Phycoremediation of Cadmium using *Chlorella vulgaris* in Photobioreactor

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Paper submit : 31 Oktober 2022, Paper publish: 31 Maret 2023

Abstract *– Chlorella vulgaris had the ability to accumulate heavy metals in their bodies, so they could be used as biosorbent in handling heavy metal pollution in waters. The effectiveness of C. vulgaris in the remediation of cadmium (Cd) would be tested in this present study. C. vulgaris were cultured for 14 days in the photobioreactor which was an enclosed chamber that was fully aerated and illuminated with LED lamps. Cadmium with concentrations of 0 (control), 0.05, and 0.1 ppm was mixed with C. vulgaris growth medium. The number of C. vulgaris cells was counted every 3.5 days using a hemocytometer to determine the growth condition. Metal concentrations were also measured on days 0, 7, and 14 using atomic absorption spectrophotometry (AAS). C. vulgaris was able to reduce cadmium levels up to 98%. The decrease in cadmium levels with the highest efficiency occurred at a cadmium concentration of 0.05 ppm.*

Keywords: *bioremediation, cadmium, Chlorella vulgaris, photobioreactor, phycoremediation*

PENDAHULUAN

The increase in industrial activities had impact on increasing energy consumption (Sembada, 2022) and the release of waste into the surrounding environment (Faizal *et al.*, 2021), including the marine environment. One of the industrial wastes discharged into sea waters was heavy metal. The increase in heavy metal concentrations in sea waters was becoming public concern, especially when the bioaccumulation of heavy metal occured in the food chain (Pandey & Madhuri, 2014). Cadmium (Cd) is heavy metal that is very toxic after mercury (Hg). Cadmium was often used as the main or additional material in industrial activities, including the nickel-cadmium battery industry, pigments, coating materials, stabilizers in the plastics and other synthetic goods industry (Rahimzadeh *et al.*, 2017). Cadmium is also type of heavy metal that is dangerous and can cause several severe diseases such as anemia, lung disorders, emphysema, and chronic renal tubular disease (Sardar *et al.*, 2013).

One of the waste treatment technologies that are environmentally friendly and have beneficial value is biological waste treatment or phytoremediation (Sembada & Suyadi, 2022). Biological waste treatment systems are still considered the cheapest way when compared to chemical methods, considering the relatively high price of chemicals. One alternative is the use of the microalgae (phycoremediation) to reduce the pollutants present in the waste. Microalgae are reliable bioremediators with biosorption capabilities because they have functional groups that can bind metal ions, especially carboxyl, hydroxyl, amine, sulfudryl imadazole, sulfate and sulfonate groups found in cell walls (Kumar *et al.*, 2015). The biomass are also easy to obtain and available in large quantities with low operational production cost, minimal sludge generated, and does not require additional nutrients.

This study aimed to evaluate the ability of *Chlorella vulgaris* to remediate cadmium. *C. vulgaris* was chosen due to the capability for multiplying its biomass rapidly (Ye *et al.*, 2018). *C. vulgaris* also easy to cultivate, can produces oxygen through the process of photosynthesis, and contains high protein with amino acids as the main component as in most plants (Panahi *et al*., 2019; Sembada & Faizal, 2022). *C. vulgaris* does not require large area for cultivation when compared to other plants which are also used as phytoremediation because of their micro size.

MATERIALS AND METHODS

1. Research subject

This study was an experimental research conducted in a laboratory. This study took place at the Plant Tissue Culture Laboratory, School of Life Sciences and Technology (SITH), Institut Teknologi Bandung (ITB), Jatinangor Campus in June – July 2019.

2. Materials

Materials used in this study were culture of *C. vulgaris*, Sodium-Phosphorus-Potassium (NPK) liquid fertilizer, Walne culture media, cadmium oxide (CdO), distilles water, glass bottles, light-emitting diode (LED) lamps, storage cabinets, hemocytometers, light microscopes, pipettes, micropipettes, aerators, hoses, spray bottles, 10 mL measuring glass, 20 mL measuring glass, 1.000 mL measuring glass, conical centrifuge tube, centrifuge, and autoclave.

METHODS AND RESEARCH DESIGNS

1. Sterilization of photobioreactor

Photobioreactor used for culture consisted of glass bottles, aerators, hoses and irradiated with LED lamps as shown in Figure 1. These photobioreactors then sterilized by autoclaving which aimed to disinfect or kill unwanted microorganisms. Furthermore, nine photobioreactors were filled with *C. vulgaris* culture, distilled water, and NPK fertilizer. Walne culture media was also added as nutrient in the culture.

a. Treatment with cadmium

The concentrations of Cd used in this study were 0, 0.05, and 0.1 ppm. Cadmium dioxide (CdO) was mixed in *C. vulgaris* growing medium with those concentrations. The repetition was carried out three times at each concentration. These treatments using heavy metal lasted for 14 days.

Figure 1. *C. vulgaris* in photobioreactor

b. Counting the number of *C. vulgaris* cells using counting-chamber (hemocytometer)

The number of *C. vulgaris* cells was counted using hemocytometer every 3.5 days (Ratomski & Hawrot-Paw, 2021). Approximately 4 mL of each culture from the photobioreactor was taken for measurement. Hemocytometer was first cleaned with alcohol then cover slip was placed over it. The culture media that had been diluted was dripped using pipette on the hemocytometer until it was completely filled. Dropping had to be careful to avoid air bubbles under the cover glass. Furthermore, the hemocytometer was observed under light microscope with a magnification of 100 or 400 times. The number of *C. vulgaris* cells was determined by counting the cells in the specified square.

c. Determining cadmium levels on the medium

Cadmium levels in the medium were tested on days 0, 7 and 14. Approximately 14 mL of each culture medium from the photobioreactor was taken for analysis. Analysis of cadmium on the medium was carried out in Environmental Laboratory Raksa Buana, Bandung, West Java. Analysis were done using atomic absorption spectrometry (AAS) (Kasa *et al.*, 2017). The efficiency of the reduction of the cadmium levels in the medium was calculated by the following equation:

$$\text{Reduction efficiency}(\%) = \frac{M_x}{M_0}$$

M_x represented the concentration of Cd (ppm) on other days such as day 7 and 14. M_0 represented the concentration of Cd (ppm) in the medium on the initial day.

d. Statistical analysis

The data obtained were then analyzed using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) with SPSS.

RESULT AND DISCUSSION

The phycoremediation process using *C. vulgaris* was evaluated by measuring the cadmium content in the medium on days 0, 7, and 14 as shown in Figure 2. The results of these measurements also became the basis for calculating the reduction efficiency as shown in Table 1. These results indicated that *C. vulgaris* was able to remediate cadmium.

Metal toxicity was influenced by culture conditions (Juneja *et al.*, 2013). High levels of nitrate and phosphate and also low temperatures would reduce the toxicity of cadmium. Accumulation of heavy metal could inhibit the cell growth when in the high concentrations (Rizwan *et al.*, 2017) because the organism's protective system was unable to offset the effects of metal toxicity.

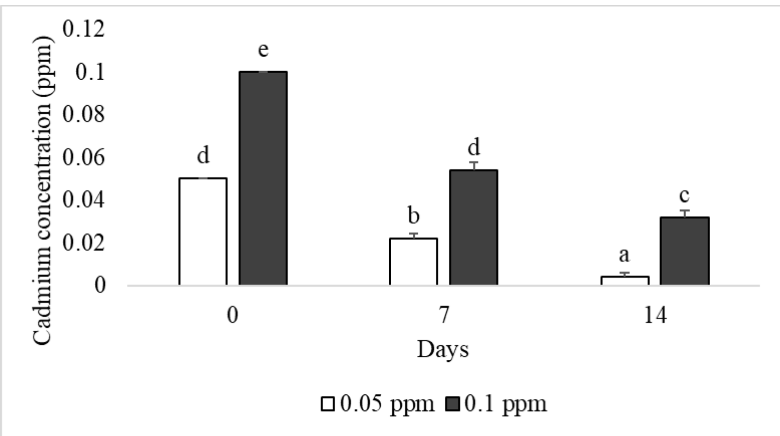

Figure 2. Cadmium concentration (ppm) observed during phycoremediation

Table 1. Reduction efficiency (%) observed during phycoremediation

Concentration of Cd (ppm)	Reduction efficiency (%)	
	Day 7	Day 14
0.05	56 ± 4.9	92 ± 4.32
0.1	46 ± 3.74	68 ± 2.94

C. vulgaris cell wall could bind cadmium ion. Saber *et al.* (2011) stated that the main key of heavy metal remediation is that the metals are non-biodegradable but can still be transformed through the process of sorption, mediation, compensation, and changes in their valence values. When heavy metal ions are scattered around the cell, metal ions will be bound to the elements contained in the cell wall based on the ability of the cell's chemical affinity (Soares & Soares, 2012). The process of absorption of cadmium by *C. vulgaris* was by the biosorption. This process indicated with the exchange of monovalent and divalent ions such as Na, Mg, and Ca contained in the cell wall and replaced by heavy metal ions and then forms complexes between heavy metals or ions (Chen *et al.*, 2018) and functional groups such as carbonyl, amino, thiol, hydroxyl, phosphate, and hydroxyl-carboxyl. The process of biosorption took place quickly and occured in both dead and living cells. This process took place effectively in the certain pH and the presence of other ions where heavy metals become insoluble salts that are precipitated (Abdi & Kazemi, 2015). The cell wall was the most important part of the cell defense mechanism because it was the first barrier against the accumulation of toxic heavy metals (Parotta *et al.*, 2015). After the biosorption process (passive uptake), the next mechanism was active uptake where *C. vulgaris* transferred metal ions that had been bound to the cell wall to deeper cell organelles (namely bioaccumulation) (Arishi & Mashhour, 2021).

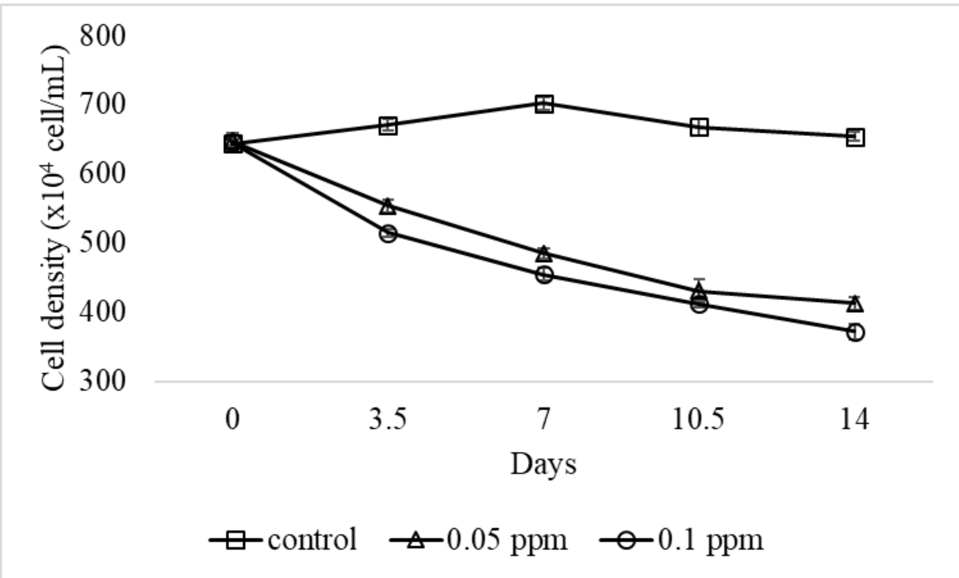

Figure 3. Cadmium concentration (ppm) observed during phycoremediation

It can be seen in Figure 3 that the population or cell density of *C. vulgaris* in all treatment variations decreased, including the control that were not treated with cadmium.

Growth of *C. vulgaris* strongly influenced by several environmental factors, including nutrients in the culture media and water quality such as salinity, pH, temperature, optimum light intensity (Metsoviti *et al.*, 2019). According to Lakaniemi *et al.* (2012), the decline in the cell number of *C. vulgaris* because the culture was carried out in the limited volume which caused the amount of nutrients contained in the media was also limited so that *C. vulgaris* could not longer able to maintain its cell density. Ras *et al.* (2013) also stated that the decrease in the growth of algal culture could be caused by three factors, such as reduced of nutrients in the media, reduced of the light intensity, and increased of fierce competition for nutrients, living space, and light during the culture.

CONCLUSIONS

Cadmium could be reduced by 92% for 14 days by using phycoremediation process with *C. vulgaris*. This indicated the potential of *C. vulgaris* to be used as a heavy metal biosorbent in the environment. Further research was needed to determine the ability of *C. vulgaris* to absorb several types of metal contaminants.

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