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Study of Cadmium Bioaccumulation in *Perna viridis* through Food Pathway and Its Decontamination

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Abstrak

Makanan laut dapat terkontaminasi oleh logam berat yang terkandung dalam air laut dan sumber makanan yang dikonsumsi biota laut. Kadmium merupakan salah satu kontaminan yang ditemukan di lingkungan laut. Studi bioakumulasi melalui jalur makanan melengkapi studi sebelumnya melalui jalur air laut. Penelitian ini juga mengupayakan dekontaminasi kadmium menggunakan asam asetat dan asam sitrat. Hasil percobaan menunjukkan kemampuan bioakumulasi total Cd oleh *Perna viridis* adalah 74,01. Dekontaminasi Cd yang terakumulasi di *Perna viridis* menurunkan level hingga 21%

Kata kunci: Bioakumulasi; *Perna viridis*; Kadmium; dekontaminasi

Abstract

Seafood can be contaminated by heavy metals that contained in seawater and the source of food that marine biotas eats. Cadmium is one of the contaminants found in the marine environment. Bioaccumulation studies via foood pathway were complement previous studies through the seawater pathway. This study also made an effort to biologically decontaminate cadmium using acetic acid and citric acid. The experimental results showed the total bioaccumulation ability of Cd by Perna viridis was 74.01. Cd decontamination which accumulates in the Perna viridis decreases the level up to 21%

Keywords: Bioaccumulation; Perna viridis; Cadmium; decontamination

Introduction

Green mussel (*Perna viridis*) is one of the marine biota that is used as the livelihood of people in Indo Pacific regions and also around Jakarta Bay Indonesia. Green mussels are widely cultivated in the Asia Pacific region. The threat to aqua culture of green mussel is the presence of heavy metals in coastal waters. Among heavy metals, Cd is generally associated with industrialization activities that produce waste from plastics, paints and metal alloys. (Jing *et al.*, 2019)

Heavy metals are easily bio accumulated by bivalves and biomagnification along food chain (Yuan *et al.*, 2020). The studies of heavy metal bioaccumulation by *Perna viridis* or other species of mussels have been carried out by

many researchers. For example, Bioaccumulation of some metals by Green mussel *Perna viridis* (Linnaeus 1758) from Pekan, Pahang, Malaysia (Kamaruzzaan *et al.*, 2011); heavy metal and its relation to the malformation of green mussel *Perna viridis* from Muara Kamal waters, Jakarta (Etty *et al.*, 2018). Moreover, effect of sublethal gradient concentration of heavy metal on poslarvae of *Perna viridis* have been studied by Nagarjuna et al. (2019). Cadmium bioaccumulation and ellimination in tissues of the freshwater mussel *Anodonta woodiana* have been studied by Jing *et al.* (2019). Most of these studies are based on monitoring results. The ability of bioaccumulation is determined from the ratio of the concentration of contaminants in biota to its concentration in water. Furthermore the bioaccumulation studies have been conducted in accumulation antibiotic and radiocesium (Prihatiningsih *et al.*, 2016; Suseno, Hudiyono and Muslim, 2016). All these studies were conducted only consider the bioaccumulation of contaminant through sea water pathways.

In this research a bioaccumulation study was carried out through food pathway. Also conducted research related to decontamination of green shells that have been contaminated due to the bioaccumulation process. This decontamination is related to seafood safety

Methods

Material and equipments used in the experiment are aquaria system, laboratory glasswares, analytical balance, NaI(Tl) Gamma Spectrometer, and Atomic Adsoption Spectroscopy.

Preparation of *Botryococcus braunii* Culture without Contaminants.

Equipment was sterilized using an autoclave with a temperature of 121^{o}C. Lighting and aeration system on Erlenmeyer was arranged in such a way that it can function properly. Microalgae seeds obtained from the BBPBAP Jepara with initial density 1x10^{5}-2x10^{5} cells/mL, with salinity of 30 ppt, lighting of 3000 lux. Furthermore, 800 mL of culture medium (seawater) was incorporated into the erlenmeyer and microalga seeds were added as much as 200 ml and 1 mL wale and then bred for 6 days. After 6 days microalgae seedling, harvesting and centrifugation at 3500 rpm for 5 minutes. The already formed microalgae slurry is added aquades to remove impurities. The contaminant culture used for the bioaccumulation process has a density of 1x10^{6} cells / mL. Breed microalgae slurry taken as much as 1 mL was added to 100 mL of Cd metal solution with concentration of 1 ppm.

Acclimatization

Green mussels (*Perna viridis*) that taken from Laki Island and Muara Kamal were about 3-4 months old, with a length of about ± 5-6 cm. The aquarium used for the acclimation process is prepared in advance with an aquaria and filtration system. Sea water of 250 L was put into a preprepared aquarium and measured its salinity by using a refractrometer of 34-35$^{o}/_{oo}$ and temperature at 32°C. The purpose of acclimatization is to allow the green mussels to adapt itself to new environments with conditions created and adapted to their original habitat conditions.

Food Pathway Bioaccumulation (Uptake)

First, 10 glass jars containing 2.5 L of sea water prepared and 3 green mussels were added into it. Centrifuged of contaminants microalgae were rinsed with aquadest and then fed on green mussels. Feeding is done daily using a food that has been contaminated with 100 mL cadmium metal for each jar. The process of bioaccumulation of metals is carried out with variations of immersion time that is for 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days. Every day, there is a replacement of sea water. Sampling is done daily until day 10. Method with modification by Metial *et al.* (2016). Green mussels used for the

bioaccumulation process are green mussels derived from Laki Island.

Determination of Metals Content

The green mussels was removed with a wet destruction process using 5 mL of HNO_3 65% and 1 mL H_2SO_4 97% that was applied to the dissection result in beaker glass. Next, heating process using hotplate with temperature 70 - 100 ^{0}C until filtrate become clear. After cooling, the destruction results are filtered and diluted to a 50 mL measuring flask using 0,1 N HNO_3. The solution is ready for measurement of metal content using Atomic absorption spectroscopy (AAS) instrumentation with wavelength of 228.8 nm (Ishak A. R. *et al.*, 2020). The determination of metal content was carried out on the samples, namely: control, bioacumulation of Laki Island, Laki Island water depuration, Muara Kamal water drainage depuration, and acid immersion depuration.

Determination of Food Concentration

Botryococcus Braunii microalgae is centrifuged and then dried in an oven at 105 ° C. A total of 0.5 g microalgae were added 10 mL of aqua regia (HNO_3: HCl, 3: 1) and 1 mL $HClO_4$. The mixture is heated over hotplate at 80 ° C. Thereafter the solution was diluted with 0.1N HNO_3 in a 50 mL measuring flask up to the boundary mark and analyzed its metal content with AAS instrumentation at 228.8 nm Wavelength.

AE / Assimilation Efficiency Determination

The ability of green mussels to accumulate Cd from the food pathway is represented as assimilation efficiency. The efficiency of assimilation is the percentage of contaminants absorbed or digested by the biota body after feeding for 24 hours. Method with modification by Metian *et al.* (2016). Green mussels are placed on a 1 L beaker glass containing contaminant-free seawater. Furthermore, green mussels were fed by Botryococcus Braunii. After feeding, the green mussels were subsequently transferred to an aquarium containing 25 L of contaminant-free seawater. Green mussels were sampled at 0, 1, 2, 3, 4, 5, 20, 21, 22, 23, and 24 hours and measured the metal content of Cd with AAS instrumentation.

Depuration

Aquarium filled with sea water as much as 40 L and sea water replaced every day for 3 days. The green mussels used is derived from the bioaccumulation process that previously have done. In the process of depuration required 9 green mussels with each day taken 3 green mussels.

Decontamination of Cd in *Perna viridis* in Acid

The process of this depuration using two solvents namely acetic acid and citric acid. Method with acid modification by Metian *et al.* (2016). This experiment was conducted on three different containers according to the variation of acid concentration used ie 0.375%, 0.75%, and 1.125% in each type of acid. Selection of green mussels is done by variation of time 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 150 minutes in each container. Acetic acid used to soak green mussels is a commonly used vinegar acid in households. Meanwhile, citric acid used to immerse shell is a citric acid commonly used in households. After the immersion process is complete, then the process of determining the metal content. Replication of biotas similar with the uptake and depuration experiment.

Results and Discussion

Observation of Green mussels from Laki Island

Bioaccumulation of Cadmium Heavy Metals in Food Pathway. The level of cadmium metal present in the body of the green mussels as a control is 0.16 mg/kg. After knowing the level of metal control on the green mussels, then heavy metal ions content of cadmium in green mussels in bioaccumulated results detected. The

cadmium content in bioaccumulation process shown in Fig. 1 below.

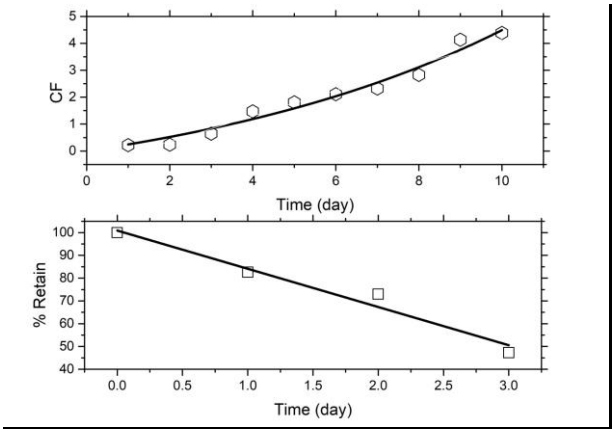

Figure 1. Bioaccumulation and elimination of Cd from food pathway

Cd accumulation through the feed path way was increased as time increases and has not experienced steady conditions. Within 10 days cadmium accumulates 10 times compared to its concentration in water. The uptake constant (k_{uf}) was obtained through this experiment is 0.478 day^{-1}. Compare with previous study conducted by Reinardy H. C. *et al.* (2011), the uptake constant of Cd for different organism i.e zebra fish is 0.12 day^{-1}. All those values shows the uptake constant of Cd were varied depend on the organism. According to Herve-Fernandez *et al.*, (2010), Cd Bioaccumulation through water pathway is very slow and steady state is reached after 43 days. At this condition, the CF state was 4.38. The Cd is quickly released from the viridis pen body for 3 days trial. The elimination of Cd in the body *Perna viridis* after undergoing the process of bioaccumulation through the feed passage lasts for 3 days. The elimination constant (k_{ef}) was 0.17 day^{-1}. The bioaccumulation factors via the food pathway (BCF_f) was calculated using the equation by Suseno *et al.* (2010)

$$BCF_f = \frac{k_{uf}}{k_{ef}} \quad (1)$$

The result of calculation was obtained the ability of *Perna viridis* to accumulate Cd through the food pathway was 2.81 with consideration this value was varied depend on the organism and its metabolism (Reinardy H. C. *et al.* 2011).

In bioaccumulation the contribution via the food path way is not yet clear and depends on the AE value. AE value is the efficiency of digestion of contaminants through the food pathway (Metian *et al.*, 2016). According to Dutton and Fisher (2011), metal bioaccumulation models often rely on the efficiency of assimilation (AE) of digestible metals and constants. Fig. 2 was shown the Cd release process stated in AE.

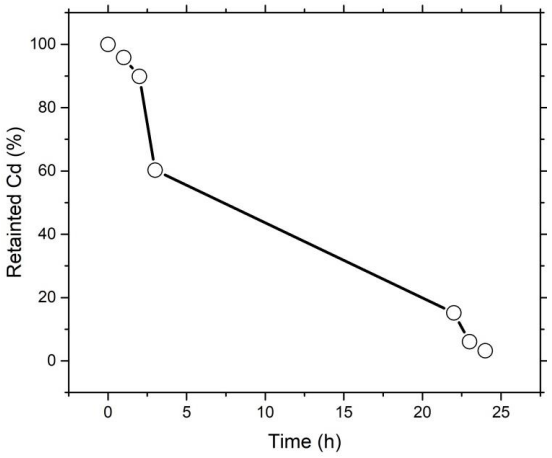

Figure 2. Determination of AE

In this experiment AE was determined after the 24 hour release process. The experimental results show that the AE value is 4.38% day^{-1}. This shows that only 4.38% of the Cd contained in the feed can be digested and bioaccumulated.

Previous experiments regarding the bioaccumulation of Cd by *Perna viridis* through the water pathway was obtained the average of value of BCF_w is 61.7 (Suseno, 2006). To determine the total bioaccumulation ability of Cd by *Perna viridis*, an equation was used (Suseno *et al.*, 2010)

$$BAF = BCF_w + AE.BCF_f \quad (2)$$

The recapitulation of parameters biokinetic Cd and BF calculation were shown in Table 1

Table 1. Biokinetic parameter

Biokinetic Parameter	Value	Reference
BCF_w	61.7	(Suseno, 2006)
AE	4.38%	This study
BCF_f	4.38	This study
BAF	74.01	This study

The BCF_w value 60.07 founded from previous study is the highest concentration of cadmium in Perna Viridis for pH 8.2 (Suseno, 2006). On the other side, founded from present study the BCF_f value are more lower at 4.38. The differences of BCF value because of pH control and its feeding pathway.

Decontamination Cd from *Perna viridis*

Reducing Cd contaminants that contained in the body *Perna viridis* can be conducted by immersing it within weak acids such as acetic acid or citric acid. The result of experiment were shown at Fig. 3.

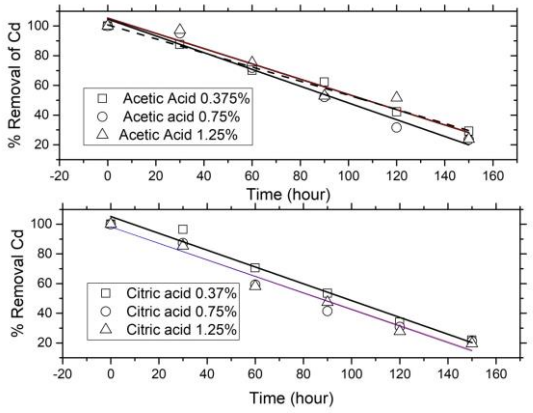

Figure 3. Decontamination process of Cd by acetic and citric acid

The experimental results showed a significant reduction in the use of both decontaminants. This can be applied to seafood processing that will be consumed by humans. Efforts to reduce Cd contamination that are conventionally carried out in seafood restaurants are soaking in a solution of vinegar and citric acid. To find out the effectiveness of this decontamination immersion was carried out for a maximum interval of 150 minutes. Decontaminants used are vinegar and citric acid (0.375-1.25%). The results of the experiment showed that the Cd content in the *Perna viridis* could be reduced to a maximum of 19.53% and 23.81 using decontaminant of citrate and acetate acids respectively

Conclusions

Total bioaccumulation ability of Cd by Perna viridis was 74.01. The best depuration method for the reduction of metal content with no significant decrease in protein is on the deposition of citric acid with a concentration of 0.375%.

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