# The Effect of Aeration and *Chlorella vulgaris* Initial Cell Density on Mercury Removal

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## ABSTRACT

Research on *Chlorella vulgaris* is vital due to its rapid growth and versatile habitat adaptability. *C. vulgaris* is a fast-growing green microalga used for phycoremediation of heavy metals such as mercury which is usually found in gold mining wastewater. This research aims to determine the best mercury removal efficiency with variations in aeration rate, medium concentration, and *C. vulgaris* inoculum concentration. This research was conducted by cultivating *C. vulgaris* and examining its response to various parameters in an artificial mercury (II) chloride ($HgCl_2$) solution. Moreover, other parameters such as cell density, pH, temperature, bicarbonate, and dissolved oxygen were monitored over a seven-day experimental period. The highest removal efficiency (61.34%) at a mercury concentration of 0.3 mg/L was found in the variant with a walne medium dose of 1 mL/L, inoculum concentration of 10%, and aeration rate of 3 L/min. This study confirmed the potential of *C. vulgaris* as an effective agent for heavy metal removal, particularly mercury, and contributed to bioremediation.

Keywords: mercury, aeration rate, walne medium, *Chlorella vulgaris.*

## Introduction

Research on microalgae is vital due to their fast and easy cultivation, as well as their ability to thrive in fresh or saltwater (Coronado-Reyes et al., 2022). The application of microalgae has numerous branches due to their different strategies according to their nutritional requirement. Maximizing its effectiveness is essential for regulating nutrient composition and various environmental factors, such as temperature, light intensity, and salinity (Cunha et al., 2023). Microalgae has been widely explored as an efficient biological method for liquid waste treatment to degrade nutrient composition while enhancing biomass and lipid concentration (Kiran et al., 2017). The biological method that uses algae to remove or change the form of contaminants into harmless products is also called phycoremediation. Phycoremediation can eliminates nutrients, toxins, and heavy metals and helps facultative aerobic bacteria mineralize organic contaminants by providing oxygen (Koul et al., 2022). Microalgae can live in various sub-habitats with general characteristics such as planktonic, epiphytic, and benthic (Arsad et al., 2020). One common microalgae species is *C. vulgaris,* which has versatile growth capabilities (Coronado-Reyes et al., 2022).

*C. vulgaris* is cosmopolitan, meaning it can thrive in a wide range of environments and has a high reproductive rate (Habir, 2021). As stated, microalgae growth is influenced by several factors, such as temperature, light intensity, and salinity (Cunha et al., 2023). Other factors are nitrogen, pH, and carbon dioxide ($CO_2$) (Slamet, 2016). Nutrients in the form of macronutrients and micronutrients are needed to stimulate biomass growth (Mufidah et al., 2017). Microalgae require micronutrients such as trace elements for growth in small and limited amounts of concentration (Siong et al., 2021). The high reproductive ability of *C. vulgaris* has attracted the attention as a bioremediation agent, including heavy metals in waters that called as phycoremediation (Hartiono, 2019). Heavy metals pose significant environmental problems due to their negative impacts and toxicity to living organisms specifically when present at atomic densities above 4 gr/L, which cannot be treated biologically. One of the most dangerous heavy metals is mercury (Hg), which is widely used

and produced in anthropogenic activities, such as gold mining wastewater (tailings) (Musa et al., 2020). Mercury contamination has a significant toxic effect on the environment and human health (Wang et al., 2020). Bioremediation offers an eco-friendly and cost-effective solution for heavy metal removal (Bentez Alvis et al., 2017). Previous research described that eliminating and absorbing heavy metals from water has been significantly effective, especially using *C. vulgaris* (Manzoor et al., 2019). Musa et al. (2020) stated that when *C. vulgaris* was exposed to mercury for 8 days with the addition of walne medium, it showed its ability to remove 43.19% of the mercury concentration in the form of mercury (II) chloride ($HgCl_2$) with maximum concentration 1 mg/L.

Previous research stated that *C. vulgaris* survived in a solution containing $HgCl_2$ and walne medium; however, it has not been further investigated regarding the optimal dose of the medium and several other factors, such as the aeration rate and *C. vulgaris* inoculum concentration on the efficiency of mercury removal (Arsad et al., 2020). Aeration rate, medium, and inoculum concentration are important factors for microalgae cultivation. Aeration can increase mass transfer, gas supplies, and control toxic concentrations if dissolved oxygen in the culture is low (>3 mg/L) (Magdaong et al., 2019). Medium is critical because it contains macronutrients and micronutrients for microalgae cultivation that can affect the quantity of inoculum biomass (Mardalisa et al., 2022). The use of Walne medium is more suitable for the growth of green microalgae than Guillard medium due to its superior nutritional content (Putri & Alaa, 2020). Therefore, this study aims to determine the mercury (II) chloride removal efficiency by varying the walne medium concentration, *C. vulgaris* inoculum concentration, and aeration rate.

## Material and Methods

### *Preparation of Microalgae Culture*

The microalgae starter culture used in this research was *C. vulgaris*, which was purchased from microalgae cultivation farmers in Tangerang, Indonesia, and to ensure their viability, it was propagated in the remediation laboratory of Institut Teknologi Sepuluh Nopember (ITS), Surabaya, Indonesia for seven days before use. The results of physical observation are shown in Figure 1.

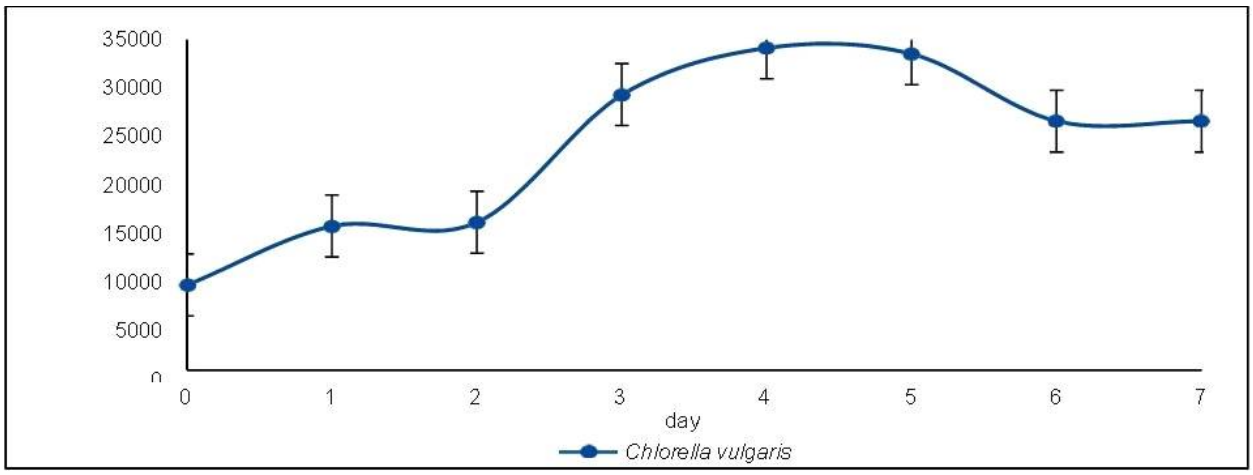


Figure 1. Cell Density During Propagation. Error Bars Indicate the Variability of Cell Density Data Based on Standard Deviation (SD) From Replicate Experiments.

The cell density observed showed varying results, with the highest ($3.41 \times 10^8$ cells/mL) on the 4th day. During propagation, pH, temperature, and $CO_2$ concentration were observed. During the seven days of cultivation, the pH of the medium was in the range of 7 - 8, the temperature was in the range of 27.6 - 28.5 °C, and the $CO_2$ concentration was in the range of 504 mg/L - 423 mg/L. Based on the growth curve, the *C. vulgaris* used for treatment were harvested on the 4 th day of propagation. The cell density was measured using a hemocytometer (Neubauer Improved, Marienfeld, Germany), and pH was monitored using a digital pH meter (Eutech pH 700, Singapore) equipped with automatic temperature compensation. The lighting during propagation used Philips LED Bulb E27 4W 6500K Cool Daylight (Indonesia) with an intensity of 90 µmol $m^{-2}$ $s^{-1}$.

### *Preparation of Mercury Solution*

The solution used was mercury (II) chloride ($HgCl_2$) (Merck, Germany), prepared by diluting the standard stock solution with a concentration of 0.3 mg/L to 10 mg/L using distilled water (aquadest).

### *Minimum Inhibitory Concentration (MIC) Test*

An MIC test was carried out to determine *C. vulgaris* resistance to mercury. Hartiono (2019) showed that *C. vulgaris* survived in a medium containing $HgCl_2$ up to a concentration of 5 mg/L, so this research was conducted using a mercury concentration close to 5 mg/L. The MIC test was performed with some modifications by varying *C. vulgaris* inoculum, aeration rate, and walne medium dosage. The experiment was carried out using $HgCl_2$ (Merck, Germany) solutions with various concentrations of 0; 0.3; 0.6; 1; 3; 6 mg/L (volume of 225 mL) and 25 mL of microalgae inoculum (cell density of 2.92 x $10^8$ cells/mL) was added in the experiment reactor (10% v/v). The total volume of each experiment reactor was 250 mL. The reactor was conditioned with a cool daylight lamp (Philips LED Bulb E27 4w 6500K Cool Daylight), light intensity of 6000 lux or 90 µmol $m^{-2}$ $s^{-1}$ for 12 hours, and it was aerated by air ambient (3 L/min) for 24 hours and seven days. Lighting conditions, aeration time, and light duration refer to the research of Berlianto (2018), which states that *C. vulgaris* can be cultivated optimally with lighting conditions of 90 µmol $m^{-2}$ $s^{-1}$, aeration time of 24 hours, and light duration of 12 hours. Microalgae cells were calculated using a haemocytometer with Average Observed Area (AOA) method (cells/mL), while temperature, $CO_2$ concentration, and pH were observed every 24 hours for seven days.

### *Experimental Set-Up*

The research used $HgCl_2$ concentrations from the previous MIC tests (0.3 mg/L) using the same artificial $HgCl_2$ solution with composition as in the MIC test. The variations included walne medium added (1 mL/L and 1.5 mL/L), aeration rate (2.5 L/min and 3 L/min), and concentrations of *C. vulgaris* inoculum (0%, 2%, 5%, and 10% v/v) (Table 1).

Table 1. Experimental Set-Up Variations

| Variation Set-Up (Walne dose; Aeration rate; Inoculum) | Designated Code | Variations Set-Up (Walne dose; Aeration rate; Inoculum) | Designated Code |
|---|---|---|---|
| 1 mL/L, 2.5L/min, 0% | A | 1.5 mL/L, 2.5L/min, 0% | I |
| 1 mL/L, 2.5L/min, 2% | B | 1.5 mL/L, 2.5L/min, 2% | J |
| 1 mL/L, 2.5L/min, 5% | C | 1.5 mL/L, 2.5L/min, 5% | K |
| 1 mL/L, 2.5L/min, 10% | D | 1.5 mL/L, 2.5L/min, 10% | L |
| 1 mL/L, 3L/min, 0% | E | 1.5 mL/L, 3L/min, 0% | M |
| 1 mL/L, 3L/min, 2% | F | 1.5 mL/L, 3L/min, 2% | N |
| 1 mL/L, 3L/min, 5% | G | 1.5 mL/L, 3L/min, 5% | O |
| 1 mL/L, 3L/min, 10% | H | 1.5 mL/L, 3L/min, 10% | P |

In this research, a 750 mL reactor containing $HgCl_2$, aquadest, and inoculum was used, and subsequently, walne medium was added. The reactor was conditioned with cool daylight lamps (90 µmol $m^{-2}$ $s^{-1}$) for 12 hours per day and operated for seven days. Parameters were measured, including microalgae cell density (cells/mL) using the Hemocytometer method (Boy Thompson Institute, 2015), while pH and temperature were measured using a pH meter with automatic temperature compensation (Eutech, Singapore) based on SNI 6989.11:2019 (National Standard Institution, 2019) and SNI 06-6989.23-2005 (National Standard Institution, 2005), $HCO_3$ concentration was measured using the titration method (Bassett et al., 1944), Dissolved Oxygen (DO) using the titration method based on SNI 06-6989.14-2004 (National Standard Institution, 2004), and mercury concentration using Atomic Absorption Spectrophotometry (AAS) iCE 3000 Series (Thermo Scientific, United States) method based on SNI 6989.78-2011 (National Standard Institution, 2011). Mercury concentration in the medium was measured on days 0 and day 8. Mercury removal efficiency was calculated using the following equation:

$$\text{Mercury Removal Efficiency} = \frac{Co - C1}{C0} \times 100\% \quad \ldots\ldots (1)$$

where Co is the initial mercury concentration of (mg/L); $C_1$ is the final mercury concentration (mg/L).

### *Statistical analysis*

Statistical analysis was carried out using the Analysis of Variance (ANOVA) method. The two-way ANOVA (p-value < 0.05) type method was used in this research to know which variable significantly influences mercury removal efficiency in microalgae growth reactors. The two-way ANOVA analysis method used Minitab 17 (free trial version) software. The test was conducted on three independent variables: walne medium dose, aeration rate, and inoculum concentration.

## Results and Discussion

### *The Growth of* C. vulgaris

Observations on the number of cells (cell density) were carried out every day for seven days. The provision of nutrients in the form of walne medium was expected to increase the growth and resistance of *C. vulgaris* cells to mercury compared to the MIC test. The results of physical observations are shown in Figure 2.

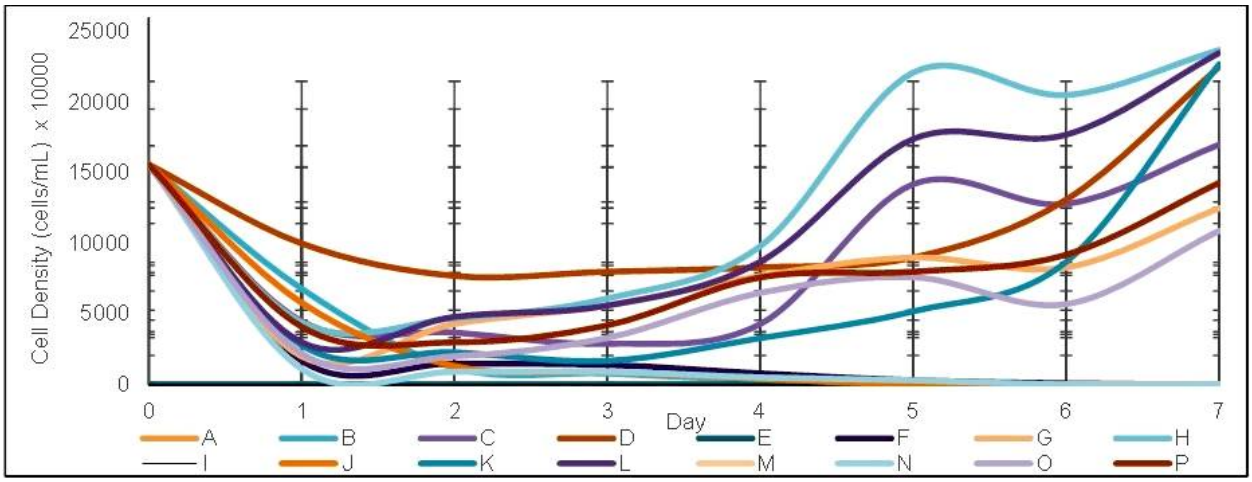


Figure 2. Cell Density During the Experiment. For variations set-up, Error Bars Indicate the Variability of Cell Density Data Based on Standard Deviation (SD) From Replicate Experiments.

The cell density observed showed varying results, with the highest (2.44 x $10^8$ cells/mL) occurring in variations (H) with the medium dose of 1 mL, aeration rate of 2.5 L/min, and inoculum concentration of 10% on the seventh day. In contrast, the lowest (1.09 x $10^8$ cells/mL) occurred in variations (O) with the medium dose of 1.5 mL dose, 3 L/min rate, and 5% inoculum. Cell growth initially increased with rising inoculum concentration but eventually slowed down due to reduced nutrient absorption and excess cell density (Ma & Jian, 2023). The difference in dose of the medium also affected the cell density. Providing excessive nutrition can lead to stressful conditions and cause a decrease in biomass. Excessive micronutrients will cause the nutrients to become toxic, disrupt cell metabolism, and cause microalgae cells to die or stop growing (Wardani et al., 2022). As depicted in Figure 2, the medium dose of 1 mL/L was optimal for the microalgae cell growth, evidenced by this variant's lowest cell density of 1.5 mL/L. These findings showed that the resulting cell density in variant (H) was 2.44 x $10^8$ cells/mL using a 1 mL/L medium dose. It was higher than the cell density in variant (P) (1.37 x $10^8$ cells/mL) using the 1.5 mg/L medium dose. The only difference between variables H and P is the Walne dose used (variable H has a Walne dose of 1 mL/L, while variable P has a Walne dose of 1.5 mL/L). Other variables of both variants were an aeration rate of 3 L/min and an inoculum concentration of 10% v/v. A simple linear regression statistical test was performed to examine the effect of the medium dose on cell density by comparing variant (H) with variant (P). The test result showed that medium dose had no significant effect on cell density (p - value > 0.05), when aeration rate and inoculum concentration are held constant (3 L/min, and 10% v/v).

### *pH*

pH was monitored daily over a period of 7 days. Qiu (2020) stated that pH in microalgae's living environment is essential because it determines the solubility and availability of $CO_2$ and nutrients, which will significantly influence microalgae metabolism. The monitored of *C. vulgaris* pH are shown in Figure 3.

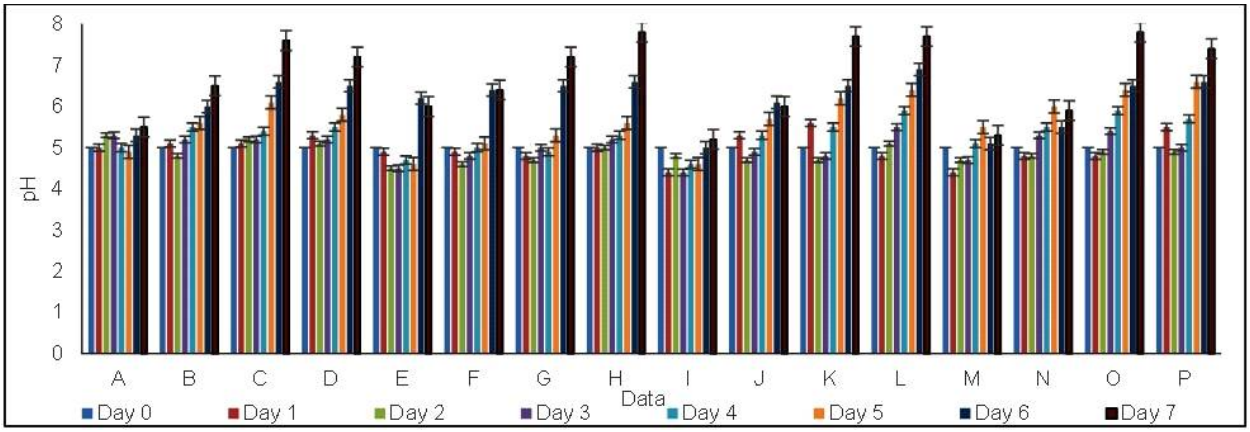


Figure 3. pH During the Experiment, Error Bars Indicate the Variability of pH Data Based on Standard Deviation (SD) From Replicate Experiments.

***Standard Deviation (SD) From Replicate Experiments.***

As depicted in Figure 3, several variants show that the pH in the *C. vulgaris* culture medium continued to increase during the research. The highest pH was observed in two variants: (H) and (O) (see Table 1). In both variants, the pH in the culture was 7.8 after the seventh day. Meanwhile, the lowest pH, 5.9, was observed in variants with the medium dose of 1.5 mL/L, aeration rate of 3 L/min, and inoculum concentration of 2%. Generally, microalgae can grow and increase their biomass between pH 6 to 10 (Nouri et al., 2021). Specifically, based on experiment, *C. vulgaris* can grow in the pH range of 4 to 10. In culture media with a pH between 9 and 10, microalgae exhibit the highest biomass productivity (Daliry et al., 2021). However, in our experiment, *C. vulgaris* cells could grow at a pH of up to 8. Variations in pH cause metabolic changes in microalgae cultures by affecting the balance of inorganic carbon (Okoro et al., 2019). Consequently, the variant with the highest pH had a greater cell density than the lowest, as shown in Figure 3. The pH in microalgae culture determines the solubility of minerals and $CO_2$ in the medium (Chowdury et al., 2020). $CO_2$ at an acidic pH will form carbonic acid; at an alkali pH, it will form a bicarbonate ion ($HCO_3^{2-}$). It showed that variant with the highest pH (pH>9) has $CO_2$ in the form of bicarbonate, it can utilize as a source of $CO_2$ (Sa'adah & Widyaningsih, 2018).

***Temperature***

Temperature observations in *C. vulgaris* were carried out daily during the research. The observation results in this research are shown in Figure 4.

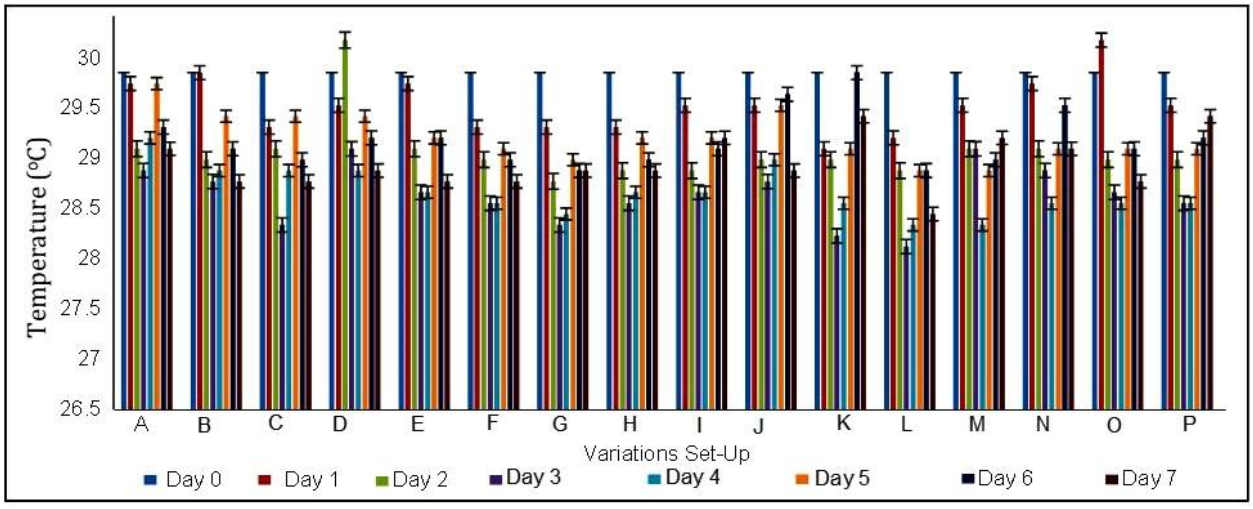


Figure 4. Temperature During the Experiment, Error Bars Indicate The Variability of Temperature Data Based on Standard Deviation (SD) From Replicate Experiments.

Microalgae are widely distributed organisms that thrive in harsh conditions, including environments with varying temperatures (Jaiswal et al., 2020). The shift beyond optimum temperature can affect or inhibit cell growth. The higher temperatures can make culture collapse because of heat stress and growth retardation of biomass, and the lower temperatures can decrease the growth of biomass (Corredor et al., 2021). Based on Figure 4, overall, no temperature was too high. All variants were still classified as room temperature. The initial temperature for all reactors on day 0 was consistent at 29.6°C. However, all reactors experienced a slight decrease from 28.6 to 29.2°C for seven days. The variant (K) and variant (L) with the highest temperature (29.2 °C) contained a dose of 1.5 ml/L Walne medium, an aeration rate of 2.5 L/min, as well as 5% and 10% inoculum concentration. In contrast, the lowest (28.6 °C) was obtained with 1 mL/L, 3 L/min, and 2% values. The results of the ANOVA statistical test showed that only the inoculum concentration had a significant effect on the mercury concentration.

## Bicarbonate Concentration

Bicarbonate concentration ($HCO_3$) was observed on the zero, third, and seventh days. This test was aimed to ascertain the presence of microalgae cells, indicated by their use of a carbon source for life. The observation results for all variants are shown in Figure 5.

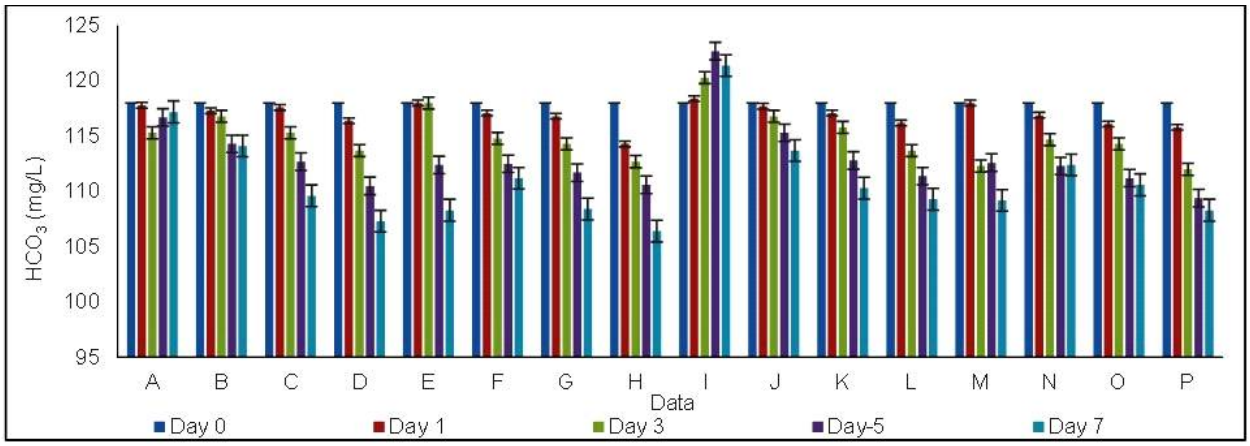


Figure 5. Bicarbonate Concentration During the Experiment, Error Bars Indicate the Variability of Bicarbonate Concentration Data Based on Standard Deviation (SD) From Replicate Experiments.

As shown in Figure 5, the initial bicarbonate concentration in the medium on day 0 is 118 mg/L, then all variants experienced a decrease up to the seventh day. The highest value (114.1 mg/L) was obtained in variation (I) with Walne medium dose of 1.5 mL/L, aeration rate of 2.5 L/min, and 0% inoculum concentration on the fifth day. The trend is different because variation (I) (control variable) did not have cell growth. As a control variable, these samples only show environmental changes (such as pH or HCO*f* ) without any biological activity from *C. vulgaris*. In contrast, the lowest $HCO_3$ concentration (106.4 mg/L) was observed in variant H of 1 mL/L, 3 L/min, and 10% on day seven. The decline in bicarbonate concentrations was accompanied by increased pH, reducing the solubility of metals in water. In the range of 6-10, $CO_2$ was used as bicarbonate ($HCO_3$) (Sa'adah & Widyaningsih, 2018). Based on the results, the variant with the lowest bicarbonate concentration had a high pH value on the seventh day, namely 7.8 (variant H) as shown in Figure 5, proving that a decrease in $CO_2$ concentrations increased the pH in the medium. The final pH of 7.8 also indicated that the dissolved $CO_2$ in the medium was in the form of bicarbonate ($HCO_3$).

## DO Concentration

During the research, observation was also carried out to test the DO content in the culture. The observation results for all variants are shown in Figure 6.

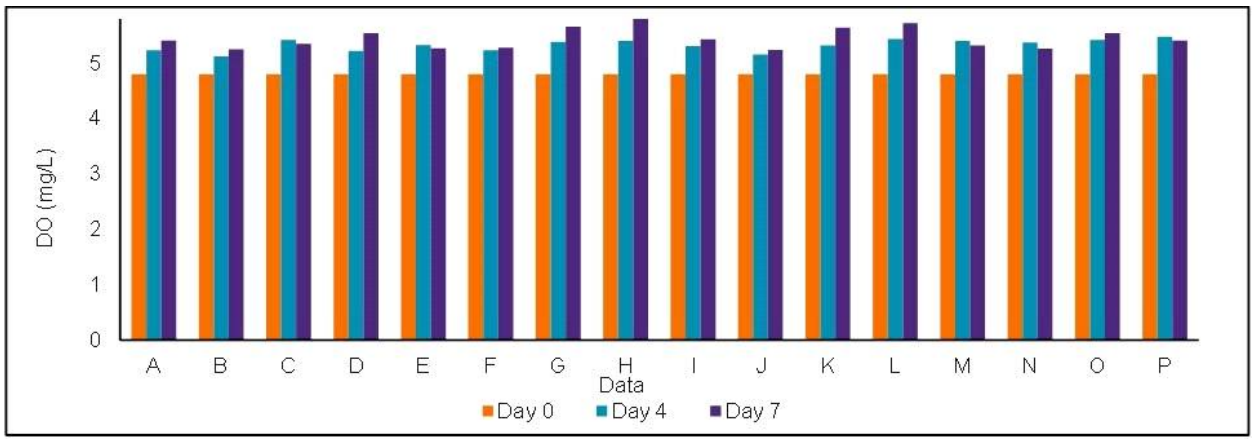


Figure 6. DO During the Experiment, Error Bars Indicate The Variability of DO Concentration Data Based on Standard Deviation (SD) From Replicate Experiments.

Phytoplankton biomass activity is reflected in the concentration of dissolved oxygen. DO concentrations should not be below 4 mg/L because it will harm almost all aquatic organisms. After all, it can affect the physiology and metabolism of plankton (Alvateha et al., 2020). Based on Figure 6, the highest DO concentration of 5.93 mg/L was found on the seventh day in the variant with Walne medium dose of 1 mL/L, aeration rate of 3 L/min, and 10% inoculum concentration. In contrast, the lowest DO concentration was obtained in the variant with a variant of 1.5 mL/L, 2.5 L/min, and 2%. The higher the DO value lead the better the conditions in a medium. The optimal DO concentration for *C. vulgaris* ranges from 6.31 mg/L to 9.22 mg/L. Elevated DO concentrations may impede photosynthetic microorganisms growth within microalgal culture systems. Based on these observations, the value obtained in the variant with Walne medium dose of 1 mL/L, aeration rate of 3 L/min, and an inoculum concentration of 10% indicated that the photosynthesis process was efficient compared to other variants. However, the value is below the optimal DO concentration for the growth of *C. vulgaris* cells.

### *MIC Test*

Observation of *C. vulgaris* cells was carried out by counting cell density every day for seven days. The results of physical observations are shown in Figure 7.

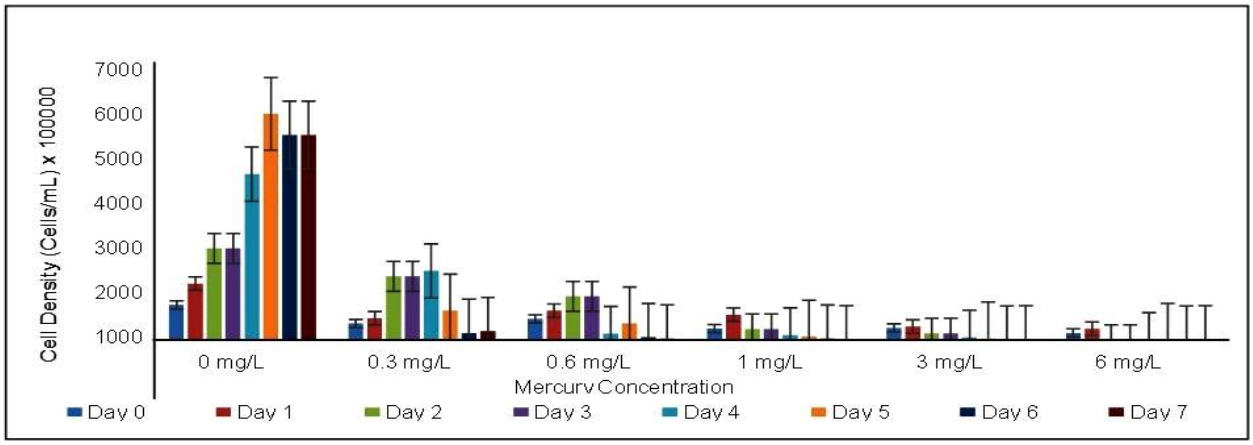


Figure 7. Cell Density *C. vulgaris* During MIC Test, Error Bars Indicate The Variability of Cell Density Data Based on Standard Deviation (SD) From Replicate Experiments.

Based on Figure 7, the highest cell density until the seventh day was only found in the variant with a Mercury concentration 0.3 mg/L while at higher concentrations (0.6 mg/L, 1 mg/L, 3 mg/L, 6 mg/L), no cell density was found on the seventh day of the MIC test. This proves that *C. vulgaris* cells can only survive at a mercury concentration of 0.3 mg/L, while higher mercury concentrations inhibit their growth.

### *Mercury Concentration*

The removal efficiency was monitored by analyzing The removal efficiency was assessed by comparing the mercury concentrations at day 0 and day 7. The observation results for all variants are shown in Figure 8.

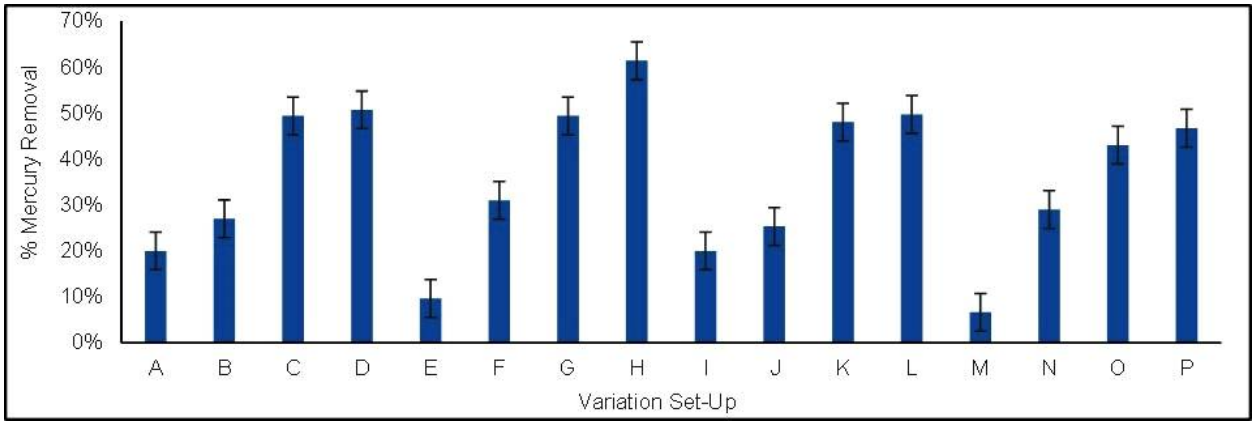


Figure 8. Mercury Removal Efficiency. Error Bars Indicate the Variability of Percentage Mercury Removal Data Based on Standard Deviation (SD) From Replicate Experiments.

Based on Figure 8, The results showed that the highest removal efficiency (61.34%) at a mercury concentration of 0.3 mg/L. the highest mercury removal efficiency is found in the variant with walne medium dose of 1 mL/L, aeration rate of 3 L/min, and inoculum concentration of 10%. In this variant, mercury concentration initially 0.3 mg/L decreased to 0.116 mg/L on the seventh day, indicating a removal efficiency of 61.34%. The variant produced the lowest mercury concentration on variant (J) with walne medium dose of 1.5 mL/L, aeration rate of 2.5 L/min, and 2% inoculum concentration. This variant experienced a decline in mercury concentration from 0.3 mg/L to 0.224 mg/L on the seventh day, implying a removal efficiency of 25.34%. These observations showed that the inoculum concentration, walne medium dose, and aeration rate influenced the ability of *C. vulgaris* to remove mercury in the medium. Wang et al. (2022) stated that mercury can exert its toxic effect on growth by inhibiting nutrient uptake and metabolism subsequently affecting the photosynthesis process. According to Hartiono (2019), heavy metals such as mercury can cause the death of aquatic organisms due to the bioaccumulation process. The variant with an inoculum concentration of 2% across all variations of walne medium dose and aeration rate showed that on the fifth day, there was no cell density in the medium (0 cell/mL). However, residual mercury concentration was still in the medium on the seventh day, confirming that bioaccumulation had occurred.

The rapid uptake of heavy metals by microalgae can happen through different methods, such as the creation of covalent bonds between the metals and the charged cell walls, the attachment of metal cations to the negatively charged uronic acids found in microalgae exopolysaccharides, and the exchange of metal ions with cations on the cell wall (Kit & Chang, 2020). The variation in inoculum concentration indicated a significant effect on the percentage of mercury removal (p-value < 0.05). This was further supported by the observation of mercury removal percentages by *C. vulgaris*, where the percentage of mercury removal consistently increased with increasing inoculum concentration, with the highest mercury removal percentage shown by the variant with the highest inoculum concentration, which is 10%. On the other hand, it can be concluded that there was no significant effect on the percentage of mercury removal resulting from variations in Walne dose (p-value > 0.05) and aeration rate (p-value > 0.05). However, the coefficient of determination ($R^2$) result was 93.92%, interpreting that the variation in Walne medium dose and aeration rate explains 93.92% of the variation in mercury concentration in the samples. The correlation coefficient (r) value of 0.969 interpreted a strong linear relationship between the variations in Walne medium dose, aeration rate, and inoculum concentration on a scale from 0 to 1.

Mercury processing is caused by enzymatic biotransformation, defined as the chemical transformation from a highly toxic to a less harmful form through oxidation-reduction reactions. Heavy metals cannot be degraded but are converted from one oxidation state to another inorganic complex form, reducing their toxic effects. Few studies highlight the role of oxidoreductase enzymes in detoxifying heavy metals by microalgae. One of the main redox enzymes known is Mercury reductase (merA), encoded by the merA gene (Danouche et al., 2021). The primary resistance mechanism is the enzymatic reduction of $Hg^{2+}$ to $Hg^0$. This enzyme is part of the merr operon (mercury resistance in the form of genetic sequence found in some bacteria that encodes proteins involved), which encodes a group of proteins associated with detecting, scavenging, transporting, and reducing Hg concentrations (Møller et al., 2014). Therefore, several factors should be considered to prevent damage to

enzymes in microalgae. In this experiment, all the parameters tested were some factors that affected microalgae growth. The explanation for that has been discussed in the previous section.

## Conclusion

The research results demonstrated that *C. vulgaris* cultured with a walne medium dose of 1 mL/L, an inoculum concentration of 10%, and an aeration rate of 3 L/min achieved a mercury removal efficiency of 61.34%. Statistical analysis indicated that the inoculum concentration significantly affected the mercury removal percentage. Furthermore, the analysis also indicated a robust linear relationship among the variations in walne dose, aeration rate, and inoculum concentration.

## Acknowledgments

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## References

Alvateha, D., Falentina, S., Kasitowati, R. D., Suherman, S. P., Sari, L. A., & Arsad, S. (2020). The performance of Chlorella vulgaris growth on mass-scale cultivation. *Tomini Journal of Aquatic Science*, *1*(2), 45–54. https://doi.org/10.37905/tjas.v1i2.8123

Arsad, S., Kholifah, S. N., Prabawati, E., Sari, L. A., Kadim, M. K., & Kilawati, Y. (2020). Effect of Mercury on Growth of Several Microalgae. *Asian Journal of Water, Environment and Pollution*, *17*(3), 13–17. https://doi.org/10.3233/AJW200029

Bassett, J., Denney, R. ., Jeffery, G. ., & Mendham, J. (1944). *Kimia Analisis Kuantitatif Anorganik*. EGC.

Bentez Alvis, S., Prez Cordero, A., & Vitola Romero, D. (2017). Removal and Recovery of Mercury in Vitro Using Immobilized Live Biomass of Chlorella sp. *Indian Journal of Science and Technology*, *11*(45), 1–8. https://doi.org/10.17485/ijst/2018/v11i45/137575

Berlianto, M. (2018). *Dekonsentrasi Chromium Pada Limbah Cair Menggunakan Konsorsium Bakteri dan Mikroalga dengan Sistem High Rate Algal Reactor*. Institut Teknologi Sepuluh Nopember.

Chowdury, K. H., Nahar, N., & Deb, U. K. (2020). The Growth Factors Involved in Microalgae Cultivation for Biofuel Production/ : A Review. *Computational Water, Energy, and Environmental Engineering*, *9*, 185–215. https://doi.org/10.4236/cweee.2020.94012

Coronado-Reyes, J. A., Salazar-Torres, J. A., Juárez-Campos, B., & González-Hernández, J. C. (2022). Chlorella vulgaris, a microalgae important to be used in Biotechnology: a review. *Food Science and Technology (Brazil)*, *42*, 1–11. https://doi.org/10.1590/fst.37320

Corredor, L., Barnhart, E. P., Parker, A. E., Gerlach, R., & Fields, M. W. (2021). Effect of temperature, nitrate concentration, pH and bicarbonate addition on biomass and lipid accumulation in the sporulating green alga PW95. *Algal Research*, *53*, 102148. https://doi.org/10.1016/j.algal.2020.102148

Cunha, E., Sousa, V., Geada, P., Teixeira, J. A., Vicente, A. A., & Dias, O. (2023). Systems biology's role in leveraging microalgal biomass potential: Current status and future perspectives. *Algal Research*, *69*(January 2022). https://doi.org/10.1016/j.algal.2022.102963

Danouche, M., El Ghachtouli, N., & El Arroussi, H. (2021). Phycoremediation mechanisms of heavy metals using living green microalgae: physicochemical and molecular approaches for enhancing selectivity and removal capacity. *Heliyon*, *7*(7), e07609. https://doi.org/10.1016/j.heliyon.2021.e07609

Habir, T. A. S. (2021). *Formula Sediaan Chlorella Vulgaris*. Universitas Hasanuddin. https://repository.unhas.ac.id/id/eprint/11685/

Hartiono, R. F. (2019). *Analisis Penurunan Kadar Logam Berat Merkuri (Hg) dengan Menggunakan Mikroalga Chlorella vulgaris*. Universitas Brawijaya. Malang.

Institute, B. T. (2015). *Algae to Energy - Using and Re-using a Hemocytometer to Count Algae Cells*. 2–4. file:///Users/emilypurvis/Downloads/e.-Algae-to-Energy-Counting-Algae-Cells.pdf

Jaiswal, K. K., Banerjee, I., Singh, D., Sajwan, P., & Chhetri, V. (2020). Ecological stress stimulus to improve microalgae biofuel generation/ : a review. *Octa Journal of Biosciences*, *8*(June), 48–54.

Kiran, B., Pathak, K., Kumar, R., & Desmukh, D. (2017). Phycoremediation: An Eco friendly Approach to Solve Water Pollution Problems. *Microbial Applications*, *1*, 1–331. https://doi.org/10.1007/978-3-319-52666-9

Koul, B., Sharma, K., & Shah, M. P. (2022). Phycoremediation: A sustainable alternative in wastewater treatment (WWT) regime. *Environmental Technology and Innovation*, *25*, 102040. https://doi.org/10.1016/j.eti.2021.102040

Ma, X., & Jian, W. (2023). Growth Conditions and Growth Kinetics of Chlorella Vulgaris Cultured in Domestic Sewage. *Sustainability (Switzerland)*, *15*(3). https://doi.org/10.3390/su15032162

Magdaong, J. B., Ubando, A. T., Culaba, A. B., Chang, J. S., & Chen, W. H. (2019). Effect of aeration rate and light cycle on the growth characteristics of Chlorella sorokiniana in a photobioreactor. *IOP Conference Series: Earth and Environmental Science*, *268*(1). https://doi.org/10.1088/1755-1315/268/1/012112

Manzoor, F., Karbassi, A., & Golzary, A. (2019). Removal of Heavy Metal Contaminants from Wastewater by Using Chlorella vulgaris Beijerinck: A Review . *Current Environmental Management*, *6*(3), 174–187. https://doi.org/10.2174/2212717806666190716160536

Mardalisa, M., Zalfiatri, Y., & Rahmayuni, R. (2022). The Effect of Culture Media Types on the Growth of Marine Microalgae Chlorella vulgaris. *IOP Conference*

*Series: Earth and Environmental Science*, *1118*(1). https://doi.org/10.1088/1755-1315/1118/1/012029

Møller, A. K., Barkay, T., Hansen, M. A., Norman, A., Hansen, L. H., Sørensen, S. J., Boyd, E. S., & Kroer, N. (2014). Mercuric reductase genes (merA) and mercury resistance plasmids in High Arctic snow, freshwater and sea-ice brine. *FEMS Microbiology Ecology*, *87*(1), 52–63. https://doi.org/10.1111/1574-6941.12189

Mufidah, A., Agustono, Sudarno, & Nindarwi, D. D. (2017). *Teknik Kultur Chlorella sp. Skala Laboratorium dan Intermediet di Balai Perikanan Budidaya Air Payau (BPBAP) Situbondo Jawa Timur*. *7*(2).

Musa, M., Arsad, S., Kilawati, Y., Hartiono, R. F., Fajrin, I., Sari, L. A., & Prasetiya, F. S. (2020). Phycoremediation of mercury in the aquatic environment. *Environment Protection Engineering*, *46*(4), 69–76. https://doi.org/10.37190/epe200405

Nouri, H., Roushandeh, J. M., Hallajisani, A., Golzary, A., & Daliry, S. (2021). The effects of glucose, nitrate, and pH on cultivation of Chlorella sp. Microalgae. *Global Journal of Environmental Science and Management*, *7*(1), 103–116. https://doi.org/10.22034/gjesm.2021.01.08

Okoro, V., Azimov, U., Munoz, J., Hernandez, H. H., & Phan, A. N. (2019). Microalgae cultivation and harvesting: Growth performance and use of flocculants - A review. *Renewable and Sustainable Energy Reviews*, *115*(July). https://doi.org/10.1016/j.rser.2019.109364

Putri, D. S., & Alaa, S. (2020). The Growth Comparison of Haematococcus Pluvialis in Two Different Medium. *Biota*, *April*. https://doi.org/10.20414/jb.v12i2.202

Qiu, R. (2020). *Growth Condition Optimization and Bacterial Control for Mass Production of Microalgae*. The University Of Arizona.

Sa'adah, N., & Widyaningsih, S. (2018). Pengaruh Pemberian CO2 terhadap pH Air pada Pertumbuhan Caulerpa racemosa var. uvifera. *Jurnal Kelautan Tropis*, *21*(1), 17. https://doi.org/10.14710/jkt.v21i1.2460

Siong, W., Gee, W., Siti, H., Munawaroh, H., Kumar, V., Ho, S., & Loke, P. (2021). Multifaceted roles of microalgae in the application of wastewater biotreatment/ : A review *. *Environmental Pollution*, *269*, 116236. https://doi.org/10.1016/j.envpol.2020.116236

Slamet, A. (2016). Peningkatan Fungsi Boezem Morokrembangan Sebagai Pengolah Air Limbah Perkotaan Menggunakan Sistem Alga-Bakteri. *Disertasi*, 1–324.

National Standard Institution. (2004). *SNI 06-6989.14-2004/ : Oksigen Terlarut*.

National Standard Institution. (2005). *SNI 06-6989.23-2005/ : Air dan air limbah – Bagian 23: Cara uji suhu dengan termometer*.

National Standard Institution. (2011). *SNI 6989.78-2011 Air dan air limbah – Bagian 78/ : Cara uji raksa ( Hg ) secara Spektrofotometri Serapan Atom ( SSA ) – uap dingin atau Mercury Analyzer*.

National Standard Institution. (2019). *SNI 6989.11-2019 Air dan air limbah – Bagian 11: Cara uji derajat keasaman (pH) dengan menggunakan pH meter*.

Thakur, M., Bajaal, S., Rana, N., & Verma, M. L. (2020). Microalgal Technology: A Promising Tool for Wastewater Remediation. In *Springer Nature Singapore* (Vol. 22). Springer Nature Singapore. http://link.springer.com/10.1007/978-981-15-2679-4

Wang, L., Hou, D., Cao, Y., Ok, Y. S., Tack, F. M. G., Rinklebe, J., & O'Connor, D. (2020). Remediation of mercury contaminated soil, water, and air: A review of emerging materials and innovative technologies. *Environment International*, *134*(November 2019), 105281. https://doi.org/10.1016/j.envint.2019.105281

Wang, S., Duo, J., Wufuer, R., Li, W., & Pan, X. (2022). The Binding Ability of Mercury (Hg) to Photosystem I and II Explained the Difference in Its Toxicity on the Two Photosystems of Chlorella pyrenoidosa. *Toxics*, *10*(8). https://doi.org/10.3390/toxics10080455

Wardani, N. K., Supriyantini, E., & Santosa, G. W. (2022). Pengaruh Konsentrasi Pupuk Walne Terhadap Laju Pertumbuhan dan Kandungan Klorofil-a Tetraselmis chuii. *Journal of Marine Research*, *11*(1), 77–85.