Journal of Scientific Research and Development 3 (5): 229-235, 2016
Available online at www.jsrad.org
ISSN 1115-7569

Characterization of chitosan as chromium adsorbent

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Abstract: The adsorption behavior of chromium ion on chitosan, extracted from crab exoskeleton, was investigated as an aid for the understanding of the adsorption process of chromium on chitosan. This study focuses on the partial characterization of the extracted chitosan and the chitosan-Cr complex. Chitosan was extracted from crab exoskeletons using a conventional chemical method. FTIR analysis offered structural validation of the extracted chitosan. The degree of deacetylation of chitosan (extent of its conversion from chitin) was approximated at about 37.2 %. Comparison of the FTIR spectra of the complexed chitosan (chitosan-Cr) against the free chitosan showed attenuation of the O-H and N-H stretching bands. This implies participation of the said functional groups on chromium ion adsorption. The zero-point charge (pHzpc) of the extracted chitosan, determined through pH-metric titration method, was estimated at pH 6.3.

Key words: Chitosan; Adsorption; Chromium

1. Introduction

The rates at which effluents are released into the environment, especially on bodies of water, have been increasing as a result of urbanization (Horsfall, 2004). These effluents contain toxic substances, specifically heavy metals, which continuously threatens the environment because of their bio-accumulating tendency, toxicity and undesirable effects to humans (Abu Al-Rub, 2003). One such substance is chromium ion.

Chromium is a naturally occurring element present in water, sediments, rocks, soils, plants, biota, animals, and volcanic emissions (Ducros, 1992). It exists in a number of oxidation states, but only trivalent, Cr(III) and hexavalent, Cr(VI) chromium are biologically and environmentally stable (Ducros, 1992). The presence of each ionic form of chromium in solution is pH dependent (EPA, 1984). Cr(VI) species are extremely toxic, whereas Cr(III) compounds are much safer (Porter, 1999). Furthermore, Cr(III) can be found in many kinds of food and are widely used as pharmaceuticals and dietary supplements to prevent diabetes, lipoprotein abnormalities, and cardiovascular diseases (Porter, 1999).

Hexavalent chromium Cr(VI) is known for its carcinogenic effects in humans (Sorahan, 1998). Pigment chromate handlers, ferrochromium production worker, stainless steel welders, and chromeplaters are more prone to lung cancer, which can be attributed to Cr(VI) exposure (Sorahan, 1998). Short term and prolonged exposure through inhalation, ingestion or topical contact can also cause adverse health effects in human (Olaguibel, 1989; Langard, 1990; Machle, 1948; Shenutt, 2007). The estimated exposure dose of Cr(VI) is limited to 0.57 mg/kg-day (Zhang, 1987). Potassium dichromate, which is one form of Cr(VI), has been reported as amplifier of some negative effects in organism (ATSDR, 1993).

Due to the high toxicity of hexavalent chromium ion to living systems, it is imperative that it must be removed from wastewater (Saifuddin, 2005). Complete understanding of the harmful effects of Cr(VI) has encouraged studies on separation of heavy metals from aqueous solution with the aid of adsorption and biosorption (Martino, 2005).

The efficient removal of toxic metal ions from wastewater is an important and widely studied research area. A number of technologies have been developed over the years. The currently available treatment technologies for metal-bearing effluents are either not effective enough or prohibitively expensive and inadequate considering the vast wastewater quantities (Volesky, 2001). Adsorption, which is the capacity of the adsorbate to adhere or attach to the adsorbent, is a well-established technique for separation, used to remove dilute pollutants as well as to recover valuable products from aqueous solution. Adsorptive removal of heavy metals from aqueous effluents, which have received much attention in recent years, is usually achieved by using biopolymer resins that can form complexes with the heavy metal ion (Martino, 2005; Crini, 2005).

In the recent years, biosorption by biologically originated materials in removing heavy metals has drawn more and more attention, largely due to the unique properties of these biomaterials being environmentally benign, low cost, effective at low metal concentration and easily reusable (Marczenko,

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1986; Dantas, 2001). Biosorption, which involves active and non-active uptake by biomass, is a good alternative to traditional processes. Biosorption of toxic heavy metals is especially suited as a 'polishing' wastewater treatment step because it can produce close to drinking water quality (Naja, 2007). Biopolymers that are abundantly available are used as alternative for biosorption mainly because they are a cheap resource (Volesky, 2011). One such biopolymer is chitosan (Fig. 1). Chitosan is obtained from the deacetylation of chitin (Fig. 1). Chitin is one of the most abundantly available natural polymers, and found in shrimp, crabs, and other crustacean shells. Chitosan is a copolymer of glucosamine and N-acetylglucosamine, and it has an amine functional group that strongly associates with metal ions specifically at pH close to neutral (Rhazi, 2001). At low pH, the amine group of chitosan is protonated, and is able to bind anions by electrostatic attraction (Kunkoro, 2005).

This natural biopolymer is industrially attractive as biosorbent since it is readily available, inexpensive, biodegradable, and possesses numerous reactive groups that are able to participate in metal ion adsorption.

This study deals with the characterization of chitosan as chromium adsorbent. Chitosan is recommended as suitable functional materials, because this natural polymer has excellent properties such as biocompatibility, biodegradability, nontoxicity, and chelating properties (Ona, 2005).

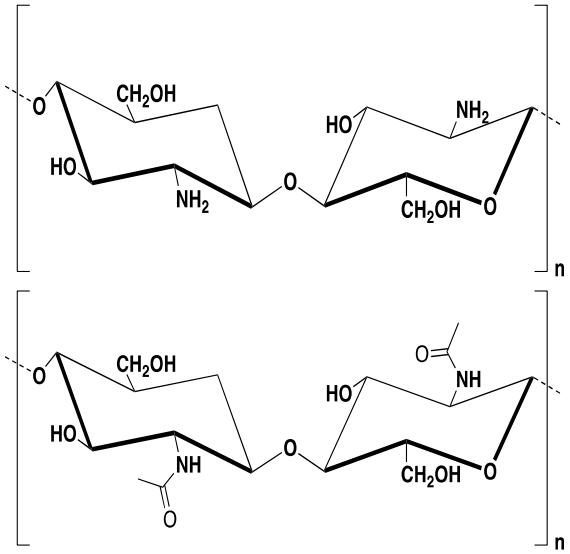

Fig. 1: Structure of chitosan (top) and chitin (bottom)

2. Methodology

2.1. Chemicals and instrument

All chemicals were of reagent grade and used as purchased. All weighing were done using a Mettler Toledo AT21 Comparator analytical balance. IR spectra were collected from a Nicolet iS-50 Analytical FT-IR spectrometer equipped with diamond ATR sample cell. The pH was measured using Fisher Accumet pH meter model 610A.

2.2. Sample collection and preparation

Crab shells were collected from Lala, Lanao del Norte. The shells were washed in running water to remove undesired particles or substances such as sand, soil and attached flesh. The washed samples were laid under sunlight to achieve complete dryness and prevent growth of molds.

2.3. Preparation of chitosan

Chitosan wass prepared by deacetylation of chitin, which is naturally found in crab exoskeleton. The purification method of chitin was based on the detailed methods of Bader, 1997. Further isolation of chitosan from chitin was done following the procedure obtained from the work of Hadi, 2013. These are briefly discussed in the following sections.

2.3.1. Preparation of crab exoskeleton

The dried crab exoskeletons were crushed and pulverized using a blender. It was then washed thoroughly with deionized water by repeated soaking, stirring and decanting until all dirt and unwanted impurities were removed. The clean pulverized sample was oven-dried at 80 ^{0}C for a few hours until stable weight was attained. The dried pulverized sample was then sieved into 1.18 mm particle size.

2.3.2. Calcium carbonate removal (Demineralization)

The crab shell sample (25.0 g) was added slowly with HCl (250 mL, 1 M) and the mixture was stirred for 6 h at room temperature until gas stopped to evolve. The sample was then filtered. To check if $CaCO_3$ is completely removed, additional HCl was added until no further generation of gas (bubbling) occurred. The residue was then washed with deionized water.

2.3.3. Protein removal

NaOH (250 mL, 1 M) was added to the previously demineralized sample. The sample was soaked for 16 h at ambient temperature. The sample was filtered and washed with deionized water to neutral pH. The sample was oven dried at 100 ^{0}C until constant weight was attained. At this point, purified chitin is obtained.

2.3.4. Preparation of chitosan from chitin by deacetylation

In the deacetylation of chitin, NaOH (250 mL, 50 % (w/v)) was added to 25 g of the previously prepared chitin and was heated at 50 ^{0}C for 4 h. The

sample was placed under the hood for 30 min. The cooled sample was washed with NaOH (250 mL, 50 % (w/v) and filtered to retain the solid matter, which was the chitosan. The prepared chitosan was oven dried at 100 ^{0}C for few hours until constant weight of the sample was attained.

2.4 Preparation of hexavalent chromium ion solution

Potassium dichromate was the source of Cr(VI) in the adsorption experiment. Cr(VI) stock solution (500 mL, 500 ppm) was prepared from $K_2Cr_2O_7$ (AR grade).

2.5. Adsorption

Batch equilibriation method was carried out for the adsorption experiment. The adsorbate (30 mg of chitosan of 1.18 mm particle size) was soaked in a solution of Cr(VI) ions (30 ppm of $K_2Cr_2O_7$) at pH of 2.0 for 45 min. After equilibration, the mixture was then filtered, and the residue was collected for further analysis.

"Complexed" chitosan was obtained from residue after chitosan was subjected to batch method according to the procedure above. The "free" chitosan was prepared using the same conditions but without chromium ions.

The degree of deacytelation of chitosan was calcualted from the IR spectra using Eq. 1,

$$\%DDA = 100 - \frac{\frac{A_{amide-I}}{A_{hydroxyl}}}{1.33} x100 \quad (1)$$

where $A_{amide\text{-}I}$ is the absorbance of amide-I band at 1655 cm^{-1}, which is a measure of the N–acetyl group content, and $A_{hydroxyl}$ is the absorbane of the hydroxyl band at 3450 cm^{-1}, which is an internal standard to correct for film thickness or for differences in chitosan concentration powder form. The factor 1.33 denotes the value of the ratio of $A_{amide\text{-}I}$ to $A_{hydroxyl}$ for fully N–deacetylated chitosan. The lower the absorbance of amide–I group, the higher the degree of deacetylation.

2.6. Determination of the zero-point charge (pHzpc)

The pH_{zpc} (pH at zero point charge) of chitosan was determined by pH-metric titration (Hosne, 2012). Briefly chitosan (0.5 g) was added to four identical portions of NaCl solution (60 mL 0.1 M at pH 7). The mixtures were equilibrated for 24 h with occasional shaking. Two of the suspensions were titrated directly with 0.05 M HCl and 0.05 M NaOH separately. The constant pH values were noted after each aliquot of titrant (acid or alkali) addition. The other two suspensions were filtered and the filtrates were titrated with the acid and alkali solutions as described above. Two combined sample titration curves of the two sets of samples – the suspensions and filtrate – were obtained by adjoining their respective acid and alkali titration curves. The net titration curve was obtained by plotting together the two combined sample titration curves. The net titration curves meet at a point (pH), which is defined as the pH_{zpc}.

3. Results and discussions

The chitosan sample used in this study was extracted from crab shells using a conventional chemical method of purification. Table 1 shows the mass and corresponding yield of chitosan after each indicated extraction step.

Table 1: Yield of Chitosan Extracted from Crab Shells (25 g)

Extraction Step	Mass, g	% Yield
Demineralization and deproteinization`` (Chitin)	13.9	55.8
Deacetylation (Chitosan)	7.14	28.6

3.1. FTIR analysis of extracted chitosan

The structure of chitosan (Fig. 1) shows three types of prominent functional groups: an amino group, and primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively.

The FTIR spectrum of the extracted chitosan (Fig. 2) shows a characteristic broad band around 3520 cm^{-1}. This is attributed to the stretching vibrations of the O–H functional group and intermolecular hydrogen bonds, overlapping with each other. Peaks at 1650 cm^{-1} and 1666 cm^{-1} indicates the amide-I and amide-II functional groups, respectively. The presence of these amide bands indicates incomplete deacetylation of chitin to chitosan, which is also observed from other studies (Bhatia, 2000; Peletier, 1990). This also led to the low intensity of the band of bending vibrations of the N–H functional group (primary amine) around 1650 – 1573 cm^{-1} which is one of the active site of a fully deacetylated chitosan for metal binding. The broad band at 3262 cm^{-1} and peak at 1382 cm^{-1} correspond to the N–H stretching and C–H bending, respectively.

The absence of bands at 1540 cm–1 and 1420–1430 cm^{-1} indicates the absence of proteins and $CaCO_3$, respectively, thus, showing the effectiveness of the method used for deproteinization and demineralization (Mohammed, 2013).

The presence of strong bands around 2350 cm^{-1} is due to the asymmetrical stretch of CO_2 (Coleman, 1993). These bands appear in samples during runs on the instruments, since CO_2 is present in the atmosphere. The CO_2 might be present in the sample compartment at a greater level when the sample was scanned than they were when the background was taken. Also, CO_2 levels in the laboratory can change during the day, and uncompensation in the ratioed spectrum will be observed from time to time. These observations are also present in Figs. 3 and 4.

Table 2: Summary of IR absorption bands of extracted chitosan compared to literature

Characteristics	Literature	Experimental
O–H stretching band (Sabnis, 1997; Ng, 2006)	3450	3520
N–H bending band (Ng, 2006)	1650 – 1580	1650 – 1573
Amide-I band (Sabnis, 1997)	1655	1650
Amide-II band (Kumirska, 2010)	1700	1666
N–H stretching band (Lima, 2004)	3529 – 3105	3262
C–H bending band (Kumirska, 2010)	1380	1382

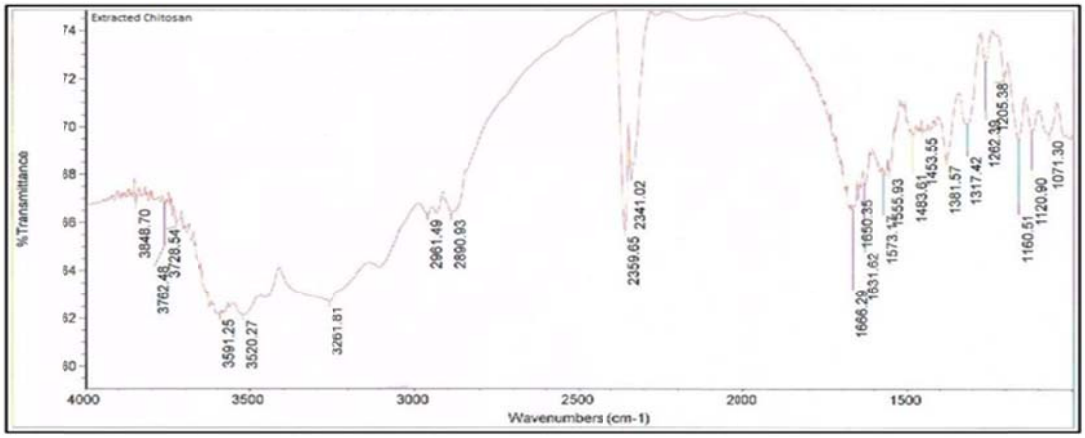

Fig. 2: FTIR spectrum of chitosan extracted from crab shells

3.2. Degree of deacetylation of chitosan

The absorbance of amide-I band at 1650 cm^{-1} is 0.17276 (%T = 67.180) and the absorbance of hydroxyl band at 3520 cm^{-1} is 0.206692 (%T = 62.131), obtained from Fig. 2. Using Eq. 1, the method used for deacetylation produced a 37.2 % DDA.

An increase in either temperature or strength of sodium hydroxide solution can enhance the removal of acetyl groups from chitin, resulting in a range of chitosan molecules with different properties and applications (Baxter, 1992). Thus, the degree of deacetylation (DDA) depends mainly on the method of purification and reaction conditions (Baxter, 1992; Li, 1997).

3.3. FT-IR analysis of "Free" and "Complexed" chitosan

The FTIR spectrum of the "complexed" chitosan (Fig. 3) shows an obvious attenuation in the characteristic broad bands of O–H and N–H stretching bands around 3200-3500 cm^{-1} relative to free chitosan (Fig. 4). Such observation indicates that these functional groups are the active sites for the binding of chromium ions. The interaction of these functional groups with chromium ions hinders the vibrational stretching motion, thus resulting to the observed attenuation.

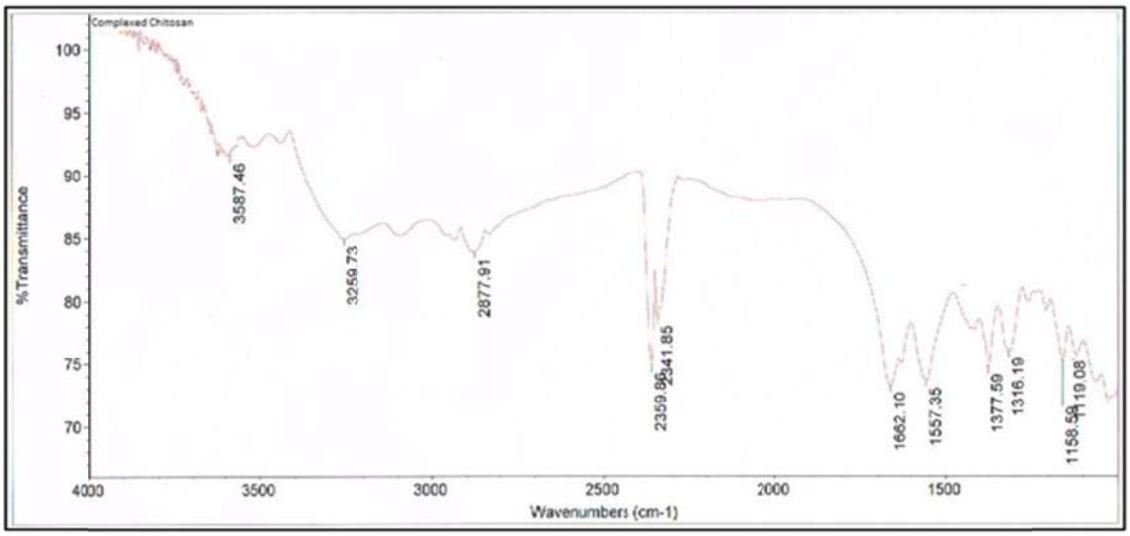

Fig. 3: FTIR Spectrum of "Complexed" Chitosan

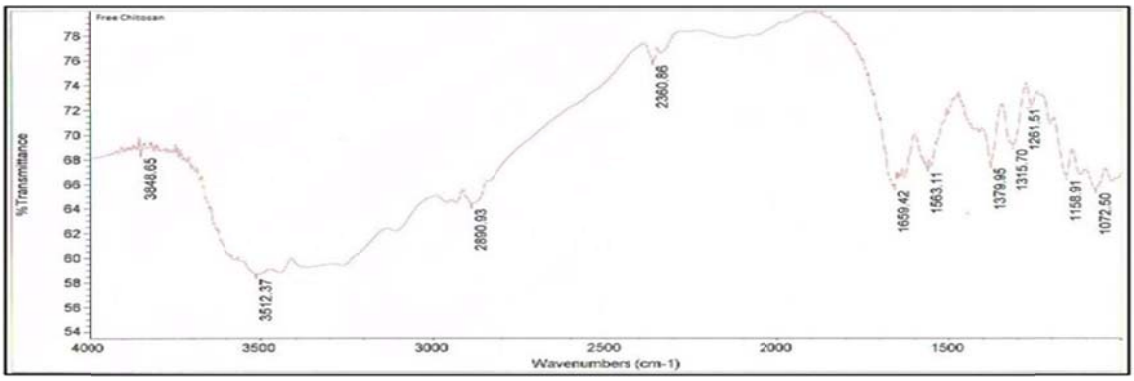

Fig. 4: FTIR Spectrum of "Free" Chitosan

3.2. pHzpc of extracted chitosan

The zero-point charge, pH_{zpc} of the surface of chitosan was determined by approximating the point at which two combined sample titration curves of the suspension and filtrate meet. The resulting net titration curve is shown in Fig. 5.

At solution pH values lower than that required for attaining the zpc, the sites become protonated and an excess positive charge develops on the surface and the contrary occurs at pH values higher than the zpc. The pH_{zpc} of the extracted chitosan is approximately 6.3 (Fig. 5). Since the adsorption of Cr(VI) on the chitosan was done using potassium dichromate solution at pH 2, the sites on the surface of chitosan was protonated and the adsorption of anions were favored. The net positive charge of chitosan surface attracts the Cr(VI) anion species, thus favoring adsorption. This was seen on the FTIR of the "free" and "complexed" chitosan.

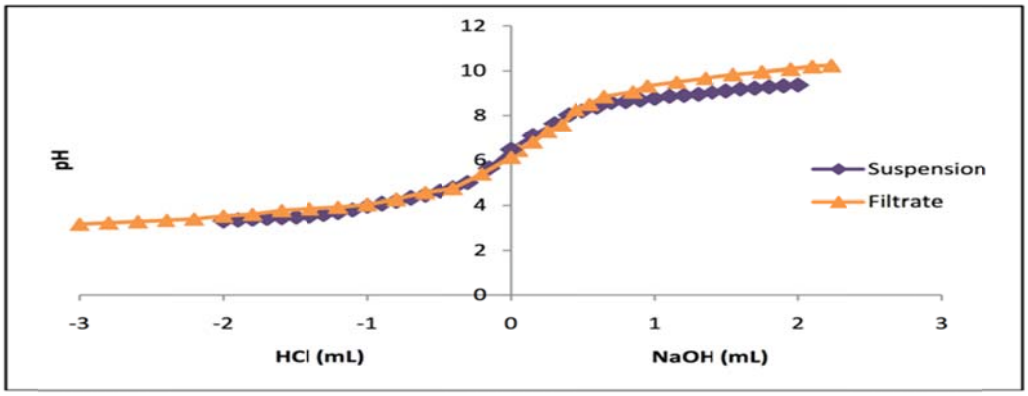

Fig. 5: Titration curve for determination of pH_{zpc}

4. Conclusions and recommendations

Chitosan was able to adsorb chromium ions through its O–H and N–H functional groups. The chitosan-chromium adsorption process was characterized by IR and titrimetric techniques. The pH_{zpc} of the adsorbent chitosan is at 6.3, thus favoring adsorption of anions at pH < 6.3. Other characterization analyses are highly encouraged such as surface area analysis and SEM analysis.

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