Eco. Env. & Cons. 26 (3) : 2020; pp. (1319-1323)

ISSN 0971–765X

Comparison of remediation performance of *Chaetoceros calcitrans* on heavy metals and diesel fuel exposure

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(Received 21 October, 2019; Accepted 1 March, 2020)

ABSTRACT

Sea pollution can be caused by various sources, one of the biggest sources of marine pollution is anthropogenic activities. Types of pollutants that are often found in pollution are heavy metal content in sea water and oil spills resulting from transportation activities. Both types of pollutants will survive for a long time and can enter the food chain. Therefore, we need an Eco-friendly solution for the purpose of the problem. Bioremediation techniques using microalgae *Chaetoceros calcitrans* and *Skeletonema costatum* are the right solution. This study compares the quality of these microalgae in degrading heavy metal Cd and diesel oil on a laboratory scale. The concentration of heavy metal test was 0.7, 1.3 and 1.9 ppm, while the amount of diesel oil was 5, 10, and 20 mL. Showing the two microalgae that can degrade all test pollutants with different percentages of degradation.

***Key words**: Biodegradation, Heavy metals, Microalgae, Diesel oil*

Introduction

The ocean is one of the largest, most complex and most dynamic ecosystems in the world. Interactions that occur between physical, chemical, and biological factors take place quickly and continuously so as to determine the condition of the ecosystem that exists in the aquatic environment. The decline in water health can be caused by adverse effects from various stressors such as climate and habitat change, invasive species, eutrophication, until the entry of contaminants into the waters (Blasco *et al.*, 2015; Bohari and Palutturi, 2018; Irhamni *et al.*, 2018; Sulastri and Tampubolon, 2019). Pollutants enter the waters through various sources due to anthropogenic activities, including industry, agriculture, transportation, and improper waste disposal practices (Pavlostathis *et al.*, 2001; Soegianto *et al.*, 2020).

Pollutants that enter the aquatic ecosystem can be immobilized and accumulated in sediments or can undergo transformation and activation processes (Martinez-Jeronimo *et al.,* 2008).

Pollutants can cause primary damage with a direct impact that can be identified on the environment, or can also cause secondary damage in the form of minor disturbance in the balance of biological food webs that can be detected in a long time (Hermawati *et al.*, 2009; Ghani, 2015). Environmental pollution due to heavy metal pollutants is a significant problem throughout the world, this is because heavy metals can accumulate in the food chain and their presence will continue in the ecosystem (Eshmat *et al.*, 2014; Aneja *et al.,* 2010; Soegianto *et al.,* 2020; Yunasfi *et al.*, 2019). Some types of heavy metals that are often found in sea water are Cadmium and Copper. The main sources of Cd (II) release into the aquatic environment are smelting, electroplating, batteries, fertilizer, mining, paint pigmentation, etc. (Iqbal and Edyvean, 2005).

Petroleum is one of the most important energy resources and raw materials for the chemical industry. The increasing global energy demand is causing an increase in crude oil extraction and transportation at sea. This makes the sea vulnerable to the risk of oil spills (Kim *et al.,* 2013). Various pollutants in the sea will cause toxic effects on the ecosystem if the concentration is increasing and is in a long time. Therefore we need the right solution to deal with this problem.

Economical and environmentally friendly bioremediation is considered as one of the effective techniques for removing pollutants from the environment. Many microorganisms are known to reduce pollutant content from the environment (Boopathy *et al.,* 2012; Fahruddin and Tanjung, 2019). Microorganisms that are often used in the bioremediation process are microalgae, for example *Chaetoceros calcitrans*, *Skeletonema costatum* (Pratiwi, 2019), and *Skeletonema* sp. (Soedarti *et al.,* 2019; Pratama *et al.,* 2020). This study aims to compare the degradation capabilities of the two microalgae against Cd, Cu, and diesel oil pollutants on a laboratory scale.

Materials and Methods

Material

Pure *Chaetoceros calcitrans* and *Skeletonema costatum* cultures were obtained from culture selectively in the laboratory and were acclimatized and confirmed to be free of pollutants. Both of these microalgae are exposed to pollutants at the age of 4 days because they are in an exponential phase. Beginning with exposure to microalgae given nutrients in the form of vitamins, silicates, and diatomic fertilizers. Seawater used in the whole testing process is sterile sea water that has been sterilized using an autoclave for at least 15 minutes with a temperature of 121 ^{o}C and a pressure of 1.5 Pa. All glassware was washed with 10% nitric acid and acetone and rinsed using distilled water.

Methods

The concentrations of cadmium and copper used in this study were control, 0.7, 1.3, and 1.9 ppm. While the diesel oil used is control, 5, 10 and 20 mL per treatment. Each concentration was repeated 3 times and during the exposure the microalgae was given good aeration and lighting for the process of photosynthesis. Exposure to heavy metals carried out for 4 days (96 hours) and exposure to diesel oil for 8 days. Every day microalgae density calculations are performed to determine the response of microalgae growth to pollutants and water quality measurements to ensure the condition of exposure to water is in a normal state. At the end of the exposure, heavy metal concentration and total diesel oil content were calculated to determine the percentage of pollutant degradation by microalgae *Chaetoceros calcitrans* and *Skeletonema costatum*.

Results and Discussion

Measurement of heavy metal concentrations in the media was carried out using Atomic Absorption Spectrophotometry (AAS) at the end of the exposure. Whereas for exposure to diesel oil, concentration measurements on the media are carried out using gravimetric test. Gravimetric method is a process of isolation and measurement of the weight of a particular element or compound. Most of the gravimetric method involves the transformation of elements or radicals into pure stable compounds which can be transformed into forms that can be weighed or examined. Elemental weight is calculated based on the formula of the compound and the atomic weight of its constituents (Ren *et al.,* 2015). Measurement of media concentration aims to determine the concentration of diesel oil remaining in microalgae culture media *Chaetoceros calcitrans* and *Skeletonema costatum*. The remaining diesel oil in microalgae maintenance media at the end of the study showed the remaining diesel oil that was not absorbed by microalgae. On heavy metal exposure, before measuring the final concentration, the media is separated from microalgae using Whatman filter paper No. 5. The results of this measurement were used to measure the percentage of heavy metal degradation in the media by using the following formula:

Percentage of degradation= ((Initial concentration-Last concentration)/Initial concentration) x100%

The results of the calculation of the percentage of heavy metal degradation and diesel oil by *Chaetoceros calcitrans* can be seen in Table 1 and *Skeletonema costatum* in Table 2.

Table 1. Degradation percentage of pollutant by *Chaetoceros calcitrans*

Chaetoceros calcitrans Treatment	Degradation percentage (%)		
	Cd	Cu	Oil
1	12.86	27.14	68
2	8.46	16.92	37.9
3	6.32	11.05	34.9

Notes: Treatment 1 (Cd dan Cu : 0.7 ppm; oil: 5 mL); Treatment 2 (Cd dan Cu : 1.3 ppm; oil: 10 mL); Treatment 3 (Cd dan Cu : 1.9 ppm; oil: 20 mL)

The efficiency of the oil biodegradation process can be influenced by several factors, including the type of media, oxygen, nutrient concentrations, environmental sensitivity and abundance of the oil decomposing microorganisms themselves (Rodriguez *et al.,* 2010). Meanwhile, the factors that influence the absorption of heavy metals include cell wall characteristics of microalgae, physical and chemical parameters such as temperature and pH, initial concentration of metal ions and biomass concentration (Aksu, 2006). Therefore, the degradation ability of *Chaetoceros calcitrans* in each pollutant is different. The same results were also obtained in the testing of pollutant degradation by *Skeletonema costatum.* Percentage value of pollutant degradation by *Skeletonema costatum* can be seen in Table 2.

Trendline of pollutant degradation by *Skeletonema costatum* microalgae can be seen in Figure 2.Based on the two gravities, the percentage of degradation between pollutants is different. Heavy metals have their respective characteristics in the media. Cadmium has a low solubility level, this metal is a metal that is lipophobic or difficult to dissolve in fat. In the process of heavy metal degradation occurs in 2 stages, namely the process of passive uptake (biosorption) and active uptake. The active uptake process occurs when microalgae cells move heavy metal ions that have been bound to cell walls into deeper cell organelles (Rugnini, 2017). Because cadmium is difficult to dissolve in fat, to be absorbed into microalgae cells requires a diffusion process facilitated with a longer time, so the amount of cadmium that can be absorbed by microalgae is lower than copper. While copper is an essential heavy metal that is needed by microalgae for its metabolic process and has the characteristic of being soluble in water, so that the process of absorption of copper by microalgae occurs more easily than cadmium.

Table 2. Degradation percentage of pollutant by *Skeletonema costatum*

Skeletonema costataum Treatment	Degradation percentage (%)		
	Cd	Cu	Oil
1	17.14	21.43	58
2	11.54	14.62	29
3	5.26	6.84	28.5

Notes: Treatment 1 (Cd dan Cu : 0.7 ppm; oil: 5 mL); Treatment 2 (Cd dan Cu : 1.3 ppm; oil: 10 mL); Treatment 3 (Cd dan Cu : 1.9 ppm; oil: 20 mL)

Some microalgae can produce enzymes that can degrade harmful organic compounds to convert petroleum hydrocarbons into less toxic compounds (Davies and Westlake, 1979). In the process of biodegradation of oil, sea water will turn more turbid because of cell growth and oil will dissolve (Zahed *et al.,* 2010). The remediation process is very necessary for oxygen and light, this is because microalgae require oxygen to optimize the body’s performance and light is needed to carry out photosynthesis. This is very supportive of microalgae in reducing diesel oil. Microalgae in culture media can increase the potential for degradation to eliminate pollutants and increase lipid biomass production so as to reduce pollution. Microalgae are able to reduce aliphatic and aromatic hydrocarbons, because they have a flexible metabolism (Hamouda *et al.,* 2016).

Diatom microalgae can live in polluted conditions and some reduce the content of heavy metals such as alkanes and polycyclic aromatic hydrocarbons so that they are very useful as bioremediation agents for crude oil and heavy metals under mixed conditions (Hamouda *et al.,* 2016). Diatoms have functional groups found in cell walls and contain carboxylic, hydroxyl, amino, sulfidril, sulfate and phosphate groups. Cell walls in diatoms contain proteins and polysaccharides that can bind to pollutants (Das *et al.,* 2008). *Chaetoceros calcitrans* and *Skeletonema costatum* are diatomic microalgae, so both have biodegradability of pollutants. However, the degradation ability between microalgae is different because the cell wall characteristics between microorganisms have differences. Therefore, the percentage of degradation between the two microalgae shows a difference.

Conclusion

Cadmium and copper are heavy metals that are often found in aquatic environments. In high concentrations this heavy metal will be toxic to the ecosystem. Another pollutant that is often found in the sea is oil, this is due to the frequent occurrence of oil spills due to transportation activities. One technique that is inexpensive and environmentally friendly to reduce the presence of pollutants is bioremediation. This technique can be done using microalgae *Chaetoceros calcitrans* and *Skeletonema Costatum*. Both types of microalgae are diatoms which contain cell walls of carboxylic groups, proteins, and polysaccharides. The content can be used to bind pollutants in the environment, so that this type of microlaga can be used as an agent for biodegradation of pollutants in water.

Acknowledgment

This project funded by Indonesia Higher Education Biro (DIKTI) through Universitas Brawijaya (PUPT Program). All the credits to all Environmental Physiology and Chemistry Laboratory members, CORECT RG (Coastal Resilience and Climate Change Adaptation- Research Group).

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