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Production and Application of Chitosan to Reduce Organic and Heavy Metal Content (Pb and Cd) on the Aquaculture Media

Arief Taslihan[1], Suryati[2], Ratna Indria Sari[3]
[1,2,3]Main Center of Brackishwater Aquaculture Development, Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries of Republic of Indonesia,
Jl. Cik Lanang Bulu Jepara 59418. Tel. +62-291-591125, Fax. +62-291-591724. [1]Email: arieftaslihan@yahoo.com

Abstract. Chitosan has an amine and hydroxyl groups which can act as coagulants to bind organic and heavy metals from industrial waste into floc forms that are very easily separated from their original media. Shrimp and crab shells waste are used as the raw material for chitosan production, which is obtained from the shrimp processing unit (cold storage) in Pati, Central Java. This research aims to increase the economic value of fishery waste. The principle of extraction for shrimp and crab shells is relatively the same both in physical and chemical treatment. The production process of chitosan is drying to a water content of 10-13%, demineralization, deproteination and deacetylation. The yield in this activity were 14.3% for shrimp shell and 20% for crab shell. The decrease of dissolved organic matter on aquaculture media (water pond) after application of chitosan at 100 mg/L is to 18%. The decrease of heavy metals in aquaculture media was reduced by 20% and 18% for each Cd and Pb metal respectively in the application of 50 mg/L chitosan. In the application of 100 mg/L, the decrease in heavy metal content of Cd and Pb was respectively 29% and 35%. Meanwhile, the decrease in Cd and Pb heavy metal content in the aquaculture media with the application of 200 mg/L chitosan was obtained by 46% and 37%.

Keywords: Chitosan, Total Organic Matter, Heavy Metal. **Running title:** Production and application of chitosan

INTRODUCTION

The intensification of the ponds results in an increasing load of organic waste, thereby accelerating the degradation of environmental quality. The high level of organic matter triggers various types of diseases. Several materials have been introduced to eliminate organic matter in ponds, however they have not been effective. The material that is considered to have the potential to bind organic materials and heavy metals is chitosan ($C_{56}H_{103}N_9O_{39}$).

Chitosan has a molecular weight of 1526.464 g / mol. Chitosan is a polymer compound that is able to bind organic materials and heavy metals because of its polyelectrolytic properties. The advantage of chitosan is that it is non-toxic and biodegradable. Chitosan is also insoluble in water, but dissolves in organic acids, such as acetic acid and formic acid. Chitosan is obtained from processing waste from the cold storage of the fish processing plant through deacetylation with a high concentration of alkaline, washing, drying and flouring/powdering.

Chitosan is a bioactive compound found in crustacean shells such as shrimp and crab. The technique of making chitosan is generally carried out by a physical process in the form of drying shrimp and crab shells, then followed by a chemical process which includes deproteination, decalcification and deacetylation. The reason for using chitosan comes from shrimp waste because of its amine and hydroxyl groups that can function as coagulants. The coagulant can bind heavy metals from industrial waste into flocks which are easily separated from the original media.

Indonesia is a country that is famous for abundant marine products and have a very wide water area and make it as one of the fisheries commodity-exporting countries such as crab and shrimp. Most of these commodities are exported in frozen conditions, where the head and shell have been separated, leaving shell waste that has the potential to pollute the environment. In 2006, Indonesia produced 11,008 ton of crab, of which 85% was shell waste.

Shrimp and crabs belong to the phylum Arthropoda and the Crustacean class, most of which have an external skeleton composed of chitin and reinforced by calcium carbonate materials. The chitin content of shrimp waste (head, skin, and tail) reaches about 50% of the weight of the shrimp (Widodo et al., 2005, in Purwanti 2014) so that this shrimp waste can be used as raw material for producing high value chitin, chitosan, and their derivatives. (Rachmania, 2011 in Purwanti 2014). One alternative to take advantage of the shell waste is to process it into a new product that has economic value, namely chitosan.

Chitosan is also a polymer material that burns easily without leaving residue. If heavy metals are easily absorbed by chitosan which then removes the absorbed metal by burning this is the most economical way without requiring elution again. Therefore chitosan is very good for reducing the metal ion content in water.

Seo et al., (2002) in their research used water-soluble chitosan (chitosan with lower molecular weight) to absorb various metal ions. As a result, heavy metal ions such as Cr, Fe, and Cu have been successfully absorbed by the water-soluble chitosan.

Considering the important role of chitosan, and the easy access to raw materials as a source of chitosan, this material has the potential to be produced through engineering activities. The objective of the activity is the production of chitosan from two raw materials, shrimp shell and crab to reduce organic matter and heavy metals Pb and Cd.

MATERIALS AND METHODS

Study Area

The raw materials used for chitosan production are shrimp shells (Figure 1) and crab shells (Figure 2) which come from the shrimp processing unit (cold storage) in Pati, Central Java. Meanwhile, the aquaculture media water used comes from the KB1 plot of National Shrimp broodstock Centre (NSBC) pond, Brackish Water Aquaculture Main Center, Jepara. Chitosan is produced by extraction method. Total organic matter (TOM) and heavy metals content were analyzed in the chemical laboratory of the Brackish Water Aquaculture Main Center, Jepara.

Figure 1. Shrimp shells from the shrimp processing unit (cold storage) in Pati, Central Java.

Figure 2. Crab shells from the. shrimp processing unit (cold storage) in Pati, Central Java.

Procedures

Physical Process

Shrimp and crab shells that will be used as raw materials are washed under running water and then dried in the sun for about 1 week. After the shrimp shell is dry, with a water content of about 10-13%, the process is followed by homogeneation using a grinding machine.

Demineralization Process

The dried shrimp and crab shell flour was soaked in 2 M HCl (technical HCl) solution at room temperature for two days. After that, it was washed to a neutral pH and then oven-dried at 80 ° C for 24 hours.

Deproteination Process

The deproteination process was carried out by soaking the shrimp and crab shell flour after demineralization in 3% NaOH solution for 30 minutes at 80 °C. After that, it was washed to a neutral pH and then oven-dried at 80 ° C for 24 hours.

Deacetylation Process

The deacetylation process was carried out by soaking the shrimp and crab shell flour after deproteination in 50% NaOH solution for 90 minutes at 120 °C. After that, it was washed until neutral pH and then oven-dried at 80 ° C for 24 hours.

Chitosan Application

Chitosan was applied by inserting 1000 mL of media water into 20 containers. 10 containers for dissolved organic matter analysis and another 10 containers for heavy metal analysis. Treatment conditions in the media water of the efficacy test of chitosan to reduce dissolved organic matter and heavy metals content are as shown in Table 1. After three days of application, a brown sediment was formed at the bottom of the container. For the next step, the media water sample was filtered using a glass microfiber filter.

Table 1. Test sample conditions.

Treatment	+ Cd	+ Pb	+ Chitosan
Control 1			
Control 2			
Spike Control 1	50 µg/L	50 µg/L	
Spike Control 2	50 µg/L	50 µg/L	
Chitosan 50 mg/L (1)	50 µg/L	50 µg/L	50 mg
Chitosan 50 mg/L (2)	50 µg/L	50 µg/L	50 mg
Chitosan 100 mg/L (1)	50 µg/L	50 µg/L	100 mg
Chitosan 100 mg/L (2)	50 µg/L	50 µg/L	100 mg
Chitosan 200 mg/L (1)	50 µg/L	50 µg/L	200 mg
Chitosan 200 mg/L (2)	50 µg/L	50 µg/L	200 mg

Note: Heavy metal spiking is done because the heavy metal content in the water is quite low. The container is left for 3 days for further analysis in the laboratory.

Dissolved Organic Matter analysis

Dissolved organic matter content was known by analyzing the total organic matter using the titrimetric method specifically Permanganate reverse Titration. The principle of analysis of total organic matter (TOM) is based on the fact that almost all organic matter can be oxidized using Potassium manganate(VII) or Potassium dichromate compounds. The oxidizing agent used in TOM determination was $KMnO_4$, acidified with H_2SO_4 and boiled for a while.

Heavy metals (Pb and Cd) analysis

Heavy metal (Pb and Cd) analysis was performed using the Graphite Furnace AAS (GF AAS) instrument. Sample preparation is done by extracting the sample into the organic phase using 1% APDC and MIBK solutions.

RESULTS AND DISCUSSION

Chitosan Production

The production of chitosan from shrimp and crab shell extraction produces chitosan chunks in beige color which are then mashed to make it easier to apply (Figure 3).

Figure 3. Chitosan product.

The yield of chitosan flour produced by extraction of shrimp shells is relatively lower than that of crab shells. The results of chitosan obtained from crab shells can reach

20%, while the chitosan obtained from shrimp shells is only 14.3% (Table 2).

Table 2. Chitosan yield obtained through the extraction process from crab shells and shrimp.

Raw Material	dry weight (kg)	Chitosan Product (kg)	Yield percentage (%)
Shrimp shells	49	7	14.3
Crab shells	25	5	20.0

Testing the reduction of dissolved organic matter content

The test aims to determine the effect of chitosan application on the reduction of organic matter in pond water, as well as to determine the effective content of chitosan that is applied. The results of the tests are presented in table 3.

Table 3. Reduction of pond organic matter through the application of chitosan.

Treatment	TOM (mg/L)	Average (mg/L)	Change of TOM
Control 1	151.08	149.28	
Control 2	147.47		
Spike Control 1	217.29	242.87	
Spike Control 2	268.45		
Chitosan 50 mg/L (1)	247.38	246.48	-1%
Chitosan 50 mg/L (2)	245.58		
Chitosan 100 mg/L (1)	180.57	200.07	18%
Chitosan 100 mg/L (2)	219.56		
Chitosan 200 mg/L (1)	255.81	237.15	2%
Chitosan 200 mg/L (2)	218.49		

Testing the reduction of heavy metal (Pb and Cd) content

The test was carried out on a pilot scale to measure the content of chitosan which were effective in reducing heavy metals Cd and Pb. Test results of chitosan application on the reduction of heavy metal cadmium (Cd) showed that giving chitosan significantly decreased the heavy metal content in water media. The amount of heavy metal that has been bonded is directly proportional to the chitosan content. The chitosan treatment at the level of 50 mg / L decreased 20% of the heavy metal Cd content in water, at the level of 100 mg / L it was able to reduce 29%, and at the level of 200 mg / L it was able to reduce up to 46% (Figure 4; Table 6).

Table 4. Reduction of heavy metal Cadmium (Cd) through application with chitosan.

Treatment	Cd (µg/L)	Average (µg/L)	Change of Cd (µg/L)	Percentage of change (%)
Control 1	0.64	0.41		
Control 2	0.18			
Spike Control 1	22.70	22.06		
Spike Control 2	21.43			
Chitosan 50 mg/L (1)	18.04	17.67	4.39	20%
Chitosan 50 mg/L (2)	17.31			
Chitosan 100 mg/L (1)	15.97	15.61	6.46	29%
Chitosan 100 mg/L (2)	15.25			
Chitosan 200 mg/L (1)	11.30	12.02	10.05	46%
Chitosan 200 mg/L (2)	12.74			

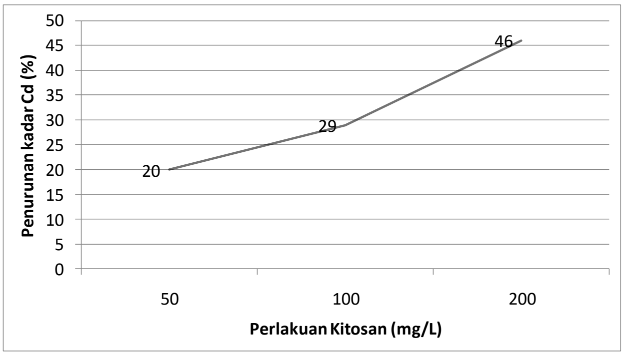

Figure 4. Reduction of heavy metal Cadmium (Cd) in pond water treatment with various doses of chitosan 50, 100, and 200 mg / L.

In addition to reducing Cd, chitosan effectiveness testing was also applied to reduce heavy metal Pb. The test results of chitosan application on the reduction of heavy metal lead (Pb) show that giving chitosan significantly reduced Pb levels in water media (Table 5).

Table 5. Reduction of heavy metal Lead (Pb) through application with chitosan.

Treatment	Pb (µg/L)	Average (µg/L)	Change of Pb (µg/L)	Percentage of change (%)
Control 1	0.265	0.25		
Control 2	0.24			
Spike Control 1	28.366	28.71		
Spike Control 2	29.061			
Chitosan 50 mg/L (1)	21.988	23.46	5.26	18 %
Chitosan 50 mg/L (2)	24.927			
Chitosan 100 mg/L (1)	16.195	18.77	9.94	35 %
Chitosan 100 mg/L (2)	21.354			
Chitosan 200 mg/L (1)	19.207	18.07	10.65	37

		%
Chitosan 200 mg/L (2)	16.927	

The Pb level that was successfully bound was directly proportional to the chitosan content. The chitosan treatment at the level of 50 mg / L reduced the heavy metal content of Pb in water by 18%, at the level of 100 mg / L it was able to reduce 35%, and at the level of 200 mg / L it was able to reduce up to 37% (Figure 5).

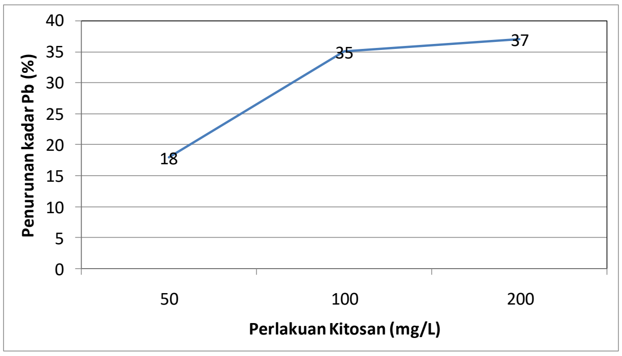

Figure 5. Reduction of heavy metal Lead (Pb) in pond water treatment with various doses of chitosan 50, 100, and 200 mg / L

Discussion

Chitosan products vary widely depending on the source of the raw material. The thickness of the shells greatly affects the amount of chitosan products that can be produced. The production of chitosan from shrimp shells in this study was relatively higher than the production of chitosan reported by Al-Manhel (2016) which obtained a yield of 12.93%, but lower than that reported by Hossain and Iqbal (2014) which was able to reach 15.40% of chitosan from waste shrimp. Even though they both use shrimp shells, the resulting yield can be different. The differences with other researchers may be due to the different procedures used. However, what was produced was not too different. The yield of chitosan in crab shells was higher than that in shrimp shells, possibly because the crab shell was thicker and denser than shrimp shells.

Testing the effectiveness of the application of chitosan in this study resulted in a new technology for eliminating organic waste in water. Chitosan is significantly able to bind both organic matter and heavy metals Cd and Pb in media water, so that it can be used in aquaculture. Chitosan contains free amino groups in the carbon chain which are positively charged so that the molecule is resistant to mechanical stress. It is this free amino group that provides many uses for chitosan (Knorr 1982). The ability to bind organic materials and heavy metals is due to chemical bonds (Seo et al., 2002). The results of the test on the absorption of organic matter, heavy metals Cd and Pb can be seen, chitosan can absorb organic matter up to more than 20%, and heavy metals up to 37% Pb, and up to 40% for Cd. The sigmoid line trend on the chitosan application curve to the reduction of heavy metal Pb indicates that the chitosan content will reach the optimum point in reducing the heavy metal Pb content. No matter how much chitosan is given, it will no longer increase the uptake of the heavy metal Pb. Meanwhile, the trend line that is still straight on the chitosan application curve towards the reduction of heavy metal Cd shows that the higher the chitosan content will be able to absorb the larger Cd heavy metal. Based on these results, it can be stated that chitosan has a good chance of reducing heavy metals in waste. This application can be used in aquaculture environments, especially for the needs of water supply for hatcheries. The source of raw water for supply in hatcheries is often contaminated with heavy metals, this is because of the potential for these metals or because of pollution from industries located around hatcheries. In addition, chitosan in shrimp ponds can

also be applied as a component of a waste treatment plant to reduce pollution of public waters. As a waste treatment material, chitosan is a material that does not cause residue and is not harmful to the environment.

CONCLUSIONS

The results of this research concluded that the chitosan yield from crab shells was 20%, higher than the chitosan from shrimp shells which was 14.3%. The application of chitosan was effective at giving 100 mg / L to the aquaculture media water with a decrease in dissolved organic matter by 18%. Application of chitosan 50, 100, and 200 mg / L in aquaculture media water (in heavy metal spikes Pb and Cd) can reduce Cd metal content by 20%, 29%, and 46%. Meanwhile, the application of chitosan 50; 100; and 200 mg / L in aquaculture media water (in heavy metal spikes Pb and Cd) can reduce Pb metal content by 18%, 35%, and 37%.

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