# The Influences of Chitosan Obtained from Waste Shrimp on pH and Humic Acid Concentrations of Peat Water

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## ABSTRACT

Chitosan is a biopolymer that abundant availability and can be transformed from shrimp shell waste. The peat water is available in much amount in several places that can be developed into clean water. Aims of the research were to transformed chitosan from chitin isolated from dried shrimp, to evaluate the degree of deacetylation of chitosan obtained, to evaluate the influence of chitosan on pH and concentration of humic acid in peat water, and to evaluate the absorptivity of its on organic and inorganic compounds in the water. The transformation of dried shrimp shells was performed in several steps, as follows: deproteinization, demineralization, decolorization, and deacetylation using NaOH 3.5%, HCl 1 N, NaClO 0.315%, and NaOH 60% solutions, respectively. 10-50 mg chitosan were added to 10 mL of peat water, mixed, stored for 24 hours and filtrated. Absorbance of sample solutions were measured using a UV spectrophotometer at λ max of 288 nm. The yield of chitosan from shrimp shell waste was 15.36 ± 0.98 %. Degree of deacetylation of chitosan obtained and standard chitosan were 73.59 ± 2.69 and 83.11 ± 3.01 %, respectively. pH of peat water was changed from 5.6 to in a range of 7-7.2. Increasing chitosan concentration, decreasing the humic acid of peat swamp water significantly ($p < 0.01$). Chitosan increased the quality of peat swamp water effectively.

Key words: chitosan, peat water, absorptivity,

## INTRODUCTION

Shrimp freezing factories (cold storage) produce a large amount of shell waste (50-60%) often discarded or only used as a mixture of animal feed. This waste causes pollution. It can affect the cleanliness of the environment so that it endangers human health (Drastinawati, 2002).

In developed countries, the waste is a potential source of raw materials for the manufacture of chitin and chitosan compounds, namely biopolymers which function as biocatalysts and are used for various purposes. Chitin, chitosan, and their various derivatives can be used as anti-coagulants and anti-cholesterol in the medical field, excipients in the pharmaceutical dosage formsr, preservatives of food processing industry, antimicrobials in the fungicide industry, paper industry and the wood industry (Majeti & Kumar, 2000: Alkhamis *et.al*, 2001).

Because of its polycationic characteristic, chitosan is also used as a clotting agent in liquid waste management. Based on the specific properties chitosan can be used as an ion exchange resin for reducing heavy metals, coagulating oil/grease, and minimizing turbidity (McKay *et.al*, 1989; Cash, 1997).

Peat swamp water is surface water in peat soils which are generally found in tidal swamp areas with the characteristics of a brownish red color, high organic matter content, low pH (2-5) and unpleasant odor. Peat swamp water contains organic compounds consisting of humic acid, fulvic acid, humic acid, carbohydrates, lignin, wax, protein and many other organic compounds (Kim, 1991).

Humic material is a dark, acidic macromolecular compound with complex chemical properties and is polyelectrolyte in nature. These compounds are formed from the chemical and biological degradation of plant debris carried out by the activity of microorganisms both in the aquatic and terrestrial environment (Lubis, 1989).

This research was designated to isolate chitin from shrimp waste, to transform chitin into chitosan, to observe the effect of chitosan on pH and humic acid in an effort to purify peat water by adding chitosan as a coagulant. The coagulant was expected to remove the turbidity of organic and inorganic matter, pathogenic bacteria, and was expected to improve the quality of peat swamp water.

## MATERIALS AND METHODS

Equipments used include: UV-Visible spectrophotometer (Pharmaspec 1700, Shimadzu), Fourier Transform Infrared FTIR (Perkin-Elmer Spectrum One) spectrophotometer, digital scales (Metler PM 2000), atomic absorption spectrophotometer, Kjeldahl micro flask, pH meter, magnetic stirrer, cuvette, and glassware commonly used in laboratories. Materials used: shrimp waste, chitosan SIGMA® 116H1465, NaOH, HCl 1 N, peat swamp water, distilled water, acetone, 0.5% sodium hypochlorite.

### Identification of Shrimp Waste Samples

Samples were identified at the Ecology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas.

### Examination of Peat Swamp Water and Chitosan Raw Materials

The peat swamp water taken from swamp land ares was identified in the Natural Material Chemistry laboratory of Universitas Andalas. The tests include odor, color and pH. The chitosan was examinated based on the standard parameters of Protan Laboratories Inc., including the degree of deacetylation, description, solubility, drying loss, and ash content (Manjang, 1993; Fahmi, 1997).

### Transformation of Chitosan (Manjang, 1993; Fahmi, 1997)

Shrimp shells are cleaned, dried, mashed and weighed. Proteins were separated using 3.5 % (1:10) NaOH solution at 65 $^{o}$C for 2 hours, filtered and washed with distilled water to neutral pH. Protein free shrimp shells was added with 1 N (1:10) hydrochloric acid, stirred for 30 minutes at room temperature, filtered and washed with distilled water to neutral pH to produce chitin. Chitin obtained was soaked with acetone then bleached using 2.5 % sodium hypochlorite solution for 2.5 hours at room temperature, filtered and washed with distilled water to neutral pH and dried in an oven at 65 $^{o}$C for 4 hours.

***Deacetylation of Chitin***

Chitin was reacted with 60 % NaOH (1:10) in a beaker glass then heated at 100 $^{o}$C while stirring for 30 minutes. The solid obtained was filtered and washed with water to a neutral pH then dried at 60 $^{o}$C for 4 hours. Chitosan obtained was weighed and stored in a plastic bag at room temperature.

**Characterization of Chitosan**

***Protein Free Test (Robinson, 1995)***

Chitin and chitosan were analyzed using the Biuret test, if no purple or pink color was formed then chitin and chitosan were stated as a protein-free chitin and/or chitosan.

***Determination of Residual $Ca^{++}$ and $Mg^{++}$ Minerals*** (Vogel, 1987)

Standard solutions of $Ca^{++}$ and $Mg^{++}$ were prepared at the various concentrations of 2.5; 5; 10; 15; and 20 mg/L and 1, 2, 3, 4 and 5 mg/L, respectively. Preparation of sample solution was created as follows. 25 mL of 1 N HCl solution was added to 0.05 g of sample, stirred and allowed to stand for 30 minutes, then filtered. The absorption of filtrated solution was measured using an Atomic Absorption Spectrophotometer at the suitable maximum absorption wavelength of each metal. The concentration of the sample solution was determined using a standard solution calibration curve.

***Determination of Moisture Content (Manjang, 1993)***

One gram sample was placed into the evaporator plate and weighted (W1), then heated in an oven at 105 $^{o}$C for 3 hours and cooled in a desiccator. Heating process was repeated until the constant weight (W2). The water content was computed using the following equation:

Moisture contain = (W1-W2)/weigh of chitosan X 100%

***Determination of Ash Content (****Manjang, 1993)*

The sample was put into a porcelain weighed dish, added concentrated H2SO4 and treated at 800 $^{o}$C in a furnace for 3 hours. The plates were cooled in a desiccator and weighed.

Ash content = (b-a)/mass of chitosan X 100 %

Where: a = weight of empty crucible, b = weight the crucible plus ash.

***Chitosan IR Absorption Examination (Sabnis, 1997; Khan et.al, 2002)***

Chitosan was prepared into a KBr pellet. One to two mg of chitosan mixed with 10 mg of finely ground KBr dried powder. The mixture was compressed using a compressing machine at a

pressure of 10 tons to obtain a transparent disc. Chitosan KBr pellet was removed from the appliance and measured using a Perkin - Elmer FTIR spectrophotometer.

***Determination of the Degree of Deacetylation of Chitosan (Sabnis, 1997; Khan et.al, 2002)***

The degree of deacetylation of chitosan was determined using the baseline method, based on the comparison of the absorbance value of the absorption band from the infrared spectrum at the wave numbers 1655 $cm^{-1}$ and 3450 $cm^{-1}$.

The value of the degree of deacetylation was calculated using the formula:

$$DD\ (\%) = 100 - (A_{1655}/A_{3450} \times 115)$$

$A_{1655}$= absorbance of the amide group, absorption band at 1655 $cm^{-1}$, $A_{3450}$ = absorbance of the hydroxyl group absorption band at 3450 $cm^{-1}$.

***Observation the Ability of Chitosan to Dissolve in Acetic Acid Solution***

The ability to dissolve chitosan was determined using a 1 % acetic acid solution. Chitosan was added to 1 % acetic acid solution and mixed for 15 minutes (Suardi, 2001).

***Humic Acid Sample Preparation and Effect of Chitosan***

*Isolation of Humic Acid from Peat Swamp Water (Desmarina, 2000)*

Five L of peat water in a beaker glass was added with 0.1 N HCl solution to pH <2, kept for 4 days. The sediment was separated using centrifuge. The precipitate was washed with 95 % alcohol and dried in a desiccator. The availability of humic acid was evaluated using a neutral NaCl salt solution. The test is positive if a brown precipitate and a grayish solution are formed. Purification of humic acid was carried out by washing the blackish brown powder using 95 % alcohol several times. Purification was continued until two stains found on the TLC plate.

***Measurement of Humic Acid Absorption***

Humic acid was dissolved in 0.1 N NaOH solution at a concentration of 10 mg/25 mL.

**Effect of Chitosan Addition on Peat Swamp Water Purification**

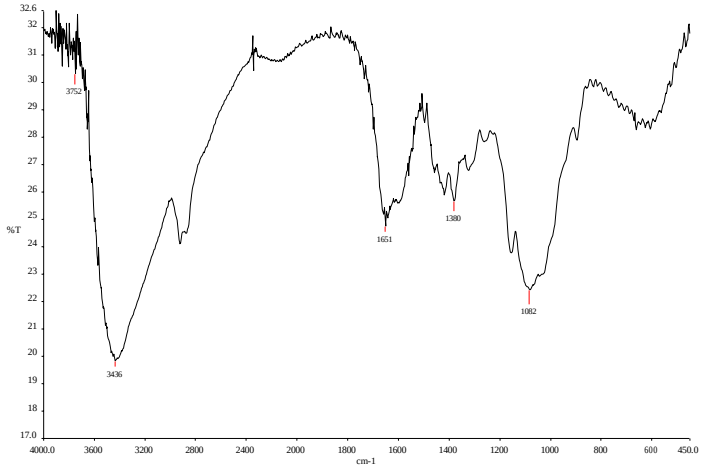
A total of 10, 20, 30, 40, 50 mg of chitosan were each added to 10 mL of peat water. The mixture was stirred homogeneously, left for 24 hours, and filtered with filter paper. Solution absorbance was measured using UV-visible at a wavelength of 288 nm.

## RESULTS AND DISCUSSION

Based on the identification result, the species of shrimp used was speckled shrimp or *Metapenaeus monoceros* fabricius (Fig. 1).

Fig. 1. Speckled shrimp *Metapenaeus monoceros* fabricius.

87.41 grams of chitin or 19.42 % was produced From 450 grams of dried shrimp shells., 69.14 grams of chitosan or 79.1 % were produced from 87.41 grams of chitin. The yield of chitosan from shrimp shell waste was 15.36 ± 0.98 %. Characterization of chitosan which includes measurement of infrared spectrum and degree of deacetylation fulfill the standard requirements issued by Protan Laboratories Inc.




Wave number ($cm^{-1}$)

Fig 2(a). Infrared spectrum of standard chitosan (Chitosan SIGMA®) in a KBr plate.

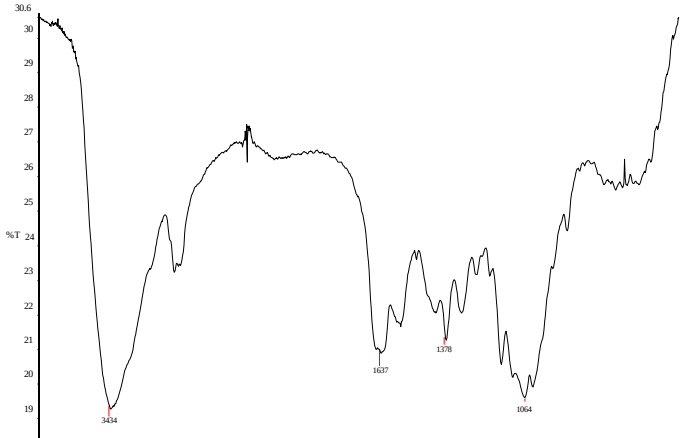



Fig. 2(b). Infrared spectrum of isolated chitosan in a KBr plate.

The evaluation of chitosan, including: description, solubility, drying loss, ash content, protein content, mineral content and deacetylation degree fulfill the standard parameters of Protan Laboratories Inc.

Table 1. The Results of Evaluation of Isolated Chitosan

| No. | Identification | Requirements (Fahmi, 1997; Sabnis & Block, 1997) | Observation |
|---|---|---|---|
| 1. | Description | Granule powder, white color - almost yellow, odorless and tasteless. | Powder, slightly yellowish white, odorless and tasteless. |
| 2. | Solubility | Practically insoluble in water, very difficult to dissolve in alcohol, soluble in acetic acid. | Practically insoluble in water, very difficult to dissolve in alcohol, soluble in 1 % acetic acid. |
| 3. | Water content | Not more than 10 % | 0.58 % |
| 4. | Ash content | Not more than 2 % | 0.24 % |
| 5. | Degree of deacetylation | More than 70 % | 73.59 % |
| 6. | Protein test (Robinson,1975 ) | - | No purple color formed |
| 7. | Magnesium metal | - | 0.108 % |
| 8. | Calcium metal | - | 0.047 % |

10.203 mg of brown powder humic acid was obtained from 5 L of peat swamp water. The maximum uptake of humic acid was found at a wavelength of 288 nm. Increasing chitosan concentration, decreasing the humic acid uptake from peat swamp water significantly ($p < 0.01$).

Shrimp shells from shrimp waste can be used as a source of chitin and chitosan. Deproteination of shrimp shells can be done by reflux with NaOH 5% solution. The protein separation stage was intended to free chitin from proteins in the tissue. In principle, this process is a hydrolysis process and avoids the chitin structure from being damaged, so the extraction process is carried out at a temperature of 65 $^{o}$C for 2 hours (Bough *et.al*, 1978; Beaulieu, 2004).

Demineralization of shrimp shell containing calcium carbonate about 25-40% can be extracted using acid solution. The shrimp shell was mixed with 1 N HCl for 30 minutes at room temperature. In this process, the use of HCl was effective to take out of almost all calcium carbonate to change into soluble calcium chloride and ionic form, thus can remove it easily and appropriately (Bough, 1978; Manjang, 1993; Fahmi, 1997).

Decolorization of chitin from the pigment is deemed necessary. The content of the color pigment can reduce the quality of chitin. Decolorization process was carried out by oxidation process using a relatively weak aqueous NaOCl solution. It was not only to removal of color

pigments adsorbed on the chitin through blanching process, but also to avoid the damage of chitin molecule (Beaulieu, 2004).

Deacetylation of chitin into chitosan using concentrated strong base, 60 % NaOH solution at a temperature of 100 $^{o}$C for 60 minutes was to remove acetyl groups. In this process chitin was hydrolyzed its amide group into an amine group producing chitosan and acetic acid. The combination of high NaOH concentrations, high mix ratios, stirring and high temperatures will produce the good quality of chitosan that high degree of deacetylation (Khan *et.al,* 2002; Beaulieu, 2004).

Characterization of chitin and chitosan includes several parameters including the determination of the ash content, moisture content, solubility, pH, nitrogen content, protein content and degree of deacetylation (Manjang, 1993; Fahmi,1997; Khan *et.al,* 2002). Chitosan obtained was white in the form of a thin sheet that has water content of 0.58 %. The moisture content of chitosan was an important parameter in determining the quality of chitosan. The water content standard of chitosan is maximum of 10% (Anonymous, 1987). The moisture content can be increased by the relative humidity of the air around the storage area because of hygroscopic nature of chitosan (Manjang, 1993; Beaulieu, 2004).The ash content of chitosan was found 0.24 %. Ash content according to the standard is not more than 2%. The color of the chitosan solution was clear, mean fulfills the requirements (Anonymous, 1987; Manjang, 1993).

Determination of the degree of deacetylation using base line method is related to the intensity of the C = O absorption band of the N-acetyl group at 1655 $cm^{-1}$ and the absorption band of NH at 3450 $cm^{-1}$ (Manjang, 1993; Fahmi, 1997: *Khan et.al*, 2002). The infrared spectrum of comparison standard chitosan and isolated chitosan is shown in Figures 1 and 2.

The degree of deacetylation of isolated chitosan was 73.59 %. The result meet the quality requirements of chitosan greater than 70 % (Sabnis & Block, 1997; Khan *et.al,* 2002). The mineral content in terms of calcium and magnesium content found were 0.047 and 0.108 %. It founded that the chitosan produced was fulfilled the standard requirements issued by Protan Lab. Inc.

The relationship between chitosan levels and humic acid was tested statistically using the Pearson correlation. Pearson correlation analysis showed a negative relationship between chitosan concentration and humic acid uptake ($p < 0.01$). It showed that, increasing concentration of chitosan, decreasing the uptake of humic acid significantly ($p<0.01$). The best concentration of chitosan to produce humic acid uptake in peat swamp water was at a minimum concentration of 0.5 %. Humic acid was almost entirely absorbed by chitosan with a decrease of 95.08 % and 42.3 %, respectively. Results of uptake of humic acid in peat swamp water with an increase in the concentration of chitosan.

The pH of the peat swamp water before treatment was 5.6. Its acidic condition is caused by organic compounds derived from bacterial growth causing fermentation in the soil (Lubis, 1989). This pH value does not meet the water quality standard because according to the standard, the allowed pH is 6 - 9. After treatment the pH of peat swamp water was increased into 7-7.2. The pH value was closer to the pH of the water quality standard compared to the initial pH. The use of chitosan increased the pH of peat swamp water was due to the reduced content of humic acids since the absorption by chitosan. Based on the results of the Pearson correlation analysis, it showed

a positive correlation between the pH of peat swamp water and the concentration of chitosan ($p<0.05$). It showed that chitosan can be used to improve the pH of peat swamp water.

## CONCLUSION

The species of shrimp used was *Metapenaeus monoceros* fabricius. The isolated chitosan meets the requirements stated by Protan Lab Inc. The yield of chitosan from shrimp shell waste was 15.36 ± 0.98 %%. Degree of deacetylation of obtained chitosan and standard chitosan were 73.59 ± 2.69 and 83.11 ± 3.01 %, respectively. The optimal amount of standard chitosan and test chitosan to reduce humic acid content in peat swamp water is 50 mg/10 mL. The pH of peat swamp water before and after treatment were 5.6 and in range of 7-7.2, respectively. The pH of the treated water was fulfill the pH of the standard water quality of 6-9. Increasing chitosan concentration, decreasing the humic acid of peat swamp water significantly ($p <0.01$). Chitosan increased the quality of peat swamp water effectively.

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