

The difference of percentage removal efficiency of heavy metal Pb (II) in leachate by *Skeletonema* sp. immobilized

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ABSTRACT

The purpose of this research was to determine the difference of heavy metal Pb (II) removal efficiency percentage in TPA leachate by immobilized *Skeletonema* sp. based on variations in contact time. In this research, *Skeletonema* sp. cells were immobilized using sodium alginate; with concentration of 0.65%, and 1-7 days of contact time variation. Observation data on the removal efficiency percentage of heavy metals were statistically analyzed using the One Way Anova test. Based on the results of the analysis, it is known that there is a difference in heavy metals Pb (II) efficiency removal percentage in landfills' leachate based on the determined contact time. The maximum contact time of the heavy metals removal Pb (II) in leachate found to occur at the 7day with efficiency percentage of 89.353%.

Key words : Bioremediation, Pb (II), *Skeletonema* sp. immobilized, Sodium alginate, Leachate

Introduction

The increase in the population in Indonesia is directly proportional to the increase in the amount of waste in each region in Indonesia. The increase in the amount of waste resulted in a high volume of waste at the final disposal site (TPA). The high landfill waste in landfill which is not directly proportional to landfill land area can cause various kinds of environmental problems. One of the problems that arises due to landfill in TPA is leachate.

Leachate has a high concentration of BOD, COD and TSS. Besides that leachate contains various kinds of heavy metals such as Pb, Hg, Cd, Cr, Fe, Mn and others (Sari *et al.*, 2017; Sumanik *et al.*, 2019).

Heavy metals are elements that cannot be decomposed and can accumulate through food chains with long-term effects that are harmful to living things (Wijaya *et al.*, 2019; Yunasfi *et al.* 2019; Hanum *et al.*, 2017; Tanjung *et al.*, 2019; Usman *et al.*, 2013; Soegianto *et al.*, 2020).

Heavy metal pollution must be handled because they can damage the ecosystem and disrupt the public health. One method to reduce the levels of heavy metals in the waters is by bioremediation. Microalgae can be used as a bioremediation agent to increase water quality and to decrease the level of heavy metals in the water (Soedarti *et al.*, 2019; Guntur *et al.*, 2019; Masithah *et al.*, 2011; Prayogo *et al.*, 2012). *Skeletonema* sp. as a member of microalgae

has the ability to absorb heavy metals in 2 ways, namely absorption and adsorption. Adsorption occurs because of *Skeletonema* sp. have cell walls that contain cellulose which is useful for absorbing metals with their hydroxyl bonds. While the absorption process occurs because of *Skeletonema* sp. contains *phytochelatin* in its vacuole which can function to induce a variety of metals (Gupta *et al.*, 2000). In bioremediation, *Skeletonema* sp. can be done by immobilizing with sodium alginate. Immobilized cells can maintain respiratory activity, photosynthesis, and metabolic processes such as algae under normal conditions (Riffiani, 2009).

Based on the description above, leachate TPA containing heavy metals can cause environmental damage and can cause health problems in humans, as well as the ability of *Skeletonema* sp. as a bioremediation agent, it is necessary to conduct research to determine the ability of *Skeletonema* sp. in conducting bioremediation of TPA leachate containing Pb heavy metals.

Materials and Methods

Tools and Materials

The tools used in this research include 350 mL glass bottles, 100 mL glass bottles, aerator hoses, aquarium aerators, cupboards, TL 40 Watt lamps, analytic scales, lux meter, autoclave, refractometer, thermometer, 10 mL volume pipette, pump pipette volume, microscope, Neubauer Hemocytometer, handcounter, drop pipette, micrometer ocular, 100 mL and 50 mL measuring cups, T and L aerator hose connectors, plastic funnels, glass funnels, bunsen, matches, pH pen, pH indicator paper, stirring spoon, cotton, aluminum foil, label paper and tissue.

The materials used in this study were TPA leachate water, *Skeletonema* sp. Culture. obtained from the Situbondo Brackishwater Cultivation Center (BBAP), sodium alginate, CaCl (2%), 70% alcohol, 1 M HNO₃, methylated, aquadest, and aquadine.

Preparation of Tools and Materials

The equipment to be used must be sterilized first by spraying alcohol and rinsing it with distilled water. While the glass bottles to be used are sterilized by autoclave at 120° C for 2 hours. Sterilization of this equipment aims to avoid contaminants by other microorganisms that can interfere with the research

treatment process.

Preparation of *Skeletonema* sp. and Leachate

Skeletonema sp. obtained from the Brackish Water Aquaculture Center (BPAP) in Situbondo, East Java, counted the number of cells using a Neubauer hemocytometer and handcounter. Number of cells obtained from the calculation is $\times 10^4$ cells/mL. Number of cells of *Skeletonema* sp. what is needed is $1,500 \times 10^4$ cells/100 mL in 2 ppm (Soedarti *et al.*, 2017). *Skeletonema* sp. Cells in the centrifuge with a speed of 5000 rpm for 7 minutes. The first leachate preparation was, leachate water that had been taken at TPA, added 1 M HNO₃ solution to pH to 6. TPA leachate water was then analyzed to determine Pb heavy metal concentration. Besides that, the pH, salinity and leachate temperature were tested. The volume of leachate used in the treatment was 100 mL.

Making of *Skeletonema* sp. Beads Using Sodium Alginate

The concentration of sodium alginate used was 0.65%. Sodium alginate is mixed with 40 mL of distilled water, so the amount of sodium alginate needed in 0.65% is 0.26 g. Prepared sodium solution, mixed with *Skeletonema* sp. with a number of cells that have been determined, then dropped into a 2% CaCl solution. Finished beads are filtered first to separate the CaCl solution. The beads are then transferred in a treatment bottle which has been filled with 100 mL leachate.

Preparation of Running and Harvesting Samples

Samples on glass bottles containing beads and as much as 100 mL of leachate water were aerated using an aquarium aerator connected to an aquarium hose. The sample is aerated according to the specified contact time (1, 2, 3, 4, 5, 6, and 7 days). Samples that have been aerated according to the time of contact, are then harvested by separating beads with leachate water. Leachate water which has been separated with beads is inserted into a sterile infusion bottle. After that leachate was analyzed for parameters of heavy metal content and other supporting parameters.

Data analysis

Calculation of Pb Heavy Metal Allowance Percentage

The percentage of Pb heavy metal absorption can be

calculated based on initial concentration and concentration after treatment. Percentage of absorption can be calculated by Equation (1)

$$\text{Percentage of absorption} = \frac{C_0 - C}{C_0} \times 100\% \quad .. (1)$$

Information:

C_0 = Initial Concentration of Pb (mg/L)

C = Final Concentration of Pb (mg/L)

Statistical Analysis

The statistical test has several steps. The first step is a normality test. If the data is normally distributed ($\alpha > 0.05$) then the homogeneity test is continued. If the data is homogeneous, one-way ANOVA test is conducted to determine whether there is a difference in efficiency of Pb removal in leachate.

If the data is not normally distributed then the Kruskal Wallis test is carried out, if the significant value is more than 0.05, then the hypothesis H_0 is accepted, which means there is no difference in the percentage of Pb allowance. Whereas, if the significant value is smaller than 0.05, the H_a hypothesis is accepted, which means that there is a difference in the percentage of Pb allowance. The data entered is the percentage data on the efficiency of removal of heavy metals Pb (II) and the cell length data of *Skeletonema* sp. before diimobil and after melting the beads.

Results and Discussion

The results of the efficiency of removal of heavy metals Pb (II) in TPA leachate by *Skeletonema* sp. Immobilization based on statistical tests on day 1 to day 7 can be seen in Table 1.

To find out whether there is a difference in the percentage of efficiency of Pb (II) heavy metals in

Table 1. Efficiency percentage of heavy metal Pb (II) in leachate water by *Skeletonema* sp. immobilized

No.	Contact time	Efficiency percentage (%)
1.	Day-1	79.276 ± 0.208 ^a
2.	Day-2	79.443 ± 0.153 ^a
3.	Day-3	85.293 ± 0.105 ^b
4.	Day-4	85.767 ± 0.152 ^c
5.	Day-5	86.240 ± 0.325 ^d
6.	Day-6	88.540 ± 0.300 ^e
7.	Day-7	89.353 ± 0.305 ^f

Description: the same letter shows no difference

leachate, Anova One Way statistical tests were carried out. The One Way Anova test was carried out after the normality test and homogeneity test. Based on the results of the One Way Anova test it is known that the p value in this research data is 0,000. If the value of $p < 0.05$ indicates that there is a difference in the data or in other words reject H_0 and accept H_a . If it is known that the data has a difference then the next test is the Duncan test. Based on the Duncan test results, it is known that there is no difference in the percentage of efficiency of removal of heavy metals Pb (II) in leachate water on days 1 and 2. Meanwhile, the percentage of efficiency of removal of heavy metals Pb (II) in leachate water on the 2nd, 3rd, 4th, 5th, 6th, and 7th days had a significant difference. Based on this, it is known that the optimum contact time for the efficiency of Pb heavy metal removal in leachate occurred on the 7th day.

The percentage of efficiency of heavy metals Pb (II) on leachate water from day 1 to day 7 continues to increase. This is due to the use of sodium alginate as an immobilization agent in the bioremediation process of heavy metals Pb (II) in leachate. *Skeletonema* sp. immobilized can maintain respiratory activity, photosynthesis, and metabolic processes such as algae under normal conditions (Riffiani, 2009). The percentage of the efficiency of heavy metal removal by *Skeletonema* sp. immobilization is caused by sodium alginate which is used as an immobilization material also has the ability to reduce heavy metals such as micro algae which are used as bioremediation agents (Rafly, 2016).

Basically *Skeletonema* sp. has the ability to absorb heavy metals in 2 ways, namely absorption and adsorption. The absorption process occurs because of *Skeletonema* sp. contains phytochelatin in its vacuole which can function to induce various metals. While adsorption occurs because of *Skeletonema* sp. have cell walls that contain cellulose which is useful for absorbing metals with their hydroxyl bonds (Gupta et al., 2000). The higher protein content possessed by phytoplankton, the better the ability to absorb heavy metals because proteins are maintained on carboxyl groups (-COOH) which are able to bind well to heavy metal ions.

Conclusion

The results showed that there were differences in the percentage of efficiency of removal of heavy metals Pb (II) in leachate water by immobilized

Skeletonema sp based on contact time where the maximum contact time occurs on the 7th day with efficiency 89,353%.

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