

Mercury depuration effectiveness on green mussel (*Perna viridis* L.) and blood cockle (*Anadara granosa* L.) from Jakarta Bay using ozone, chitosan and hydrodynamic technique

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Abstract. Depuration has been used to eliminate microorganism and toxic chemical contaminants in bivalve. However, scientific research still needs to discover the effectiveness of depuration. This research aimed at assessing the best depuration effectiveness in decreasing mercury (Hg) concentration level in two species of bivalves, green mussel (*Perna viridis* L.) and blood cockle (*Anadara granosa* L.). The depuration treatments applied 1.5 ppm ozon, 0.5 ppm chitosan, hydrodynamic technique (1.3 m/s), combination between hydrodynamic-ozon, hydrodynamic-chitosan and ozon-chitosan. The experiment were conducted in mini aquaria for 60 minutes. Mercury concentration was measured in 10 g dry weight of green mussel (4.05 ± 0.020 ppm) and blood cockle (3.27 ± 0.666 ppm). The result showed that mercury depuration were highly effective by combination of ozone-chitosan in green mussel (96.51%) and 1.5 ppm ozone in blood cockle (87.06%).

1. Introduction

The abundance of Indonesia marine resources was nowadays putting fisheries sector as a major concern. Annual statistical reported by the General Directorate of Fisheries, Ministry of Marine Affairs and Fisheries (DJPB KKP RI) in the latest version that the mariculture is on the top position with 8,3 billion ton production while others are the 1-2 million ton. Bivalve molluscs (e.g. mussels, oysters and clams) commonly named as shellfish is the top commodity produced from the coastal Indonesia following fish and seaweed commodities. Shellfish commodity [1] as many as 48, 449 ton national product within which 25, 935 ton were produced throughout Jakarta Bay, the northern coast of Java.

Despite of its major contribution for shellfish production, Jakarta Bay was founded to be chemically polluted, Riyadi *et al.* reporting that. The concentration of mercury (Hg) is as high as 0.067 ppm in Jakarta Bay while [2] the standard concentration of mercury for security marine product should be less than 0.001 ppm [3]. Therefore, Jakarta Bay is not safe for marine production, especially for the shellfish commodity. This problem becomes a concern for the seafood consumers.

Shellfish are filter feeder organisms. They feed on filtered seawater and accumulate food in their mantle. Like other filter feeder organisms, green mussels (*Perna viridis* L.) inhabit substrate by



attaching to wood or bamboo in the industrial cultivation, while the blood cockles (*Anadara granosa* L.) stay in the sediment. Chemical pollution can be found in the shellfish bodies as a contaminant. The shellfish that have been contaminated with mercury are not safe for consumption. This leads to a variety of health problems due to accumulation of heavy metals in the human body. Therefore, the Food and Drug Monitoring Agency (BPOM) [4] has set the value of 1.0 ppm as the maximum levels of mercury contaminant on shellfish to be safe for consumption.

In order to eliminate the contaminants, such as microorganism and their residual toxicity, depuration is commonly conducted on marine products. Depuration, on the lab scale, has been conducted to eliminate heavy metals. The conventional methods applied were soaking, washing and boiling. The latest result was reported by Anacleto *et al.* [5] by using filtered seawater and UV radiation in the three species, *Ruditapes philippinarum*, *Mytilus galloprovincialis* and *Scrobicularia plana*. This research resulted on decreasing level of mercury up to 32.2% in the shellfish body within 6 days of depuration. However, the research on depuration effectiveness are still need to be observed.

Human exposure to mercury contaminants can be endangered with comparative risk of fetal developmental neurotoxicity [6]. The mechanism of mercury exposure come from sea food consumed that contaminated. The aim of the research is to find the effectivity of depuration method by treatments ozone, chitosan, hydrodynamic technique and combination both of treatments.

2. Materials and methods

2.1. Materials

Materials used in this research were green mussel (*Perna viridis* L.) and blood cockle (*Anadara granosa* L.), HNO₃ 65%, HClO₄ (60%), aquades, 1.5 ppm of ozone, and 0.5 ppm of chitosan. Digital refractometer HACH sension5 and Atomic Absorption Spectrophotometer (AAS).

2.2. Methods

2.2.1. Sample collection. Green mussel (*Perna viridis* L.) and blood cockle (*Anadara granosa* L.) were freshly collected from Cilincing and Marunda (Jakarta Bay), DKI Jakarta (Picture 1). Samples were brought to laboratory using container and ice box. Dead or damaged specimens were eliminated prior to mussel size standardization. The green mussels with 2-3 cm in length, 1-1.5 cm in width, and 0.75-1.25 cm in thickness were used as samples. The blood cockle with 4-7 cm in length, 3-4 cm in width, and 1.5-2 cm in thickness were also used as samples.

2.2.2. Depuration experiment. The experiment was conducted in mini aquarium for 60 min with two replications. Weight of fresh sample were 500 g of green mussel and 400 g of blood cockle in used. The treatments were ozon, chitosan, hydrodynamics, combining between hydrodynamics-ozon, hydrodynamics-chitosan, ozon-chitosan, and control. Ozon liquid concentration was 1.5 ppm. Chitosan liquid concentration was 0.5 ppm. The technique of hydrodynamic was approached by pumping the water to constant velocity of 1.3 m/s. Combination of two treatments was conducted together. Physical parameters was measured three times, at 9 am, 2 pm, and 9 pm. Flesh samples were separated from cockle and scaled for preparation. Preparation samples were dried at temperatures of 40 °C for 24 hours, and average water concentration was scaled.

2.2.3. Mercury concentration analysis [7]. Depurated samples (10 g) was added 10 mL HNO₃ 65% and HClO₄ 60% solutions. Samples were soaked for 48 h and warmed for 4 h. Afterwards samples were filtered and reduced into 100 mL liquid measure. Mercury concentration was measured by Atomic Absorption Spectrophotometer (AAS).

2.2.4. Statistical analysis. The results were presented as descriptive statistic. One-way analysis of variance (ANOVA) [8] was used to test the significance of depuration treatment. Post hoc tes was

used to analyze the multiple comparisons among control, treatment of ozone, chitosan, hydrodynamics, and combined between both. Stastical anaylses were performed using the SPSS 22.0 software.

3. Results

3.1. Mercury concentration

Mercury concentration was measured on green mussel and blood cockle under laboratory experiment. Depuration experiment was conducted in mini aquaria set for these conditions, i.e., temperature (28 ± 1 °C), salinity (34.83 ± 2.369 ‰), and pH (7.63 ± 0.152). Mercury concentration was decreased after 60 minutes of depuration experiment and presented in the Table 1.

Table 1. Mercury concentration in green mussel and blood cockle

Treatment	Mercury concentration (mean \pm SD)			
	Green mussel (ppm)		Blood cockle (ppm)	
	Dry weight	Wet weight	Dry weight	Wet weight
Control	4.05 \pm 0.020 ^a	0.83 \pm 0.004	3.27 \pm 0.666 ^a	0.53 \pm 0.109
1.5 ppm Ozone	0.90 \pm 0.024 ^c	0.13 \pm 0.003	0.33 \pm 0.005 ^d	0.07 \pm 0.001
0.5 ppm Chitosan	0.85 \pm 0.004 ^d	0.17 \pm 0.000	0.39 \pm 0.011 ^d	0.09 \pm 0.002
1.3 m/s Hydrodynamic	3.22 \pm 0.032 ^b	0.50 \pm 0.005	0.75 \pm 0.006 ^c	0.16 \pm 0.000
Hydrodynamic-Ozone	0.50 \pm 0.003 ^e	0.17 \pm 0.001	2.09 \pm 0.004 ^b	0.42 \pm 0.000
Hydrodynamic-Chitosan	0.20 \pm 0.002 ^f	0.03 \pm 0.000	0.43 \pm 0.022 ^d	0.06 \pm 0.003
Ozone-Chitosan	0.15 \pm 0.004 ^g	0.31 \pm 0.000	0.45 \pm 0.011 ^d	0.10 \pm 0.002

Note :Mercury concentration was measured in 10 g dry weight and converted in wet weight

The highest concentration of mercury was found in control sample (without any treatment, see Table 1). Concentration of mercury in dry weight was 4.05 \pm 0.020 ppm in green mussel and 3.27 \pm 0.384 ppm in blood cockle. Mercury concentration level decreased in the treatment of ozone-chitosan combination in green mussel and in dissolve ozone in blood cockle (1.5 ppm) .

The effect of depuration method on mercury level in green mussel is depicted in Figure 1. Mercury concentration is significantly ($P < 0.000$) decreased in the order of control > ozon > chitosan > hydrodynamic-ozon > hydrodynamic-chitosan > ozon-chitosan. Combination of ozone-chitosan treatment is the most effective depuration method, decreasing mercury concentration by 96.51%, and then followed by the treatment of hydrodynamic-chitosan (94.94%), hydrodynamic-ozone (87.79%), chitosan (79.11%), ozone (77.46%). Hydrodynamic has the least effective method, decreasing mercury concentration only by (21.53%).

The effect of depuration method on mercury concentration in blood cockle is presented in Figure 2. Concentration mercury are significantly ($P < 0.000$) decreased in the order of control > hydrodynamic-ozone > hydrodynamic > ozone-chitosan > hydrodynamic-chitosan > chitosan > ozone. Combination of ozone treatment is the most effective depuration method, decreasing mercury concentration by 87.06%, followed by the treatment of chitosan (85.07%), hydrodynamic-chitosan (83.71%), ozone-chitosan (82.28%), hydrodynamic (70.03%) hydrodynamic-ozone has the least effective methode, decreasing mercury concentration only by (17.25%).

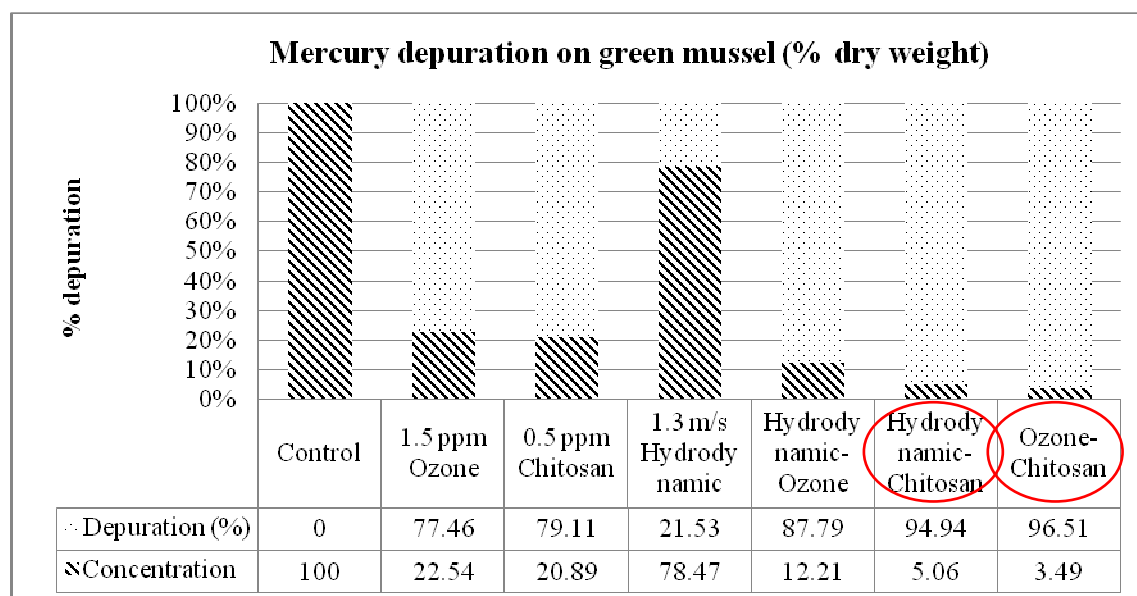


Figure 1. Effect of depuration method on mercury concentration in green mussel

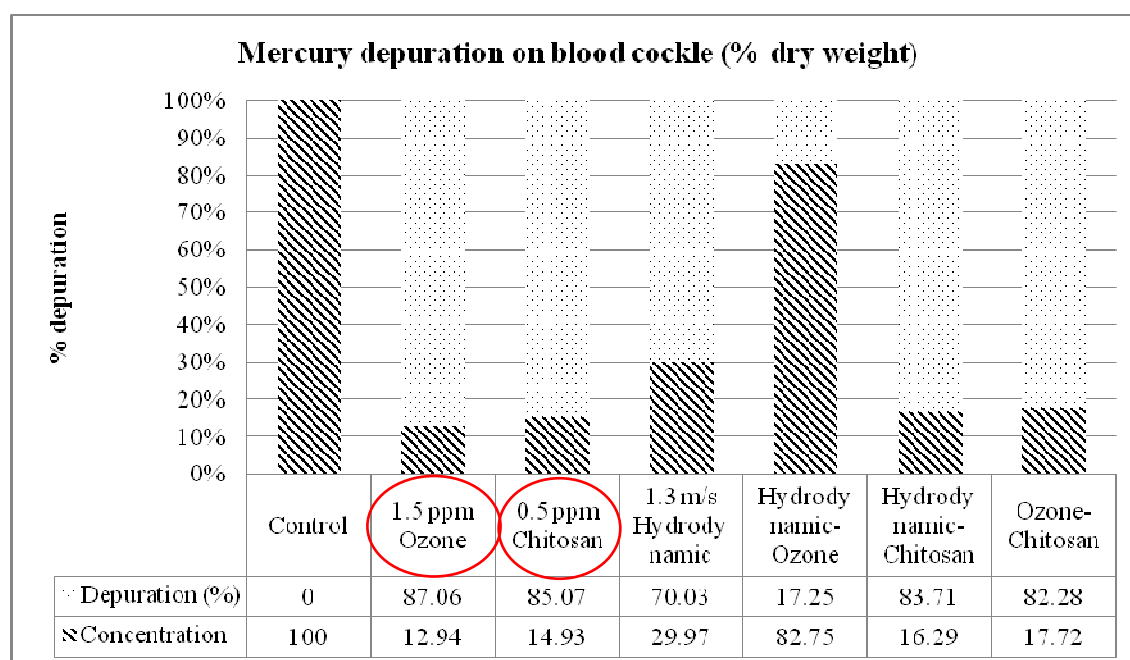


Figure 2. Effect of depuration method on mercury concentration in blood cockle

4. Discussion

Depuration technique was conducted in the laboratory to eliminate microorganism contaminants and their chemical toxic levels. Depuration treatment was reported by Yokohama and Park [9] using the temperature treatment (15°C and 25°C), in five days might reduce Cyanobacterial toxicity. The latest result was reported by Anacleto et al [5] that mercury levels in three species were also reduced, *Ruditapes philippinarum*, *Mytilus galloprovincialis* and *Scrobicularia plana*. The treatment were using filtered seawater and UV radiation and could decrease the mercury levels up to 32.2% within six days of depuration. In this study, three depuration methods were tested. These were hydrodynamic technique, ozone and chitosan in this research. The combination among three types of depuration are used in the group of treatment to find out the depuration effectiveness.

Ozone has been used in the pesticides eliminating process of agricultural production [10] (e.g. on fruits and vegetables). The use of dissolved ozone (1.4-2.0 ppm) is reported that it can eliminate pesticide residues from the surface of vegetable. The low concentration of dissolve ozone are effective to reduce 60-99% residual pesticide in the first 5 minutes. The concentration of dissolved ozone used in this research treatment is low (1.5 ppm).

Chitosan has been used to eliminate mercury from contaminated water. Chitosan particles are reported can decrease the mercury concentration in the water up to $0.29 \pm 91.55\%$, [11], while Kyzaz and Kostoglou [12] reported that effective absorbent chitosan used to adsorb heavy metals from polluted water was microsphere cross-linked chitosan. Its derivative of chitosan, i.e., chitosan beads, has a weak dilute acid in matrix of chitosan bead. This causes $-NH_2$ of chitosan beads has higher affinity than normal chitosan and can increased the heavy metal adsorption. Chitosan beads can be made by dissolving chitosan powder into 5% acetic acid to form a gel. Thus, the use of low chitosan was determined by 0.5 ppm in this research treatment.

The technique of hydrodynamic was approached by pumping the water to constant velocity of 1.3 m/s. Hydrodynamic technique has been used [13] in combination with UV radiation to decrease contaminant in type of bivalves. However, this technique is only intended to remove microorganism contaminants while its use in heavy metals depuration has not been carried out.

The depuration of the experiment was fast and resulted satisfying outcomes. Statistical test conducted on the depuration rate showed statistical different ($P < 0.000$) between control and the treatment in both samples. Mercury concentration in green mussel were decreased with percentage up to 96.51%, from 4.05 ppm to 0.15 ppm, by the treatment of ozon-chitosan. Meanwhile, the treatment of ozone in blood cockle decreased mercury concentration from 3.27 ppm to 0.33 ppm, with percentage up to 87.06% are decreased by the treatment of ozon-chitosan up to 96.51% (4.05 ppm to 0.15 ppm) in green mussel and the treatment of ozone in blood cockle can be decrease mercury level up to 87.06% (3.27 ppm to 0.33 ppm). The depuration treatment in green mussel were significantly different among control and groups of treatment. The result in blood cockle showed different response ($P < 0.000$) between control and groups of treatment while variance of ozon and chitosan, also combination between hydrodynamic-chitosan and ozone-chitosan have not significantly different. However, the high depuration rate was showed in ozon-chitosan treatment (green mussel) and ozone treatment (blood cockle). The low depuration rate were on hydrodynamic (green mussel) and hydrodynamic-ozone (blood cockle).

5. Conclusion

The effective of mercury depuration in green mussel were by the treatment of ozone-chitosan, hydrodynamic-chitosan and hydrodynamic-ozone, in respect to 96.51%, 94.94% and 87.79%. The treatment of ozone, chitosan, hydrodynamic-chitosan and ozone-chitosan, in respect to 87.06%, 85.07%, 83.71% and 82.28% were effective to decrease mercury levels in blood cockle.

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