The Reduction of Cadmium Concentration by *Oscillatoria* Microalgae in a Culture Media

The Reduction of Cadmium Concentration by *Oscillatoria* Microalgae in a Culture Media

Hentiana[1], Doni Setiawan[1], Risfidian Mohadi[2], Hermansyah[2], and Hilda Zulkifli[1,*]

[1]Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Jalan Raya Palembang Prabumulih KM32, Inderalaya, South Sumatera, Indonesia 30662.
[2]Chemistry Department, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Jalan Raya Palembang Prabumulih KM32, Inderalaya, South Sumatera, Indonesia 30662.

*Corresponding author: hilda.zulkifli@yahoo.com

Abstract. Microalgae has functional group such as carboxylic (-COOH) and hydroxyl (-OH) which play an important role as adsorbent of heavy metal. Reduction of cadmium concentration by *Oscillatoria* microalgae in a culture medium has been carried out. This research aimed to study the ability of *Oscillatoria* microalgae as a biological adsorbent agent of cadmium heavy metal using BG-11 media. There were four treatments culture media with six repetitions consisting of P_0 = 100 mL medium + 10,000 individual *Oscillatoria*; P_1 = 1 mg/L Cd(II) + 100 mL medium + 10,000 individual *Oscillatoria*; P_2 = 3 mg/L Cd(II) + 100 mL medium + 10,000 individual *Oscillatoria*; and P_3 = 5 mg/L Cd(II) + 100 mL medium + 10,000 individual *Oscillatoria*. The result has shown the increasing of *Oscillatoria* in BG-11 culture media starting from day 4 to day 7 day was around 1208-1599 ind/L/day. P_3 treatment which consist of 1 mg/L show the highest reduction of cadmium absorption with percentage of 24.05%, compared to the others.

1. Introduction

Heavy metal is a metal that has not more than 5 g/cm^3 of density. Heavy metal could react to form coordination bonding with ligands, i.e. OH, -COO-, -OPO_3H-, -C=O, -SH, -S-S-, -NH_2 and =NH [12]. Based on toxicology explanation, non-essential heavy metal is toxic metal in which their existence in the human body does not have any function yet such as Hg, Cd, Pb, Sn, and Cr(VI) and As. These heavy metals bring a negative effect on human health [20]. The effects of heavy metal could directly impact the body if it accumulated on food chain although in a very small concentration. Even though it needs a long time to transferred, in the end it will be harmful.

Cadmium is one of the heavy metals with a wide range of dissemination in nature. This metal has 48 atomic numbers, 112.40 atomic mass with 321°C of melting point and 765°C of boiling point [13]. In the natural water, cadmium (Cd) form complex bonding with organic or inorganic ligands which are Cd^{2+}, $Cd(OH)^+$, $CdCl^+$, $CdSO_4$, $CdCO_3$, and Cd organic [17]. Cadmium has a low boiling point and easy to concentrate in the atmosphere. Water could be contaminated by sediment and mining waste that contain cadmium. Meanwhile, when contacted with fume, it will produce air pollution [9].

There were many papers about heavy metal waste treatment to resolve the environmental contamination and poisoning risk for living organisms. Adsorption process could remove the heavy metal from water. This technique is more valuable compared to other; it is more economical and does not produce any toxic substances. Generally, the adsorption method worked because of the interaction between the metallic ion and functional group that exist in the surface of adsorbent through complex

formation reaction. Moreover, it always happens in the surface of solid which has functional groups of –OH, -NH, -SH and –COOH [3]. In reality, the process of heavy metal waste handling could be solved by using microalgae as the absorption agent of the metal.

Microalgae is a eukaryotic autotrophic protista and it differentiated by cell wall flagella, chloroplast and food reserves. Microalgae has green photosynthetic pigment (chlorophyll), brown (fucoxanthin), greenish blue (phycobilin), and red (phycoerythrin). Moreover, microalgae life supplies 50% of aquatic oxygen and the main constituents of plankton in the humidity surface [2]. The morphology structure of microalgae can be a single cell or colony and survives in the various aquatic habitats. Sometimes, microalgae have unicellular or multicellular structure. The difference between microalgae and plant is that algae cell does not have clear function [15]. In this research, *Oscillatoria* is the microalgae that utilizes for metal absorption.

Oscillatoria is an alga from Cyanophyceae class included in Cyanophyte division which has clorophyll combination of green and blue (fucoxanthin) usually discovered in freshwater. Generally, this genus can adapt and survive in every environmental condition because it has good metabolism in adjusting the amount of chlorophyll with their cell [5,18]. *Oscillatoria* has specific, bluish-green color and forms long, straight and soft filament. Fragmentation is the way of *Oscillatoria* to multiple. When one of the cells died in the long filament, this cell will seperated into 2 or more pieces. The process is known as hormogonium. Fragmentation also comes from cell wall separation with trachoma [11]. *Oscillatoria* develops in culture media to produce the abundance cell of it.

Media is the main material that utilized for isolation and culture which composed by the mixing of food substances or nutrients as the energy source for microorganism [21]. Sylvester [19] stated that the purpose of microalgae cultivation in media culture is to reproduce new individual. The materials were absorbed by cell that utilized for metabolism. In the bioenergy process, nutrient has a function as electron acceptor. The chemical energy from metabolism was spent on reproduction, spore formation, and biosynthesis.

The mechanism of heavy metal intake divided in two ways which are adsorption and absorption. The processes are ion exchange and ion bonding of heavy metal on the surface by the functional group. Generally, *Oscillatoria* cell wall made from cellulose that has functional group such as hydroxyl which can bind together with heavy metal [6]. Absorption was worked slower than adsorption through active transport. Heavy metal was bonded with protein and it collected in vacuoles of *Oscillatoria* [4].

2. Experimental Method

2.1. Treatment Test

This treatment was done in different place with six times of repetitions:
Treatment P_0 = 1000 mL media + 10,000 *Oscillatoria*
Treatment P_1 = 1 g/L Cd(II) + 1000 mL media + 10,000 *Oscillatoria*
Treatment P_2 = 3 g/L Cd(II) + 1000 mL media + 10,000 *Oscillatoria*
Treatment P_3 = 1 mg/L Cd(II) + 1000 mL media + 10,000 *Oscillatoria*

2.2. BG-11 Media as Culture

The synthesize of BG-11 media, as shown in Table 1, was made by adding 10 mL of stock solution 1 and 2, 1 mL of trace metal solution into Erlenmeyer continue by adding water until 1000 mL and finally homogenized. This solution mixture was then heated in an autoclave at 121°C with 15 lbs pressure for 15 minutes.

2.3. Glassware Sterilization

All glassware are washed, cleaned and then sterilized in an autoclave at 121°C with 15 lbs pressure for 15 minutes.

Table 1. The Composition of BG-11 Media.

Composition	Amount (g/mL)
Stock 1 (Per 500 ml):	
$NaNO_3$	75.0 g
Stock 2 (per 500 ml):	
K_2HPO_4	2.0 g
$MgSO_4.7H_2O$	3.75 g
$CaCl_2.2H_2O$	1.80 g
Citric acid	0.30 g
Ammonium ferric citrate green	0.30 g
EDTANa2	0.05 g
$Na2CO_3$	1.00 g
Trace metal solution (per 1.000 ml):	
H_3BO_3	2.86 g
$MnCl_2.4H_2O$	1.81 g
$ZnSO_4.7H_2O$	0.22 g
$Na_2MoO_4.2H_2O$	0.39 g
$CuSO_4.5H_2O$	0.08 g
$Co(NO_3)_2.6H_2O$	0.05 g
Medium	
Stock solutions 1	10 mL
Stock solution 2	10 mL
Trace metal solution	1.0 mL
Aquadest	1.000 mL

2.4. Cadmium Stock Solution

Cadmium stock solution was made based on dilution formula:

$$M_1 \times V_1 = M_2 \times V_2 \quad (1)$$

note:
M_1: initial concentration
M_2: calculated concentration
V_1: initial volume
V_2: calculated volume

2.5. Oscillatoria Cultivation in BG-11 Media

The pristine algae cultivation prepared from 6 bottles that filled up with BG-11 media until 995 mL which has been sterilized. 10,000 *Oscillatoria* germ put in each bottle and each of it connected with a generator and illuminated by neon light 36 watts with 3000 lux of light intensity for seven days continuously.

2.6. Oscillatoria Inoculum Calculations

The initial germ of *Oscillatoria* is 10.000 ind/L. The population of culture stock was counted by microscope with 10 times of magnification using Sedgewick Rafter Counting (SRC) formulae:

$$V_1 = N_2 \text{ x } V_2 / N_1 \quad (2)$$

note:
V_1= Calculated inoculum volume (mL)
V_2= Culture media volume (1.000 mL)
N_1= Stock abundance (unit/mL)
N_2= Calculated abundance cell (unit/mL)

2.7. Oscillatoria Treatment Step

Twenty-four bottles were initially prepared, 6 bottles for control contain only BG-11 media and another 18 bottles contain 995 BG-11 media mixed with prepared cadmium. Next, all bottles were sterilized in an autoclave at 121°C with 15 lbs pressure for 15 minutes. 10,000 of *Oscillatoria* were then placed in every sterilized bottle and the growing rate of *Oscillatoria* was counted every day for seven days.

2.8. Oscillatoria Growing Rate Calculation

The growing rate of *Oscillatoria* was counted using SRCC, while the specific algae growth rate is counted by the Vonshak formula:

$$\mu = (\ln N_t - \ln N_0)/t \quad (3)$$

note:

μ = Specific growing rate (days^{-1})
N_0 = Initial *Oscillatoria* cell solidity (ind/mL)
N_t = Calculated *Oscillatoria* cell solidity (ind/mL)
t = time interval N_0 to N_t (days)

The concentration of cadmium was analyzed by Atomic Absorption Spectroscopy (AAS) AA-7000 Shimadzu.

3. Result and Discussion

3.1. The growing Rate of Oscillatoria

Oscillatoria growing rate was carried out using *Sedgwick Rafter Counting Cell* (SRCC) under the microscope with 40 times magnification. The observation of *Oscillatoria* growing rate finished in day 7 inside BG-11 media. Figure 1 describes the growing rate of *Oscillatoria* at P_0 treatment during the research. From the beginning until the last days of observation, *Oscillatoria* growth was increased, from 136 Ind/L/day in day 1 to 1599 Ind/L/day in day seventh. These number of *Oscillatoria* growth is proof that BG-11 media is suitable for algae cell growth. Based on Figure 1, the optimum condition of *Oscillatoria* harvest time is the seventh day. Isnanstyo and Kurniastuti [10] explain that the best harvest time for microalgae is in the last exponential phase. Currently, in this phase, microalgae are in the optimum condition, it has high nutrient content of the cell. Between day 4th and 7th, the total abundance of *Oscillatoria* cell is significantly increases and it called as the exponential phase. The cells are actively multiply through the division in the exponential phase, it causes the rapid cell growth.

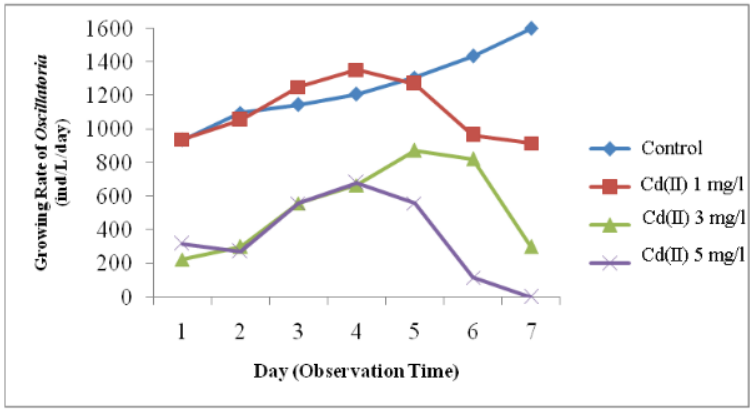

Figure 1. The growing rate of *Oscillatoria (*Ind/L/day) in BG-11 media.

The abundance of *Oscillatoria* was calculated each day for seven days. The growing rate was counted by adding heavy metal Cd(II) using variation: 0; 1; 3; 5 mg/L. The purpose of this growth

data retrieval was to know the ability of *Oscillatoria* to absorb Cd(II) and the effect Cd(II) on *Oscillatoria*. The algae growth was measured by BG-11 media with six repetitions. At the beginning of culture, 1000 Ind/L/day of *Oscillatoria* was used and the average of algae growth that obtained in the first day reach 938 Ind/L/day with P_0 (control) treatment. P_0 (control) test in the second day was produced 1098 Ind/L/day and slightly increase in the third day. However, the increasing number was not significantly happened because *Oscillatoria* was in [7] the adaptation phase with the culture media. [16] It was concluded that in the adaptation phase by adding inoculum into the culture media in the population will not giving any changes. The population did metabolism without cell division, so the cell density was not increased quickly.

In the fourth day of *Oscillatoria* cell growth in BG-11 media which contain 1 mg/L of Cd(II) represent its optimum condition or exponential phase. In fact, *Oscillatoria* can tolerate that amount of Cd(II) and create 1345 Ind/L/day of a cell. Explain that, exponential phase duration depends on inoculum volume, growth speed, media and the surrounding which support the algae development. Exponential phase marked by rapid growth period, cell division with a constant rate, the equilibrium between nutrient absorption, and the increasing number of microalgae. Moreover, Cd(II) almost has the same characteristic with zinc (Zn) and this is the reason why Cd(II) can substitute Zn in enzymatic reaction through enzymatic structure changing and affect the activation process. Moreover, a small amount of Zn could stimulate the development of the plant. These abilities of Cd(II) could replace the function of Zn in the synthesis of carbonic anhydrase enzymes and generate hydrogen ion for cell division [1].

The growth of *Oscillatoria* between day fifth and sixth with 3 mg/L of Cd(II) was likely stable. This static condition caused by the lacking supply of nutrient for *Oscillatoria* that used for the division process. Compared to day seventh, the amount of *Oscillatoria* was rapidly decreased to 300 Ind/L/day. In a constant condition, the composition of microalgae was significantly changed because the limited amount of nitrate in culture media which multiplied the carbohydrate compound, compared to that of protein. This affect cell reproduction rate equal with the dead rate, which means cell addition and elimination was insignificantly different.

The growth of *Oscillatoria* algae with 5 mg/L was decreased dramatically until the day of observation. After a deep observation through the microscope, the cell was burst and died which was proved by the changing of color of the cell. At the beginning, the culture color was green and turned into light green to eventually pale green. The increasing number of Cd and Hg concentration will disturb the growth of algae cell. Furthetmore, the standar deviasion each treatment (control, Cd(II) 1, 3, and 5 mg/L) are lower than the average value. The avarage value are 1247; 1107; 535; and 358 Ind/L/day while the number of standar deviasion are 221; 180; 265; and 252 Ind/L/day. This data explain the small posibility of error in the conducted experiment.

Based on Figure 1, the conclusion of this research was the adaptation process happened in the first to third day. Some parameters which influence the cell growth were the size of inoculum and the condition of growth media. If a cell was placed in a lack of nutrients media, it will need a longer time of adaptation because the cell needs to produce an enzyme which is the same as the nutrient.

Oscillatoria growth depends on the given nutrients and environmental condition. The environmental conditions are temperature, aeration, light intensity, and pH. In this research were used 6 as constant pH for each test. Moreover, aeration also applied in the observation with water circulation to deliver the same amount of nutrient for each alga in the culture media. Microalgae also utilized the light for photosynthesis because the light intensity is important for the growth of algae [14]. The culture was finished at 25ºC as the optimum temperature of *Oscillatoria* growth likewise every species has its optimum temperature. Hariati [8] stated that temperature is an important factor for the green blue algae spreading and behavior. Generally, 20ºC-30 ºC is the optimum temperature in most of green blue algae. BG-11 media as a place for growing the alga made from phosphor and potassium which came from KH_2PO_4 and K_2HPO_4. The function of KH_2PO_4 compound was as the source of phosphor for the synthesis of energy activation cell substances. Meanwhile, potassium role was to strengthen the algae cell, algae adding cell, nutrient absorption, and metabolism process.

3.2. *The percentage of Cd(II) in the culture media*

The observation of decreasing Cd(II) concentration in each volume 1 mg/L, 3 mg/L, dan 5 mg/L of culture media was analyzed by Atomic Absorption Spectrophotometer (AAS) and it is presented Table 2.

Based on Table 2, the highest decreasing concentration of Cd(II) was 1 mg/L with reduction percentage approximately 24.05%. The higher concentration of heavy metal was inversely proportional to the capability of *Oscillatoria* to absorb it. The dimension of concentration was declining from 1 ppm, 3 ppm, 5 ppm in a row because the absorption of the metal ion was decreasing. This data became proof when the concentration of heavy metal is high it will obstruct the growth of *Oscillatoria.*

The mechanism of Cd(II) absorption by *Oscillatoria* was divided into two processes which are adsorption and absorption. The processes are ion exchange and ion bonding of heavy metal in the surface by the functional group. Generally, *Oscillatoria* cell wall made from cellulose that has functional group such as hydroxyl that binds together with heavy metal. Cellulose has good potential as metal ion catcher because OH cluster interacts with adsorbate like Cd(II). Absorption happens through active transport. Heavy metal was accumulated in the cell and bind with protein i.e. metallothionein and phytochelatin in the vacuole.

Cadmium is a heavy metal that pollutes environmental if the amount exceeds the normal tolerance. Microalgae were utilized as bio-sorbent in bioremediation agent and there were so many works that observe about algae i.e. *Scenedesmus dimorphus* sp. Fauziah [4] reported that *Scenedesmus dimorphus* sp could be used as cadmium absorbent.

Table 2. The percentage of Cd(II) reduction in day seventh.

No	Concentration of Cadmium Cd(II) mg/L.			Reduction
	Initial	Final	Absorb	percentage (%)
1	1	0.7595	0.2405	24.05 %
2	3	2.8385	0.1615	5.38 %
3	5	4.8826	0.1174	2.34%

4. Conclusion

Oscillatoria has the ability in absorbing cadmium with absorption percentage of 24.05% for 1 mg/L of Cd(II). The growth rate in BG-11 media was relatively good in the day seven in which it can duplicate themselves into 1599 Ind/L/day especially in control media.

References

[1] Anderson R A 2005 Algal culturing technique London 245-250 *Elsevier Academic Press* London
[2] Barsanti L and Gualtieri P 2014 *Algae: Anatomy, Biochemistry, And Biotechnology Second Edition* (Jakarta: Widya Graha *LIPI*)
[3] Blais J F B, Dufresne, and G Mercier 2000 *Revuedes Sciences de l'Eau* **12(4)** 687-711
[4] Fauziah 2011 (Jakarta: Universitas Syarif Hidayatullah)
[5] Guiry M D and Guiry G M 2014 *Algaebase* World-wide Electronic Publication (National University of Ireland: Galway)
[6] Gupta R, P Ahuya, S Khan, R K Sakena, and H Mohapatara 2000 *Current science* **78(8)**
[7] Hadiyanto and Azim M 2012 *Mikroalga Sumber Pangan Dan Energi Masa Depan* (Semarang: Undip Press)
[8] Hariati R 2008 *Jurnal Biologi.* **10(1)** 19-22
[9] Herman D Z 2006 *Jurnal Geologi Indonesia* **(1)1** 31-36
[10] Isnanstyo, Alim, and Kurniastuty 1995 *Teknik Kultur Phytoplankton Zooplankton Pakan Alam Untuk Pembenihan Organisme Laut. (*Yogyakarta*)*
[11] Khoirul A A 2013*Cyanobacteri (Alga hijau-biru) (*Malang: Universitas Brawijaya)
[12] Nugroho A 2006 *Makar Teknologi* **10(2)**
[13] Palar H 2004 *Pencemaran dan toksikologi logam berat* (Jakarta: Rineka cipta)
[14] Regisata M, Litaay M, and Umar R M 2017 *Bioma: Jurnal Biologi Makassar* **2(1)**
[15] Ronaldo, Silalahi I H, and Wahyuni N 2013 J. K. K **2(3)** 148-152

[16] Sahabuddin, Amin M, and Jompa H 2013 Respon Pertumbuhan Populasi *Oscillatoria* sp. Pada Peningkatan Konsentrasi Karbodiosida Antripogenik (*Prosiding Forum Inovasi Teknologi Akuakultur*)
[17] Sanusi H S 2006 *Kimia Laut Proses Fisik Kimia dan Interaksinya dengan Lingkungan (*Bogor: Departemen Ilmu dan Teknologi Kelautan Fakultas Perikanan dan Ilmu Kelautan Institut Pertanian Bogor)
[18] Sari E W 2011 Isolasi Dan Identifikasi Mikroalga Cyanophyta Dari Tanah Persawahan Kampung Sampora, Cibionong, Bogor *Skripsi* (Jakarta: Fakultas Sains Dan Teknologi Universitas Islam Negeri Syarif Hidayatullah)
[19] Sylvester B D, D Nelvy, and Sudjiharno 2002 *Dalam* Seri Budidaya Laut No. 9.
[20] Vouk V 1986 *General Chemistryof Metals In: Freiberg L, Nordberg G F and Vouk V B (Eds) (*New York: Handbook on the Toxicology of Metals)
[21] Wijoseno T 2011 Uji Pengaruh Variasi Media Kultur Terhadap Tingkat Pertumbuhan dan Kandungan Protein, Lipid, Klorofil, dan Karotenoid pada Mikoalga *Skripsi* (Depok: Universitas Indonesi)

The Reduction of Cadmium Concentration by Oscillatoria Microalgae in a Culture Media

ORIGINALITY REPORT

15%
SIMILARITY INDEX

PRIMARY SOURCES

1	A Nabilah, S Handayani, S Setiasih, D U C Rahayu, S Hudiyono. " Emulsifier and antimicrobial activity against and of oxidized fatty acid esters from hydrolyzed castor oil ", IOP Conference Series: Materials Science and Engineering, 2020 Crossref	87 words — 3%
2	Ahmed Issa, Esmat Ali, R. Abdel-Basset, M.F. Awad, A.M. Ebied, S.A. Hassan. "The impact of nitrogen concentrations on production and quality of food and feed supplements from three cyanobacteria and potential application in biotechnology", Biocatalysis and Agricultural Biotechnology, 2020 Crossref	87 words — 3%
3	M I Fitranda, Sutrisno, S Marfu'ah. "Physicochemical Properties and Antibacterial Activity of Castor Oil and Its Derivatives", IOP Conference Series: Materials Science and Engineering, 2020 Crossref	81 words — 2%
4	www.e3s-conferences.org Internet	52 words — 2%
5	eprints.itn.ac.id Internet	49 words — 2%

6	www.jocpr.com Internet	36 words — 1%
7	lib.unnes.ac.id Internet	31 words — 1%
8	Xiaorong Meng, Jiawen Li, Yongtao Lv, Yeyuan Feng, Yuanyuan Zhong. "Electro-membrane extraction of cadmium(II) by bis(2-ethylhexyl) phosphate/kerosene/polyvinyl chloride polymer inclusion membrane", Journal of Hazardous Materials, 2020 Crossref	18 words — 1%
9	Aman Santoso, Anugrah Ricky Wijaya, Chandra Fetty Purwaningtyas, Dedek Sukarianingsih, Rini Retnosari, Sumari Sumari. " The Effect of K O Concentration in K O/Al O Catalyst on Methyl Ester (Biodiesel) Synthesis from CPO Off Grade with Ultrasonic Wave ", IOP Conference Series: Materials Science and Engineering, 2020 Crossref	14 words — < 1%
10	digilib.uinsgd.ac.id Internet	10 words — < 1%
11	docplayer.net Internet	10 words — < 1%
12	www.scribd.com Internet	10 words — < 1%
13	etheses.uin-malang.ac.id Internet	8 words — < 1%
14	Gu, S.. "Comparison of transport and separation of Cd(II) between strip dispersion hybrid liquid membrane (SDHLM) and supported liquid membrane (SLM)	6 words — < 1%

using tri-n-octylamine as carrier", Separation and Purification Technology, 200610
Crossref

EXCLUDE QUOTES	OFF	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	ON		