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Cadmium and copper removal using microalgae *Chaetoceros calcitrans* for bioremediation potential test

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ABSTRACT

Cd and Cu are often found as a result of anthropogenic activities and can be toxic if the levels are excessive. All the pollutant materials can proceed naturally. However, with maximizing the existence of microalgae it can make faster the cycle of heavy metals pollution. *Chaetoceros calcitrans* is one of the microalgae that is tested as a bioremediation agent. The purpose of this research is to understand the ability of the microalgae to absorb heavy metals in a controlled environment. In this study, the microalga of *Chaetoceros calcitrans* tested using Cd and Cu with concentrations of each control, 0.7, 1.3, and 1.9 ppm for 96 hours. Microalgae density measurements are carried out every 12 hours to monitor the density of microalgae during exposure. The final of heavy metals concentration on media and microalgae was carried using AAS. In addition, the calculation of BCF (Bioconcentration Factors) was also determined to measure the potential absorption of *Chaetoceros calcitrans* to accumulate heavy metals.

Keywords : Absorbance, Bioconcentration factors, Pollution

Introduction

Environmental pollution due to heavy metal pollutants is a significant problem throughout the world, this is because heavy metals can accumulate in the food chain and their existence will continue in the ecosystem (Aneja *et al.,* 2010). Some types of heavy metals that are often found in marine waters are Cadmium and Copper. The main sources of release of Cd (II) into the aquatic environment are smelting, electroplating, batteries, fertilizers, mining, paint pigmentation, etc.. Cadmium is a type of toxic and dangerous heavy metals for humans and the environment (Iqbal *et al.,* 2007). Copper is an essential type of heavy metal that functions as a micronutrient important for growth, metabolism, and various enzyme activities in algae, cyanobacteria, and other organisms. However, copper can also be an inhibitor of algal growth at high concentrations (Erickson, 1972).

Conventional techniques that can be used to remove heavy metals from waters include chemical precipitation, chemical reduction, adsorption, ion exchange, evaporation and membrane processes. The biosorption process has advantages compared to other techniques, such as low operational costs, minimization of chemical and biological sludge, high efficiency in removing heavy metals from solu-

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tion, biosorbent regeneration, metal recovery possibilities and being environmentally friendly (Ahluwalia and Goyal, 2007).

One biosorption technique that has been widely used in various countries is the Bioremediation technique. Bioremediation is an innovative technology that uses living things or dead biomass to eliminate heavy metals or other pollutants from the marine environment. Various biomass that can be used in this technique include bacteria, yeast, fungi, algae. Microalgae is one organism that is often used for metal biosorption (Vieira and Volesky, 2000). In the biological structure, microalgae have a high binding capacity of metal ions because the cell wall structure contains polysaccharides, proteins, and lipids (Davis *et al.*, 2003).

Diatoms are a type of microalgae that is often used as an environmental bioindicator because of its high diversity, sensitivity to changes in good water quality, and its fundamental role in the food chain (Stevenson and Pan, 1999). Microalgae *Chaetoceros calcitrans* is one of the representative microalgae from the Bacillariophyceae class and is the basis of the dominant seafood chain. *Chaetoceros calcitrans* is also a fucoxanthin-producing diatom which accounts for more than 10% of the estimated total production of carotenoids in nature (Foo *et al.*, 2015). The purpose of this study was to determine how the influence of Cd and Cu heavy metals on the growth of *Chaetoceros calcitrans* and how much the microalgae *Chaetoceros calcitrans* is able to absorb heavy metals from the environment.

Materials and Methods

Pure microalgae *Chaetoceros calcitrans* is obtained from a selective culture in the laboratory and has been acclimatized and is ensured to be free of pollutants. Microalgae used are 4 days old or in the exponential phase. Nutrients used during exposure are vitamins, silicates, and diatom fertilizers. Seawater used in the entire testing process is sterile seawater that has been sterilized using an autoclave for at least 15 minutes at 121 ^{o}C and a pressure of 1.5 Pa. All glassware was washed with 10% nitric acid and acetone and rinsed using distilled water.

Stock solutions of 1000 ppm Cd and Cu heavy metals were diluted to concentrations of 0.7, 1.3, and 1.9 ppm. Dilution was carried out using sterile seawater and each concentration was put into Erlenmeyer 500 mL. Each concentration was repeated three times and during the exposure, microalgae were given aeration and good lighting for photosynthesis. Microalgae density is calculated every 12 hours using a microscope and hemocytometer to determine the growth response of microalgae during exposure. Exposure was carried out for 96 hours and at the end of the exposure, a calculation of the concentration of heavy metals in water and microalgae was carried out to determine how much heavy metal absorbance could be carried out by *Chaetoceros calcitrans*.

Results and Discussion

The results of the calculation of the microalgae density of *Chaetoceros calcitrans* on exposure to Cd (Table 1) and Cu (Table 2) both showed a decrease with increasing exposure time. In Cd exposure, the decrease in microalgae occurred faster when compared to Cu exposure.

At the first stage of exposure, Cd was able to reduce the density of microalgae in large quantities. The most significant cell decline in Cd exposure occurred at the highest concentration of 1.9 ppm. Cd is a non-essential heavy metal or heavy metal whose function is not known biologically, so this metal is a foreign substance for the microalgae *Chaetoceros calcitrans*. This causes the *Chaetoceros calcitrans* to respond quickly to the presence of this metal in the environment. The influence given by Cd heavy metals increases with increasing concentration. The same results were also obtained by Thomas *et al.*, (1980) that heavy metals Cd can reduce the level of cell density of Chaetoceros sp. significantly.

The calculation results of *Chaetoceros calcitrans* on Cu exposure also showed the same thing as exposure to Cd, namely the density of the *Chaetoceros calcitrans* showed a trend that decreased with increasing exposure time and also increased concentration.

At the end of exposure, microalgae density of *Chaetoceros calcitrans* on Cu exposure showed a smaller amount of density than Cd. Heavy metals Cu are essential heavy metals, namely heavy metals that function as micronutrients in small concentrations. Cu is needed for the growth and metabolism of organisms in levels of 5-20 μg/g. However, if the levels exceed 20 μg/g then copper will change to be toxic to the organism (Bradl, 2005). Toncheva-Panova *et al.* (2006) said that although Cu is an essential metal, if the Cu content exceeds the tolerance limit of phytoplankton it will cause disruption of growth and photosynthesis, destroy photosynthetic organs and disrupt the permeability of phytoplankton plasma membranes.

Heavy metal contamination in water can be toxic to diatoms. Many studies have shown that high concentrations of heavy metals in the waters can reduce productivity, diversity, and changes in community diatom species (Sabater, 2000). In addition, the presence of heavy metals can also cause changes in cell morphology and a decrease in growth rates (Morin *et al.*, 2007). Based on this, the *Chaetoceros calcitrans* decreased density due to exposure to heavy metals Cd and Cu.

At the end of the exposure measurements of heavy metals in water and microalgae were measured using AAS (Atomic Absorption Spectrophotometry). The results of measuring absorbance levels can be seen in Table 3.

The level of decreasing levels of Cu heavy metals in water shows a more specific level of decline than heavy metals Cd. This is because Cu heavy metals are essential metals needed by *Chaetoceros calcitrans*, so heavy Cu metals are easier to absorb into *Chaetoceros calcitrans* cells. This causes heavy metal content in the water to decrease faster. Whereas heavy metals Cd is a type of metal that has a fat-soluble property. The cell wall of the *Chaetoceros calcitrans* consists of lipids and is impermeable, so heavy metals Cd are difficult to absorb into *Chaetoceros calcitrans* cells. Therefore the rate of reduction of Cd heavy metals in water is smaller than

Table 3. The results of measuring absorbance

Treatment (ppm)		Water (ppm)	Body (Mg/Kg)
Cd	0.7	0.61	6.68
	1.3	1.19	3.06
	1.9	1.78	2.68
Cu	0.7	0.51	9.30
	1.3	1.08	5.46
	1.9	1.69	4.16

Table 1. The results of the calculation of *Chaetoceros calcitrans* cell density at exposure to Cd

Time (hours)	Growth of Microalgae (x 10^4 Cell/mL)			
	O	X	Y	Z
0	89.00 ± 1.00	91.33 ± 2.08	89.83 ± 2.75	90.67 ± 2.52
12	87.67 ± 0.76	89.83 ± 2.25	87.83 ± 3.01	87.33 ± 4.16
24	91.00 ± 1.00	74.50 ± 1.50	71.50 ± 0.50	69.17 ± 1.04
36	87.67 ± 1.26	72.00 ± 1.32	67.83 ± 1.04	64.67 ± 3.82
48	97.17 ± 1.26	68.33 ± 1.04	61.33 ± 1.04	60.33 ± 2.75
60	98.83 ± 0.76	54.17 ± 1.26	51.83 ± 0.76	48.33 ± 1.15
72	104.33 ± 3.55	45.67 ± 2.36	39.83 ± 1.61	33.50 ± 1.80
84	112.17 ± 1.26	36.33 ± 2.75	30.50 ± 1.50	23.00 ± 2.78
96	120.83 ± 1.89	17.33 ± 6.33	15.33 ± 2.36	12.50 ± 2.00

Notes : (± : STDEV; O : Control; X : 0.7 ppm; Y : 1.3 ppm; Z : 1.9 ppm)

Table 2. The results of the calculation of *Chaetoceros calcitrans* cell density at exposure to Cu

Time (hours)	Growth of Microalgae (x 10^4 Cell/mL)			
	O±STDEV	X±STDEV	Y±STDEV	Z±STDEV
0	90.17 ± 1.76	94.00 ± 4.60	89.83 ± 4.24	92.00 ± 2.18
12	92.00 ± 0.35	97.17 ± 4.24	88.83 ± 4.95	85.17 ± 2.36
24	95.00 ± 1.06	100 ± 3.89	86.17 ± 2.47	73.67 ± 2.57
36	99.33 ± 1.77	87.33 ± 1.77	81.83 ± 4.24	61.67 ± 2.57
48	104.17 ± 0.35	75.67 ± 3.89	72.67 ± 3.18	52.17 ± 4.86
60	117.33 ± 6.36	57.67 ± 3.18	57.00 ± 1.06	44.67 ± 4.25
72	129.00 ± 3.18	37.00 ± 3.01	36.67 ± 2.47	34.17 ± 3.55
84	132.33 ± 1.77	24.67 ± 3.01	16.33 ± 3.55	14.17 ± 2.75
96	134.17 ± 1.06	11.83 ± 1.26	7.33 ± 2.02	2.67 ± 2.52

Notes : (± : STDEV; O : Control; X : 0.7 ppm; Y : 1.3 ppm; Z : 1.9 ppm) the decrease in Cu heavy metals.

According to Moreno-Garrido *et al.,* (2000), Chaetoceros sp. an example of a diatom that has a functional group found on the cell wall. The functional groups are carboxylic, hydroxyl, amino, sulfhydryl, sulfate, and phosphate groups. On the cell wall, there are proteins and polysaccharides that can bind metal ions. In addition, diatoms have a fairly high ability in binding heavy metals to cell walls (adsorption) and absorption of heavy metals into cells (absorption).

Based on the absorbance value of heavy metals, Bioconcentration Factors (BCF) values were calculated (Figure 1). The BCF value serves to determine the potential of *Chaetoceros calcitrans* in accumulating heavy metals.

The BCF value of heavy metals Cd and Cu decreases when the concentration of heavy metals in water increases. Based on the graph above shows that BCF values with exposure concentrations of Cd and Cu heavy metals have a negative correlation, where high BCF values in Cd and Cu are possessed by low concentration exposure. This shows that the potential of Chaetoceros calcitrans in accumulating heavy metals will be greater in low concentration exposures

Conclusion

Heavy metal pollution in the aquatic environment is a familiar thing. This will have a negative impact on the sustainability of the ecosystem. One technology that can be used to handle this problem is the bioremediation technique. This technique utilizes organisms, such as microalgae, bacteria, fungi, etc. *Chaetoceros calcitrans* is one of the microalgae that has the ability to accumulate heavy metals from the environment. But when exposed to heavy metals, microalgae density experienced a significant decrease in both Cd and Cu exposure. At the end of the exposure, *Chaetoceros calcitrans* absorbs Cu better than Cd, this is due to the different properties of the two metals. The potential of *Chaetoceros calcitrans* to accumulate heavy metals will increase if the concentration of heavy metals in the media gets lower.

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