The effects of using shell filters in the process of depuration for the survival of *Anadara* sp.

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Abstract. *Anadara* sp. is one of the shellfish that has a source of animal protein is high and has a high economic value. However, to obtain a safe source of food, the products must meet the standard by the government, one of which is the limitations in heavy metals in the shells. In the standard of sanitation of shellfish is required to do the depuration process to remove the contaminants be it bacteria or heavy metals. In this study, randomized design with 5 treatments was used: P0 (control / without filter), P1 (25 % filter with shells), P2 (50 % filter with shells), P3 (75 % filter with shells), P4 (100 % filter with shells). Each treatment was replicated 4 times. The results showed that filtering of shell in depuration process could cause the highest shell death for 24 hours occurred in P4 of 24.39 % and the highest death during 48 hours also happened at the treatment of P4 which was equal to 61.71 %. During the research, water quality measurement was measured at 29-30 °C, pH 7.2-7.7, dissolved oxygen (DO) 4-4.4 mg/L and salinity 28-30 ppt.

1. Introduction

Cockles are potentially contaminated by heavy metals due to their filter feeder nature, so this biota is often used as a test animal to monitor the degree of the accumulation of heavy metals in marine organisms [1]. One type of shellfish that is abundantly found in Indonesian waters is the blood cockle (*Anadara* sp.). The blood cockle is a type of shellfish that has a high economic value and in general is a source of seafood in Southeast Asia and some Pacific regions [2]. Blood cockles have several uses, one of which is processed as food, however its shells can cause considerable waste. The discarded shells can act as a source of calcium. There has not been much utilization of blood cockle shells, except as raw materials for making local souvenirs.

Cockle shells contain 66.70% calcium carbonate [3], the high content of calcium carbonate can make use of them to be used as a water purifier. The calcium carbonate in shellfish can clean water, and can even reduce iron, manganese and other metals [4].

This method of depuration is principally a purification step of a biota under a controlled condition. The shellfish that are contaminated by heavy metals should be cleaned or depurated, the purpose of this depuration process is to reduce the risk of bacterial contaminants and some heavy metals harmful to human health [5].

Toxic depuration in shellfish is one of the efforts to avoid the impact of lead accumulation in shellfish that has the potential to harms humans who consume it [6]. According to the Decree of the Minister of Marine Affairs and Fisheries Number: KEP.17 / MEN / 2004, the depuration process is a
cleaning process that uses a purifier with a recirculation system. This research uses a depuration method by using powdered shells as the filter. The shell contains 66.70 % calcium carbonate so as to absorb the heavy metal lead [3]. The high content of calcium carbonate can make the shellfish shells be used as a water purifier. The calcium carbonate in shellfish can clean water, and can even reduce iron, manganese and other metals [4].

2. Methodology

This research was conducted on 30th August to 30th September 2016 at the Faculty of Fisheries and Marine of the University of Airlangga, Surabaya. The testing of heavy metal content was conducted by using an Atomic Absorption Spectrophotometer (AAS).

The tools used during research were five tubs (95 cm x 70 cm x 75 cm), 20 plastic racks (42 cm x 33 cm x 14 cm), five plastic tubes with a height of 45 cm and a diameter of 26 cm, five water pumps, five flow meters, five UV meters, sponge filtration, pipes, hoses, scooters, measuring cylinders, digital scales and water quality gauges (thermometers, refractometers, pH pens, DO test kits, nitrate and nitrite test kits).

The materials used in the study where blood cockles (A. antiquata) originating from Sedati, Sidoarjo. The blood cockles used were 7-8 grams in weight and 10 kg of the shellfish were placed in each tub. Four shelves were placed in each tub along with sea water with a salinity of 30 ppt.

2.1. Work procedures

The tubs were cleaned by using liquid soap followed by chlorine and then rinsed with clean water. The washed equipment was soaked with chlorine solution at 150 mg / l for 12-24 hours and rinsed with fresh water until the chlorine odor was gone [7]. The dried tubs were filled with 400 liters of seawater in each tub. After filling in the water, the installation of aeration, UV, water pumps, flowmeters and filters on each tub was carried out. The outer shells that were used as a filter were firstly washed and then pounded into a powder to a size of 0.5-2 cm. Next, the shell powder was placed in each tube as a filter. The amount of shell powder used was 15.3 kg for the 100 % filter, 11.5 kg for the 75 % filter, 7.7 kg for the 50 % filter and 3.8 kg for the 25 % filter.

The method used in this research was the experimental method to find out the effects of the shell filter in the depuration process in decreasing the cadmium content of the shellfish. The experimental design was performed using Completely Randomized Design with a single source of diversity ie percentage of shell powder. This study used five treatments with four replications in each treatment. The treatments in this study are as follows:

P0: Without Filter Shells
P1: Using Filter Shells 25 %
P2: Using Filter Shells 50 %
P3: Using Filter Shells 75 %
P4: Using Filter Shells 100 %

3. Results and Discussion

Shellfish are biota that are potentially contaminated by heavy metals due to their filter feeder nature, so it is often used as a test animal to monitor the degree of accumulation of heavy metals in marine organisms [1]. The heavy metal cadmium will turn into poison for aquatic life when dissolved in the waters at a certain concentration. Heavy metals that enter the body of animals such as shells are generally unremoved from the shell's body [1]. The accumulation of heavy metals in the body of aquatic animals occurs because the rate of the collection of heavy metals by aquatic organisms is faster than the release process[8].

Based on the results of the research, the blood cockle Anadara sp. samples contained an average Cd content of 0.3598 ppm and Pb content of 2.144 ppm prior to being decontaminated. According to the Decree of the Minister of Marine Affairs and Fisheries Number: KEP.17 / MEN / 2004 regarding sanitary sanitation systems in Indonesia, there is a standard of the quality of live shellfish and its
Processed products which are consumed directly. The threshold limits of heavy metals in processed products for consumption must meet the requirements of Cadmium (Cd) at a maximum of 1.0 ppm and lead (Pb) at a maximum of 1.5 ppm.

The process of depuration was conducted by using shell powder of different percentages as filters which was carried out for 48 hours [9]. Seawater was circulated continuously throughout the depuration process for about 48 hours. The percentage of death or mortality is calculated every 24 hours for 48 hours. The value of the mortality gap percentage is the number of dead shellfish divided by the number of shellfish at the beginning, as in the formula:

\[ Mo = \frac{Mt}{N_0} \times 100\% \] (1)

Information:
Mo: mortality
Mt: number of dead
N0: population

Table 1. Mortality of blood cockles.

| Treatment | Mortality ± SD 24 hours | Mortality ± SD 48 hours |
|-----------|-------------------------|-------------------------|
| P0 (Control) | 1.04 % c ± 0.6          | 3.12 % c ± 1.89          |
| P1        | 6.33 %b ± 3.82          | 30.61 % b ± 5.16         |
| P2        | 6.65 %b ± 5.35          | 31.33 % b ± 12.44        |
| P3        | 10.98 %b ±1.01          | 58.01 % a ± 0.84         |
| P4        | 17.71 %a ± 5.04         | 58.65 % a ± 3.06         |

Description: Different superscript on the same column shows a noticeable difference (p <0.05).

Based on the analysis of variance (ANOVA) and Duncan's test, the results showed that the highest mortality found in the 48 hour treatment of P4 (58.65%) was not significantly different to the 48 hour treatment of P3 (58.01%). While the lowest mortality was found in the 24 hour treatment of P0 (1.04%).

Figure 1. Mortality graphics.

High mortality was caused by the muddier water in the maintenance media so that the respiration process of the shellfish was disrupted and evidenced by the increasing Total Suspended Solid (TSS) content. The water quality measured during research is presented in table 2.
Table 2. Water quality.

|       | Before |          |          |          |
|-------|--------|----------|----------|----------|
|       | TDS (mg/L) | TSS (mg/L) | BOD (mg/L) | COD (mg/L) |
| P1    | 26280   | 12       | 185.73   | 3241.07  |
| P2    | 26280   | 12       | 185.73   | 3241.07  |
| P3    | 26280   | 12       | 185.73   | 3241.07  |
| P4    | 26280   | 12       | 185.73   | 3241.07  |
| P5    | 26280   | 12       | 185.73   | 3241.07  |
|       | After   |          |          |          |
|       | TDS (mg/L) | TSS (mg/L) | BOD (mg/L) | COD (mg/L) |
| P1    | 27150   | 13.5     | 121.945  | 1410.715 |
| P2    | 27300   | 19       | 118.87   | 1525.51  |
| P3    | 26830   | 39.5     | 140.64   | 1761.48  |
| P4    | 25370   | 142.335  | 158.925  | 1908.165 |
| P5    | 27150   | 62       | 171.285  | 2207.905 |

The above table shows that in the treatment of P4 the amount of Total Suspended Solid (TSS) at the 48th hour was 142.335 mg / L. TSS waters can reduce the value of waters and affect the lives of the organisms [10]. The value of TSS 81-400 mg/L indicates that the suspended solid content was unsuitable for fishery[11]. TSS will affect the clarity of water, which in turn will affect the penetration of light and ultimately will affect primary productivity. High TSS can block the entry of sunlight into the water, which will interfere with the process of photosynthesis and cause the decrease of dissolved oxygen released by plants into the water [10].

4. Conclusion
The use of blood cockle shells as a filter had an effect on the number of deaths of the shellfish during the depuration process. The highest death occurred in P4 where 100% of the shells were used as a filter.

5. Reference
[1] Yennie Y, Murtini JT 2016 J Mar. Fish. Sci. Ind 2 27–32 (In Indonesia)
[2] Shigeru S 2009 ICMSS09 1-9
[3] Ulysses M, Lawrence RM, Valeriano B and Shigeru S 2009 Comparative PSP toxin Accumulation in Bivalves, Paphia undulata and Perna viridis in Sorsogon Bay, Philippines (France: Nantes) p 56
[4] Siregar S M 2009 Utilization of shells and epoxy resins on the characteristics of polymer concrete (MS thesis) p 89
[5] Simaremare S R 2013 Healthy Work Envi. 3 (In Indonesia)
[6] Gabr HR, Gab-Alla A-F 2008 Oceanologia 50 83–93
[7] Chen CY, Chou HN 2001 Toxicon 39 1029–34.
[8] Isnansetyo A Kurniajutty 1995 Culture technique phytoplankton zooplankton : natural feed for the seeding of marine organisms (Yogyakarta : Kanisius) p 67–71 (In Indonesia)
[9] Darmono 2006 Environment and pollution: relation to toxicology of metal compounds (Jakarta : University of Indonesia) p 67
[10] Murdinah M 2009 Bull. Mar. Fish. Postharvest Biotechno. 4(2) 61–71
[11] Sumawidjaja K 1973 Limnology (IPB: Bogor) p 88 (In Indonesia)