The effect of pH and salinity on the capability of marine microalgae biomass for removing Cd and Pb

Lestari¹, S Permadi¹, A Bayu¹, A A Sari², Harmesa¹, D Yogaswara¹ and F Budiyanto¹*

¹ Research Center for Oceanography-LIPI, Jl. Pasir Putih 1, No. 1, Ancol Timur, Jakarta, Indonesia 14430
² Research Center for Chemistry-LIPI, Kawasan PUSPIPTEK, Serpong, Banten, Indonesia

*Corresponding author’s email: fitri.budiyanto@lipi.go.id

Abstract. The increase of heavy metals pollution requires a novel approach as an effective and eco-friendly removal strategy. The marine microalgae biomass was a strong candidate; however the study on local marine microalgae from Indonesia to be upgraded as metals biosorbent was limited. Thus, this study was aimed to utilize marine microalgae biomass as biosorbent for Cd and Pb removal in various pH and salinity value. The microalgae biomass was selected from diatomae, i.e. Chaeroceros sp., and Nitzschia sp., and green algae, i.e. Tetraselmis sp. The removal test was carried out at various pH and salinity range with initial metals concentration at 5 ppm and the measurement of metals concentration was utilizing Flame Atomic Absorption Spectrophotometer. The result exhibited the maximum Cd removal at pH 7 using Tetraselmis sp. (83.1%) and maximum Pb removal at pH 4 (85.4%) using Tetraselmis sp. and Chaetoceros sp. The removal of Cd and Pb was indicated a similar trend for the low removal capability at high salinity. Thus, this study revealed the best removal ability of the three marine microalgae was at pH 7 and low salinity.

1. Introduction

The increasing concern of environmental issue is demanding a comprehensive approach in either minimizing or remediating pollution in the last decades [1]. Among those pollutants, many types are categorized as hazardous one like heavy metal. The characteristics of non-essential heavy metals, i.e. toxic, persistent, bioaccumulate and biomagnification, assign them to the most observed pollutant [2]. Cadmium (Cd) and Lead (Pb) are reported to stimulate human health problems relating to acute and chronic exposure of them. The ability of cadmium to replace any major elements in human bone is triggering the disruption of the bone’s microstructure causing a serious problem like itai-itai disease in Japan. The acute and chronic exposure of cadmium is also accused of central and peripheral nervous system failure, lung and kidney damage [3]. Meanwhile, the characteristic of Pb to compete with Fe in erythrocyte production is blamed for the low level of haemoglobin. Other hazards related to Pb exposure are central nervous, kidney and liver failure [4]. Considering the impact, the level of Cd and Pb in the environment should be kept in check in order to minimize the exposure problem to human.

Many technologies were invented to keep heavy metals concentration to their minimal level in the environment. Those technologies include various proven processes like biological remediation using microorganism, and physicochemical processes like membrane and adsorption technology [5]. Those
The promising technologies claimed to have their advantages; however most of them are expensive, especially in the case of environmental remediation for pollutants [6]. The recent study on that technology leads to a cost-wise and eco-friendly aspect of technology and one of the most studied technology is adsorption using natural biomass [7]. The natural biomass as a base for biosorber is intensively modified from the waste like a banana peel, tea leaves, rice husk, etc. [8]. The use of biomass of microalgae is also become a superior alternative as biosorbent since they have a high affinity to the pollutant.

The attractive points of microalgae as a potential biosorbent are due to their convenient reproducibility and many types of biosorbent can be generated according to the species selection. The use of dead microalgae as biosorbent of pollutant is preferred compared to life microalgae, though both dead and life microalgae exhibit an ability to remove pollutant. However, in the term of applicability, the dead microalgae show their superiority, i.e. 1) the rapid removal of metal in the first contact, 2) the ability to be modified by simple chemical/physical treatment, 3) The independence from pollutant toxicity, 4) the potency of reusability of biosorbent, and 5) the convenience in storage [9].

Indonesia is harbouring a high potency on marine microalgae biomass since it is an archipelagic country. The exploration of many potencies of local marine microalgae is undergoing throughout the country. However, the study on the utilization of local marine microalgae biomass as biosorbent for pollutant Cd and Pb has limited especially the influence of physicochemical condition like pH and salinity. Thus, this study was aimed to evaluate the capability of selected marine microalgae biomass Chaetoceros sp., Nitzschia sp., and Tetraselmis sp. as biosorbent for Cd and Pb under the variation of pH and salinity.

2. **Materials and methods**

2.1. **Chemical specification**

All chemicals used in this study were analytical grade purchased from Merck, Darmstadt, Germany, i.e., HNO\(_3\), NaOH, Pb(NO\(_3\))\(_2\), CdCl\(_2\). The f/2 media for marine microalgae culture was prepared using various chemicals purchased from NaNO\(_3\), NaH\(_2\)PO\(_4\).H\(_2\)O, Na\(_3\)SiO\(_3\).9H\(_2\)O, FeCl\(_{3}\).6H\(_2\)O, Na\(_2\)EDTA.2H\(_2\)O, CuSO\(_4\).5H\(_2\)O, Na\(_3\)MoO\(_4\).2H\(_2\)O, ZnSO\(_4\).7H\(_2\)O, CoCl\(_2\).6H\(_2\)O, MnCl\(_2\).4H\(_2\)O, Vitamin B12.

2.2. **The media**

The culturing media for marine microalga was applying f/2 media, which is firstly introduced by [10]. The media was dominantly composed by filtered seawater with the addition of trace element and vitamin solution. The nutrient was added into 1 L of filtered seawater, i.e. 1 mL NaNO\(_3\) (8.82 x 10\(^{-4}\) M), 1 mL NaH\(_2\)PO\(_4\).H\(_2\)O (3.62 x 10\(^{-5}\) M), 1 mL Na\(_3\)SiO\(_3\).9H\(_2\)O (1.06 x 10\(^{-4}\) M), 1 mL of trace metal and 0.5 mL of vitamin solution. The trace metal solution was prepared by added trace element into 1 L of demin water, i.e.; 3.15 g FeCl\(_3\).6H\(_2\)O, 4.36 g Na\(_2\)EDTA.2H\(_2\)O, 1 mL CuSO\(_4\).5H\(_2\)O (3.93 x 10\(^{-8}\) M), 1 mL Na\(_3\)MoO\(_4\).2H\(_2\)O (2.60 x 10\(^{-8}\) M), 1 mL ZnSO\(_4\).7H\(_2\)O (7.65 x 10\(^{-8}\) M), 1 mL CoCl\(_2\).6H\(_2\)O (4.20 x10\(^{-8}\) M), and 1 mL MnCl\(_2\).4H\(_2\)O (9.10x10\(^{-7}\) M). Meanwhile, the vitamin solution was prepared by adding the following vitamin into 1 L of demin water, i.e., 200 mg of thiamine HCl (vit B1), 1 mL biotin (vit H)(2.05 x 10\(^{-9}\) M) and 1 mL cyanocobalamin (vit B12) (3.26 x 10\(^{-10}\) M).

The artificial seawater (ASW) for salinity test was prepared using the following procedure (10). In 1000 mL of deionized water, 31 g NaCl, 10 g MgSO\(_4\).7H\(_2\)O and 0.05 g NaHCO\(_3\).H\(_2\)O were added. The final salinity of the ASW was 34 ppt then the ASW was diluted to obtain the specific salinity, i.e., 5, 10, 15, 20 and 30 ppt.

2.3. **The biomass stock**

This study was using 3 species of marine microalgae, i.e. Chaetoceros sp. (diatomae), Nitzschia sp. (diatomae) and Tetraselmis sp. (green algae). Chaetoceros sp. and Nitzschia sp. was species isolated from Simeulue waters, Aceh meanwhile Tetraselmis sp. was isolated from Ambon Bay. The
2.4. The experiment on metals removal
The test on Cd removal was carried out using liquid media containing 5 ppm of metals in 50 mL of media, and the test of Pb was conducted in the same way in the separate test set. The test was conducted by varying pH and salinity in constant room temperature and time contact. The pH was varied at 7, 6, 5, 4, 3, and 2, meanwhile the salinity was varied at 5, 10, 15, 20 and 30 ppt. Then 25 mg of biomass was added into each adjusted media and shook for 2 hours. At the end of the experiment, the media was filtered using cellulose nitrate grade, 20 µm in pore diameter, and the filtrate was then injected into Flame Atomic Absorption Spectrophotometer for heavy metals measurement. All test was carried out in triplicate. The removal percentage was calculated as the following:

\[
\% \text{removal} = \frac{100(C_0 - C_e)}{C_0}
\]  

The removal percentage in equation 1 was computed using the data of initial concentration \(C_0\) and concentration at equilibrium \(C_e\) in mg/L [6].

3. Results and discussions

3.1. The effect of pH on metals removal
The test on Cd and Pb removal at different pH were carried out at salinity 0 and initial metals concentration at 5 ppm. The removal profile for both metals, implying the similar result for the decrease in removal percentage at lower pH, i.e. pH 2 and pH 3 as presented in Figure 1. Tetraselmis sp. was superior to remove Cd compared to Chaetoceros sp. and Nitzschia sp. almost at all tested pH value since it was indicated a constant Cd removal at pH 7–pH 4 in the removal range of 83.1-81.3%. At pH range of 7-4, the removal capability of other biomasses started to decrease. The removal of Cd using Chaetoceros sp. was 77.4% at pH 7 then gradually decrease to 52.5% at pH 4 before it jumped to 0.96% at pH 3. The removal profile of Cd using Nitzschia sp. was similar with Chaetoceros sp., it effectively removed 83.9% of Cd at pH 7 then slightly decrease to 68.3% at pH 4 before it jumped to 4.2% at pH 3. Uniquely, the removal of Cd using the three microalgae biomass was ineffective to remove Cd at pH 2 since the removal capability was down to 0.8-4.01%.

The removal profile of Pb was slightly different compared with the removal profile of Cd, especially the Chaetoceros sp. and Nitzschia sp. The removal profile of Chaetoceros sp. indicated relatively constant removal capability at the range of pH 7- pH 4 before its extreme decrease at pH 3. The removal profile of Pb using Chaetoceros sp. was relatively constant in 78.5-85.4% at pH 7- pH 4 before its extreme decrease to 7.7% at pH 3. The removal profile of Pb using Tetraselmis sp. was similar to Chaetoceros sp. for its constant value in 77.5-85.4 at pH 7 – pH 3 before its declined to 11.6% at pH 2. Meanwhile, the removal profile of Nitzschia sp. was exhibiting the most different in trend compared with the other two biomasses. The Pb removal using Nitzschia sp. steadily decreased in the value of 60.09-32.79% at pH 7- pH 3 before its ineffective removal to 11.6% at pH 2.

The pH is one of fundamental factor controlling Cd and Pb adsorption onto the biomass surface. At lower pH, the proton concentration is increase resulting in the positive charge at the biomass surface to inhibit metals adsorption. Meanwhile, the increasing pH stimulates the increase of negative charge on the biomass surface for better metals adsorption [12]. The capability of the three biomasses
uses to remove Cd and Pb in this study was comparable with alga *Anabaena sphaerica* in its maximum removal of Cd and Pb at 84.5% and 88.3%, respectively [12].

**Figure 1.** Removal profile of (a) Cd and (b) Pb in different pH value using: (−−−) *Chaetoceros* sp., (−−−) *Nitzschia* sp., (−−−) *Tetraselmis* sp.

3.2. The effect of salinity on metals removal

The test on Cd and Pb removal using the three microalgae biomass was carried out at constant pH 7 and initial metals concentration at 5 ppm. Interestingly, the three biomasses were less effective in removing either Cd or Pb at high salinity, as presented in Figure 2. The removal profiles of Cd and Pb using the three biomasses were exhibiting similar profile, decreasing toward the saline environment. The decrease of removal capability of metals using the biomasses was expected due to the competition between targeted metals (Cd and Pb) and the presence of ions in the media [13]. The slight difference among removal profile was due to the variability of cell composition [7].

Cadmium profile indicated a removal superiority of green algae *Tetraselmis* sp. toward diatom *Chaetoceros* sp. and *Nitzschia* sp. in higher salinity. However, the anomaly was observed at lower salinity as 5 ppt where the Cd removal by *Tetraselmis* sp. (40.8%) was lower than *Nitzschia* sp. (52.7%) and higher than *Chaetoceros* sp. (35%). Uniquely, Cd removal by *Nitzschia* sp. was higher than *Chaetoceros* sp. in most of the salinity level. At higher salinity, *Tetraselmis* sp. exhibited its higher effectiveness to remove Cd compared with *Chaetoceros* sp. and *Nitzschia* sp. Interestingly, the removal of Pb was relatively similar among biomass fluctuated based on salinity level. However, *Chaetoceros* sp. exhibited a relatively constant removal of Pb range from 80.3%-73.8% at salinity 5-20 ppt.

**Figure 2.** Removal profile of (a) Cd and (b) Pb in different salinity using: (−−−) *Chaetoceros* sp., (−−−) *Nitzschia* sp., (−−−) *Tetraselmis* sp.
This biochemical composition of each strain has been observed from secondary sources since this study indirectly analyzed the biochemical composition as presented in Table 1. The *Tetraselmis* sp. was relatively contained higher carbohydrate content compared with *Chaetoceros* sp. and *Nitzschia* sp. Uniquely, Protein content in *Tetraselmis* sp. was comparable with *Nitzschia* sp. but higher than *Chaetoceros* sp. Thus, the difference of biochemistry content in those three microalgae biomass contributed to the difference in their metal adsorbance capability.

### Table 1. Biochemistry composition of *Chaetoceros* sp., *Nitzschia* sp., and *Tetraselmis* sp. from secondary data.

| Biomass   | Lipid (%) | Protein (%) | Ash (%) | Carbohydrate (%) | Salinity media | Reference |
|-----------|-----------|-------------|---------|------------------|----------------|-----------|
| *Nitzschia* sp. | 18.36±0.28 | 43.16±0.13  | 19.60±0.06 | 18.88            | 30 ppt         | [14]      |
| *Chaetoceros* sp. | 19.28±0.20 | 34.99±0.1   | 34.95±0.12 | 10.78            | 30 ppt         |           |
| *Tetraselmis* sp. | 4.86±1.00  | 40.49±1.34  | 8.04±0.15  | 46.52±1.12       | 5 ppt          | [15]      |
|              | 5.58±0.06  | 41.10±0.09  | 8.22±0.30  | 45.10±0.27       | 10 ppt         |           |
|              | 8.54±0.09  | 42.69±0.42  | 7.53±0.52  | 41.23±1.44       | 20 ppt         |           |

4. Conclusion

This study gave valuable information on the capability of marine microalgae *Chaetoceros* sp., *Nitzschia* sp., and *Tetraselmis* sp. for removing Cd and Pb in a batch process at various pH and salinity. The best removal capability for each marine alga biomass was expressed at pH 7 and 0-5 ppt. The green microalgae *Tetraselmis* sp. was exhibiting its superiority towards *Chaetoceros* sp. and *Nitzschia* sp. over Cd and Pb removal at either pH or salinity fluctuation. Using 5 ppm of Cd and Pb as initial concentration, the maximum removal of Cd was 83.09% at pH 7 and salinity 0 using *Tetraselmis* sp.; meanwhile the maximum removal of Pb was 85.4% at pH 4 and salinity 0 using *Chaetoceros* sp. Considering the high removal capability, the marine biomass *Chaetoceros* sp., *Nitzschia* sp., and *Tetraselmis* sp. was potential to be used as biosorbent for Pb and Cd.

Acknowledgment

The research and the publication was funded by INSINAS project No. 084/P/RPL-LIPI/INSINAS-1/II/2019. The authors appreciate all laboratory attendances, Mr. Abdul Rozak, Mr. M. Taufik Kaisupy, Mrs. Serly Sapulete and Mr. Nurjamin, for their technical support during the research.

Reference

[1] Sundararajan S, Khadanga M K, Prakash J P, Kumar J, Raghumaran S, Vijaya R and Jena B K 2017 *Mar. Pollut. Bull.* **114** 592–601
[2] Sakellari A, Plavšić M, Karavoltsos S, Dassenakis M and Scoullos M 2011 *Estuar. Coast. Shelf Sci.* **91** 1–12
[3] Dean R J, Shimmield T M and Black K D 2007 *Environ. Pollut.* **145** 84–95
[4] Gu Y-G, Lin Q, Yu Z-L, Wang X-N, Ke C-L and Ning J-J 2015 *Speciation and risk of heavy metals in sediments and human health implications of heavy metals in edible nektom in Beibu Gulf, China: A case study of Qinzhou Bay Mpb*
[5] Matheickal J T, Yu Q and Woodburn G M 1999 *Water Res.* **33** 335–42
[6] Boubakri S, Djebbi M A, Bouaziz Z, Namour P, Amara A B H, Ghorbel-Abid I and Kalfat R 2017 *Environ. Sci. Pollut. Res.* **24** 27879–96
[7] Montazer-Rahmati M M, Rabbani P, Abdolali A and Keshtkar A R 2011 *J. Hazard. Mater.* **185** 401–7
[8] Sheng P X, Ting Y-P, Chen J P and Hong L 2004 *J. Colloid Interface Sci.* **275** 131–41
[9] Gautam R K, Mudhoo A, Lofrano G and Chattopadhyaya M C 2014 *J. Environ. Chem. Eng.* **2** 239–59
[10] Guillard R R L and Ryther J H 1962 *J. Microbiol.* **8** 229–39
[11] Adamakis I, Lazaridis P A, Terzopoulou E, Torofias S, Valari M, Kalaitzi P, Rousonikolos V, Gkoutzikostas D, Zouboulis A, Zalidis G and Triantafyllidis K S 2018 *Environ. Sci. Technol.* **25** 23018–32
[12] Abdel-Aty A M, Ammar N S, Ghafar H H A and Ali R K 2013 *J. Adv. Res.* **4** 367–74
[13] Luna A S, Costa A L H, da Costa A C a and Henriques C a 2010 *Bioresour. Technol.* **101** 5104–11
[14] Rodriguez-Nunez K and Toledo-Aguero P 2017 *Grasas Y Aceites* **68** 1–8
[15] Trovao M, Pereira H, Silva J, Jaime P, Quelhas P, Silva J T, Machado A, Gouveia L, Barreira L and Varela J 2019 *Heliyon* **5** 1–6