JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(5), 129–138
https://doi.org/10.12911/22998993/200703
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Received: 2025.01.04
Accepted: 2025.02.11
Published: 2025.03.16

Response of the *Chlorella vulgaris* microalgae response in heavy media contaminated with lead

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ABSTRACT

Water is a fundamental resource for life, essential for domestic, agricultural, and industrial use. The increasing demand for clean water due to population and industrial growth has led to significant pollution challenges, particularly from untreated wastewater and the excessive use of chemicals. Heavy metal contamination, specifically lead (Pb), poses severe risks to aquatic ecosystems and human health. Pb pollution in coastal and port waters, such as Tanjung Emas Port in Indonesia, has been documented to exceed permissible limits, necessitating effective remediation strategies. Microalgae offer sustainable and cost-effective solutions for heavy metal removal through biosorption and bioaccumulation mechanisms. This study explored the potential through the responses of *Chlorella vulgaris*, a green microalga, in saline and Pb-contaminated media. The research was conducted in a laboratory using a controlled photobioreactor with 20‰ salinity and varying Pb concentrations, 0 mg/L as control, 1 mg/L, 3 mg/L, 5 mg/L, 7 mg/L, and 9 mg/L. The response of *Chlorella vulgaris* was monitored and analyzed by the key parameters, including cell density, growth rate, pH, temperature, and CO_2 concentration over 14 days to assess the ability of *Chlorella vulgaris* to survive in saline and contaminated media. The research found that *Chlorella vulgaris* with an initial cell density of $\pm$ 347 × 10^4 cells/mL can survive and grow in media with 20‰ of salinity and a heavy metal concentration of up to 9 mg/L. Over 14 days, cell density observations revealed optimal growth in a reactor with 3 L/min aeration, 20‰ salinity, and a Pb concentration of 3 mg/L. The cell density value was 16.58 × 10^6 cells/mL, the same as the cell density in the control reactor, 18.78 × 10^6 cells/mL.

Keywords: *Chlorella vulgaris*, lead, microalgae, salinity.

INTRODUCTION

Water is the primary resource needed by living things. Water is widely used for individual needs, groups, domestic activities, agriculture, and industrial-scale needs. Population and industrial growth increase the need for clean water. All forms of human activity that utilize water cannot be separated from the residue or waste produced. Environmental contamination, particularly water pollution, can result from the usage of water without the waste being processed. Water pollution can result from all forms of water utilization activities above. Pollution can occur due to a lack of processing of the wastewater produced and using chemicals or other materials in massive quantities, so they become the primary source of water pollution. The presence of heavy metals in aquatic systems became one of the water contaminations.

Pb is one of the environmental pollutants in soil, water, and air, and it is widely discussed because of its impacts. The Pb pollution in water can come from various activities, such as leachate from final disposal sites, industrial activities, byproducts from industrial processes, and daily human activities, which then pollute surface water, such as reservoirs, rivers, and marine pollution (Adani et al., 2018; Chowdhury et al., 2022;

Handriyani et al., 2020; Putra et al., 2020). Many human activities are also conducted in sea waters, especially ports. Some examples of activities include loading and unloading coal, ship passenger activities, and other activities that can pollute seawater quality in ports or coastal areas. Recent research states that there has been Pb pollution in the waters of Tanjung Emas Port, Semarang City, Central Java (Sulistyo et al., 2024). The concentration of Pb found ranged from 0.390 - 0.640 mg/L. This concentration value has exceeded the applicable quality standards. Referring to Attachment VIII of the Government Regulation of the Republic of Indonesia Number 22 of 2021 concerning the Implementation of Environmental Protection and Management (Lampiran VIII Peraturan Pemerintah Republik Indonesia Nomor 22 Tahun 2021 Tentang Penyelenggaraan Perlindungan Dan Pengelolaan Lingkungan Hidup), the maximum limit for the concentration of Pb in seawater for port use is 0.05 mg/L.

The presence of Pb is also a concern for living things, especially fish. Several types of marine fish can accumulate heavy metals in massive quantities, which can become a food chain pathway until human consumption. Various kinds of marine fish also have heavy metal quality standards to fulfill the requirements of ingredients that humans can consume. Another study stated that there has been a significant accumulation of lead in green mussels that live in seawater with a concentration of Pb that does not exceed the quality standard (Simbolon, 2018). Therefore, Pb can also cause unfavorable conditions at small concentrations and be dangerous for other living things. Accumulation of Pb in the human body can cause various negative impacts such as inhibiting enzyme activity, disrupting metabolism, inhibiting fetal development, and causing kidney damage (Malik et al., 2021).

On the basis of the conditions of Pb pollution in port and coastal waters, a form of wastewater treatment and environmental management of water contaminated with heavy metals is needed. In managing an environment contaminated with heavy metals, the treatment must focus on the contaminated media, such as seawater in the port area. From the perspective of sustainable environmental management, addressing seawater contamination by heavy metals is a critical priority, as it poses significant threats to both aquatic ecosystems 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 (SDGs), which are currently being widely promoted. Indonesia is one of the countries that support and participate in realizing the 17 Global SDGs. One of the goals for managing seawater pollution is the SDGs number 14, Marine Ecosystems (BAPPENAS, 2023).

There are various methods for managing contamination with heavy metals in seawater. One of the management methods that can be applied is the remediation method. Remediation technology provides multiple methods to restore water contaminated with heavy metals. This management can be done physically, chemically, biologically, and integrated between them. Remediation methods have also been widely developed so that existing natural resources could be can utilized in the processing of contaminated media. Several considerations need to be taken into account in choosing a remediation method, such as the amount of pollution, the contaminated area, environmental conditions, the remediation method, and the application of the selected method. On the basis of those considerations, remediation by utilizing living things became an 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.

Many types of microalgae can be used to remediate the water contaminated with heavy metals, especially in seawater. *Chlorella vulgaris* is one type of microalgae that can be used to remediate the water contaminated with Pb. Research on the ability of *Chlorella vulgaris* has begun to be conducted to determine its ability to remove heavy metals. *Chlorella vulgaris* can also remediate heavy metals, such as Cr and Hg. *Chlorella vulgaris* can remove 11% chromium (VI) at a concentration of 5 mg/L (Maysitha and Titah, 2024), and remove mercury content by 61.34% at a mercury concentration of 0.3 mg/L (Dienullah and Titah, 2023). These microalgae can survive in the media contaminated with heavy metals and high salinity. In addition, *Chlorella vulgaris* can reduce the concentration of heavy metals in water media through several mechanisms. Microalgae emerge as a favorable option for remediation methods that prioritize cost-effectiveness and ecological sustainability (Chakravorty et al., 2023).

Microalgae are single-celled organisms capable of harnessing light energy through

photosynthesis, using CO_2 from their growth medium and converting it into chemical energy (Esteves et al., 2024; Razzak et al., 2024). They play a vital role in environmental quality restoration, and numerous studies have examined their potential to remediate polluted environments. Microalgae have several mechanisms or strategies for protecting themselves, such as metallothionein synthesis, biosorption, antioxidant production, and anticancer potential (Hamai-Amara et al., 2024). The strategies used by microalgae in removing heavy metals include passive extracellular adsorption/biosorption on the cell surface and active intracellular diffusion and accumulation/bioaccumulation (Chakravorty et al., 2023).

Chlorella vulgaris is included in the group of single-celled green microalgae and contains chlorophyll, which is used for photosynthesis. *Chlorella vulgaris* can also accumulate high amounts of carbohydrates under certain conditions (Ammar, 2016). *Chlorella vulgaris* has walls composed of cellulose with a chemical structure consisting of OH groups. Therefore, cellulose in *Chlorella vulgaris* can be an adsorbent in capturing heavy metal ions (Dewi and Nuravivah, 2018). *Chlorella vulgaris* is one of the most abundant and easily found algae in waters. *Chlorella vulgaris* can be found in both freshwater and seawater. *Chlorella vulgaris* has the following classification:

- Domain: Eukaryota,
- Kingdom: Plantae,
- Division: Chlorophyta,
- Class: Tre-bouxiophyceaefig,
- Order: Chlorellales,
- Family: Chlorellaceae,
- Genus: Chlorella,
- Species: *Chlorella vulgaris.*

On the basis of the explanation above, *Chlorella vulgaris* has excellent potential to restore environmental quality. Therefore, a study was conducted to determine the response of microalgae in media with salinity levels and heavy metal concentrations of Pb. This study served as the first stage in determining the potential that could be produced from *Chlorella vulgaris*.

MATERIAL AND METHODS

The research was conducted at the environmental remediation laboratory of the Environmental Engineering Department at Institut Teknologi Sepuluh Nopember, Surabaya. The study was conducted on a laboratory scale using a plastic reactor with 1 L capacity.

Microalgae stock

The *Chlorella vulgaris* microalgae were obtained from *Balai Perikanan Budidaya Air Payau* (Brackish Water Aquaculture Development Center), Situbondo Regency, East Java, Indonesia. As a source of nutrition for microalgae, additional nutrients are used in the form of walne, vitamins, and trace metals. The number of *Chlorella vulgaris* microalgae stocks used in this study was 10% of the total observation volume with the initial cell density being $\pm$ 347 $\times$ 10^4 cells/mL. Nutrients were added at a dose of 1 mL/L on day 0 and day 7.

Media preparation

The heavy metal lead media contamination used in this test was artificial. The media consisted of distilled water, NaCl as a source of media salinity, lead heavy metal, and added nutrients. The salinity level used in this test was 20‰, prepared by diluting NaCl salt in distilled water. The 20‰ salinity was chosen regarding the previous research which stated that the *Chlorella vulgaris* microalgae can grow well at a salinity level of 20‰ (Maysitha et al., 2024). The concentrations of heavy metals used in this study were 0 mg/L as a control, 1 mg/l, 3 mg/l, 5 mg/l, 7 mg/l, and 9 mg/l. The difference in the concentration of Pb was carried out using the dilution method from the stock solution. The Pb stock solution was diluted by 0.16 grams of $Pb(NO_3)_2$ in 100 mL of distilled water. In addition, additional nutrients were also used in the media. The nutrients used consist of walne, vitamins, and trace metals. The composition of walne nutrients consisted of $NaNO_2$, NaH_2PO_4, Na_2EDTA, H_3BO_3, $MnCl_2$, and $FeCl_3$. The vitamins consisted of B_1 and B_{12}. The trace metal consisted of $ZnCl_2$, $CoCl_2{\cdot}6H_2O$, $(NH_4)_6{\cdot}M_{O7}O_{24}{\cdot}4H_2O$, and $CuSO_4{\cdot}5H_2O$. The nutrients were added at a dose of 1 ml/L on days 0 and 7. The nutrients consisting of walne, vitamin, and trace metal were added at an amount of 0.5 L for each reactor. The total volume of the research was 500 mL for each reactor, consisting of 10% or 50 mL of microalga stock, and 90% or 450 mL of lead media contaminated and nutrients.

Photobioreactor

The research used a plastic reactor equipped with additional lighting and an aerator as shown in Figure 1. The aerator operated continuously (24 hours) at a rate of 3 to 4 L/minute. LED cool daylight lights, providing approximately 1400 lux, enhanced illumination with a daily cycle of 12 hours on and 12 hours off. Microalgae metabolism was greatly influenced by the availability of light or energy sources in carrying out the photosynthesis process (Acién et al., 2017). The supply of light in the cultivation process was the factor that most influenced the growth process of autotrophic microalgae (Esteves et al., 2024). Light was utilized by microalgae to produce ATP (adenosine triphosphate), which was used for cell synthesis (Razzak et al., 2024). For a certain purpose, artificial light sources, such as LED lamps were used to produce high-value products of microalgae and to facilitate the control of the required light (Mahari et al., 2024).

The monitoring of *Chlorella vulgaris* and parameters in the media

The microalgae cell density of *Chlorella vulgaris*, pH, temperature, and CO_2 concentration in the reactor are all examined in the research. A Yazumi microscope, made in China, was used to observe the number of microalgae cells. Microalgae counting field was carried out using a Neubauer-improved hemocytometer made in Germany. An EZ 9909 pH meter was used to measure the temperature and pH of the media. The CO_2 concentration in the reactor was measured using a Lutron GC-2028, which was made in Taiwan. To determine the influence and effect of the changes on *Chlorella vulgaris* growth rate, all parameters were tracked every 24 hours for 14 days. The cell density and the growth rate of microalgae can be calculated using the collected data of cells counted in a hemocytometer through Equation 1 and Equation 2.

$$Cells\ density = \left(\frac{number\ of\ cells\ from\ each\ square\ together}{number\ of\ square}\right) \times \frac{}{Volume\ of\ square} \times Dilution\ factor \quad (1)$$

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

where: N_2 and N_1 – number of cells in the exponential phase; T_2 and T_1 – time from N_1 to N_2.

Statistical analysis

The statistical analysis in this study was conducted using the ANOVA method with the Design Expert 13 by Stat-Ease 360 Trial program. ANOVA was employed to determine whether the input data were statistically significant based on the applied model. The primary inquiries in this research were whether variations in aeration influence cell density and whether different concentrations of lead (Pb) impact cell density over a 14-day observation period. There were 3 factors analyzed for the ANOVA, i.e. aeration, day of observation, and Pb concentration. There were 2 aerators with capacities of 3 L/min and 4 L/min. The observation times were days of 0, 1, 2, 5, 6, 7, 8, 9, 12, 13, and 14, and the Pb concentrations were 0 mg/L as control, 1 mg/L, 3 mg/L, 5 mg/L,7

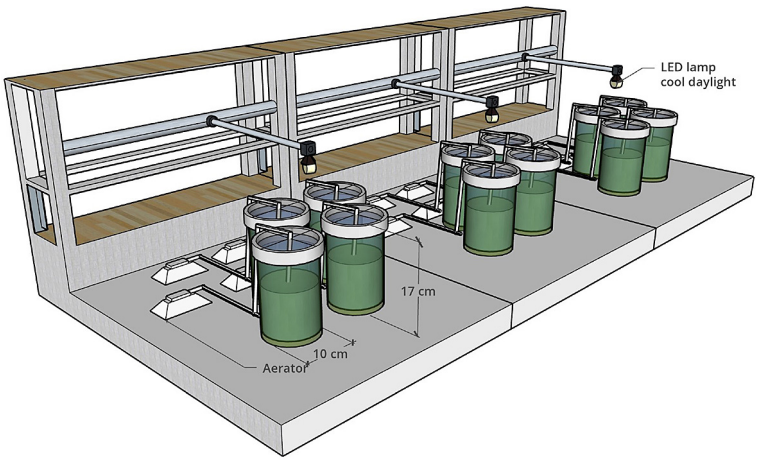

Figure 1. Design photobioreactor for the observation

mg/L, and 9 mg/L. There were 132 responses to the microalgae cell density values. The responses were recorded and analyzed using ANOVA.

RESULT

The observation of the number of microalgae cells shows the fluctuation and increases in the growth rate of microalgae. During the 14 days of observation, the number of *Chlorella vulgaris* cells increased and fluctuated. Compared to the control photobioreactor (the reactor without additional lead heavy metal), *Chlorella vulgaris* in media with the lead concentrations 1 mg/L, 3 mg/L, 5 mg/L, 7 mg/L, and 9 mg/L showed a lower increase of cells. Despite this, *Chlorella vulgaris* is still capable of surviving and proliferating. The results of this study are similar to the previous study, which stated that *Chlorella vulgaris* with an initial cell density of 10×10^4 cells/mL can survive in media with a Pb concentration of up to 9 mg/L (Anggraini et al., 2022), and also survive and grow in seawater media with a concentration of heavy metal Pb reaching 10 mg/L (Halima et al., 2020).

Daily observations were made on the growth rate of microalgae cells and media parameters to determine the differences in responses produced by microalgae to saline media contaminated with lead heavy metals. In addition to the data presented in graphical form, the response data of *Chlorella vulgaris* microalgae, consisting of maximum cell density, microalgae growth rate, and parameters produced during the observation, are also presented in Table 1 and Table 2.

The response of microalgae in media contaminated with heavy metal lead with a 20‰ salinity level and aeration of 3 L/min has various data. The maximum number of cells produced varies. The maximum number of cells of 16.58×10^6 cells/mL was produced by microalgae living in saline media contaminated with heavy metal Pb of 3 mg/L. The maximum number of cells was produced on the 13th day of observation. Then, at concentrations of 4 mg/L, 7 mg/L, and 9 mg/L, microalgae could produce a smaller maximum number of cells sequentially. The maximum number of cells was produced on the 13th day, 14th day, 8th day, and 9th day, respectively. In general, microalgal cells experienced an increase in the number of cells until the 7th day, then experienced a decrease on the 8th day, and again experienced an increase in the number of cells on the 9th day after the re-addition of nutrients to the microalgal growth medium.

The response of microalgae in the media contaminated with lead, a salinity level of 20‰, and aeration of 4 L/min also yields various data. The graph shows the growth of microalgae cells, which tends to increase daily. The maximum number of cells produced varies. The maximum number of 15.12×10^6 cells/mL is produced by microalgae living in saline media contaminated with Pb in an amount of 3 mg/L. *Chlorella vulgaris* microalgae in the media with heavy metal concentrations of 1 mg/L, 5 mg/L, 7 mg/L, and 9 mg/L have a smaller maximum number of cells. The smallest maximum number of cells produced is in media with a lead-heavy metal concentration of 9 mg/L, 8.86×10^6 cells/mL. Observations were also conducted using a control reactor to determine the difference in response in the media with and without lead. The maximum number of cells produced with 3 L/minute aeration was 16.58×10^6 cells/mL, while with 4 L/minute aeration, it was 15.12×10^6 cells/mL.

Tables 1 and 2 show the maximum number of cells produced from each variation of heavy metal concentration. In addition to determining the maximum number of cells produced during the observation period, the growth rate of microalgae cells was also calculated in this research. the growth rate of microalgae was calculated during the logarithmic growth phase. The parameters of the study were also measured daily. The results of observations of CO_2 parameters in the air in the research reactor show a fluctuating graph with a downward trend. Observations on pH parameters show results that increase every day. While conducting observations on temperature parameters, the data obtained fluctuated during the observation. The graph of data from observations of CO_2, pH, and temperature parameters is presented in Figure 4. Data showing the most minor and most significant data ranges of the parameters produced are also presented in Tables 1 and 2.

Figures 2 and 3 show the response of microalgae in the study with variations in Pb concentrations in the media and variations in aeration discharge. The observation results showed that aerator use affected the growth rate of microalgae cells within 14 days of observation. Figure 3 shows the reactor with 4 L/minute aeration, the *Chlorella vulgaris* microalgae continued to grow until the 14th day of observation. Figure 2 indicates, the

Table 1. Empirical data on the response of the Chlorella vulgaris microalgae to media contaminated with lead at 3 L/minute

Parameter	Concentration of heavy metal Pb (mg/L)					
	0	1	3	5	7	9
Growth rate (μ) (cells/mL/day)	0.263	0.514	0.412	0.280	0.251	0.360
Maximum cell density (× 10^6 cells/mL)	18.78	16.24	16.58	10.44	8.92	8.88
pH	7.07–8.69	6.9–8.68	7.21–8.62	6.98–8.61	7.1–8.6	7.12–8.5
Temperature (°C)	28.9–30.9	28.8–30.9	28.4–30.1	28.7–30.3	28.8–30.4	28.1–29.8
CO_2 concentration in the air of the reactor (ppm)	423–518	425–524	429–521	412–523	414–521	420–528

Table 2. Empirical data on the response of the *Chlorella vulgaris* microalgae to lead-contaminated media at 4 L/minute

Parameter	Concentration of heavy metal Pb (mg/L)					
	0	1	3	5	7	9
Growth rate (μ) (cells/mL/day)	0.370	0.306	0.510	0.399	0.204	0.195
Maximum cell density (× 10^6 cells/mL)	25.46	14.24	15.12	11.36	11.78	8.68
pH	6.8–8.72	6.92–8.67	7.01–8.7	7.06–8.61	7.26–8.7	7.13–8.82
Temperature (°C)	28.5–30.4	28.4–30.7	28.6–30.6	28.7 - 30.5	28.6–30.8	29.2–31.4
CO_2 concentration in the air of the reactor (ppm)	424–536	430–538	431–541	433–538	431–543	429–543

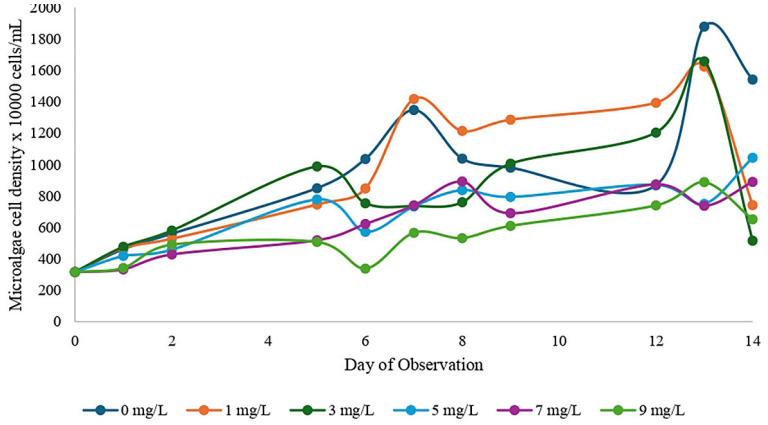

Figure 2. Microalgal responses by the 3 L/minute of aeration

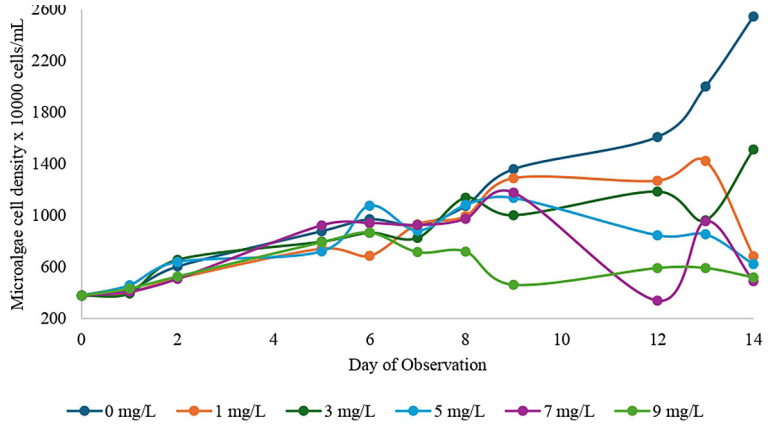

Figure 3. Microalgal responses by the 4 L/minute of aeration

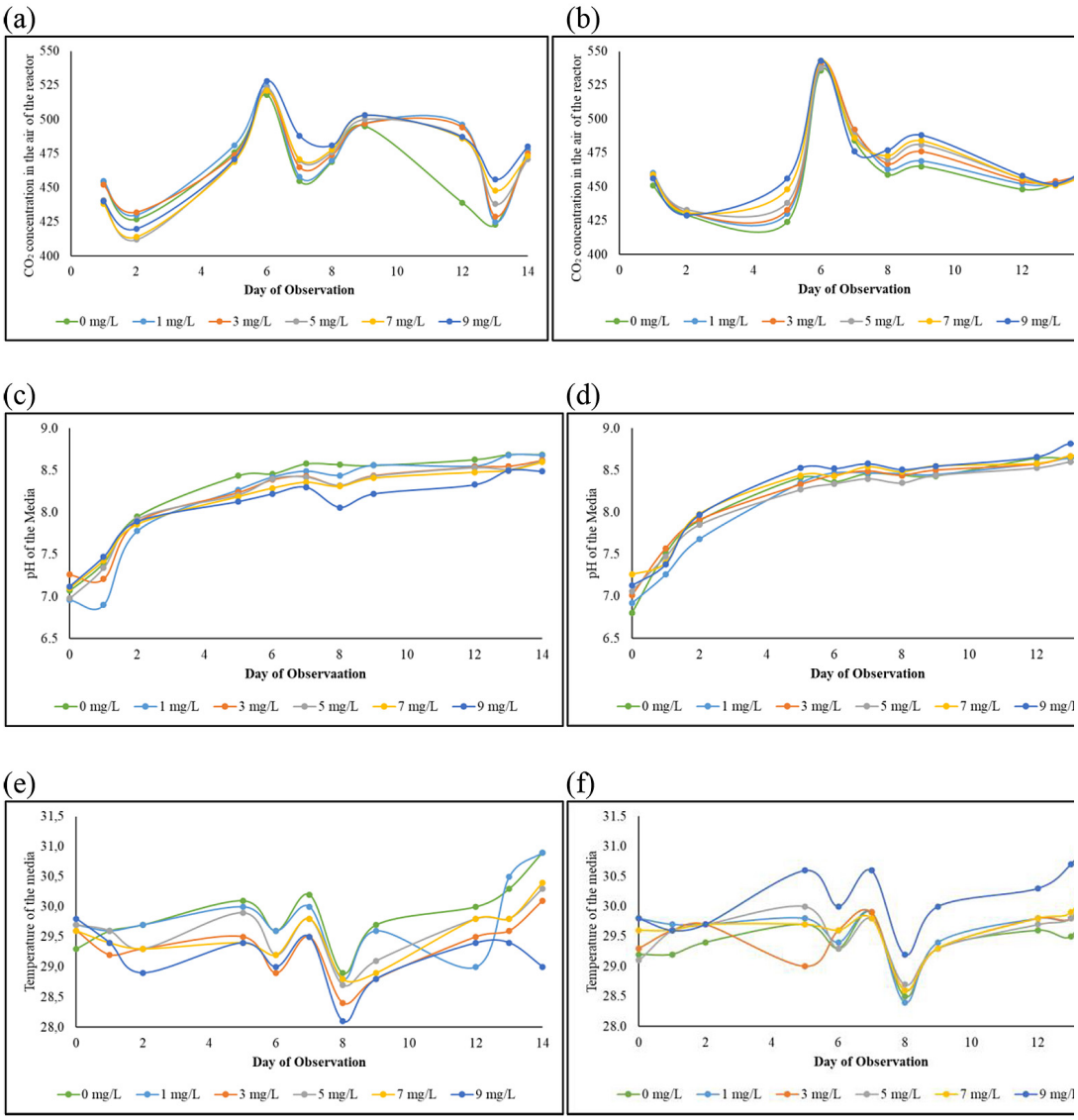

Figure 4. *Chlorella vulgaris* microalgae response on the parameters: (a) CO_2 concentration with 3 L/minute of aeration, (b) CO_2 concentration with 4 L/minute of aeration, (c) pH with 3 L/minute of aeration, (d) pH with 4 L/minute of aeration, (e) Temperature with 3 L/minute of aeration, and (f) Temperature with 4 L/minute of aeration

reactor with 3 L/minute aeration, the *Chlorella vulgaris* microalgae began to die after the 13th day of observation. This condition is related to carbon availability in the microalgal growth medium. In addition, the stirring process caused by aeration and nutrient content also affects the growth rate of microalgae in controlled reactors. Similar studies also stated that the growth rate of microalgae increased along with the airflow rate in the batch reactor and the increase in nutrient concentration in the reactor (Ammar, 2016). The observation of the parameters can be seen in Figure 4. There was a decrease in CO_2 concentration in all the reactors. The decrease in CO_2 is related to microalgal mechanism in photosynthesis. Most microalgae are photosynthetic and use CO_2 as well as light energy to synthesize carbohydrates and reduce greenhouse gas emissions (Devi et al., 2023). The pH value in the media increased during 14 days of observation. This is related to the mechanism of microalgae in carrying out photosynthesis. Microalgae can produce oxygen, which can bind to free H^+ ions in the media to form OH^- ions and increase the pH value in the media (Ding et al., 2020). The pH directly affects microalgal biochemical and physiological activities, which impacts their respiration, growth, and productivity. In addition, pH also plays a role in regulating the availability of CO_2 and the absorption of nutrient ions. During photosynthesis, using CO_2 and HCO_3^- often causes an increase in pH to exceed 11. Too high pH can inhibit the growth of microalgae (Devi et al., 2023).

DISCUSSION

The *Chlorella vulgaris* microalgae with an initial cell density of ± 347 × 10^4 cells/mL can survive in the media contaminated with lead up to a concentration of 9 mg/L. This is a reference or guideline for further research on the ability of the *Chlorella vulgaris* microalgae to remediate media contaminated with heavy metals in water media. Microalgae have several forms of mechanisms and strategies for protecting themselves i.e. metallothionein synthesis, biosorption, and antioxidant production (Hamai-Amara et al., 2024). The mechanisms used by microalgae in bioremediation consist of biosorption, bioaccumulation, and biodegradation (Abdelfattah et al., 2023). With these mechanisms, the *Chlorella vulgaris* microalgae can survive in the media contaminated with Pb.

The results of this study were also in line with the findings of the research that used the *Chlorella vulgaris* microalgae with an initial cell density of 100 × 10^4 cells/mL, which was able to remediate the seawater contaminated with Pb at concentrations up to 5 mg/L (Dewi and Nuravivah, 2018). On the basis of the values and results of this study, the *Chlorella vulgaris* microalgae also has the potential to remediate the seawater contaminated with Pb in Tanjung Emas Port, Semarang City, Central Java which has a Pb concentration of up to 0.64 mg/L (Sulistyo et al., 2024), textile industry wastewater with 0.058 mg/L of Pb concentration (Febriyanti et al., 2023), pulp and paper wastewater with 0.864 mg/L of Pb concentration (Novita et al., 2012), and also the tanning wastewater with 0.83 mg/L of Pb concentration (Nabila and Ibrahim, 2020). Further research can be designed by evaluating the initial and final concentrations of Pb throughout the study. Various previous studies have explored the role of this microalga in removing heavy metals from water.

The control reactor with 4 L/min aeration showed a higher maximum cell density value than the control reactor with 3 L/min aeration. The maximum cell density values achieved by 4 L/min and 3 L/min aeration are 25.46 × 10^6 cells/mL and 18.78 × 10^6 cells/mL, respectively. The highest maximum cell density in the reactor with Pb was produced with 3 L/min aeration at a Pb concentration of 3 mg/L, 16.58 × 10^6 cells/mL, while in the reactor with Pb concentrations of 1 mg/L, 5 mg/L, 7 mg/L, and 9 mg/L had a lower value. The maximum cell density values were not much different between the reactor with 3 L/min aeration and the reactor with 4 L/min aeration. The results of this study indicate that the use of 3 L/min aeration yields reliable results and is sufficient to support the growth rate of *Chlorella vulgaris* microalgae cells. In addition, the results of this study indicate that for the need to increase the number of microalgae cells/propagation purposes, 4 L/minute aeration can be a viable choice. This study also supports the results of previous studies which stated that aeration with 3 L/minute resulted in a better cultivation of the *Chlorella vulgaris* microalgae in a controlled reactor than the one with 2.5 L/minute (Dienullah and Titah, 2023). However, for aeration needs with the aim of supporting the growth of microalgae in the media contaminated with Pb, 3 L/minute aeration can be a more appropriate choice. The *Chlorella vulgaris* microalgae with 3 L/minute aerations can still grow well in an environment with a salinity level of 20‰ and a heavy metal concentration

Table 3. ANOVA Result for the cell density of the *Chlorella vulgaris* microalgae

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	1.264E+07	6	2.107E+06	39.04	< 0.0001	significant
A - aeration	1.328E+05	1	1.328E+05	2.46	0.1193	
B - day of observation	6.364E+06	1	6.364E+06	117.93	< 0.0001	
C - lead concentration	3.285E+06	1	3.285E+06	60.87	< 0.0001	
AB	2793.73	1	2793.73	0.0518	0.8204	
AC	1785.45	1	1785.45	0.0331	0.8560	
BC	1.929E+06	1	1.929E+06	35.75	< 0.0001	
Residual	6.691E+06	124	53961.14			
Cor total	1.933E+07	130				

of 3 mg/L. In future research, selecting the appropriate aeration method is a crucial factor to consider. Additional factors, i.e. cost and operational efficiency, can aid in determining the optimal aeration specifications.

ANOVA analysis

ANOVA analysis was conducted using a design expert. It can analyze the significance of each data obtained based on the variables. The fit model for microalgal cell density was two factors interaction (2FI) model. The ANOVA analysis was presented in Table 3. The model showed a significant value, with a p-value < 0.0001. In addition, the observation time and Pb concentration had a significant effect on the density of *Chlorella vulgaris* cells with p-values < 0.0001 and < 0.0001, respectively. Meanwhile, the aeration factor had a p-value of 0.1193. It indicated an insignificant value (p-value > 0.05). It was suggested that aeration with 3 L/minute and 4 L/minute did not have a significant effect on the microalgal cell density produced in the observation. The results of the ANOVA analysis supported the statement that the presence of heavy metals in the media can affect the growth and number of *Chlorella vulgaris* cells. In other considerations i.e. cost and maintenance, aeration of 3 L/minute was considered sufficient to be used in microalgal cultivation.

CONCLUSIONS

On the basis of the results of this research, *Chlorella vulgaris* with an initial cell density of $\pm$ 347 × 10^4 cells/mL could still survive and grow in media with a salinity of 20‰ and a heavy metal concentration of 9 mg/L. The response of *Chlorella vulgaris* was shown through microalgal cell density data for 14 days of observation. The best response of the *Chlorella vulgaris* microalgae was demonstrated in a reactor with aeration of 3 L/minute, a salinity level of 20‰, and a lead concentration of 3 mg/L.

Acknowledgements

The author gratefully acknowledges funding support for the research provided by The Ministry of Education and Culture, Directorate of Higher Education, under contract numbers No. 038/E5/PG.02.00.PL/2024 and 1756/PKS/ITS/2024.

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