Conference Paper

Bioremediation of Mercury (II) Contaminated Seawater Using The Diatom *Skeletonema costatum*

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Abstract

The mercury contaminated seawater can pollute fish pond. Bioremediation is an effective process for the removal and recovery of mercury (II) from seawater using organism as an agent of biological degradation. The aim of this study was to know the optimum contact time and concentrations of the *Skeletonema costatum* cell inoculation on the bioremediation in mercury (II) contaminated seawater. This study has used the concentrations of the cell inoculation (5000; 10000; and 15000 cells/mL), the mercury (II) (0; 0.5; 1; and 2 mg/L), the contact time (24, 48, 72, 96, and 120 hours), and its replicated five times. The maximum bioremediation capacity of mercury (II) was 2 mg/L at 15 000 cells/mL and contact time 96 hours, with bioremediation efficiency 86.83%. Diatom *Skeletonema costatum* was efficient at removing 2 mg/L mercury (II) 79.5% for 5 000 cells/L at 72 hours, 83.3% for 10 000 cells/L at 72 hours and 85% for 15 000 cells/L at 72 hours. The optimum contact time and concentrations of the *Skeletonema costatum* cell inoculation on the bioremediation in mercury (II) contaminated seawater for 2 mg/L, i.e. 5 000 cells/L for 72 hours (79.5%).

Keywords: *bioremediation; mercury (II); seawater; Skeletonema costatum*.

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1. Introduction

Heavy metal pollution in the waters of Indonesia increased with increasing industrialization. One of the heavy metals is mercury. Mercury is classified into hazardous and toxic materials as a form of a single element (metal) or in the form of compounds. According to Waldichuk, 1974 [1], Hg^{2+} is the most dangerous. Mercury can block the action of the enzyme and deform the membrane in the cell wall or cell membrane because mercury can form a strong bond with the sulfur cluster. This group of enzymes present in the membrane and cell wall or cell membrane. The most toxic mercury compound is methyl mercury for humans [2]. Seawater pollution by

Hg in several regions in Indonesia have exceeded the quality standard.

One of microalga whose existence is abundant in nature, i.e., *Skeletonema costatum*. Microalgae belong to the group of diatoms, which are unicellular microalgae. *Skeletonema costatum* known to have the ability in bioremediation of heavy metals that are able to accumulate heavy metals to produce metallothionein to bind ions into stable elements [3]. Other results indicate that green algae accumulate *T. suecica* Cd^{2+} in vacuoles and cell walls. Besides heavy metal accumulation also occurs in chloroplasts and mitochondria. Furthermore, heavy metal complexes and metallothionein class III (MtIII) can be transported to other organelles (not organelles that accumulate heavy metals) or settle into organelles where the accumulation of heavy metals [4].

According to Leonard [5], *Skeletonema sp.* able to grow on the environmental conditions that contain heavy metals mercury (Hg) at a concentration of 0,06 ppm as nutrients.

There are several methods used to treat heavy metal waste. Bioremediation is a process that uses an organism as an agent of biological degradation [6]. According to Priadie [6], bioremediation is the result of the development of environmental biotechnology field by using biological processes in controlling pollution and quite attractive and cost-effective. Basically, there are two processes in bioremediation technologies that have been developed, namely the process of bioremediation in-situ and ex-situ.

The process of heavy metal bioremediation is generally composed of two mechanisms that involve making process is active uptake and passive uptake [7] Passive uptake is a process that occurs when heavy metal ions bind to the cell wall in two different ways, ways the first is the exchange of monovalent and divalent ions such as Na, Mg and Ca in the cell walls are replaced by ions of heavy metals; The second way is the formation of a complex between the ions of heavy metals with functional groups such as carbonyl, amino, thiol, hydroxy, phosphate, and carboxyl hydroxy located on the cell wall; This process is alternating and fast; While active uptake can occur in various types of living cells; This mechanism simultaneously occurs in line with the consumption of the metal ion to the growth of microorganisms and intracellular accumulation of the metal ion; The heavy metals can also be deposited in the metabolism and excretion process on the second level; Factors that influence the uptake passive process, i.e., pH and the presence of other ions. The passive uptake process is influenced by pH and the presence of other ions; While, the active uptake process is influenced by pH, temperature, the ionic bonds strength, and light [8].

Based on the above it was necessary to know the optimum contact time and inoculation concentration of cell A in marine bioremediation contaminated with mercury (II).

2. Material and Methods

2.1. Material

The materials used in the study is to isolate microalgae *Skeletonema costatum* from BBAP (Brackish Water Aquaculture Centres) Situbondo, East Java. Then, its have been identified using the book [9]. XMU media (KNO3, Na2HPO4.12H2O, Na2SiO3, and FeSO4.7H2O) is a culture media for *Skeletonema costatum* [10], metallic mercury used is HgCl.H2O, distilled water, artificial seawater, and sea-soil.

2.2. Instrumentation

Light microscopy (400x), 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 Absorption 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.

2.3. Procedure

2.3.1. Preparation: sea-soil supernatant

Sea soil 1 Kg mixed with distilled water 1000 mL, then stirred. After, it was boiled approximately 60 minutes. After two days, it was filtered by double filter paper. The supernatant obtained was stored in the refrigerator [10].

2.3.2. Preparation: XMU media

Sea soil supernatant 15 mL mixed with salt (KNO_3 400 mg, Na2HPO4.12H2O 40 mg, 20 mg Na2SiO3, and FeSO4.7H2O 14 mg) and 1000 mL of artificial seawater, then stirred for approximately 10 minutes (the pH was measured from 7.8 to 8.5). Then, it was sterilized by autoclave at a temperature of 121°C for 15 minutes [10 and 11].

2.3.3. *S. costatum* culture

S. costatum was entered into XMU media 250 mL. The culture was placed under the light of 3199 lux (fluorescent lamp 40 watts) and at a temperature of 25°C [12].

2.4. Experimental design

This study uses a 3 × 3 × 5 factorial design. That is three variations of *S. costatum* cells inoculation {5 000 (S1), 10 000 (S2) and 15 000 (S3) cells/mL}, four variations of mercury concentrations {0.5 ppm (P1); 1 ppm (P2); and 2 ppm (P3)} and five variation of time contacts {24 (T1), 48 (T2), 72 (T3), 96 (T4), 120 (T5) hours}. The treatment was consisted 60 treatments by five times repetition.

2.5. Observation and data collection

10 mL *S. costatum* was centrifuged at a speed of 5000 rpm for 5 minutes, in order to obtain filtrate and supernatant. The filtrate was filtered through filter paper and dried in the oven. Then, it was destructed. After that, the filtrate and the supernatant were analyzed using AAS with a wavelength of heavy metals.

The calculation of the absorbed heavy metal concentrations using Langmuir method in [13], which calculates the efficiency of entrapment by the following formula:

$$Ep = C_s \times 100\%$$

$$Cs = C0 - Cf$$

Description: Ep = adsorption efficiency (%); Cs = Concentration of metal adsorbed (mg/L); Co = Concentration of metal prior to contact (mg/L); Cf = Concentration of metal after contact (filtrate) (mg/L).

2.6. Data Analysis

The data have been tested by Manova (Multivariate analysis of variance) at α = 0,05. If there was a difference of removal efficiency, then continued by Duncan's test at α = 0.05.

3. Result and Discussion

S. costatum was dead after 72 hours or for 96 hours of contact (fourth day) at concentrations of 0.5, 1 and 2 ppm mercury (II). The control is still alive. This indicates that mercury (II) is highly lethal. However, S. costatum has had the highest adsorbent efficiency (Tabel 1).

Table 1: The adsorption efficiency (%) of mercury by *Skeletonema costatum*.

Number of cell inoculum	Concentration of Mercury II (mg/L)	Time contacts (hours)			
		24	48	72	96
5000	0.5	38.00 ± 2.00^{ab}	70.00 ± 22.50^{fghij}	70.67 ± 3.05^{ghij}	74.67 ± 4.16^{hijklm}
cells/mL	1	34.00 ± 2.00^{a}	69.00 ± 1.00^{fghi}	77.00 ± 2.64^{ijklmnop}	78.33 ± 2.08^{jklmnopq}
	2	52.00 ± 2.18^{cd}	66.13 ± 1.61^{efgh}	79.50 ± 0.50^{jklmnopq}	81.00 ± 0.50^{klmnopq}
10000	0.5	45.33 ± 3.05^{bc}	66.00 ± 2.00^{efg}	76.00 ± 2.00^{ijklmno}	80.00 ± 2.00^{klmnopq}
cells/mL	1	51.67 ± 3.05^{cd}	75.33 ± 1.53^{ijklmn}	82.00 ± 1.00^{lmnopq}	84.00 ± 1.00^{nopq}
	2	59.33 ± 0.57^{de}	69.33 ± 0.76^{fghi}	83.33 ± 0.76^{mnopq}	85.17 ± 1.26^{pq}
15000	0.5	58.00 ± 2.00^{de}	74.00 ± 2.00^{ghijkl}	80.67 ± 1.15^{klmnopq}	84.67 ± 1.15^{opq}
cells/mL	1	58.67 ± 1.53^{de}	82.00 ± 2.01^{lmnopq}	84.67 ± 0.57^{opq}	86.67 ± 1.52^{q}
	2	62.00 ± 1.00^{ef}	73.00 ± 1.32^{ghij}	85.00 ± 0.50^{opq}	86.83 ± 0.76^{q}

Description: a number followed by the same letter are not significantly different.

S. costatum bioremediation capabilities could be seen from the value of adsorption efficiency (%). Table 1 showed that inoculation of *S. costatum* 15 000 cells/mL in 96 hours of the most highly rated efficiency of absorption. So we could say the effectiveness of microalgae to absorb heavy metals has depended on the ability of microalgae to adaptation. *S. costatum* able to absorb heavy metals such as microalgae, because *S. costatum* has a negative charge while the heavy metals in waters have a positive charge, so *S. costatum* able to absorb heavy metals [5].

Well, *S. costatum* able to absorb heavy metals in two ways: absorption and adsorption. Adsorption occurs because this diatom has cell walls with cellulose content consisting of hydroxyl functional groups capable of binding to heavy metals [8 and 13] or replace Zn contained within the cell wall [14]. The absorption was undertaken by *S. costatum* because it has produced cytokeratin. In addition, The microalgae produce phytochelatin, namely the metallothionein class III (MtIII). This phytochelatin serves to detoxify heavy metals [4]. And that MtIII was in S. costatum vacuole [14].

4. Conclusion

Based on the research results, we conclude that the optimum contact time and concentrations of the *Skeletonema costatum* cell inoculation on the bioremediation in mercury (II) contaminated seawater for 2 mg/L, i.e. 5 000 cells/L for 72 hours (79.5%).

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