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PHYCOREMEDIATION OF MERCURY IN THE AQUATIC ENVIRONMENT

Phycoremediation refers to the technology of using microalgae to reduce pollutants in the aquatic environment. The purpose of this study was to analyze the reduction of mercury heavy metal in the media by using several species of microalgae such as *Spirulina maxima, Nannochloropsis oculata*, *Chlorella vulgaris*, and *Porphyridium cruentum*. The algae were exposed to mercury during eight days of cultivation. A randomized design was set with three different concentrations of mercury, namely 1, 3, and 5 mg/dm^3, with three replications for each concentration. The initial concentration of microalgae was set to 10 000 cells/cm^3 for *S. maxima* and *N. oculata*, while the concentration for *C. vulgaris* and *P. cruentum* was set to 100 000 cells/cm^3. The concentration of mercury was measured at the beginning (1st day), the middle (4th day), and the end of microalgae cultivation (8th day) by using the atomic absorption spectroscopy (AAS) tool. The result demonstrated a reduction of mercury concentration during the experiment in all experimental media, where the highest reduction was found at 1 mg/dm^3 ($p < 0.05$). In conclusion, microalgae have their limited ability to absorb and adsorb heavy metals. Therefore, the utilization of low-concentration microalgae on reducing heavy metal such mercury is recommended and merits further investigation.

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1. INTRODUCTION

Heavy metals pollution is an environmental serious problem because of its toxic and negative impact on living organisms [1, 2]. Heavy metals belong to a group with atomic densities higher than 4 g/cm^3 and cannot be degraded biologically, yet it can be accumulated [3]. There are several harmful toxic heavy metals including arsenic (As), lead (Pb), mercury (Hg), and cadmium (Cd) [4]. Their presence in nature usually presents in both dissolved or suspended form (bound to solids) as well as an ionic form. One of the most dangerous wastes is mercury. Its production is considerably large and its use in various fields is relatively extensive. Mercury may come from the natural and/or from anthropogenic activities. The source of mercury from natural activities includes volcanic and rock weathering, while that from human activities comes from the alkaline chlorine industry, thermometers and batteries, electrical equipment, gold mining, and other kinds [5, 6]. The impact of heavy metals could be even greater because the accumulation of heavy metals could increase by the biomagnification process that could lead to a negative effect on aquatic organisms [7]. Through the food chain cycle [8], the human can be exposed to heavy metals and it will affect human health due to its chronic (skin damage, DNA and nervous system disturbance, disability order) and acute effects (lethal) [1, 2].

This study aimed to analyze the percentage of mercury heavy metal reduction by using microalgae. Phycoremerediation refers to biotechnology on using microalgae to minimize the concentration of heavy metals in the aquatic ecosystem. Phycoremediation process has several advantages compared to physical (lime precipitation) or chemical (ion exchange) remediation process. To our knowledge, conventional technology sometimes ineffective, relatively expensive, and even creates another form of pollutant. Nowadays, the bioecological technology approach that has more benefit (phycoremediation), offers an alternative solution to decrease the waste from the aquatic environment such as environmentally friendly, easy to cultivate, available naturally on waters, cheap, and relatively easy to apply [5].

Microalgae, the microscopic autotroph organisms, live cosmopolite and commonly found in freshwater, brackish water, and seawater. They play an important role as a primary producer by doing the photosynthetic process and provide oxygen to the water environment. They require sunlight [10], carbon dioxide, and nutrients in the form of mineral salts to grow and reproduce. They obtain the advantage from the presence of heavy metals as trace elements to support their growth [11].

2. MATERIALS AND METHODS

Microalgae cultivation. In this study, several microalgae, i.e., *Spirulina maxima*, *Nannochloropsis oculata*, *Chlorella vulgaris*, and *Porphyridium cruentum* were used as

phycoremediators. These strains were obtained from the brackish water cultivation center (BBAP) Situbondo Regency located in East Java, Indonesia. All species were initially acclimated and cultivated 3–4 days before the experiment, in sterilized 2 dm^3 flasks containing 1 dm^3 of Walne medium. Microalgae were cultured at a light intensity of 3200 lux (TL lamp 18 Watt, light meter LX-101A) from 25 to 28 °C (DO meter Lutron PDO-519), salinity was 34–38 ppt (refractometer), and pH was approximately 7–8 (pH meter ATC).

Laboratory setup. The experimental method was realized by using a completely randomized design. Three different concentrations of mercury heavy metal (1, 3, 5 mg/dm^3) were applied to four microalgae species and control (microalgae without mercury exposure) with three replications for each concentration (Fig. 1).

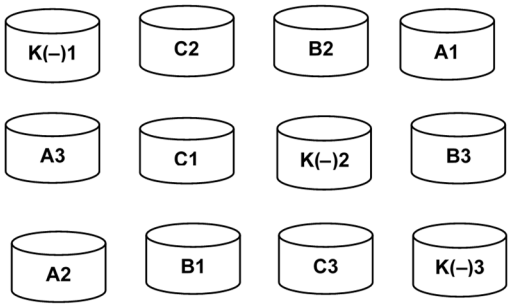

Fig. 1. Completely random design; A, B, and C – treatment with the medium containing microalgae and mercury heavy metal of 1, 3, and 5 mg/dm^3, respectively, K(–) – medium containing mercury heavy metal without microalgae

Laboratory experiment. $HgCl_2$ was tested on media culture as a pollutant. The initial cell of microalgae was set to 10 000 cells/cm^3 (*S. maxima* and *N. oculata)* and 100 000 cells/cm^3 (*C. vulgaris* and *P. cruentum*). On the first day of the experiment, mercury was added into the medium. The mercury concentration was measured three times: on the first, 4th, and 8th day of the experiment by the absorption spectroscopy (AAS) as described in [12]. The mercury concentration was measured in the culture media. The fraction absorbed C_A was calculated from [13, 14]

$$C_A = \frac{C_0 - C_1}{C_0} \times 100\%$$

where C_0 is the initial concentration of heavy metal, and C_1 is its final concentration after absorption.

The cell densities of microalgae were measured daily by using Neubauer's hemocytometer and a light microscope Olympus CX21LED (40× magnification). Water quality parameters were maintained and measured daily or when required.

Statistical analyses. Data were analyzed by using the ANOVA test with the level confidence of 95% by using SPSS 16.0 and Microsoft Office Excel 2010 for Windows. Additionally, when the significance between the treatments was found, further statistical analysis was conducted using the least significance difference (LSD) test.

3. RESULTS AND DISCUSSION

A slight decrease in the mercury concentration was found during the observation. It was varying among microalgae species (Table 1). Control media did not show a change of concentration and remained stable. However, as compared to other treatments, there was a decrease in mercury concentration in treatment A, B, and C with a small variation.

Table 1

Concentration of mercury [mg/dm^3] exposed to microalgae culture media

Species	Experiment[a]	Day 0	Day 4	Day 8
N. oculata	A	0.994	0.976	0.954
	B	2.998	2.967	2.947
	C	5.002	4.974	4.922
S. maxima	A	1.011	0.990	0.968
	B	3.010	2.985	2.974
	C	4.979	4.941	4.931
C. vulgaris	A	0.996	0.762	0.642
	B	2.995	2.782	2.678
	C	4.995	4.985	4.979
P. cruentum	A	0.995	0.979	0.965
	B	3.005	2.980	2.968
	C	4.996	4.985	4.971
Control	A	1.002	1.002	1.002
	B	3.001	3.002	3.001
	C	4.999	4.999	4.999

[a]Initial concentration of mercury: A – 1 mg/dm^3, B – 3 mg/dm^3, C – 5 mg/dm^3; control (without microalgae).

ANOVA analysis showed that there was a significant absorption of mercury in *N. oculata* in the water media. Additionally, the LSD test indicated that A and B treatments were the best to absorb the mercury. Furthermore, a similar result was found in *S. maxima* culture media where this species influenced the absorption process. The LSD analyses revealed that A treatment was different from B and C treatments. As for *C. vulgaris*, the addition of these microalgae in water media containing mercury appeared to be significant. In line with that, *P. cruentum* showed results similar to those for the

previous species. The highest decrease of mercury concentration was found in treatment A for all microalgae species, while the lowest decrease was found in treatment C. In contrast, as for *C. vulgaris*, the highest decrease of mercury concentration was found in treatment A, while the lowest was found in treatment C. In this study, the tolerability of microalgae to mercury exposure appeared to be species-specific. Also, the density of *C. vulgaris* was higher than *S. maxima* and *N. oculata*. This could affect the capacity of mercury absorption by these microalgae.

3.1. ABSORPTION OF MERCURY

The percentage of mercury absorption by microalgae was calculated on the 4th and 8th days of the experiment (Table 2). The highest absorption occurred after 4 days in *C. vulgaris* culture media on A treatment, while the lowest absorption was found in treatment C. The highest and the lowest absorption after 8 days occurred also in the culture media containing *C. vulgaris* in A (1 mg/dm^3) and C treatment (5 mg/dm^3), respectively.

Table 2

Absorption of mercury [%]

Species	Treatment[a]	Day 0–4	Day 4–8	Day 0–8
S. maxima	A	1.77	2.25	3.74
	B	1.03	0.69	1.70
	C	0.56	1.03	1.58
N. oculata	A	2.08	2.22	2.58
	B	0.83	0.37	0.89
	C	0.76	0.20	0.89
C. vulgaris	A	23.49	15.75	43.19
	B	7.13	3.75	9.46
	C	0.20	0.11	0.31
P. cruentum	A	1.61	1.43	4.00
	B	0.83	0.40	1.00
	C	0.22	0.27	0.00
Control	A	0.00	0.00	0.00
	B	0.00	0.00	0.00
	C	0.00	0.00	0.00

[a]Initial concentration of mercury: A – 1 mg/dm^3, B – 3 mg/dm^3, C – 5 mg/dm^3; control (without microalgae).

C. vulgaris is a type of one-celled green algae. The cell stands alone with a round or oval formation with a diameter of 3–8 μ in diameter. The algae have cup-shaped chloroplasts and hard walls [24]. This species has adsorption and absorption ability by utilizing the content of organic and inorganic compounds derived from heavy metals for its metabolism. The cell wall of *C. vulgaris* is composed of cellulose [15] and contains

pectin, which has a weak acid group (–COOH) thus easy to release H^+. The content of organic or inorganic material contained in heavy metals will be used by this microalga as a nutrient. This microalga species can use Hg as a building block in their metabolic activities. Nevertheless, when the heavy metal concentration is too high, the microalgae cell could be damaged and the cell function to absorb will no longer work. Cellulose in cell walls of microalgae contains hydroxyl groups that could bind to heavy metals. Through the adsorption process, heavy metals bind to hydroxyl groups found in cell walls. When the concentration of heavy metals in extracellular organs is higher than in the intracellular ones, the heavy metals will be absorbed into the cells. Heavy metals that infiltrate into the cells will bind with the biomolecules containing exchangeable ions or phytochelatin (heavy metal-binding proteins) [2, 16]. Phytochelatin is a low molecular-weight polypeptide that is rich in cysteine and could form a chelate with heavy metals through thiol groups (–SH) and amino acids. The complexes of phytocrystalline bonds with these metals end up on microalgae vacuoles and may cause death from biomass [11]. Therefore, the utilization of *C. vulgaris* as a phycoremediation agent in reducing mercury concentration merits further investigation. A longer cultivation time could be applied to determine the effectiveness of microalgae biosorption process.

Mercury is a heavy metal belonging to the highest toxic heavy metal group among others. This substance could damage the structure of cell tissues and lead to cell death [17]. The higher the concentration of heavy metals, the more it inhibits the cell growth because cells cannot compensate toxic effects caused by heavy metals.

3.2. ANALYSES OF MERCURY HEAVY METAL ABSORPTION

ANOVA was carried out to determine the effect of microalgae species on the absorption ability of mercury. Our ANOVA analyses for all microalgae species displayed an influence of microalgae availability on the reduction of mercury concentration in the medium (*S. maxima* 9.991 > 9.55, *N. oculata* 18.924 > 9.55, *C. vulgaris* 17.018 > 9.55, and *P. cruentum* 23.482 > 9.55). Following LSD test analyses, all microalgae species used in this study significantly reduced the concentration of mercury in A treatment (1 mg/dm^3). The *S. maxima* exposed to 3 and 5 mg/dm^3 present decay of cell growth. Furthermore, other species demonstrate similar results for the Hg concenrations of 3 and 5 mg/dm^3. A higher concentration of mercury could inhibit the population growth and cell damage of microalgae. A previous study [18] showed that at 10 mg/dm^3 of mercury exposure could kill the entire microalgae population in less than 24 h, while a concentration of 5 mg/dm^3 of mercury exposure could lead to sub-lethal effects on the microalgae population. These results could be due to the alteration of cell division or cell damage that causes the decrease of cell division. A study [18] showed a decrease in the concentration of chlorophyll due to the increasing concentrations of mercury. In addition, cell density also decreases slowly with the presence of mercury exposure to microalgae.

3.3. IMPACT OF WATER QUALITY PARAMETERS ON HEAVY METAL ABSORPTION

Culture success depends on optimal water quality parameters because they support the growth of microalgae in reducing heavy metal concentration in the medium. The relevant value for dissolved oxygen (DO) concentration for microalgae ranges from 6.31 to 9.22 mg/dm^3. An increase in DO concentration indicates the photosynthesis process is going well. Furthermore, temperature also plays an important role in the growth of microalgae [19]. The optimal temperature limit for microalgae growth is around 20–30 °C. The optimal pH is 7–9 [20]. This value can support the growth of microalgae optimally. The pH in this study was in the range of 7–8. The pH of 5–6 is the optimum pH for the mercury biosorption process [21]. A study [22] revealed that the optimum salinity for microalgal growth ranging from 33 to 40 ppt. The intensity of the light obtained in this study was 3200 lux. This is consistent with the authors of [23] who found that the light intensity for optimal microalgae growth ranged from 2500 to 5000 lux. Therefore, water quality parameters could affect the mechanism of heavy metal biosorption.

4. CONCLUSION

The present study demonstrates that the species with the highest percentage of mercury reduction during the study was *C. vulgaris* with treatment of 1 mg/dm^3 heavy metal inside. Mercury concentration 1 mg/dm^3 is still tolerable for the growth of microalgae.

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