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BIOREMEDIATION OF LEAD [Pb II] CONTAMINATED SEA WATER BY MARINE DIATOM *SKELETONEMA COSTATUM*

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ABSTRACT

Water contaminations is frequently occurred by heavy metals as a consequence of human activities become a key concern in environmental and health problem. Sea water pollution by heavy metals can contaminate fish pond. Therefore, it is necessary to remove heavy metals from seawater before entering the pond. Lead (Pb) is heavy metal. The aim of this study was to know the ability of growth and bioremediation of marine diatom *Skeletonema costatum* in lead [Pb II] contaminated sea water. Experiment has been designed 3X4, i.e. concentrations of the cell inoculation (5 000 cells/mL; 10 000 cells/mL; and 15 000 cells/mL) and Pb II (0; 0.5; 1; and 2 ppm), and three times replication. The Data analyzed by multivariate ANOVA and then Duncan test (α = 5%). During five-day exposure time, every day, cell density has been observed by microscope (400 x), while filtrate has been analyzed by Atomic Absorbation spectroscopy (AAS).

The results have shown that: (1) *S. costatum* highest growth was at 15000 cells/mL at 2 ppm in the second day and still able to grow up to the fifth day at 2 ppm; and (2) Bioremediation of Pb II by *S. costatum* highest was on the first day and then progressively decreasing. The adsorption efficiency of Pb II *S. costatum* was highest at 80.50 ± 0.50 % (at inoculation of 5 000 cells/mL in 2 ppm on the first day).

Keywords: Adsorption efficiency; Bioremediation; Growth, Lead; *Skeletonema costatum*

1. INTRODUCTION

Heavy metal pollution in the environment is an important issue because of the impact it may cause interference for living things, including health problems in humans. Presence of heavy metals in the water can come from various sources, one of which is industrial waste. Lead (Pb) including heavy metal that is naturally present in the earth's crust. Industries that use lead, the battery industry, metal plating, and ceramic industry. These metals are often found in the waters. Heavy metals are contaminants that have harmful effects because it is not biodegradable and can be stable (Palar, 2008). According to Ahamed & Siddiqui (2007), lead can cause nerve damage, the risk of cancer, anemia, birth is not perfect, delayed growth, and others. Garcia-Leston et al. (2010) states, that lead causes gene damage. Similarly, the US Agency for Research on Cancer classifies lead as a possible human carcinogen.

Sumiyani et al. (2005) reported that, in Coastal Kenjeran Surabaya been contaminated by heavy metals lead (Pb II). It is characterized by the presence of biota Anadara

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antiquate containing lead about 29,636 ± 2.096 mg/g dry weight. The results of several studies, the content of Pb in Water Kenjeran from 0.3065 to 0.5182 mg/L (Yulita, 2007), and amounted to 1.2246 to 2.0713 mg/L (Dyah, 2007), while according to Woro (2011) of 0.22 mg/Kg. The concentration is higher than the threshold value set by the Minister of Environment (KEMENLH) No. 51 of 2004, which is the quality standard for marine life for lead of 0.008 mg/L.

This pollution also occurs in some waters in Indonesia. This was reported by Fitriyah (2007), that the metal content of lead in Coastal Lekok of 1,308 ppm. Arsad, et al. (2012) also reported that the sea water in coastal Port Taipa - North Palu contains lead of 0.703 mg / L-0.919 mg / L.

Organisms in marine waters, particularly the fish is an export commodity in Indonesia. Exports of tuna in 2014 amounted to 101 111 tons and the shrimp 148519.4 tons (Badan Pusat Statistik, 2016). Meanwhile, the content of metallic lead in fish and shrimp living in the sea or in ponds need to be considered. Therefore, it affects human health as well as for export. It is therefore necessary techniques to process waste or polluted sea water before use for the pond.

One such technique is remediation. These techniques transform the heavy metal into an harmless element (Romimohtarto & Juwana, 2001). Bioremediation is one of remediation technique that uses elements of biology as remediator. This technique does not cause environmental damage and death on the biota in polluted waters (Jamil, 2001). From several studies it is known that algae capable of being bioremediator. Red algae, *Porphyra leucosticta*, able to remediate Pb (II) with an efficiency of 90% (Ye, et al., 2015). Marine diatom, *Skeletonema sp*. able to remediate Pb with an efficiency of 24.7% (Wisudyawati, 2014). *Skeletonema sp*. including microalgae are aquatic organisms that have the molecular mechanisms to distinguish non-essential heavy metals from heavy metals essential to growth.

Skeletonema sp. able to absorb heavy metals in two ways: absorption and adsorption. Adsorption occurs because *Skeletonema sp.* have cell walls. The cell walls *Skeletonema sp.* consists of cellulose. Cellulose in the cell walls have functional groups such as hydroxyl which can bind with heavy metals (Knauer & Sigg, 1997; Gupta et al., 2000) or replace Zn contained in the cell wall (Fauziah, 2011). Absorption was undertaken by *S. costatum* because its has produced fitokelatin. Fitokelatin bond with metal has observed in vakuola of *Skeletonema sp* (Nassiri et al., 1997). *Skeletonema sp*. like other microalgae that also produces fitokelatin, namely peptides metalotionin class III 3 (MtIII) to detoxify heavy metals (Perales-Vela et al., 2006). Biosynthesis MtIII can be induced by the presence of heavy metals such as Cd^{2+}, Ag^{2+}, Bi^{3+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, $^{Hg2+}$ and Au^{2+} both in vivo and in vitro (Shaw, 1989).

Based on this it needs to know the growth and the ability of bioremediation marine diatom *Skeletonema costatum* in lead-contaminated sea water ex situ. So that sea water before entering into the fish pond free of heavy metals lead. Thus, the *Skeletonema costatum* can be given to a sea water will be used for fish ponds.

2. METHODOLOGY/ EXPERIMENTAL

The material used in the study was: (1) isolate microalgae *Skeletonema costatum* was obtained from the BBAP (Brackish Water Aquaculture Centres) Situbondo. *S. Costatum* has been identified using the book "Examination of Diatom: Found on The Surface of The Sea of Java" (Cleve, 1873); (2) XMU (i.e. KNO 3, Na2HPO4.12H2O, Na2SiO3,

and FeSO47H2O) as a culture medium (Soedarti et al., 2009); $Pb(NO_3)_2$, distilled water, sea water, sea soil (as a medium of treatment).
The instrumentation in study were Ponar grab, aerator 6 volts dc, ice box, hoses, light microscopy, culture bottles of 350 mL, glass beaker 500 mL, measuring cups 250 mL, tube Erlenmeyer 500 mL and 100 mL, pH indicator paper, thermometer, Hand refractometer, Atomic Absorbation spectroscopy (AAS), Bunsen, laminar air flow, electric stove, shaker, analytical balance, measuring pipette, tip, micropipette, vein, autoclave, 40 watt fluorescent lamp, and a hand counter.

2.1. Preparation of media

2.1.1. Sea soil supernatan

Sea soil 1 kg mixed with distilled water 1000 mL, then stirred. After, it boiled approximately 60 minutes.After two days, it filtered by double filter paper. The supernatant obtained was stored in the refrigerator (Soedarti et al., 2005).

2.1.2. XMU media

Sea soil supernatant 15 mL mixed with salt (KNO 3 400 mg, Na2HPO4.12H2O 40 mg, 20 mg Na2SiO3, and FeSO47H2O 14 mg) and 1000 mL of pure sea water, then stirred for approximately 10 minutes (the pH was measured from 7.8 to 8.5). Then, it sterilized by autoclave at a temperature of 121^0C for 15 minutes (Soedarti et al., 2009).

2.1.3. Lead stock solution

Pb II concentration used in this study was 0.5; 1; and 2 ppm. Dilution concentration of 1000 ppm stock solution of formula (1).

$$V_1 N_1 = V_2 N_2 \quad (1)$$

Description: V_1 = volume of the stock has been known (mL); N_1 = the stock concentration has been known (ppm); V_2 = volume of the stock is unknown (mL); and N_2 = the stock concentration is unknown (ppm).

2.2. *S. costatum* culture

S. costatum was entered into XMU media 250 mL. *S. costatum* in order to grow well, the culture was placed under the light of 3199 lux (fluorescent lamp 40 watt) and in a temperature of 25^oC (Soedarti et al., 2005).

2.3. Stage treatment

This study uses a 3 X 4 factorial design that is 4 kinds of varying concentrations of lead and three variations cell inoculation. The treatment consisted of 12 treatments with each treatment there were 3 repetitions. Each treatment was inoculated with different numbers of cells at 5×10^3 (S1), 10×10^3 (S2) and 15×10^3 (S3) cells/mL *S. costatum* in a total volume of 250 mL each treatment were exposed to heavy metals lead at concentrations of 0 (C); 0.5 ppm (P1); 1 ppm (P2); and 2 ppm (P3). The treatment consists of several groups as follows.

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Table 1 Experimental design (factorial 3X4)

No.	Concentration of Lead (Pb II) / Number of cell inoculation	C	P1	P2	P3
1.	S1	CS1	P1S1	P2S1	P3S1
2.	S2	CS2	P1S2	P2S2	P3S1
3.	S3	CS3	P1S3	P2S3	P3S1

Description:
C : 0 ppm, P1 : 0,5 ppm, P2 : 1 ppm, P3 : 2 ppm
S1: 5000 cells/mL, S2 : 10 000 cells/mL, S3 : 15 000 cells/mL

2.4. Measurement of the physical condition of the culture medium

Measurements were made every 24 hours during the study (the first day to the fifth day), including room temperature (^oC) by using a thermometer, pH using pH indicator paper, light intensity using a lux meter and salinity using a hand refractometer.

2.5. Data of growth and efficiency of adsorption

Observations growth of *Skeletonema costatum* conducted directly by counting the number of cells per mL using a haemocytometer under a microscope (400 X). Sampling was done from the first day to the fifth ..Observations and data collection capabilities lead bioremediation by *S. costatum* .Each sample on day 2, 3, 4, and 5 are drawn as many as 10 specimens *S. costatum* mL of growth media by centrifugation at a speed of 5000 rpm for 5 minutes, in order to obtain filtrate and supernatant. The filtrate is filtered with filter paper and dried in an oven. The dried filtrate was ready for destruction. The filtrate that has been destroyed, then the levels of lead were analyzed using AAS with a wavelength of heavy metals (283,3 nm) (Wisudyawati, 2014). The supernatant was also analyzed the heavy metal content in order to know the concentration of heavy metals remaining in the media (Hala et al., 2012).
The calculation of the absorbed heavy metal concentrations using Langmuir method in (Hala et al., 2012).which calculates the efficiency of entrapment by the following formula (2) and (3):

$$C_s = C_0 - C_f \quad (2)$$

$$E_p = \frac{C_s}{C_0} \times 100\% \quad (3)$$

Description: E_p = adsorption efficiency (%); C_s = Concentration of metal adsorbed (mg/L); C_0 = Concentration of metal prior to contact (mg/L); and C_f= Concentration of metal after contact (filtrate) (mg/L).

2.6. Data analysis

Data obtained in the form *S. costatum* cell density and the concentration of lead that is absorbed by the cells *S. costatum*. The data has tested F test - multivariate on $\alpha = 0.05$ to determine the different growth and uptake of heavy metals by *S. costatum* the combination treatment of heavy metal concentrations and the number of cell inoculation. If there is a difference, then continued with Duncan test at $\alpha = 0.05$.

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3. RESULTS

3.1. Growth of *Skeletonema costatum*

This study, microalgae *Skeletonema costatum* proved able to grow on media treatment given lead until the fifth day (Fig. 1).

Figure 1 has shown that the optimum growth of *S. costatum* in the second and third day. Optimum growth on the third day of inoculation found in 5 000 cells/mL and 10 0000 cells/mL for all treatments, except the control treatment on 10 000 cells/mL inoculation (the second day). Optimum growth medium on the second day of inoculation was found at 15 000 cells/mL.

In the treatment (P1, P2 and P3), the highest growth was the inoculation of 15 000 cells/mL (S3) on the 2nd day, amounted to 13.30 x 10^4 cells/mL in media which added 0.1 ppm lead (P2). This amount was more than inoculating 5000 cells/mL (S1) and 10 000 cells/mL (S2), that was amounted to 12.07 x 10^4 cells/mL (in the media of lead 1 ppm (P2) and on the third day) and 10.70 x 10^4 cells/mL (at media of lead 0.5 ppm (P1) and on the third day).

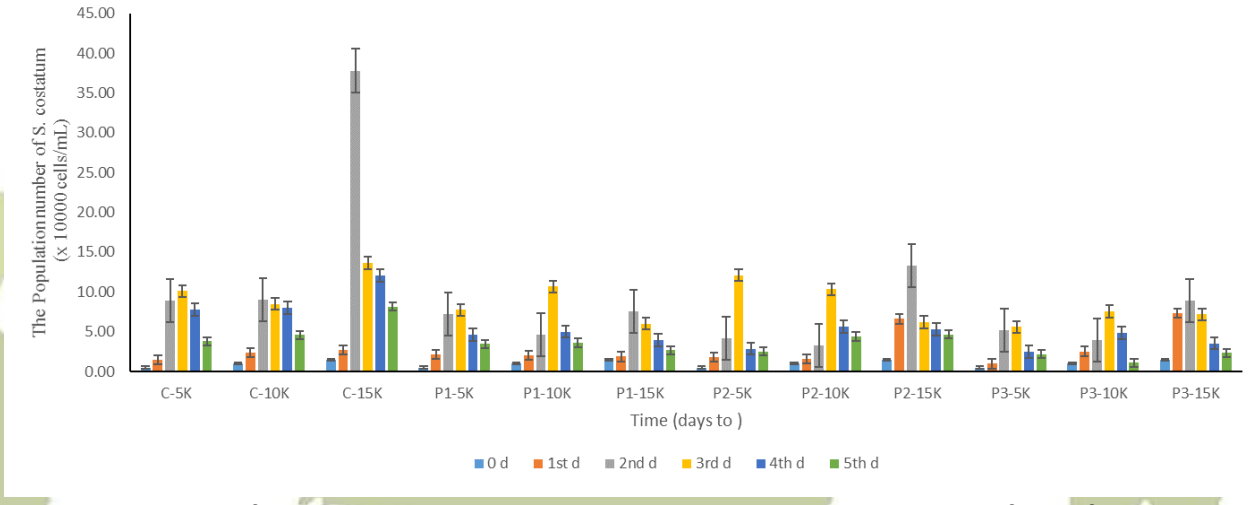

Figure 1 Charts of *Skeletonema costatum* growth in various concentrations of lead for five

Days. C-5K: Control, inoculation of 5 000 cells/mL; C-10K: Control, inoculation of 10 000 cells/mL; C-15K: Control, inoculation of 15 000 cells/mL; P1-5K: lead 0.5 ppm, inoculation of 5 000 cells/mL; P1-10K: lead 0.5 ppm, inoculation of 10 000 cells/mL; P1-15K : lead 0.5 ppm, inoculation of 15 000 cells/mL; P2-5K: lead 1 ppm, inoculation of 5 000 cells/mL; P2-10K: lead 1 ppm, inoculation of 10 000 cells/mL; P2-15K : lead 1 ppm, inoculation of 15 000 cells/mL; P3-5K: lead 2 ppm, inoculation of 5 000 cells/mL; P3-10K : lead 2 ppm, inoculation of 10 000 cells/mL; P3-15K : lead 2 ppm, inoculation of 15 000 cells/mL

The result of statistical test has shown that inoculation of 15 000 cells/mL in treatment of P2 (the concentration of lead (Pb II) 1 ppm) on the second day were able to live as well as inoculation of 15 000 cells/mL in the control treatment on the fourth day.

3.2. Adsorption of lead

Skeletonema costatum bioremediation capabilities could be seen from the value of adsorption efficiency (%) (Table 2). Table 2 has shown that inoculation of *S. costatum* 5

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x 10^4 cells/mL in media of lead 2 ppm (S1P3) on first day had the most highly rated efficiency of adsorption (80,5 ± 0,5 %).

Table 2 has shown that the efficiency of adsorption of lead on the first day (after one day or after 24 hours) was the highest number. Adsorption ability of lead of *S. costatum* at treatment S1P3 (inoculation of 5000 cells/mL in the lead 2 ppm) on the fifth day wasn't different from treatment S3P3 (inoculation of 15 000 cells/mL in the lead 2 ppm).

Table 2 Average of Lead (Pb II) Adsorption Efficiency in *Skeletonema costatum*

Treatment of cells number	Days to	Adsorption efficiency (%)			
		C (Lead 0 ppm)	P1 (Lead 0,5 ppm)	P2 (Lead 1 ppm)	P3 (Lead 2 ppm)
S1 (5 000 cells mL)	1st	0 ± 0.00^{a}	71.33 ± 4.16^{r}	76.67 ± 1.15^{s}	80.50 ± 0.50^{t}
	2nd	0 ± 0.00^{a}	62.00 ± 2.00^{op}	61.00 ± 1.00^{op}	71.50 ± 0.50^{r}
	3rd	0 ± 0.00^{a}	50.00 ± 2.00^{l}	55.33 ± 2.52^{m}	65.00 ± 1.00^{pq}
	4th	0 ± 0.00^{a}	40.67 ± 1.15^{jk}	40.00 ± 2.00^{jk}	62.00 ± 1.00^{op}
	5th	0 ± 0.00^{a}	37.33 ± 2.31^{ij}	37.67 ± 2.52^{ij}	55.33 ± 4.91^{m}
S2 (10 000 cells/mL)	1st	0 ± 0.00^{a}	60.67 ± 1.15^{m}	56.33 ± 1.52^{mn}	68.17 ± 1.26^{qr}
	2nd	0 ± 0.00^{a}	49.33 ± 1.15^{l}	48.00 ± 2.00^{l}	62.00 ± 0.50^{op}
	3rd	0 ± 0.00^{a}	40.00 ± 2.00^{jk}	40.33 ± 0.57^{jk}	58.33 ± 0.76^{mno}
	4th	0 ± 0.00^{a}	29.33 ± 3.05^{efg}	34.33 ± 2.08^{hi}	55.83 ± 0.76^{m}
	5th	0 ± 0.00^{a}	26.67 ± 2.31^{def}	30.67 ± 0.58^{fgh}	50.33 ± 2.56^{l}
S3 (15 000 cells/mL)	1st	0 ± 0.00^{a}	48.00 ± 5.29^{l}	34.00 ± 2.64^{hi}	60.00 ± 1.00^{op}
	2nd	0 ± 0.00^{a}	42.67 ± 5.03^{k}	29.00 ± 1.00^{efg}	55.33 ± 0.58^{m}
	3rd	0 ± 0.00^{a}	32.00 ± 2.00^{gh}	25.67 ± 0.58^{de}	51.17 ± 1.61^{l}
	4th	0 ± 0.00^{a}	24.00 ± 4.00^{cd}	21.00 ± 1.00^{bc}	50.17 ± 2.84^{l}
	5th	0 ± 0.00^{a}	20.00 ± 4.00^{b}	19.00 ± 1.73^{b}	47.67 ± 2.56^{l}

Information: The number followed by the same letter are not significantly different

4. DISCUSSION

The adsorbed Pb II amount has shown the ability of *Skeletonema costatum* doing bioremediation (Table 2). This is due to the cell wall of *S. costatum* have functional groups such as hydroxyl which can bind heavy metals (Knauer & Sigg, 1997; Gupta et al, 2000). The functional groups can bind metal ions due to the reaction between the negative charge functional groups contained within the cell wall with the positive charge of the metal ion Pb.

Naturally, the bioremoval of metal ions consists of two mechanisms involving the passive uptake and active uptake. The passive uptake was known as biosorption. This process occurs when the heavy metal ions bound to the cell wall in two ways. The first, exchange of monovalent and divalent ions, such as Na, Mg, and Ca, on the cell wall with heavy metal ions; and the second, Complex formation between heavy metal ions with functional groups such as carbonyl, amino, thiol, hydroxyl, phosphate and hydroxy-carboxyl located on the cell wall. The bonding process of heavy metal ions on the surface of these cells can occur in dead cells and living cells (Suhendrayatna, 2001).

The S. costatum growth was lower than control, although S. costatum was still able to live in a medium containing lead (Fig. 1). This was because the Pb concentrations were not yet toxic. Besides, *S. costatum* is one of the phytoplankton that high-protein, ie 37% (Erlina et al., 2004). This protein is composed of a carboxyl group (-COOH) which is able to bind well with heavy metal ions (Sembiring et al., 2009). Thus *S. costatum* can adsorb heavy metal ions. So, if more and more *S. costatum* have a carboxyl group, the

adsorption increases. Thus, *S. costatum* can be given to a sea water will be used for fish ponds.

5. CONCLUSION

Based on the research results, we have conclude that (1) *Skeletonema costatum* could grow in lead exposure until 2 ppm, and (2) the ability bioremediation *S. costatum* in lead [Pb II] contaminated sea water were until 2 ppm and the adsorption efficiency of lead was highest at 80.50 ± 0.50 % (at inoculation of 5 000 cells/mL in 2 ppm of lead in the first day).

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