INSOLOGI: Jurnal Sains dan Teknologi
https://journal.literasisains.id/index.php/INSOLOGI
ISSN 2828-4984 (Media Online) | ISSN 2828-4992 (Media Cetak)
Vol. 2 No. 4 (Agustus 2023) 703-711
DOI: 10.55123/insologi.v2i4.2278
Submitted: 19-07-2023 | Accepted: 10-08-2023 | Published: 28-08-2023

Phycoremediation Using Microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* to Remove Lead Heavy Metals

Chia Fifin Nafi'ah[1], Okik Hendriyanto Cahyonugroho[2*]
[1,2*]Department of Environmental Engineering, Faculty of Engineering, UPN "Veteran" Jawa Timur, Surabaya, Indonesia
Email: [1]chiafifin26@gmail.com, [2*]okikhc@upnjatim.ac.id

Abstract

Heavy metals are pollutant parameters that contain high toxicity and are dangerous which can accumulate in the tissues of living things. One of the heavy metals contained in wastewater is lead (Pb). This metal is included in the extremely toxic category which can cause health problems. One way to treat waste containing the heavy metal Pb is by bioremediation using microalgae (phycoremediation). In this study, phycoremediation used the microalgae Chlorella vulgaris and Nannochloropsis oculata, as well as a consortium of both. This study aims to determine the ability of each microalga and the consortium of the two microalgae in removing lead heavy metals. The phycoremediation potential was measured based on the ratio of microalgae varied (100:0, 50:50, and 0:100) and the concentration of Pb waste (1, 5, and 10 mg/L) within 14 days on a batch scale. The results showed that the microalgae Chlorella vulgaris in reactor 10B (100% Chlorella vulgaris, 0% Nannochloropsis oculata) was able to remove 85.0% from a concentration of 10 mg/L to 1,5 mg/L.

Keywords: *Lead Waste, Microalgae, Chlorella Vulgaris, Nannochloropsis Oculata, Consortium*

1. INTRODUCTION

The emergence of pollution can occur because liquid waste is discharged into water bodies without any treatment, or has been processed, but has not met quality standards (Rahmi & Sajidah, 2017). One of the causes of water pollution that is currently rife is due to the presence of industry, namely heavy metal pollution, one of which is lead (Pb). This metal belongs to the extremely toxic category that can cause health problems (Pranudta et al., 2021). Lead that accumulates in the human body can inhibit growth and development, cause physical disabilities, and affect nerves and bones (Ridwan et al., 2019), because Pb2+ ions can replace the presence of Ca2+ ions in the bone tissue and cause paralysis (Saeni, 1997). One of the economical wastewater treatment methods is bioremediation using microalgae (phycoremediation) (Wetipo et al., 2015). One of them is the microalgae *Chlorella* sp. which is able to remove 80.08% of Pb in water in textile waste (Soeprobowati & Hariyati, 2013). In addition, *Nannochloropsis* sp. also able to remove Pb around 11.46% (Masithah et al., 2011).

Therefore, in this study, the microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* were used to determine the ability of each microalgae and the consortium of the two microalgae to remove lead heavy metal. The potential for phycoremediation was measured based on the ratio of microalgae which was varied to see the best composition of microalgae in removing lead heavy metal. The levels of lead-containing waste were also varied to determine how much resistance the microalgae had against lead contaminants in artificial wastewater.

2. MATERIALS AND METHODS

2.1 Materials and Equipment

The materials needed in this research were microalgae cultures of *Chlorella vulgaris* and *Nannochloropsis oculata*, with nutrients in the form of walne fertilizer, vitamins, and tracemetal solutions. These materials were obtained from BPBAP Situbondo. The growing media used sterile seawater. Pb artificial liquid waste from $Pb(NO_3)_2$ crystals dissolved in distilled water.

The equipment required for the study included 2 glass aquariums with a capacity of 5 L, 45 glass jar reactors with a capacity of 1 L, aerators, aeration hoses and aeration stones, and 30 watt TL lamps with an intensity of 10.000 lux. Meanwhile, the test requires a pH meter, thermometer, microscope, haemocytometer, and spectrophotometry.

2.2 Research Methods

The research method used in this study is an experimental method by conducting experiments to see a result. The treatment was given in the form of differences in Pb concentrations of three levels, namely 1 mg/L, 5 mg/L, and 10 mg/L, the ratio of the addition of microalgae was treated in reactor A (control), reactor B (100% *Chlorella vulgaris*, 0% *Nannochloropsis oculata*), reactor C (50% *Chlorella vulgaris*, 50% *Nannochloropsis oculata*) and reactor D (0% *Chlorella vulgaris*, 100% *Nannochloropsis oculata*). Sampling was carried out on days 0, 3, 6, 9, and 12. The reactor used was a glass jar with a capacity of 1000 mL under aeration conditions and given a light intensity of 10.000 lux. The experiment was carried out in batches with each reactor having a volume of 500 mL.

2.2.1 Microalgae seeding

Microalgae seeding was carried out as a way to increase the number of *Chlorella vulgaris* and *Nannochloropsis oculata* microalgae. The growth medium uses sterile seawater that has been boiled, with a media ratio: inoculum = 70: 30 (Kawaroe et al., 2019). The nutrients used were walne, vitamins, and trace metal fertilizers in the amount of 1 mL/1 L of media. Nutrition adjusted to the initial condition of microalgae seeds obtained from BPBAP Situbondo, Indonesia. Seeding was observed for 14 days to determine the exponential phase of the microalgae.

2.2.2 Acclimatization

Acclimatization was carried out after the microalgae were in the exponential phase and had a deep green color. Acclimatization is carried out so that the microalgae adapt and do not experience shock loading the first time they come into direct contact with the waste during the main research. The ratio of microalgae to waste is 1:1, with an acclimatization volume of 3 L. Changes in the condition of microalgae during the acclimatization process can be seen visually from the change in color which becomes clear.

2.2.3 Calculation of The Number of Microalgae Cells

The number of microalgae cells was counted every day at 10.00 a.m. for 14 days to determine the exponential phase of microalgae. The number of cells was counted using a haemocytometer and microscope. The formula for calculating the number of cells is as in Equation 1 (Abotero, 2021).

$$\text{Number of microalgae cells} = \frac{\text{Counted number of cells}}{\text{Counted number of squares}} \times 10^4 \quad (1)$$

2.2.4 Pb Concentration Analysis

Lead (Pb) concentration measurements were tested using an Atomic Absorption Spectrophotometer (AAS) based on Indonesian National Standard SNI 6989.8:2009. This measurement was carried out to determine the concentration of Pb contained in the reactor that had been contacted with microalgae. The final Pb concentration indicated the remaining heavy metals which were not absorbed by the microalgae. The absorption efficiency calculation uses the formula Equation 2 (Wiyarsi & Priyambodo, 2013).

$$Efficiency = \frac{C0-C1}{C0} \times 100\% \quad (2)$$

Information:
Efficiency : Absorption efficiency (%)
C0 : First concentration (mg/L)
C1 : Final concentration after absorption (mg/L)

3. RESULTS AND DISCUSSION

3.1 The Effect of Number of Microalgae Cells on Microalgae Growth Rate

The number of microalgae cells at the time of seeding was counted using a haemocytometer and microscope for 14 days. By knowing the number of microalgae every day, it can determine the life phase of microalgae and the optimum conditions for harvesting when entering the exponential phase. This calculation can directly provide the content of the number of microalgae cells in the liquid because what is counted is the number of organisms from microalgae (Tangahu et al., 2019). The growth rate of the two microalgae can be seen in Figure 1.

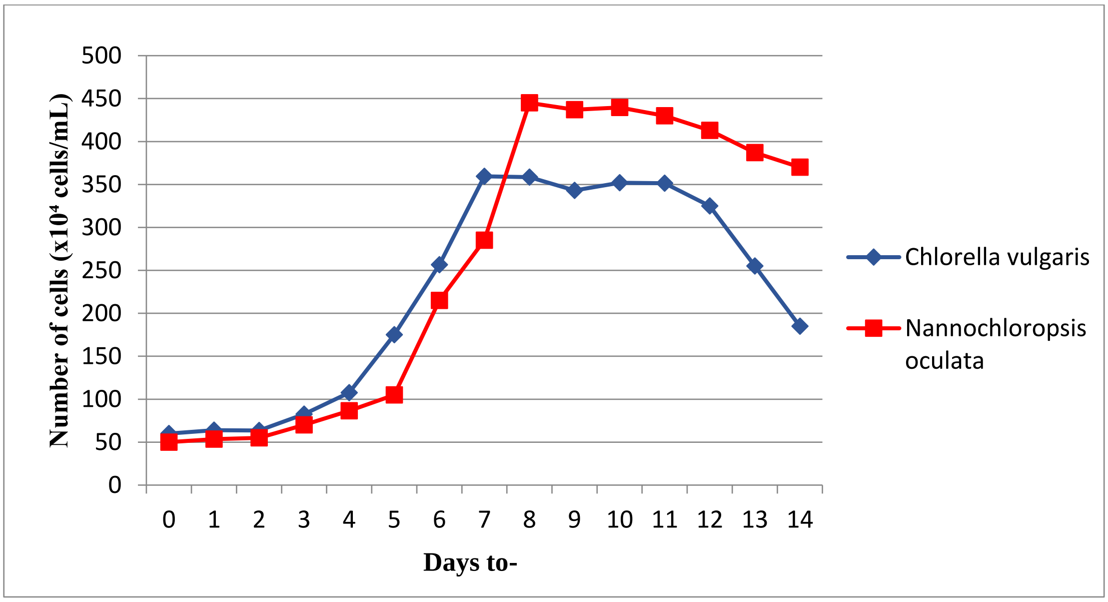

Figure 1. The growth rate of microalgae based on the number of cells in *Chlorella vulgaris* and *Nannochloropsis oculate*

The graph in Figure 1 shows 4 phases in microalgae growth, namely: lag phase, exponential phase, stationary phase, and death phase. In the *Chlorella vulgaris* microalgae, days 0 to 2 show a lag phase where the microalgae adapt and the addition of cells looks little or even tends to be absent (Halima et al., 2019). Day 3 shows the exponential phase until the peak of growth on day 7, days 8 to 10 is the stationary phase, which is characterized by a stable number of cells and no cell addition (Kurniawan & Aunurohim, 2014). Days 11 to 14 is the death phase which is characterized by a decrease in the number of cells. The number of cells on day 0 was 60×10^4 cells/mL and at the peak of growth, the number of cells was 359.5×10^4 cells/mL. This amount increases by 5× from the initial amount.

Whereas in *Nannochloropsis oculata*, the exponential phase occurs on days 3 to 8. The initial density of this microalgae is 50×10^4 cells/mL and at the peak of growth the number of cells is 445.5×10^4 cells/mL, so this number increases by 9× from the initial density. Days 0 to 7 show the lag phase, days 9 to 11 are the stationary phase and days 12 to 14 are the death phase. After reaching optimum conditions, the growth of microalgae will naturally decrease, because the nutrients decrease along with the increasing number of cells (Kurniawan & Aunurohim, 2014).

3.2 Removal of Heavy Metal Lead (Pb) by Microalgae

In the research to test the ability of microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* to remove heavy metal Pb, a batch reactor with a capacity of 1000 mL was used. Parameters of heavy metal lead (Pb) were measured on days 0, 3, 6, 9, and 12 during the main study to determine the percentage of Pb removal by microalgae. The Pb testing method uses the Atomic Absorption Spectrophotometer (AAS) method. The results of the test analysis on the 12th-day sampling obtained data as in Table 1.

Table 1. Analysis of Heavy Metal (Pb) Concentration and Percentage of Removal

No.	Reactor	Initial Pb Concentration (mg/L)	Pb Concentration Final (mg/L)	Percentage Removal (%)
1	1B	1	0,16	84,0%
2	5B	5	0,93	81,4%
3	10B	10	1,5	85,0%
4	1C	1	0,28	72,0%
5	5C	5	0,9	82,0%
6	10C	10	1,66	83,4%
7	1D	1	0,19	81,0%
8	5D	5	0,95	81,0%
9	10D	10	1,7	83,0%

Information :
Reactor B : (100% *Chlorella vulgaris*, 0% *Nannochloropsis oculata*)
Reactor C : (50% *Chlorella vulgaris*, 50% *Nannochloropsis oculata*)
Reactor D : (0% *Chlorella vulgaris*, 100% *Nannochloropsis oculata*)

The results of the analysis in Table 1 show that the highest percentage of removal for Pb concentration of 10 mg/L in reactor 10B (100% *Chlorella vulgaris*, 0% *Nannochloropsis oculata*) was 85.0% from a concentration of 10 mg/L to 1.5 mg/L. The graph of removal of each Pb concentration at each sampling time can be seen more clearly in Figure 2, Figure 3, and Figure 4.

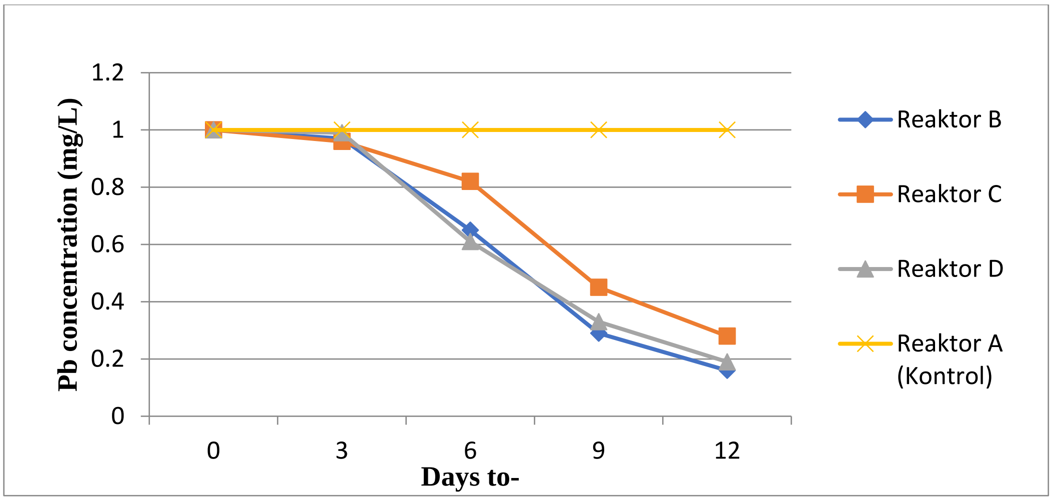

Figure 2. Lead Removal Concentration 1 mg/L

Information :
Reactor A : Control without microalgae (Pb waste concentrations of 1, 5 and 10 mg/L)
Reactor B : (100% *Chlorella vulgaris*, 0% *Nannochloropsis oculata*)
Reactor C : (50% *Chlorella vulgaris*, 50% *Nannochloropsis oculata*)
Reactor D : (0% *Chlorella vulgaris*, 100% *Nannochloropsis oculata*)

Based on the graph in Figure 2, data on the reduction of Pb concentrations were obtained at the time of sampling on days 0, 3, 6, 9, and 12 with three variations in the ratio of microalgae added to the reactor. The trend in the graph of Pb removal at a concentration of 1 mg/L in Figure 2 shows a decrease in Pb concentration in each variation. When compared between variations, the variations in reactor B experienced a greater decrease in Pb concentration than reactor D, followed by reactor C. The highest percentage was found in reactor B with an elimination percentage of 84%. Variation of reactor B containing 100% *Chlorella vulgaris*, and 0% *Nannochloropsis oculata* showed that the addition of *Chlorella vulgaris* microalgae in various concentrations of Pb waste was able to set aside Pb waste concentration of 1 mg/L to 0.16 mg/L.

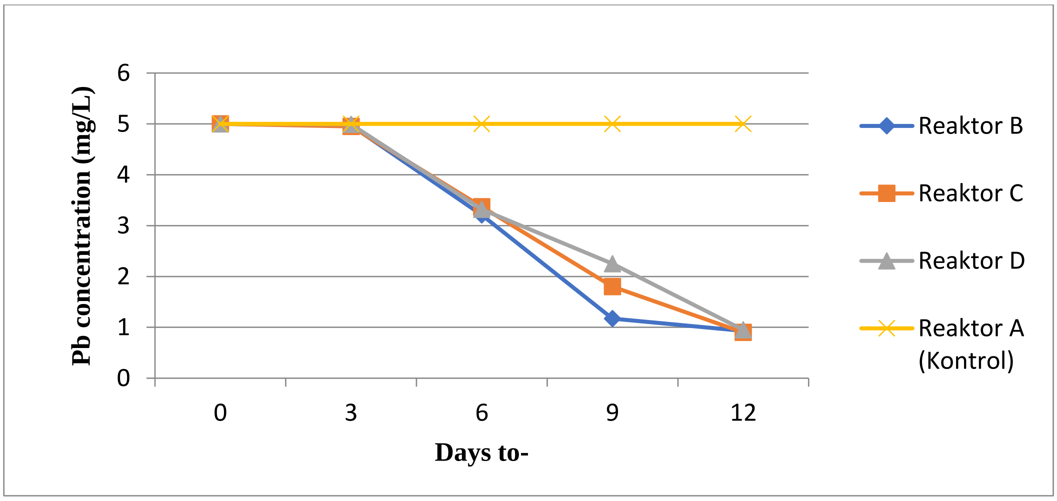

Figure 3. Lead Removal Concentration 5 mg/L

Figure 3 presents a graph of Pb removal at a concentration of 5 mg/L which shows the highest percentage in reactor C with 82% removal, followed by reactors B and D. The variation of reactor C containing 50% *Chlorella vulgaris*, 50% *Nannochloropsis oculata* shows that the consortium of microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* is effective in removing Pb waste at a concentration of 5 mg/L to 0.9 mg/L.

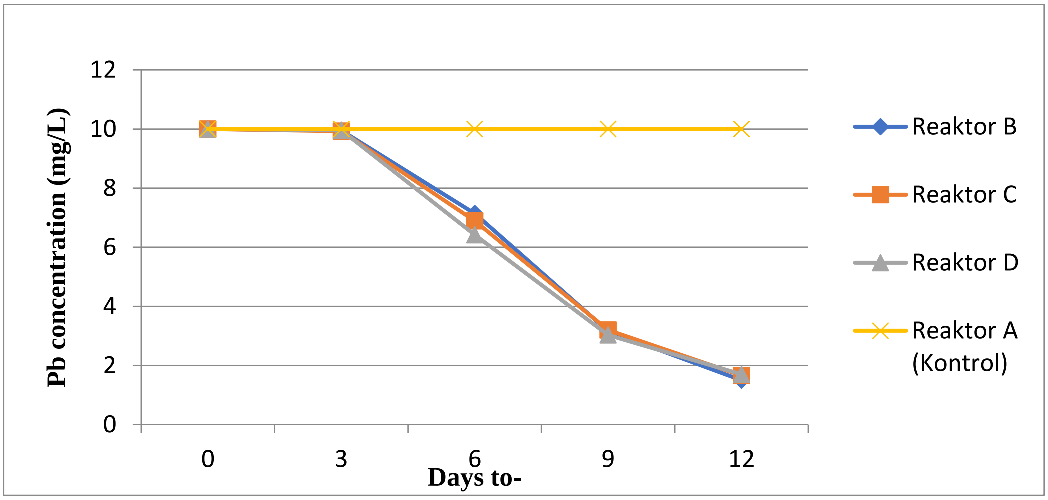

Figure 4. Lead Removal Concentration 10 mg/L

Figure 4 presents a graph of Pb removal at a concentration of 10 mg/L which shows the highest percentage in reactor B with a removal percentage of 85%. The variation of reactor B containing 100% *Chlorella vulgaris*, and 0% *Nannochloropsis oculata* showed that the addition of *Chlorella vulgaris* microalgae in various concentrations of Pb waste was able to set aside Pb waste concentration of 10 mg/L to 1.5 mg/L.

The same thing also happened in research (Kurniawan & Aunurohim, 2014) that *Chlorella vulgaris* microalgae were able to adsorb Pb metal with an efficiency of 60.5% at the first concentration of 50 mg/L. In the application of the use of the *Chlorella vulgaris* microalgae for industrial waste, these microalgae can reduce the content of pollutant parameters in plastic liquid wastewater by 83.08% which contains lead (Soeprobowati & Hariyati, 2013).

Each type of microalgae has a different ability to absorb and accumulate heavy metals. *Chlorella* sp cells. in a study (Nacorda et al., 2007) it was easier to absorb Pb than Cd and Cr because Pb metal has a higher affinity so it is easily absorbed by the active site on the microalgae cell wall. The absorption ability of *Chlorella vulgaris* is greater than *Nannochloropsis oculata* because *Chlorella vulgaris* can adapt better than other microalgae even in extreme environments, fluctuating pH, and high concentrations of heavy metal ions (Merizawati, 2008).

Nannochloropsis sp. is a type of *Chlorophyta* that can be used to adsorb metal ions. *Nannochloropsis* sp. has a considerable adsorption capacity because there are amine, amide, and carboxylic functional groups that bind to metal ions. In addition, proteins and polysaccharides also play an important role in the absorption of heavy metal ions due to the presence of covalent bonds with carboxyl groups and amine groups (Putra, 2007). *Chlorella* sp. cell wall. consists of cellulose, hemicellulose, glycoprotein, and pectin. This compound has functional groups consisting of thiol and carboxylic groups which are important in binding heavy metal ions (Asiandu & Wahyudi, 2021). *Chlorella vulgaris* also contains polysaccharides containing alginate which will bind to heavy metals (Kurniawan & Aunurohim, 2014).

3.3 Relationship between Pb Removal and Number of Microalgae Cells

In the main research, the number of microalgae was also analyzed every time a sample was carried out to test the concentration of Pb waste. Data on the number of microalgae can be seen in Table 2.

Table 2. Analysis of the number of microalgae cells during the main study

Days to-	Number of Microalgae Cells (×10^4 cells/mL)								
	Reactor B			Reactor C			Reactor D		
	1 mg/L	5 mg/L	10 mg/L	1 mg/L	5 mg/L	10 mg/L	1 mg/L	5 mg/L	10 mg/L
0	301	301	301	350	350	350	402	402	402
3	300	307	304	361	358	368	405	407	402
6	322	316	312	422	390	405	429	426	414
9	344	326	323	491	470	494	439	417	426
12	328	314	275	445	432	385	422	391	366
15	303	290	231	307	280	236	362	338	323

Information :
Reactor B : (100% *Chlorella vulgaris*, 0% *Nannochloropsis oculata*)
Reactor C : (50% *Chlorella vulgaris*, 50% *Nannochloropsis oculata*)
Reactor D : (0% *Chlorella vulgaris*, 100% *Nannochloropsis oculata*)

Based on Table 2, the initial number of cells on day 0 of the main study in reactor B was 301 × 10^4 cells/mL, reactor C was 350 × 10^4 cells/mL, and reactor D was 420 × 10^4 cells/mL. The optimal initial number of microalgal cells inoculation was 106 cells/mL (Haryoto & Wibowo, 2004) The maximum number of microalgae cells was found in reactor C with a concentration of 10 mg/L on day 9 of 494 × 10^4 cells/mL. This number is more than other reactors because this reactor contains two types of microalgae in one reactor. Both of them carry out cell division with each other so that their number is greater than the number of cells in reactors B and D which are only filled with one type of microalgae, and the consortium can quickly hydrolyze exogenous proteins in the growth media (Kiani et al., 2023).

The increase in the number of cells in microalgae after being contacted with lead indicates that there has been an accumulation of heavy metals in the microalgae bodies. This accumulation occurs because microalgae are able to absorb heavy metals up to a certain concentration limit by accumulating heavy metals as biosorbents without causing poisoning in the body. Microalgae have a larger surface area than their volume ratio, thereby supporting their ability to accumulate briefly (Rahmadiani & Aunurohim, 2013).

4. CONCLUSION

The conclusion of this study showed that the microalgae Chlorella vulgaris and Nannochloropsis oculata were able to reduce the concentration of the heavy metal lead (Pb), but there were differences in their absorption abilities. The most effective composition for reducing Pb levels was in reactor 10B (100% Chlorella vulgaris, 0% Nannochloropsis oculata) at 85.0% from a concentration of 10 mg/L to 1.5 mg/L, so that these microalgae can be used as a phycoremediation agent.

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