growth include macronutrients such as carbon, nitrogen, phosphorus, and micronutrients like trace metals, chloride, and other vitamins [10].

This research conducted several focus discussions to determine the percentage of microalgae inoculum that produces the best value in cultivation. The value is analyzed by comparing the number of cells produced and the growth rate of the microalgae.

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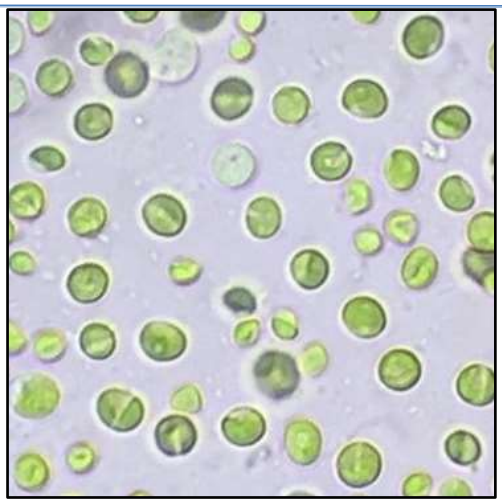


Figure 1. *Chlorella vulgaris*
Source: Taken and modified from Ramaraj, 2016 [11]

2. Material and Methods

This research was conducted in the Environmental Remediation Laboratory, Environmental Engineering Department, Institut Teknologi Sepuluh Nopember, Surabaya.

Photobioreactor. The reactor used for the research is made of plastic and equipped with an aerator and additional lighting, as shown in **Figure 2**. The aerator specifications are 3 L/minute and 4 L/minute, operating for 24 hours. Lighting is added using LED cool daylight lamps (±1.400 lux), operated with a lighting cycle of 12:12 hours on and off per day.

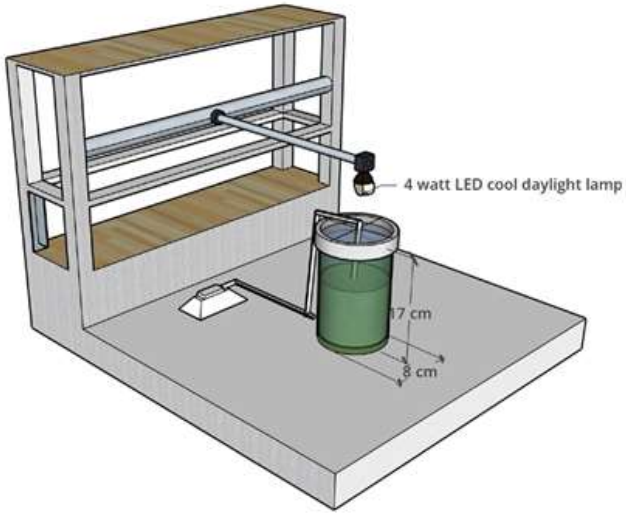


Figure 2. The Cell Density of *C. vulgaris* on First Photobioreactor

Microalgae Culture. The *Chlorella vulgaris* stock and nutrients were obtained from Balai Perikanan Budidaya Air Payau (Brackish Water Aquaculture Development Center), Situbondo Regency, East Java, Indonesia. The microalgae stock variations used in the research are 30%, 20%, and 10% of the total cultivation volume.

Media Preparation. The media used in the cultivation is distilled water and distilled water with 20‰ salinity. The final volume for the cultivation is 500 mL. The media for cultivation consist of aquades, NaCl for salinity, and nutrients. Nutrients containing walne, vitamins, and trace metals are added to the reactor as a source of nutrients for microalgae. The doses of walne, vitamins, and trace metals added are 1 mL/L,

respectively, and have been added on days 0 and 7 for 14 days of the cultivation. Aerators and lighting are also used in microalgae cultivation.

The Cultivation. The cultivation process is conducted in two stages.

- The first cultivation is carried out in an aquades medium with the variation of the percentage of the microalga inoculum added. The percentage of microalgae inoculum used in the first cultivation is 30% and 20%. The additional nutrients were added on the first day of cultivation. The aeration used in this stage is 3 L/minute.
- The second cultivation uses aquades media with a 20‰ salinity. The percentage of microalgae inoculum used in the second cultivation is 10%. The additional nutrients were added with the repetition on days 0 and 7 of cultivation. The variation in this stage is the aerator, 3 L/minute, and 4 L/minute, respectively.

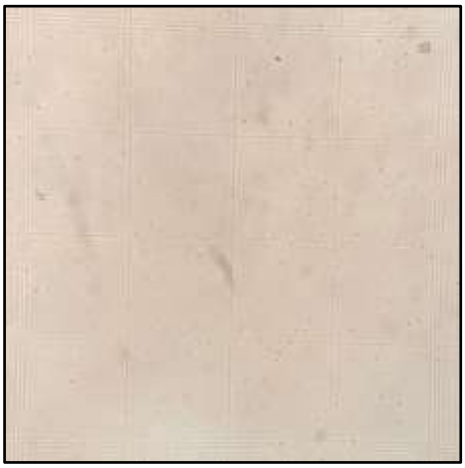


Figure 3. Cell count by Haemocytometer under Microscope

The Monitoring of *C. vulgaris* and Parameters in The Media. The main parameters tested in this research are the density of *C. vulgaris* microalgae cells, pH, temperature, and CO_2 concentration in the reactor. *C. vulgaris* microalgae cells were counted using a Neubauer-improved hemocytometer made in Germany as shown in **Figure 3**, under a microscope Yazumi brand made in China and using a hand counter. Temperature and pH were measured by pH meter Gauge EZ 9909. The CO_2 concentration was measured using the CO_2 meter GC-2028 Lutron brand made in Taiwan. All parameters were measured every 24 hours to determine the variations' effect and impact. The density of cells and microalgae growth rate were calculated through **Equation 1** and **Equation 2**.

Data Analysis. The research data consisted of the number of microalgae cells, temperature and pH in the media, and CO_2 concentration in the air inside the photobioreactor.

$$Cells\ density = \frac{\left(\frac{number\ of\ cells\ from\ each\ square\ together}{number\ of\ square}\right)}{Volume\ of\ square} x\ Dilution\ factor \qquad \textbf{Equation 1}$$

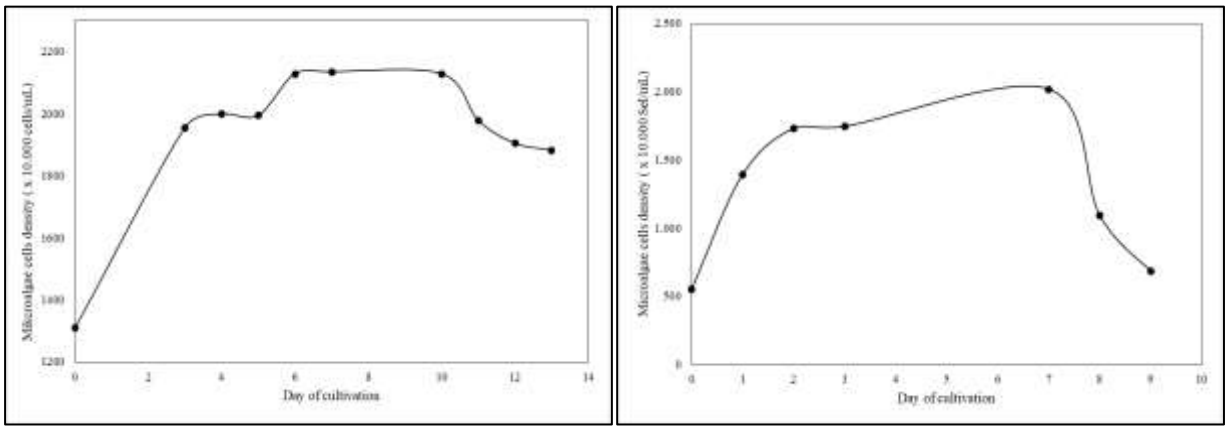
$$Microalgae\ growth\ rate\ (\mu) = \frac{Ln(\frac{N2}{n1})}{T2-T1} \qquad \textbf{Equation 2}$$

N_2 and N_1 = Number of cells in the exponential phase
T_2 and T_1 = Time from N_1 to N_2

3. Results and Discussion

The first stage of the research was observations on *Chlorella vulgaris* growth with 30% inoculum and 20% inoculum. The *C. vulgaris* initial cell density is 13.1 x 10^6 cells/mL and 5.5 x 10^6 cells/mL, respectively. The growth rate is 0.133 cells/mL/day and 0.927 cells/mL/day, respectively. The growth rate

was counted through the exponential phase from day 0 to day 3 of cultivation, as shown in **Figure 4**. The pH is around 7.95 – 8.92, and the temperature is around 28.3ºC – 31.5ºC. The empirical data for the first stage of *Chlorella vulgaris* cultivation consist of growth rate (µ), maximum cell density, pH, temperature, and CO_2 concentration shown in **Table 1**.



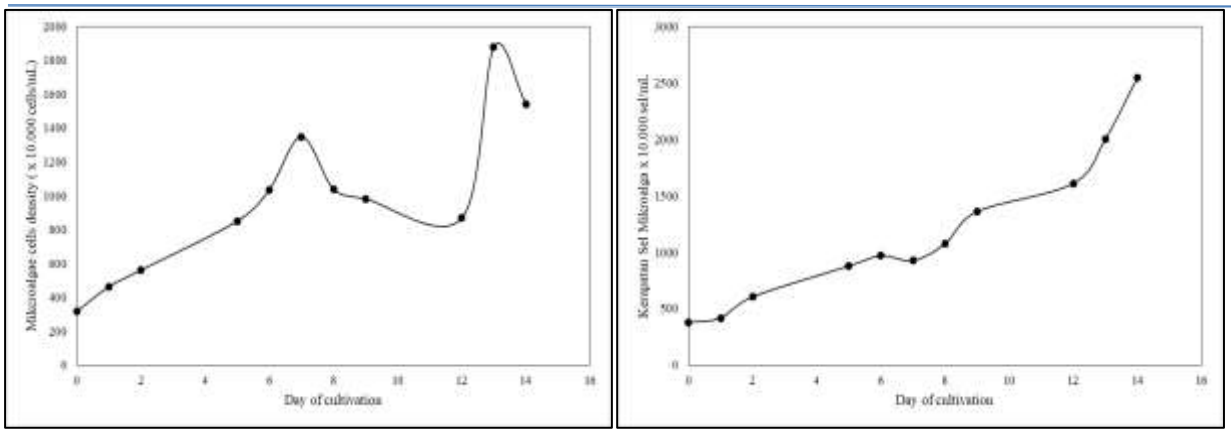
(a) (b)

Figure 4. The Cell Density of *C. vulgaris* in The First Stage of Cultivation (a) Cultivation with 30% of Microalgae Inoculum, and (b) Cultivation with 20% of Microalgae Inoculum

Table 1. Empirical Data for the First Stage of *Chlorella vulgaris* Cultivation

	Cultivation with aquades as media 3 L/min of aeration and 30% of Inoculum stock	**Cultivation with aquades as media 3 L/min of aeration and 20% of Inoculum stock**
Initial cell density	13.1 x 10^6 cells/mL	5.5 x 10^6 cells/mL
Growth rate (µ)	0.133 cells/mL/day	0.927 cells/mL/day
Maximum cell density	21.34 x 10^6 cells/mL (on the day 7 of cultivation)	20.16 x 10^6 cells/mL (on the day 7 of cultivation)
pH and Temperature	8.14 – 8.68 and 28.3ºC – 31.5ºC	7.95 – 8.92 and 28.8ºC – 31.2ºC
CO_2 concentration in the air	412 – 524 ppm	430 – 493 ppm

The second stage of the research was observations on *C. vulgaris* growth with 20‰ salinity and repetition of additional nutrients (walne, vitamins, and trace metal) on days 0 and 7. The variation in this stage is the aeration, 3 L/min, and 4 L/min. The *C. vulgaris* initial cell density is 3.78 x 10^6 cells/mL and 3.16 x 10^6 cells/mL, respectively. The growth rate is 0.263 cells/mL/day and 0.236 cells/mL/day. The growth rate has been counted through the exponential phase from day 6 to day 7 and from day 8 to day 9, respectively, as shown in **Figure 5.**



(a) (b)

Figure 5. The Cell Density of *C. vulgaris* in The Second Stage of Cultivation (a) Cultivation with 3 L/min of aeration, and (b) Cultivation with 4 L/min of aeration

Table 2. Empirical Data for the Second Stage of *Chlorella vulgaris* Cultivation

	Cultivation with 20‰ salinity 10% of Inoculum stock 3 L/min of aeration	**Cultivation with 20‰ salinity 10% of Inoculum stock 4 L/min of aeration**
Initial cell density	3.16 x 10^6 cells/mL	3.78 x 10^6 cells/mL
Growth rate (μ)	0.263 cells/mL.day	0.236 cells/mL.day
Maximum cell density	18.78 x 10^6 cells/mL (on the day 13 of cultivation)	25.46 x 10^6 cells/mL (on the day 14 of cultivation)
pH and Temperature	7.00 – 8.69 and 29.0ºC – 30.9ºC	7.00 – 8.72 and 29.0ºC – 30.4ºC
CO_2 concentration in the air	423 – 512 ppm	424 – 536 ppm

Chlorella vulgaris in the first stage can get almost the exact value of maximum cell density on day 7. 20% and 30% of inoculum stock used in the cultivation gave practically the same value for the cultivation, 21.34 x 10^6 cells/mL and 20.16 x 10^6 cells/mL. After that, microalgae become in the death phase after day 7. From the cell density in the second stage of cultivation, it can be concluded that the repetition of additional nutrients on day 7 can prevent the death phase of microalgae. *C. vulgaris* still increases its cells until day 14. Aeration with 4 L/min gave a better cell density than the 3 L/min. *C. vulgaris* cultivated with 3 L/min became in the death phase after day 14, while in cultivation with 4 L/min, *C. vulgaris* still increased its cells. Based on the results of the observations, cultivation with a microalgae percentage of 20% produced the best growth rate value. In addition, through measuring the concentration of CO_2 in the air inside the photobioreactor, the value of CO_2 concentration decreased during the cultivation process. This can be a concern, as microalgae can also maintain environmental quality by reducing CO_2 in the air.

The pH in the first stage photobioreactor is around 7.95 – 8.92, and the temperature is around 28.3ºC – 31.5ºC. In the second stage of the photobioreactor, the pH is around 7.00 – 8.72, and the temperature is around 29.0ºC – 30.9ºC. This value is still within the optimum range for microalgae growth. The optimum pH value for microalgae growth is in the range of 7-10. The pH in microalgae growth media is influenced by the microalgae mechanisms that occur during the cultivation process, such as the consumption of carbon and nitrogen and the concentration of dissolved carbon such as CO_3^{2-}, HCO_3^-, CO_2 [12], [13]. Adjustment of the pH value in microalgae growth media can be adjusted by adding air or CO_2 gas [12]. Temperature can affect microalgae's physical, chemical, and biological processes in removing pollutants. Increasing

temperature can increase the metabolic and respiration processes of microalgae [14]. Microalgae can grow optimally in the temperature range of 20ºC – 35ºC [12].

After knowing the characteristics of reasonable growth rate and cultivation in microalgae, further utilization of microalgae can be done. Other benefits that can be obtained from microalgae are the production of lipids, biofuel energy sources, the remediation of the environment, and other functions in various aspects. Microalgae play an essential role in restoring environmental quality. Several studies have been conducted to determine their ability to restore polluted environments. Phycoremediation is a bioremediation technique using algae to remediate hazardous contaminants such as hydrocarbons, pesticides, and heavy metals.

The ability of microalgae to absorb heavy metals and the ability to survive in water contaminated with heavy metals with moderate concentrations makes microalgae one of the remediation agents for water contaminated with heavy metals. Microalgae also have intracellular and extracellular mechanisms that protect themselves from heavy metal toxicity. Biosorption and bioaccumulation mechanisms are the main methods that occur in microalgae to remove heavy metals. In addition to being environmentally friendly and economically and energy efficient, microalgae can also provide benefits such as biomass production that has good nutritional content and is a renewable material in technology [5].

Domestic wastewater treatment using *C. vulgaris* can reduce COD concentration by 84%, ammonia by 95%, and phosphorus by 97% [15]. *C. vulgaris* can also remediate heavy metals such as Cr and Hg. *C. vulgaris* can remove chromium (VI) by 11% at a concentration of 5 mg/L [16] and remove mercury content by 61.34% at a mercury concentration of 0.3 mg/L [1]. C. vulgaris can also reduce heavy metal Pb by up to 90% [17]–[19]. Further research can be conducted to determine microalgae's ability to reduce various environmental pollutants.

4. Conclusion

The results showed that different percentages, initial cell densities, and aerators used in cultivation affect the development of microalgae and produce different maximum numbers of cells. Considering the maximum number of cells obtained, aeration of 3 L/min and a microalgae percentage of 20% can produce the best cultivation result. Microalgae can grow well in 20‰ saline media and potentially manage contaminated saline media as a phycoremediation agent. The repeated addition of nutrients to microalgae cultivation can prevent the death phase, increase cell production, and create a potential for further utilization of microalgae in phycoremediation.

5. Acknowledgment

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