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## Utilization of Chitosan Extracted from Shrimp Shell Waste in Wastewater Treatment as Low Cost Biosorbent

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## Abstract

Chitin, which is the second most abundant biopolymer next to cellulose, and can be extracted commercially from the shells of crustacean (such as crabs, shrimps, prawns, krill), insects, fungi, and yeast. It can be converted into chitosan by partially deacetylation process. chitosan, has unique and distinctive features like, biodegradability, nontoxicity, high reactivity, excellent chelation behavior due to the presence of amine and hydroxyl functional groups which show high adsorption potential for various aquatic pollutions, and low cost in comparison with other adsorbents. Chitosan was successfully produced in the laboratory from shell waste. The yield was about 42.34 gm dry chitosan with ratio of 15.3% from the dry shells. The produced chitosan has good solubility in 1% acetic acid solution, it achieved up to 96.3%. It has % D.D.A. with value of 63% to 80%. However, it has good performance in removal of metals from industrial wastewater, it is not recommended to use it in the raw form due to presence of undesirable characters; such as low mechanical strength, swelling, and solubility in acidic media. chitosan/Activated carbon composite overcome these undesirable features in chitosan and gave good performance in the remediation of wastewater.

*Keywords:* Shrimp shell*; Chitosan*; composite; Chitin; deacetylation; Wastewater

## 1. Introduction:

Egypt is one of the countries facing water scarcity. As reported by the Ministry of the Environment [1], Egypt`s total available water resources are estimated to be of about 64.4 billion cubic meters per year, while the total water usage is estimated to be of about 77.8 billion cubic meters per year, this will result in an annual water deficit of about 13.4 billion cubic meters per year. Given a constant supply of water, and rapidly growing of population, the average ratio of water share per capita in Egypt decreases dramatically by time. The average per capita freshwater availability in Egypt has been steadily declining from about 1,893 $m^3$/year in 1959 to about 900 $m^3$/year in 2000, and 700 $m^3$/year in 2012. It has been expected to continue dropping to 534 $m^3$/year by 2030, taking into consideration, the World Bank`s water scarcity and absolute water scarcity (water poverty) limits are of 1,000 $m^3$/year/capita and 500 $m^3$ /year/capita respectively [2,3,4]. Accordingly, one of the most vital issues
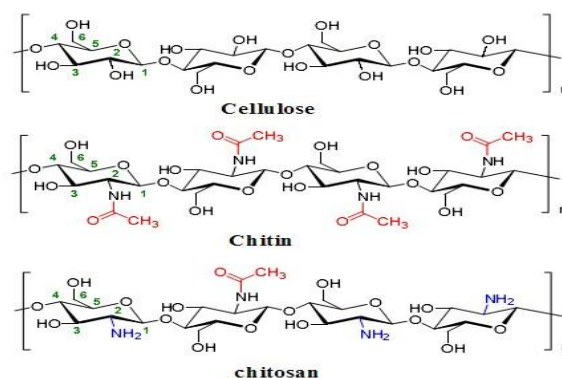


Figure (1): chemical structure of cellulose chitin and chitosan

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confronting Egypt as it will be threatened by water shortage are good management of water resources, control the water pollution, develop its irrigation system, getting rid of its wastewater in a manner that is environmentally acceptable. Table (1), shows the annual discharge of wastewater to the Nile as reported in "State of the Environment in Egypt, Report of 2015", by Ministry of the Environment in Egypt [1].

Table (1): The annual discharge of wastewater to the Nile [1].

| Type | Amount (Billion m3/year) |
|---|---|
| Agriculture discharge, contains chemicals & pesticides, and domestic discharge from village deprived of sanitation | 13.7 |
| Domestic discharge, from treatment plants | 1.06 |
| Cooling water of power plants, confirming with guidelines | 4.2 |
| Industrial wastewater, confirming to guidelines | 0.0045 |
| Industrial wastewater, non-confirming to guidelines | 0.1455 |
| Total | 19.11 |

Several remediation techniques have been employed to remediate the industrial wastewater. The commonly used techniques are chemical precipitation, coagulation-flocculation, phytoremediation, reverse osmosis, ion exchange, carbon adsorption, resin sorption, electro-dialysis, and adsorption [5-6]. However, most of these technologies are not eco-friendly or economically viable and have significant demerits such as hazardous sludge formation which convert the aquatic problem into solid problem, incomplete metal removal, and high cost at large scale [7-8]. Motivated by cost consideration, the efficiency of low cost adsorbents like agricultural wastes and seafood processing wastes is the promising alternative. Recently, a considerable attention has been focused on the biosorbents (sorbent materials with biological

origin) as an efficient, low cost, and eco-friendly materials for the treatment of wastewater containing heavy metals [9]. One of such kinds is chitosan, $\beta$-(1$\rightarrow$4)-linked -D- glucosamine units (deacetylated units) together with some proportion of N-acetyl –D- glucosamine units (acetylated units), which can be produced from fishery waste by partially alkaline deacetylation of Chitin; $\beta$-(1$\rightarrow$4)- linked-N-acetyl-D-glucosamine, which is the second most abundant biopolymer next to cellulose, and can be extracted commercially from the shells of crustacean (such as crabs, shrimps, prawns, krill), insects, fungi , and yeast [10-11].

Several techniques have been used to extract chitin from crustacean shells and convert it to chitosan, the most common one is the chemical method. The traditional chemical method for chitosan preparation, involves number of major steps; including washing, grinding, and sieving of raw shells, followed by extraction of chitin in demineralization, and deproteination steps from shrimp shells, and then production of chitosan by alkaline deacetylation of the extracted chitin [10,12,13]. Chitosan has unique and distinctive features like, biodegradability, nontoxicity, high reactivity, excellent chelation behavior due to the presence of amine and hydroxyl functional groups which show high adsorption potential for various aquatic pollutions, and low cost in comparison with other adsorbents [14-16]. However, it cannot be used in the raw form due to the presence of undesirable characters such as low mechanical strength and poor chemical resistance like swelling and solubility in acidic solutions. Physical and chemical modifications methods have been used to modify raw chitosan. These methods include crosslinking, grafting, and blending with low cost materials (e.g. activated carbon prepared from agriculture waste, sand, ceramic alumina, and cotton fibers) in composite form. The modifications were proposed in order to improve pore size, mechanical strength, chemical stability, and hydrophilicity [17]. This work aims to prepare chitosan from shrimp shell waste in the laboratory scale, determine the chitosan yield then study its characters like (solubility in 1 % acetic acid solution, bulk density, surface area, FTIR analysis, and % DDA), preparing a composite from it and activated carbon (CH/AC), and using both of them in bioremediation of industrial wastewater from glass manufactory.

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## 2. Material and Methods

### 2.1. Material

Raw shrimp shell waste collected from the local market, Hydrochloric Acid (HCl) with (ACS) reagent grade and concentration of 37%, Sodium Hydroxide (NaOH) salt with (ACS) reagent grade and assay ≥ 98 %, Acetic Acid (CH₃COOH) With (ACS) reagent grade and concentration of 96 %, and activated carbon (AC) granular shape.
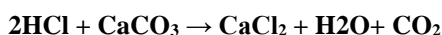
### 2.2 Preparation of chitosan

The method which used was adapted from the method of (S.Z. Islam, *et.al.,* 2016) [18] with slight modification. It includes: extracting of chitin in two steps (demineralization of the grinded dry shrimp shells followed by deproteination of the demineralized product) then partial deacetilation of the produced chitin.

### Extraction of chitin by chemical method:
a) Demineralization of the shrimp shells

A total of 275 grams of the crushed dry shells were treated with 1.0 M HCl solution at (1:10) solid to solvent ratio. Acid was slowly added to avoid foaming due to gas formation occurring because of calcium carbonate content of shell, which reacts with the acid and form carbon dioxide. Then, the mixture was heated on a hot plate with stirring at (60-70 $^{O}$C) for one hour, after that, it was allowed to stay with slow stirring overnight at room temperature (22 ± 2$^{O}$C). Subsequently, the acid -mineral solution was drained off, and the residue was washed with distilled water several times till it reached neutral pH (6.5±0.5). Finally, the solid material was dried in a drying oven at (60-70$^{O}$C) overnight.

**Chemical reaction of this step:** the minerals in the shells (mainly calcium carbonate) are decomposed into calcium chloride along with the discharge of carbon dioxide, by dilute hydrochloric acid at room temperature

$$2HCl + CaCO_3 \rightarrow CaCl_2 + H2O + CO_2$$

b) Deproteination of the shrimp shells (Chitin production)

At this step, heating is an essential factor, because of, without heating, protein cannot be efficiently withdrawn from the shells and the other stages such as N-deacetylation cannot perform effectively and conversion of shrimp shells into chitosan will be restricted **(de Man, 1999) [19].** The deproteination process was conducted; by soaking the dried decalcified shells, which prepared in the previous step in 1.0 M NaOH, solution (at solid to solvent ratio of 1:10). The mixture was heated at (80-90 $^{O}$C) with continues stirring for 6 hours. After cooling, the mixture was filtered, the residue was washed with distilled water, and dried in a drying oven at (60-70 $^{O}$C) overnight. The end product of this step is known as chitin.

**Chemical reaction of this step**: the chemical bonds between protein and chitin are disrupted in aqueous dilute alkaline solution with the presence of heating.

### c) Deacetylation of chitin (chitosan production)

At this step, the produced chitin was treated with 12.5 M NaOH solution at (solid to solvent ratio of 1: 15). The mixture was boiled for 12 hours. After then, the solution was filtered, and the residue was washed with distilled water several times till it reached neutral pH (7.5±0.5) and dried in a drying oven at (60-70 $^{O}$C) overnight. This product is known as chitosan; it was checked by the solubility in 1% acetic acid.

**Chemical reaction of this step** The alkaline deacetylation step is usually achieved by removing enough acetyl groups (CH₃-CO) from chitin by using strong concentrated alkaline solution; NaOH or KOH (40-50%) at temperature above 100 $^{0}$C. By heating chitin with concentrated sodium hydroxide solution, the N-acetyl groups (-NHCOCH3) of the molecular chain of chitin will change to amine groups (-NH2) forming the product chitosan, as in the following equation.
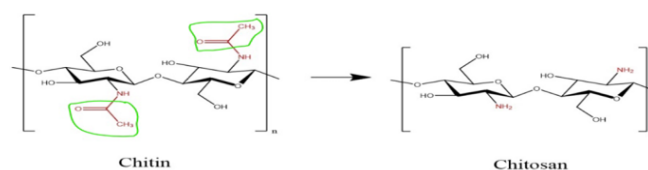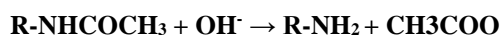
$$R\text{-}NHCOCH_3 + OH^- \rightarrow R\text{-}NH_2 + CH3COO$$



**Figure (2):** N-Deacetylation of chitin into chitosan, with 12.5 M NaOH and heat

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**Figure (3):** Mediterranean Sea Red Shrimp



**Figure (4):** Dry Shrimp shells



**Figure (5):** Crushed shrimp shells



**Figure (6):** Laboratory prepared chitin



**Figure (7):** Laboratory prepared chitosan

### 2.3. *Preparation of CH/AC composite*

The CH/AC composite with ratio of 3:1 (CH.: AC.) was prepared from the laboratory prepared chitosan and activated carbon granules. 15.0 grams of Chitosan was dissolved in 250 ml of 5% (v/v) acetic acid solution under continuous stirring at 45-50℃ until formation of (CH) gel. About 5 grams of granular (AC) was added slowly to the (CH) gel and stirred for 24 hours at 45-50℃. Then, the (CH/AC) composite beads were prepared by injecting the (CH/AC) gel in droplets through a syringe nozzle into a 500 ml of 0.1 M NaOH precipitation bath. After that, the beads were separated from NaOH solution by filtration, and washed several times with deionized water to a slightly neutral pH (7.5 ± 0.5).

Finally, the beads were dried in air oven at 60℃ for 24 hours, and grinded.

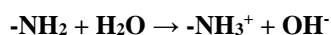### 2.4. *Characterization of laboratory prepared chitosan:*

➤ *Chitosan yield:*

the yield at each step was calculated by the following equation

Yield (%) = Dry weight of the yield (gm)/ Dry weight of the shrimp shell (gm) X 100 %

➤ *Solubility in 1% acetic acid solution:*

The main physical difference between chitin and chitosan is the ability of chitosan to be soluble in dilute aqueous solutions of organic acids such as acetic, formic, lactic, citric, and propionic acids due to protonation of amino group ($-NH_2$) at the C-2

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position of the D-glucosamine repeat unit in its molecular structure according to the following equation [20]:

$-NH_2 + H_2O \rightarrow -NH_3^+ + OH^-$

Briefly, 1.0g of the laboratory prepared chitosan was dispersed in 100 ml of 1% (v/v) acetic acid solution at 25°C and stirred by magnetic stirrer until a homogeneous solution was obtained. Then, the solution was filtered through Whatman™ Grade # 54 Quantitative filter paper (pore size 22 um) to determine the insoluble content. The insoluble content and the percentage of solubility were calculated by the following equations:

Insoluble content (g) = final weight of filter paper (g) – initial weight of filter paper (g)

$$Insoluble \ (\%) = \frac{insoluble \ weight \ (g)}{sample \ weight \ (g)} \ x \ 100 \ \%$$

Solubility (%) = 100 - % insoluble

The procedure was repeated three times and the mean value of the results was taken

> ### Bulk density:

To measure bulk density, a pre-weighted 10 ml cylinder was filled to a specified volume with dried chitosan; (the dry material was freely settled). Then the cylinder was weighted with the dry material, and the weight of the dry material was calculated as:

Weight of dry material (g) = weight of the cylinder with the material - weight of the empty cylinder

The bulk density calculated as following equation in reference [21]:

$$Bulk \ density = \frac{weight \ of \ the \ dry \ material \ (g)}{volume \ of \ the \ dry \ metrial \ (cm3)}$$

> ### BET surface area:

It was determined from adsorption-desorption isotherm of nitrogen gas at 77K, by using a Micrometrics –ASAP 2020 surface area and porosity analyzer. Prior to the analysis, the samples were degassed at 60 °C for 4 hours under a vacuum of 10 um Hg. The specific surface area was calculated using Brunauer – Emmett- Teller (BET) method, while pore volumes and average pore diameter were calculated by the Barrett- Joyner- Halenda (BJH) method.

> ### FTIR analysis

The analysis was conducted for chitosan to study the main functional groups in its structure, as well as to calculate its % degree of deacetylation (%DDA) as one of the most important parameter in chitosan characterization. It was carried out using a (JASCO

4100 FTIR spectrometer, Japan), with resolution of 4 $cm^{-1}$, in the range of 400-4000 $cm^{-1}$, and scanning speed of 2mm/second. The sample was analyzed by sample/KBr pellets technique with ratio of 1:100.

> ### % Degree of deacetylation (%DDA)

The % Degree of deacetylation (%DDA) of the prepared chitosan was calculated by the following formulas:

**Domszy and Roberts, 1985 [22] :** DDA(%) = 100- [ ($A_{1655}$ / $A_{3450}$) X 100) / 1.33]

**Where,** $A_{1655}$ is the absorption of band at 1655 $cm^{-1}$ of the amide I (primary amide) band as a measure of the N-acetyl group content and $A_{3450}$ is the absorption of band at 3450 $cm^{-1}$ due to hydroxyl group as an internal standard

**Baxter A., et.al.1992 [23] :** DDA (%) = 100- [ ($A_{1655}$ / $A_{3450}$) x 115]

**Where,** $A_{1655}$ is the absorbance value of the primary amide at 1655 $cm^{-1}$, and $A_{3450}$ is the absorbance value of hydroxyl group at 3450 $cm^{-1}$ as internal standard.

**Brugnerotto J., et.al., 2001 [16,24]:** DDA (%) = 100- [ ($A_{1320}$ / $A_{1420}$) -0.3822]* 1/0.03133]

**Where,** $A_{1320}$ absorption of band at 1320 $cm^{-1}$m it is a characteristic band of the acetylated amine or amide which measure the extent of N-acetylation of chitosan, and $A_{1420}$ absorption of band at 1420 $cm^{-1}$, it is peak for amine group, which is used as the reference band

## 3. Results and discussion
- ### Chitosan yield
The study has successfully produced about 42.34 gm dry chitosan from 275 gm dry shrimp shells with ratio of 15.3%. The chitosan produced has off white color (slight brownish to white), with flakes shape.

- ### Characterization of chitopsan:
- ### Solubility:
Solubility characterization is one of the most important parameter to determine the quality of chitosan, where higher solubility means better chitosan produced [16]. The most commonly used is 1% acetic acid solution at about ~ pH4.0. Mechanism of chitosan solubility in acetic acid is attributed to the amine groups of chitosan. As the amine groups contain hydrogen ions which make chitosan can easily interact with water through hydrogen bonding in addition with the presence of carboxyl groups in acetic acid would facilitate the dissolution of chitosan due to hydrogen interaction between the carboxyl group and the amine group of chitosan [25]

The average solubility of the prepared chitosan in

**Table (2):** Chitosan yield from 275 gm of dry shrimp shell

| *Material* | *Weight (gm)* | *yield %* |
|---|---|---|
| Wet shrimp shells | 1,800 | |
| Dry shrimp shells | 275 | 15.2 ( wt.% from the wet shrimp shells |
| Demineralized shells | 95.15 | 34.6 (Wt. % of dry shells) |
| Chitin | 57.88 | 21.05 (Wt. % of dry shells) |
| chitosan | 42.34 | 15.3 (Wt. % of dry shells) |

- **Physical characteristics**

The bulk density, surface area, pore volume, and pore size of the prepared chitosan and the composite are shown in Table (3). The obtained results showed Significant increase in bulk density, surface area and pore volume of the composite in comparison with the raw chitosan. The moderate value of bulk density and surface area of the composite in comparison with CH. give it desirable character in remediation performance in the treatment plants.

**Table (3):** Physical characteristics of the laboratory prepared chitosan and CH/AC composite

| Parameter | CH/AC composite | CH. |
|---|---|---|
| Bulk density (g/cm3) | 0.32 | 0.164 |
| BET surface area ( m2/g ) | 287.352 | 0.452 |
| Total pore volume (cm3/g) | 0.160 | 0.001 |
| Average pore (diameter ) nm | 2.232 | 10.173 |

- **FTIR analysis**

FTIR analysis of chitosan shows the band at 3435.56 cm$^{-1}$  is related to the stretching vibration of NH2 and OH groups, band at 2912.95 cm$^{-1}$ is related to C-H stretching in CH2OH, band at 1640.16  cm$^{-1}$ is related to C=O stretching and NH of amide I group
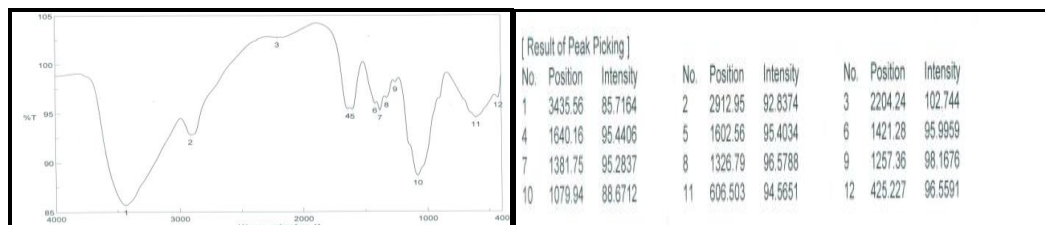
1% acetic acid solution was 96.3 % (±0.08)

which represent the presence of resedual N-acetyl group, and band at 1421.28 cm$^{-1}$ is related to CH bending  and OH in CH$_2$OH group.

**Degree of deacetylation :**

Of the various analytical techniques used in the determine of % DDA of chitosan, infrared spectroscopy (FTIR) is at the center of attention; because of, it is convenient and relatively quick, has low cost, and allows the DA value of chitin/chitosan to be determined in solid state. The % Degree of deacetylation (%DDA) of the prepared chitosan was calculated by the following formulas:

**Table (4):** % DDA of the laboratory prepared chitosan by different FTIR formulas

| # | Formula | % DDA |
|---|---|---|
| 1 | **Domszy and Roberts, 1985** | 68.07 |
| 2 | **Baxter A., *et.al.*,1992** | 63.28 |
| 3 | **Brugnerotto J., *et.al.,* 2001** | 80.1 |

(Rayane S. *et al.* 2017) **[11],** compared between the three equations (**Domszy and Roberts, 1985**), **Baxter A., *et.al.*1992, and Brugnerotto J., *et.al.,* 2001**) in calculating of DDA of chitosan samples. He reported that, "the equation proposed by Brugnerotto et. al. resulted in the most appropriate results for determining of the DA of chitosan samples by FTIR"

- **Industrial wastewater treatment**

The laboratory prepared chitosan and its composite (CH/AC) composite were used in the treatment of wastewater sample from glass manufactory. Although the performance of chitosan is good in metals removal, it appears bad performance in turbidity, color, total suspended solids, and COD. This bad performance is attributed to "presence of undesirable characters such as low mechanical strength and poor chemical resistance like swelling and   solubility in acidic solutions" as reported in the literatures. Its composite (CH/AC) gave good performance in metals removal and in physical character of the sample



**Figure (8):** FTIR spectra of laboratory prepared chitosan

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**Table (5):** Wastewater sample treatment by (CH./AC.) composite

| Parameter | | Unit | (1)Raw Sample | (2)After Settling Tank followed by sand filter | (3) after treatment with composite in (batch reactor)followed by sand / gravel filter |
|---|---|---|---|---|---|
| pH | | - | 8.42 | 8.42 | 7.32 |
| TDS | | mg/l | 320 | 290 | 305 |
| TSS | | mg/l | 442 | 154 | 12 |
| total COD | | mg/l | 127 | | 61 |
| total BOD5 | | mg/l | 99 | | 47.5 |
| chloride (cl-) | | mg/l | 30.5 | | 26 |
| Turbidity | | FTU | 482 | 140 | 35 |
| apparent Color | | Pt. Cobalt | 1505 | 915 | 193 |
| Heavy Metals | Lead (Pb) | mg/l | 31.5 | 30.1 | 0.464 |
| | Copper (Cu) | mg/l | 0.566 | 0.464 | 0.051 |
| | Cadmium (Cd) | mg/l | ND | ND | ND |
| | Chromium Total | mg/l | 0.139 | 0.121 | ND |
| | Cobalt (Co) | mg/l | 0.125 | 0.118 | 0.037 |
| | Nickel (Ni) | mg/l | 0.7 | 0.653 | 0.023 |
| | Iron (Fe) | mg/l | 0.586 | 0.553 | 0.025 |
| | Zink (Zn ) | mg/l | 1.152 | 1.137 | 0.007 |

**\*ND: Not Detected**

### 4. Conclusion

1- The study has successfully produced chitosan from shrimp shell waste in the laboratory scale.

2- The chitosan yield was about 42.34 gm dry chitosan with ratio of 15.3% from the dry shells. The produced chitosan has off white color (slight brownish to white), and flakes shape.

3- The produced chitosan has good solubility in 1% acetic acid solution, it achieved up to 96.3%.

4- The DDA value obtained was high ranged from 63% to 80%.

5- However, it has good performance in removal of metals from industrial wastewater, it is not recommended to use it in the raw form due to presence of undesirable characters; such as low mechanical strength, swelling, and solubility in acidic media.

6- Blending of it with hard and cheap material (activated carbon) in composite form improved its features and minimize its undesirable characters.

7- Chitosan/Activated carbon composite gives good performance in the treatment of industrial wastewater.

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