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Difference of Hg (II) removal efficiency in leachate by Immobilized *Skeletonema* sp.

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ABSTRACT

The purpose of this research is to find out the difference of efficiency of removal of Hg (II) ions in leachate using immobilized *Skeletonema* sp. based on contact time variation. This research was conducted with seven variations of contact time. The contact time used were one day, two days, three days, four days, five days, six days, and seven days. The data were analyzed by descriptive and statistic analysis. The One- Way Anova was used to determine the difference of efficiency of removal of Hg (II) ions in leachate using immobilized *Skeletonema* sp. based on contact time variation. The result showed that there was different among efficiency removal of Hg (II) ions in leachate using *Skeletonema* sp. immobilized based on contact time variation. The optimum value of removal efficiency of Hg (II) was 85,18% obtained at six days contact time.

Key words :*Mercury, Leachate, Landfill, Skeletonema*

Introduction

Leachate water generally contains organic and inorganic compounds with concentrations of 1000 to 5000 times higher than concentrations in ground water (Sumanik *et al.*, 2019, Ashar *et al.*, 2016; Ginting *et al.*, 2018). Organic compounds that are commonly found are hydrocarbons, humic acid, sulfuric acid, tannic and gallate, while inorganic compounds contained in sodium leachate water, potassium, calcium, magnesium, chlorine, sulfate, phosphate, phenol, nitrogen and high heavy metal compounds (Maramis, 2008; Artiningsih *et al.*, 2018).

Heavy metals commonly found in leachate water are Fe, Cu, Cr, Hg, Zn, As, Pb, Ni. One of the heavy metals content in leachate that is dangerous and needs to be considered is mercury (Hg). The maximum concentration of mercury in leachate water according to the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number 59/MENLHK/Setjen/Kum.1/ /2016 is 0.005 mg /L (Anonymous, 2016)

Mercury has toxic effects on all organ functions that found in the human body, although only a small amount is absorbed by the body (Mirdat *et al.*, 2013; Soegianto *et al.*, 2010; Yeanny and Muthawali, 2018; Soegianto *et al.*, 2020). Mercury can adversely affect organ functions, such as disorders of the kidneys, liver, digestive and reproductive organs (Herman, 2006; Berniyanti and Hariyani, 2008; Puspitasari, 2019). The high risk of pollution due to mercury in living creatures above causes the need of treatment that can reduce the concentration of heavy metals to a level that can be tolerated by the environment and at a relatively low cost (Suheryanto, 2001). One of the effective and low-cost method for that is bioremediation. One of the microorganisms that can be used for bioremediation of heavy metals is microalgae *Skeletonema* sp. (Priade, 2012).

Based on Soedarti's research results (2014), *Skeletonema* sp. has capability to absorb mercury with an allowance percentage of 79.5%. According to Permata (2018), the bioremediation process with immobilized *Skeletonema* sp. has a value of allowance efficiency greater than with non-immobilized *Skeletonema* sp. The study showed that the removal efficiency was obtained by immobilizing *Skeletonema*

Species with sodium alginate 0.65%

Based on the description of the hazards of mercury in leachate water and the advantages of bioremediation processes with immobilized *Skeletonema* sp., research with variations of contact time to determine the ability of immobilized *Skeletonema* sp. in setting aside mercury in leachate water is carried out.

Materials and Methods

The materials used in this study were leachate, *Skeletonema* sp. obtained from the Center for Brackish Water Cultivation (BBAP), sodium alginate, CaCl 2%, NaOH 0.05 M, alcohol, methylated spirits, and distilled water, aqua Demin.

The instrumentations used in this study were aerator aquarium (Recent AA/350), 250 mL glass bottle (ABC sauce), 5 meter aquarium hose, aquarium hose tee, rack, TL 40 Watt lamp (Philips), analytical balance, lux meter, refractometer, thermometer, 10 mL volume pipette (HBG), microscope (Olympus), Hemocytometer Neubauer (Assistant), hand counter (Kw-Trio), dropper pipette, 100 mL glass bottle (infusion), 100 mL glass beaker (Iwaki), glass funnel (Iwaki), 100 mL and 50 mL (Pyrex) measuring cup, fume hood, petri dish, Bunsen, lighters, pH indicator paper (Merck), nylon mesh filter, stirring spoon and tissue.

Preparation of *Skeletonema* sp.

The cell diameter of *Skeletonema* sp. was measured and the number of cells measured in each 1 mL. Calculation of the number of cells is done using a Neubauer hemocytometer and hand counter. The number of cells *Skeletonema* sp. needed is 15 × 106 cells in every 100 mL of waste with a concentration of 2 ppm (Soedarti *et al.*, 2016). *Skeletonema* sp. culture is centrifuged for 5 minutes at 7000 rpm. Treatment by centrifuge is intended to separate *Skeletonema* sp. with growing media.

Preparation of Leachate

Leachate water obtained from the Jombang Regency landfill is filtered with filter paper to further test the initial Hg concentration. The initial concentration was used as a control concentration in the study. Measurements of pH, salinity and temperature are also carried out in the preparation phase of leachate. Characteristics of known leachate are then placed in a bioreactor bottle with a volume of 100 mL.

Beads of *Skeletonema* sp. Making Process

Skeletonema sp. beads are made by mixing biomass of *Skeletonema* sp. with sodium alginate solution up to a volume of 40 mL. The concentration of sodium alginate used is 0.65% so that the mass of alginate used in 40 mL of distilled water is 0.26 g. Making beads was done by dropping a mixture of biomass *Skeletonema* sp. and sodium alginate into a 2% CaClsolution.

Bioreactor Preparation for Hg (II) Bioremediation with Immobilized *Skeletonema* sp.

A bioreactor consisting of a 250 mL glass bottle in a sterile state filled with beads and 100 mL of leachate water. Hoses, tees, and aerators are connected to the leachate aeration process which has been contacted with beads *Skeletonema* sp.

Running Process

The aeration process lasts for 24 hours without stopping. Running bioreactor takes place according to the specified contacttime.

Calculation of Percentage of Heavy Metal Allowance for Hg (II)

The percentage of absorption of heavy metal Hg can be calculated based on the initial concentration and concentration after treatment. The percentage of absorption can be calculated by the following equation:

Removal Efficiency = ((Co-C)/Co) x100%

Where Co = Initial Hg concentration (mg / L) C

= Final Hg concentration (mg / L)

Statistical Analysis

Statistical analysis was used to determine differences in the percentage of Hg (II) removal based on variations of contact time using *Skeletonema* sp. which was immobilized. Statistical tests have several steps. The first step is the normality test, homogeneity test, then One-WayANOVA. If the significance value is more than 0.05, then the hypothesis H0 is accepted, which means there is no difference in the percentage of Hg allowance. Meanwhile, if the significance value is less than 0.05, then the Ha hypothesis is accepted, which means there is a difference in the percentage of Hg allowance.

Results and Discussion

The results of Hg (II) removal in the bioremediation process using *Skeletonema* sp. immobilized with variations in contact time of one to seven days can be seen in Table 1.

Table 1. Result of Hg (II) Removal Efficiency

Contact Time (day)	Removal Efficiency (%)
1	22.22 ^{a} ± 5.55
2	27.77 ^{a} ± 5.55
3	38.89 ^{b} ± 5.55
4	64.82 ^{c} ± 8.48
5	66.67 ^{c} ± 5.55
6	85.18 ^{d} ± 3.21
7	89.05 ^{d} ± 3.20

The data were analyzed statistically to determine the presence or absence of differences using the One- Way ANOVA. The results of the normality test showed that the Hg (II) removal efficiency data based on variations in contact time were normally distributed. Homogeneity test results showed that homogeneous data with a value of $p > 0.05$ ($p = 0.763$). One- Way ANOVA results showed the value of $p < 0.05$ ($p = 0.000$) which means that there was a difference in the efficiency of the removal of Hg (II) by *Skeletonema* sp. immobilized in contact time variations.

The results obtained from the One-Way ANOVA showed a difference in the efficiency of the removal of Hg (II) in leachate liquid waste by *Skeletonema* sp. immobilized. Duncan's test is then performed to determine the significance of the differences. The results of the Duncan test can be seen in Table 1, where the same letter shows no significant difference between existing data. The efficiency of the Hg (II) removal on day 1 and day 2 did not have a significant difference, but had a significant difference with efficiency on day3.

Existing efficiency data is determined based on initial Hg (II) concentration in leachate water and Hg (II) concentration after treatment based on the determined contact time. The initial concentration of Hg (II) in leachate reaches 0.018 mg/L, this value is higher than the standard quality of Hg concentration in leachate according to the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number 59/MENLHK/Setjen/Kum.1/7/2016, i.e., 0.005 mg/L. Bioremediation process with *Skeletonema* sp. immobilized can set aside the metal Hg (II) to meet existing quality standards with a yield of 0.002 mg/L or set aside 89.05% of the initial concentration of Hg (II) found in leachate water.

Provision efficiency obtained shows the ability of *Skeletonema* sp. immobility in reducing Hg (II) in leachate water. *Skeletonema* sp. has a carboxyl functional group that is able to absorb heavy metal ions which are also found in many other microalgae. The carboxyl group is the main constituent of proteins in cells *Skeletonema* sp. The carboxyl group can bind to metal ions through ion exchange or complex formation. Ion exchange occurs when divalent or monovalent ions in the cell wall exchange with heavy metal.

Conclusion

The results of research and data processing indicate that there are differences in the efficiency of the Hg (II) removal in the bioremediation process using immobilized *Skeletonema* sp. with variations in contact time.

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