CHARACTERIZATION of CHITOSAN fromthe WASTE of SHRIMP'S SHELL: POTENTIAL as METAL ADSORBENTS

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ABSTRACT: This study aims to obtain a product of chitosan from shrimp shell waste as environmentally friendly adsorbent through the isolation and characterization process. Chitosan is obtained from shrimp shell waste (Peneausmonodon) through deproteinase, demineralization, depigmentation, and deacetylation. The results showed the yield obtained at stage of deproteinase51,72%, demineralization 24.98%, depigmentation 22.71%, and deacetylation 17.73%. The results of moisture test is 6.48%, ash content 0.40% and the degree of deacetylation 73.88%. Test of solubility in water, ammonia and sodium sulfate are insoluble and in hydrochloric acid, nitric acid are slightly soluble while in acetic acid is soluble.
KEYWORDS*:* Chitosan and Peneausmonodon

1. INTRODUCTION

One natural adsorbent that can be used is chitosan.Chitin and Chitosan were abundantly found in crustaceans such as shrimp and crab [1].

The availability of cheap shrimp shell and abundant, many found in traditional markets, have the biodegradable function[2]. Chitin and Chitosan are non toxic and biodegradable [3-4]. According to Lukum, Chitosan which obtained from the waste of shrimp's shell that comes from Gorontalo has deacetylation degree of 80%, as well as able to absorb Pb (II) metal liquid waste of Tolangohula sugar factory in Gorontalo [5-6]. Other researchers have reported that chitosan coagulant is effective in lowering levels of COD and turbidity wastewater of textile industry amounted to 72.5% and 94.9% [1]. Efficiency of chitosan to remove the turbidity of the sea water is greater than the ferrous sulfate and have the same efficiency as alum [7].

Chitosan and its derivatives showed good adsorption ability to arsenic, its products are cheap and easily obtainable [8]. Adsorption of Hg (II) by chitosan and its derivatives are assumed to occur through the interaction of single or mixed: either a chelate or coordination by hanging on the amino group or in combinationwith vicinal hydroxy, electrostatic attraction force occurs in acidic media or ion exchange with protonated amino group [9].

Chitosan and its derivatives are effective and inexpensive as heavy metal absorbent agents [10]. Shrimp is the abundant natural resources in the province of Gorontalo. Observations made in some traditional markets in Gorontalo Province shows that the sales of shrimps in the market was limited to the sale of the meat while the shell is discarded and are left to rot without any utilizationcausing environmental pollution and would damage the aesthetics of the environment. An alternative to overcome this phenomenon is to utilize shrimp shellinto chitosan products. Chitosan is biopolymers that are widely used in various chemical industries, among others, used as a coagulant in waste water treatment, moisturizing ingredients, coating the seeds to be planted, the metal ion adsorbent, anti-cancer/anti-tumor, anti-cholesterol, an additional component of animal feed, as contact lenses, fats solvents and food preservatives [6].

Chitin is not able to stand on its own but join with other compounds in nature. Chitin acetylation is often don't complete naturally, while chitosan is usually still contain acetyl groups with different levels. Therefore, actual chitin or chitosan is basically a co-polymer of N-acetyl-D-glucosamine and D-Glucosamine. The degree of deacetylation of chitin usually have less than 10%. In general, the degree of deacetylation of chitosan is about 60% and about 90-100% for the full deacetylation of chitosan experiencing. This price depends on the raw material of chitin that being used and on the process that being undertaken [12].

Biopolymer mutually bonded with bond β (1 → 4) on N-acetyl-D-glucosamine is chitin. Physical and chemical properties of chitin are white colored, tasteless, odorless, insoluble in water, organic solvents, and aqueous absa. Chitin is derived from the outer frame of Crustaceans (shrimp, crab, lobster, and a small crab attaching), insects, mushrooms and Mollusks [19, 21].

This research aims to make chitosan from shrimp shell waste as a environmentally friendly natural adsorbent. Chitosan produced in this study can adsorbs Hg potentially.

1. RESEARCH METHODS

Materials

Shell samples are obtained from shrimp that come from Gorontalo. The chemicals used in this research is standard solution of $Hg(NO_3)_2$ p. a., hydrochloric acid (HCl) p. a, sodium hydroxide (NaOH) p. a., hydrogen peroxide (H_2O_2), acetic acid (CH_3COOH), ammonia (NH_3) p. a, sodium sulfate (Na_2SO_4) p. a., nitric acid (HNO_3) p. a., aquadest, filter paper, and universal indicator.

Tools

The instrument used in this study are glassware, oven, magnetic stirrer, hotplate stirrer, universal indicator, centrifuge, sieve of 90 mesh, desiccator, furnance, infrared spectrophotometer (FTIR), analytical balance, mortar pestle, porcelain bowls.

Procedure

Isolation of chitin from shrimp shells (Peneausmonodon)

Shrimp shell washed and dried in the open air until it dries. Next crushed using a mortar and filtered with 90 mesh sieve. Isolation of chitin becomes Chitosan [8] this study was conducted with the following stages deproteinasi, demineralization, deacetylation, depigmentation.

Deproteinase process

Dried shrimp shell powder soaked with NaOH 3.5% with 1:10 (w / v) ratio between solvent and sample. The mixture was put into a beaker, heated with temperature of 90°C for 4 hours while stirring with a speed of 50 rpm. The obtained solids washed with distilled water several times until pH is neutral. The solids obtained were dried in an oven at a temperature of 60°C for 24 hours, then cooled in a desiccator and weighed.

Demineralization process

Shrimp shell powder deproteinase results soaked with HCl 1 N ratio of 1: 7 (w / v) at room temperature while stirring for 1 hour. The obtained solids washed with distilled water several times until neutral pH. The solids are dried in an oven at a temperature of 60°C for 24 hours.

Depigmentation process

Powder of demineralized shrimp marinated with H_2O_2 2% ratio of 1:10 (w / v) at room temperature for 5 minutes with stirring. The obtained solids washed with distilled water several times until neutral pH. The solids are dried in an oven at a temperature of 60°C for 24 hours.

Deacetylation process

Synthesis of Chitosan done by deacetylase of the obtained chitin. Results obtained from the process deproteinase, demineralization and depigmentation, and deacetylation process is then performed by soaking the samples into NaOH 50% with a ratio of 1:10 (w / v) at a temperature of 120°C for 1 hour while stirring with speed of 50 rpm. The solids obtained washed with distilled water several times until neutral pH. The solids are then dried in an oven at a temperature of 60 ° C for 4 hours. The calculation of the degree deacetylation do with baseline method Sabnis and Block by FTIR analysis results using the following equation [17].

$$\%\,DD = 100 - \left[\left(\frac{A_{1655}}{A_{3450}}\right) \times 115\right]$$

With :

Value of A (A) = $\log(P_0/P)$

A_{1655} = Absorbance at a wavelength of 1655 cm^{-1} for absorption amide group/acetamide ($CH3CONH^{-1}$)

A_{3450} = Absorbance at a wavelength of 3450 cm^{-1} for the absorption of hydroxyl group (-OH).

Characterization of Chitosan

Characterization of chitosan include: texture, moisture content, ash content, and solubility.

Data analysis

Data analysis in this study uses qualitative descriptive analysis.

2. RESULTS AND DISCUSSION

Isolation of Chitin from shrimp shells waste(Peneaus Monodon)

Shrimp shells obtained from some of the shrimp farms are cleaned, washed until no more dirt sticking together, then dried and pulverized using a blender or mortar and pestle, and sieved to 90 mesh sieve to obtain pink powder of shrimp shells. The results of the sieve is used to obtain chitin. In this study chitin produced by 241 g of 243.5 grams of shrimp shells.

Deproteinase Process

Deproteinase aims to eliminate binding proteins on chitin namely by using the solution of NaOH 3.5%, where the Na^+ ions bind to the end of the negatively charged protein chain and form Na-proteinase. Protein in shrimp shells dissolve in alkaline causing the protein which are covalently bonded with the functional groups of chitin are separated [18]. Stirring and heating processes aims to accelerate the binding of the end of protein chain with NaOH so that the process of degradation and protein deposition perfectly lasted [22]. Solids formed in the process deproteinase has brown color with a mass of 124.66 g resulting in a mass reduction of 116.34 g from 241 g of chitin. The yield generated in the process of deproteinase is 51.72%. These results are consistent with previous studies which stated that the shrimp shell chitin levels above 20% [23].

Demineralization Process

Demineralization process aims to separate the minerals contained in shrimpshells that is calcium carbonate ($CaCO_3$) and calcium phosphate ($Ca_3(PO_4)_2$) [19]. Minerals in shrimp shells is more easily separated than protein because it is only bound physically [24].The demineralization process of 124.66 g chitin of deproteinase result, after this process the demineralized chitin obtained as much as 60.21 g. In this process a reduction in the mass of shrimp shell powder amounted to 64.45 g, so that the yield generated on the demineralization 24.98% dark brown colored. This Indicates that the amount of mineral salts dissolved in shrimp shells in the demineralization process is 75.02%. The process that occurs on a demineralization stage are the mineral contained in shrimp shells react with HCl resulting in the separation of minerals from the shrimp shells. Mineral separation process is shown by the formation of CO_2in gas bubbles form when a solution of HCl is added in the sample [25]. Thereactions between minerals with HCl is as follows:

$$Ca_3(PO_4)_2\,(s) + HCl \rightarrow 3CaCl_2(aq) + 2H_3PO_4(aq)$$
$$CaCO_3(s) + 2HCl \rightarrow CaCl_2\,(aq) + H_2CO_3(g)$$
$$H_2CO_3(g) \rightarrow CO_2\,(g) + H_2O\,(l)$$

Depigmentation process

This stage aims to eliminate the content of the dye in the chitin which include types of carotenoids, the is red-orange astaxanthin[20]. The elimination of dyes made with the addition of H_2O_2 of 2% (1:10 ratio) at room temperature for 5 minutes with stirring. Chitin depigmentation results in powder form is light brown with the mass of 54.75 g of 60.21 g demineralized chitin, mass decreased of 2.46 g happens, so that the obtained yield of 22.71%.

The process of deacetylation of chitin into chitosan

Deacetylation process aims to eliminate the acetyl group (-$COCH_3$) by using strong alkaline solution of NaOH 50% in order to turn into an amine group (-NH_2). The product obtained from this process is called chitosan. The use of alkaline solution with high concentrations and high temperatures during the process of deacetylation can affect the magnitude of the resulting degree of deacetylation [26]. According to [27] that the greater concentration of more and more substances react and the greater the possibility of collision. The loss of an acetyl group on chitin produce positively charged chitosan that can dissolve in organic acids such as acetic acid and formic acid [28]. Reaction formation of chitosan from chitin is an amide hydrolysis reaction by a base [24]. Chitin acts as amides and NaOH as alkaline. First occurs an addition reaction, in this process the -OH enter into $NHCOCH_3$then elimination of CH_3COO^- occurs to produce an amine that is chitosan [29]. In the process of deacetylation of the chitosan produced 42.72 g of 54.75 g chitin depigmentation results are used in this process. Amount of reduction in mass is 12.03 g. Thus the percentage obtained by the transformation of chitin into chitosan amounted to 78.03% with the appearance of brownish white powder. Other researchers [24] produces Chitosan in amount of 67,08%. Percentage of chitosan obtained in this study in accordance with the previous studies that levels of chitosan from chitin of shrimp shells is greater than 50%[23].

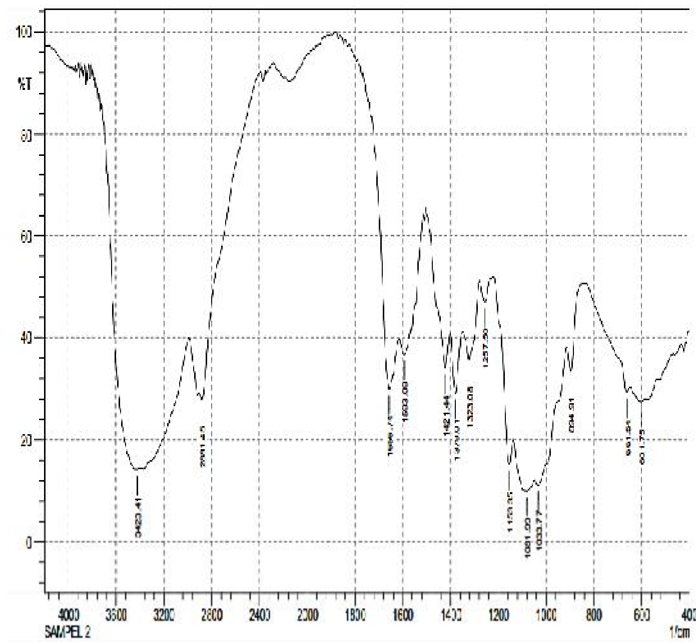

***Figure 1. FTIR spectra of Chitosan research results*.**

The generated chitosan is characterized by FTIR to identify the functional groups. FTIR spectra of chitosan compound formation can be seen in Figure 1. Based on the spectra in the figure shows that the uptake take place in the wave number area of 3423.41 cm^{-1} (O-H *stretching*), 2881.45 (CH (-CH_2-) *strecchingasym*), 1656 , 74 cm^{-1} (*Mainly*-NH *in plane deformation*), 1593.09 cm^{-1} (NH *stretching*, 1421.44 cm^{-1} (CH (-CH_2 -) *bending asym*), 1379.01 cm^{-1} (CH (-CH_2 -) *bending sym*), 1081.99 cm^{-1} (C-O (-C-O-C-) *strecchingasym*) and 1033.77 cm^{-1}. Thus the identification of active groups of chitosan research is very similar to chitosan literature. Chitosan generated in this study have a degree of deacetylation 73.88% very fulfilling the quality standards of trade chitosan about 60% [15]. Chitosan is said to have perfectly deacetylate if the degree of deacetylation greater than 90% [30]. The low degree of chitosan deacetylation generated in this study may be caused by some factors such as temperature, the duration of stirring and types of shrimp that used. Rahayu and Purnavita [15] reported that the greater the degree of deacetylation product of chitosan expected to greatly affect its use as an adsorbent (chelating) the metal ion, because the higher the degree of deacetylation chitosan, means more amine group (-NH_2) in the polymer that serves as the site of chelating, so it will be even enlarge the ability of chitosan to bind metal ions. Chitosan is a polymer that is more effective in terms of capacity and capability of the metal ion adsorption (mercury) compared with chitin. This is possible because the amount of free amine group (comparable to a large degree of deacetylation) in chitosan available for chelating, more than in chitin, so the ability of chitosan to bind metal ions is greater than chitin.

Characterization Of Chitosan

To determine the quality of the resulting Chitosan, characterization that includes test of water content, ash content and solubility are performed. Data from the chitosan characterization compared with the standard chitosan presented in Table 1.

Tabel 1. Data hasil karakterisasi kitosan dari limbah kulit udang

Parameter	Chitosan standard values	Chitosan experiment result values
Water content	≤ 10 %	6,48 %
Ash content	≤ 3 %	0,40 %
Degree of deacetylation	≥ 60 %	73,88 %
Solubility:		
- Water	Not dissolved	Not Dissolved
- Conc. HCl	Slightly soluble	Slightly soluble
- HNO_3	Slightly soluble	Slightly soluble
- CH_3COOH 1%	Soluble	Soluble
- Conc. NH_3	Not Dissolved	Not Dissolved
- Na_2SO_4 2%	Not Dissolved	Not Dissolved

Source : Rahayu & Purnavita (2007) [15], Lukum & Usman(2009) [8]

The chitosan product has water content of 6.48% andincluded in low categories. The magnitude of the moisture content on Chitosan is undesirable in utilization in various fields, as it will affect the durability against microorganisms [15].The water content in chitosan affected by the process at the time of drying, drying time, the amount of dried chitosan and the surface area of chitosan where dried.

The chitosan productin this research perfectly soluble in acetic acid1%. Solubility of chitosan in acetic acid can be used as standard quality of Chitosan[31, 32]. Thus the result of Chitosan in this research have meets the Chitosan quality standard values so that can be potentially as a natural adsobent against heavy metal.

3. CONCLUSION

The yield obtained at stage of deproteinase 51,72%, demineralization 24.98%, depigmentation 22.71%, and deacetylation 17,73%. The results of water content test is 6.48%, ash content 0.40% and the degree of deacetylation 73.88%. Test of solubility in water, ammonia and sodium sulfate is insoluble and hydrochloric acid, nitric acid is slightly soluble while in acetic acid is soluble.

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