BIOSORPTION OF Zn^{2+} AND Cd^{2+} IN A TWO-METAL SYSTEM BY *NANNOCHLOROPSIS SALINA*

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Keywords: *Nannochloropsis salina*, zinc, cadmium, two-metal system, Conwy, biosorption.

Although Zn^{2+} and Cd^{2+} ions in waters are pollutant with a large amount due to mining and industrial activities, information relating to the use of marine microalgae *Nannochloropsis salina* to absorb both types of metals is still very little. A marine microalga, *N. salina*, was used as biosorbent for the metal ions in water. Concentration of each ion (Zn^{2+} and Cd^{2+}) was 10 mg L^{-1} and the concentration ratio of Zn^{2+} to Cd^{2+} in the two-metal system (a combination of Zn^{2+} and Cd^{2+} in the culture medium) was 1:1. Experimental conditions were as follows: salinity = 25 ‰, temperature = 20 ºC, and pH = 7. Addition of each ion individually or the combination of ions was conducted when the optimum growth of microalgae was achieved (i.e. at the 8^{th} day after cultivation). The *N. salina* growth after the exposure of metal ions was observed daily and the metal ion concentration adsorbed was determined by using atomic absorption spectrophotometer. Results showed that *N. salina* can be used as biosorbent for Zn^{2+} and Cd^{2+} both in single metal ion and two-metal system. Biosorption of Zn^{2+} by *N. salina* was higher than that of Cd^{2+} in the single metal ion. The amount of Zn^{2+} adsorbed in the two-metal system was higher than the one in the single metal ion. However, the opposite trend was observed in the case of Cd^{2+} ion.

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Introduction

Heavy metals are main contaminants for environment due to the rapid development of industrialization to meet the desires of human life. Heavy metals enter to the human body through the process of digestion and respiration. Chromium, zinc, copper, and manganese are essential metals needed for a variety of physiological functions.[1] In the group of heavy metals, cadmium is recognized as one of the metals considered very toxic and has no role in biological functions. Naturally, the abundance of cadmium is small, but the activities of mining, industry and agriculture have increased the concentration of this metal in the environment.[2] However, zinc is one of the essential metals needed by the human body to function metalloenzyme, but in large quantities, these metals are potentially toxic[1] and inhibit the process of diversity in bacteria.[3] The recovery of heavy metals from industrial waste is important to the community as the effort to recycle and conservate the essential metals,[4] but metal remediation by physico-chemical methods is still expensive and not environmentally friendly. Biotechnological approaches are an attractive alternative and acceptable as a means of bioremediation in waters recently.[5] Natural materials are potential to be used for environmental cleanup of heavy metal contamination.

Microalgae, as aquatic microorganisms, are able to adsorb and accumulate heavy metals in their bodies. Previous research suggested that there was an interaction occurs between Zn ions with microalgae, where Zn showed significant toxic effects, followed by Ni and Pb.[6] Marine microalgae, *N. salina* was significantly able to adsorb Pb, Cd, and Zn ions,[7] and Zn^{2+} inhibited the growth of *N. salina* especially at the beginning time of contact. Microalgae can be used as biosorbent for Zn^{2+} ions with removal efficiency of 94.10% at a concentration of 10 mg L^{-1}.[8]

In a multi-metal ion system, antagonistic and synergic interaction may occur in relation to the increased growth of microalgae in marine waters.[9] In the three biosorbents system, adsorption of Pb ions decreased with the presence of Cd and Zn ions in the system, due to competition, both between Cd and Pb ions, as well as between Zn and Pb ions.[10] The presence of other metal ions slightly increases the total biosorption capacity of biomass.[11]

Based on the above description, research on biosorption of Zn^{2+} and Cd^{2+} ions in the two-metal system has been done using marine microalgae, *N. salina*, through the observation of its growth and determination of metal concentrations adsorbed after the optimum growth was achieved. The study was expected to be one of the alternative solutions for the heavy metals pollution in waters.

Materials and Methods

Materials

Material used in this research was sterile sea water, a unialgal strain of *N. Salina*, and Conwy cultivated medium with the composition shown in Table 1, obtained from the Research Institute for Coastal Aquaculture (Balitbang BAP) Maros Indonesia; HNO_3 solution (p.a grade); double distilled water; aluminium foil. The stock solution of Zn^{2+}

and Cd^{2+} ions were prepared by dissolving given amounts of $Zn(NO_3)_2$ and $CdSO_4·8H_2O$, respectively, in double distilled water. The solutions of different concentrations used in various experiments were obtained by dilution of the stock solutions.

Table 1. Composition of Medium Conwy[12]

Materials	Amount, g
Stock A	
$FeCl_2·6H_2O$	1.3
$MnCl_2·4\,H_2O$	0.36
H_3BO_3	33.6
EDTA (Na-salt)	45
$NaH_2PO_4·2H_2O$	20
$NaNO_3$	100
Double distilled water	1 L
Stock B	
$ZnCl_2$	2.1
$CoCl_2·6H_2O$	2
$(NH_4)_6MoO_{24}·4H_2O$	0.9
$CuSO_4·5H_2O$	2
Double distilled water	100 mL
Stock C	
Vitamin B_{12}	10
Vitamin B_1	200
Double distilled water	100 L
Stock D	
$Na_2SiO_3·5H_2O$	4.00
Double distilled water	100 mL

Equipment used is Marienfeld LOT-No 4551 haemocytometer, hand counter, Amara aerator, All American No. 1925X autoclave, atomic absorption spectrophotometer (AAS), Buck Scientific Model 205 VGP, Phase Contrast Microscope Olympus IX71 with magnification of 40 times, Hettich Micro 22R stirrer, Memmert oven, and Millipore cellulose nitrate membrane filter (0.45 μm).

Methods

Optimum growth of *N. salina*

Sterile sea water with salinity of 25 ‰ was put into 3 Erlenmeyer flasks. One mL of medium (Conwy) and a unialgal strain *N. salina* with initial population of approximately 26 x 104 cell mL^{-1} were added into each Erlenmeyer flask.8 Each mixture was added with the sterile sea water until the volume of solution become 500 mL. The solution was stirred, connected to the aerator and covered with aluminium foil. The growth of *N. salina* was observed with a haemocytometer everyday to obtain the optimum growth of *N. salina*.

Exposure of metal ions into the *N. salina* culture

When the optimum growth was achieved, each metal ion (Zn^{2+} and Cd^{2+}) with a concentration of 10 mg L^{-1} and a mixture of the ions in two-metal system with the ratio of 1:1 were added into the 3 Erlenmeyer flasks containing cultured marine microalgae of *N. salina*, respectively. Further growth of *N. salina* was observed everyday. After that, separation of solution from the residual marine microalgae was conducted by using a stirer Hettich Micro 22R for 15 min at 4 °C and 6000 rpm. Furthermore, 2 drops of 2 M HNO_3 was added into each Erlenmeyer flask to prevent precipitation of metal ions. The concentration of each ion in the solution was measured by the AAS. The concentration of metal ion adsorbed (C_a) can be calculated by equation (1) and the removal efficiency (R_e) was determined by equation (2). C_a is the difference between initial metal ion concentration (C_i) and metal ion concentration in the solution after biosorption (C_f).

$$C_a = C_i - C_f \qquad (1)$$

$$R_e = \frac{C_a}{C_i}100 \qquad (2)$$

Result and Discussions

Optimum growth of *N. salina*

The optimum growth of marine microalgae, *N. salina*, was achieved on the 8th day with a population number of 105 x 104 cells mL^{-1}. Figure 1 shows the relationship between population numbers of *N. salina* against days of growth. Naturally, the growth of *N. salina* will decrease after the optimum growth is achieved. This is because the amount of nutrients in the medium decreases with increasing population number of *N. salina*. The decrease in population is also supported by the accumulation of organic compounds from the dried biomass settling at the bottom of the media. Biomass serves as a new competitor to the *N. salina* cells that are still alive for dissolved oxygen and nutrients in the growth medium.[9]

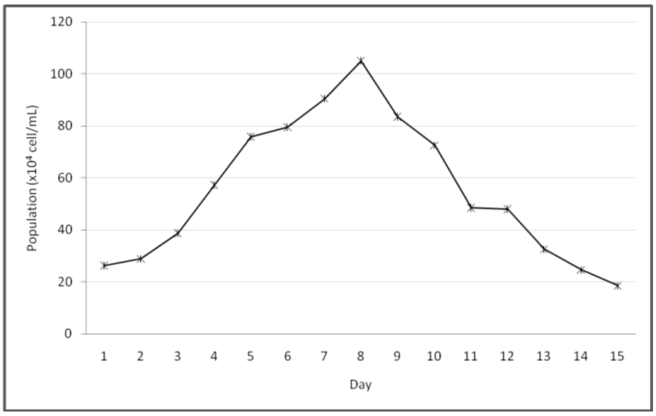

Figure 1. The population number of marine microalgae, *N. Salina*, as a function of the growth time.

Natural water is often contaminated by dissolved organic materials that could potentially form complexes with metal ions. Complex organic materials and metals that form can alter the toxicity of heavy metals. The composition of organic matter depends on the environment, it can contain various ligands, such as, $-SO_4$, $-PO_4$, $-OH$, $-COOH$, $-SH$, and $-NH_2$.[2]

Exposure of Zn^{2+} and Cd^{2+} ions individually and both ions in the two-metal system causes the drastic decrease in the *N. salina* growth compare to the control (Figure 2). This is caused by the binding of metal ions on the surface of microalgae cells containing a layer of fat, because the body surface of microalgae is covered by cell membranes so that the potency for interaction with metal ions in water is high.[13] The high surface area of cells containing various functional groups, such as the N-terminal of the $–NH_2$, C-terminal from the group –COO-, S-terminal of the –SH side chain functional groups of amino acid residues, causes the potency of active sides to bind metal ions.[14]

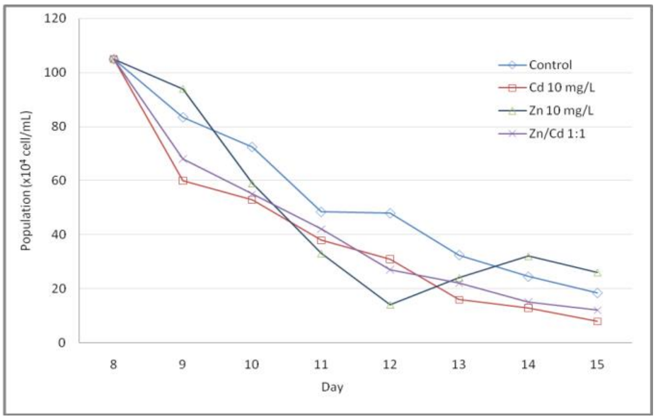

Figure 2. The growth of marine microalgae *N. salina* after exposure: Zn^{2+} 10 mg $L^{–1}$; Cd^{2+} 10 mg $L^{–1}$; and combination of Zn^{2+}/Cd^{2+} 1:1.

The presence of Cd^{2+} ions both in the single metal ion and two-metal system showed a mixture of metal ions have the lower growth trend compared to the control, but Zn^{2+} showed an increase in the population at the 13th day and the 14th day after cultivation. This is because Zn^{2+} is one of essential metals that give contribution to the growth of *N. salina* after equilibrium is achieved. Interaction of Zn^{2+} ion with functional groups of –OH and –COO- on *N. salina* will form more stable complexes than Cd^{2+} ion. According to Lesage et al.,[15] metal concentration at equilibrium (2 h) in the two-metal system is lower than the initial concentration, due to competition on the active site of biosorbent. In the previous study, exposure of Zn^{2+} ion showed dominant adsorption occurred at the early contact between the metal ions with *N. salina*.[8] This is because the adsorption of metal ions by the active group, takes place at the cell surface followed by a slow transport step of the ion into the cell membrane and then into the cytoplasm.[14] The particle size was not a significant effect on the total sorption capacity.[16]

In addition, the metal ions in solution undergo equilibrium with ligands produced and excreted by algae. [17] The antagonistic effects between cadmium and essential metals could result in an inhibition of the different enzyme activities. The toxic effects of cadmium will increase with the deficiency of essential metals.[2] Accumulation of some metals on algae occurs through active biological intracellular transport. Toxic heavy metal ions to be sequestered from the cytoplasm of cells through three possible ways, namely: by chelating intracellular biological polymers; deposition of heavy metals on the surface of the cell wall, or by surface adsorption of metal ion binding by chemical functional groups in the cell wall.[18]

Removal efficiency of metal ions

According to equation (1), the concentration of metal ions adsorbed and removal efficiency for each metal ion individually and in the two-metal system is presented in Table 2. Table 2 shows that the removal efficiency of individual Zn^{2+} ion is higher than that of individual Cd^{2+}, while in the two-metal system, the removal efficiency of Zn^{2+} ion is slightly higher than that in the single metal ion. In contrast, Cd^{2+} shows the different result, in which the removal efficiency of Cd^{2+} ion in the two-metal system is considerably lower than that in the single metal ion.

Table 2. Removal efficiency of Zn^{2+} and Cd^{2+} adsorbed individually and in the two-metal system after biosorption by *N. salina*

Initial conc., C_i (mg $L^{–1}$)	**Conc. after biosorption (mg $L^{–1}$)**		**Metal ions**	**Removal efficiency, R_e (%)**
	Solution, C_f	**Adsorbed, C_a**		
Zn^{2+}: 10	0.42	9.58	Zn^{2+}	95.77
Cd^{2+}: 10	0.71	9.29	Cd^{2+}	92.92
Zn^{2+}/Cd^{2+}	0.11	9.89	Zn^{2+}	98.89
1:1	1.31	8.69	Cd^{2+}	86.92

The high concentration of cadmium in the medium results in the decrease of the growth of microalgae and physiological changes associated with the *N. salina* growth. Inhibition of the microalgae growth is caused by inhibition of more specific metabolic processes, particularly those having metalloenzyme or enzymes with sulphydryl functional group.[2]

Conclusion

The optimum growth of *N. salina* with a population of 105 x 104 cells $mL^{–1}$ was obtained at the 8th day after cultivation. Marine microlalga of *N. salina* can be used as biosorbent for Zn^{2+} and Cd^{2+} both in single metal ion and two-metal system. Biosorption of Zn^{2+} by *N. salina* was higher than that of Cd^{2+} in the single metal ion. The amount of Zn^{2+} adsorbed in the two-metal system was higher than the one in the single metal ion. However, the amount of Cd^{2+} adsorbed in the two-metal system was lower than that in the single metal ion.

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Received: 19.12.2012.
Accepted: 13.01.2013.