Original Research Article

Extraction characterization and application of bio-coagulant for treating dyes containing solution using *aristeus antennatus* and *aristaeomorpha foliacea* red shrimps

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Chitosan is a biopolymer having huge possibilities when it comes to chemical and mechanical structural changes. These physicochemical properties confer to the polymer numerous areas of applications, in particular in the area of water treatment. From the environmental point of view, this will result in a much lower risk of toxicity for the treated waters. In addition, the sludge produced would be in lesser quantity, with a better biodegradability and a low metal content. Shells of red shrimp (*Aristeus antennatus* and *Aristaeomorpha foliacea*) fished locally 45 km to the west of Algiers, were used as raw materials for the extraction of chitosan by deproteinization, demineralization and deacetylation. The final product was characterized by different methods (FTIR, potentiometric titration, SEM, DRX); the deacetylation degree of chitosan was found to be around 75%, which was compared in the rest of this study with commercial chitosan at 95% deacetylation degree. In most cases, this polymer is used as adsorbent in its solid form, but it can also be used in the dissolved state in the coagulation-flocculation process. This work is concerned with the use of dissolved chitosan for the removal of sulfonated azo dyes. Amongst the important parameters affecting the coagulation-flocculation process are the coagulant dose and the initial pH. The best removal rates were found to be between 50% and 55% in acidic media around pH 3. The dye coagulation mechanism appears to be governed by charge neutralization. The dye sulfonic groups being attracted to protonated amino groups of chitosan in the colored solution.

Key words: Red shrimp, chitosan, characterization, coagulation-flocculation, sulfonated azo dyes.

INTRODUCTION

In recent years, great attention has been paid to the bioactivity of natural products obtained from plant, animal and in addition of marine origin, mainly to the concern on the environmental problems regarding the disposal of marine processing shellfish wastes consisting of crustacean exoskeletons. Chitin is a major component of the carapaces, crusts and shells of crustaceans such as shrimps and crabs, its estimated consumption is 4 million tons per year (planetscope.com). Chitin and chitosan are of commercial interest because of their high nitrogen content (6.89%) and their excellent properties such as biocompatibility, biodegradability, non-toxicity and adsorptive abilities (Majeti et al., 2000 and Muzzarelli et al., 2005). Recently, however, chitosan has come back into the
Table 1: Physico-chemical parameters for extraction process

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Takaguchi method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of acid</td>
<td>2N HCl</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Shells to acid ratio</td>
<td>1N NaOH</td>
</tr>
<tr>
<td>Concentration of deproteinizer</td>
<td>Chitin</td>
</tr>
<tr>
<td>Product</td>
<td>33.21</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>40% NaOH</td>
</tr>
<tr>
<td>Concentration of deacetylizer</td>
<td>1:10</td>
</tr>
<tr>
<td>Shells to deacetylizer ratio</td>
<td>120°C/6h</td>
</tr>
<tr>
<td>Temperature/Time</td>
<td>Chitosan</td>
</tr>
<tr>
<td>Product</td>
<td>65.6%</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>Insoluble</td>
</tr>
<tr>
<td>Solubility in water</td>
<td></td>
</tr>
</tbody>
</table>

Natural polymers as coagulant present a particular interest compared to synthetic products for their negative environmental consequences and potential health impacts, for which doubts were raised about their toxicity. Several studies have focused on the use of chitosan for dye waste water (Majeti et al., 2000. Crini et al., 2008 and Szygula et al., 2008), microalgae (Ahmad et al., 2011), and colloidal particles (Divakaran et al., 2001. Huang et al., 1996. Huang et al., 2000. Roussy et al., 2005. Roussy et al., 2005. Ruhsing et al., 2005 and Wang et al., 1996). Because of its cationic behavior and molecular weight, may be used both for coagulation and flocculation, resulting in its natural combination of organic polymer with an inorganic matrix (chitosan / limestone).

Chitosan is produced by deacetylation of chitin; the source of natural chitin used to produce chitosan affects the production parameters and chitosan preparations (Kumirska et al., 2010).

However its natural origin makes it an economic and ecologic coagulation agent, it generates less sludge than alum salt, which can be used as an organic soil without risk.

The aim of this study is to investigate and characterize (by various methods) the extracted chitosan from *aristeus antennatus* and *aristaeomorpha foliacea* red shrimps through demineralization deproteinization and deacetylation during the month of April to June 2015. Shrimp shells were collected from Bouharoun port, 45 km west of Algiers.

The shells obtained were washed thoroughly with distilled water and dried in an oven to constant weight at a temperature of 35°C. The dried shells were ground and sieved to smaller size (0-0.25mm).

The chitin was extracted from shells following the method of (Takaguchi, 1991a) for chitosan production, the chitin was deacetylated following (Takaguchi, 1991b) method (Table 1).

The obtained samples were washed continuously with 50% NaOH and filtered in order to retain the solid matter, the prepared chitosan was then left uncovered and oven dried at 120°C for 24 h.

The obtained chitosan was characterized by Fourier Transform Infrared (FT-IR), Scanning Electron Microscope (SEM), X-Rays Diffraction (XRD) and potentiometric measurement for molecular weight (MW) and viscosity.

To prepare chitosan solution, 100 mg of chitosan were dissolved in 1 ml of acetic acid (85% w/w) with purity of 99.8 %, under agitation and subsequently hydrated overnight at room temperature in 99 ml of demineralised water. The final polymer solution was maintained at pH 4. All reagents used in the experiment were of laboratory grade. They were diluted to the concentration required for the steps with distilled water. All chemicals were used...
without further purification.

Deacetylation degree of extracted chitosan (Ex-Ch) was found to be around 75 %, which was compared in the rest of this study with commercial chitosan (Com-Ch) purchased from Sigma® at 95% deacetylation degree.

The used dyes Brilliant Green (B.G) and Methylene Blue (M.B) were purchased from Biochem Chemopharma. All of dyes were used without purification; this purity was taken into account when calculating the true concentrations. Characteristics and wavelength of different studied dyes are summarized in the Table 2, structures are reported in Figure 1.

The residual concentration was measured by UV-Visible spectrophotometer (SHIMAZDU) at the maximum absorbance peak.

**RESULTS AND DISCUSSION**

**Extraction and characterization of chitosan**

The % yield of chitin and chitosan found are similar with those reported in literature (Das et al.,1996) observed that chitin content were 16.7% in Scylla serrata and 20.19% in Portunus pelagicus, (Das and Anand Ganesh., 2010) the percentage yield to be found in the range of 27.35 to 28.1% of chitin from trash crab, Podophthalmus vigil (Hongpattarakere et al., 2008) reported that the chitin content in carapace of black tiger shrimp Penaeus monodon was 19.9% and produced chitosan 73.6%. In this study, it was investigated to extract chitin and chitosan from aristaeus antennatus and aristaeomorpha foliacea red shrimps using chemical method. The average yield % of chitin is 33.21% and that of chitosan which is produced from the resultant chitin is 65.6%.

% yield of chitin is based on dried and ground shrimp carapace, but % yield of chitosan is based on chitin weight before deacetylation.

**Characterization by FTIR**

The FTIR spectra for extracted chitin and chitosan respectively were presented in Figure 2; the FTIR spectra gave characteristics bands of –NH₂ at 3447 cm⁻¹ and carbonyl group band at 1477 cm⁻¹. It should be noted that
The degree of deacetylation was calculated using the baseline equation as used by (Jiao et al., 2011), the wavelengths 1655 and 3450 cm⁻¹ were the baseline bands used in calculating the degree of deacetylation using the (Younesa et al., 2012) formula.

\[
DA \% = \frac{\text{Abs}_{1655} - \text{Abs}_{3450}}{\text{Abs}_{3450}} \times 100 \times \frac{1.33}{1} \tag{I.1}
\]

* \(\text{Abs}_{1655}\) absorbance at the wavelength of 1655 cm⁻¹ (amide I 1655 cm⁻¹)

* \(\text{Abs}_{3450}\) absorbance at the wavelength of 3450 cm⁻¹ (Hydroxyl 3450 cm⁻¹)

According to Figure 2 and the formula (I.1), the extract chitosan DA is of the order of 25% where the DDA is equal to 75%. The extracted chitosan (Ex-Ch) obtained was categorized as chitosan acceptable degree of deacetylation.

(Zhang et al., 2012) reported that chitin and chitosan contain three characteristic band which are 1577 cm⁻¹, 1654 cm⁻¹ and 2932 cm⁻¹ corresponding to vibration of -NH, -C=O and -CO-CH₃ group. Meanwhile, the content of polysaccharide is represented by bands between 890 and 1156 cm⁻¹ (Liu et al., 2012). Furthermore, chitin has more intense band for 2932 and 1577 cm⁻¹ than chitosan; this difference is the evidence of deacetylation (Zhang et al., 2012).

**Characterisation by potentiometric analysis for degree of deacetylation, molecular weight (MW) and viscosity characterization**

The degree of deacetylation was also determined using potentiometric titration by acid-base as described by (Tolimate et al., 2000) calculated using Czechowska-Biskup formula (I.2) (Czechowska-Biskup et al., 2012) using (OHAUS Starter 2C) pH meter with a sensitive electrode.

\[
DDA\% = 2.03 \times \frac{V_2 - V_1}{m + 0.0042(V_2 - V_1)} \tag{I.2}
\]

- \(m\): mass of chitosan (g).
- \(V_1\) and \(V_2\): volume of NaOH corresponding to two points of inflection (ml)
- 2.03: coefficient of molecular weight of the chitosan monomer unit
- 0.0042: coefficient difference between molecular weight of the acetylated monomer and the molecular weight deacetylated.

The resulting curve Figure 2 contains two points of inflection corresponds to \((V_1\) and \(V_2\)), the DDA calculated using (I.2) is around 77%, is close to that obtained by FTIR (75%).

The viscosity was carried out using (ANDA VIBRO viscometer SV-10), with a capacity from 0.3 to 10,000 mPa.s. the extrapolation of \(C = 0\) gives a viscosity equal to 1.54 dl g⁻¹, where the average viscometric molecular weight is approximately 191.5 kDa (Figure 4). According to (Dambies et al., 2000) this molecular weight provides good power coagulant for extracted chitosan (Ex-Ch). Chitosan usually involved in this type of treatment have a molecular weight on the order of 100 to 500 kDa (Abdou et al., 2008).

**Characterization by X-ray diffraction (XRD)**

XRD analysis was used to detect the chitosan crystallinity between Ex-Ch and Com-Ch. Crystallinity of chitin and chitosan was generated from hydrogen bond between corresponding hydroxyl and N-acetyl groups (Bartnicki-Garcia, 1988). Each crystalline peak characterizes crystallographic structure, which is generated from
parallel and antiparallel alignments of polymeric chains or sheets.

Figure 5 shows the difference between the diffraction patterns of the extract and commercial chitosan.

As for Com-Ch, the strongest three peaks are at 19.92° with intensity of 3838 counts, 22.04° with intensity of 2387 counts and 10.40° with intensity of 1704 counts. Then, the strongest three peaks for Ex-Ch are 19.96° with intensity of 5913 counts, 22.46° with intensity of 3581 counts and 10.44° with intensity of 2515 counts. Ex-Ch has two significant peaks more approximate to 10° and 20°. The broad diffraction peaks 10° and 20° are typical identification for semi-crystalline chitosan. Semi-crystalline chitin and chitosan have amorphous and crystalline regions (Jung, 2013). The difference in the semi-crystalline morphology of chitin and chitosan means, that chitosan obtained in a solid-state reaction has a heterogeneous distribution of N-acetyl groups along the molecular chains (Aiba, 1991).

Furthermore, the diffractogram shows the crystallinity of the commercial chitosan is less than extracted chitosan, the decrease in peak reflection intensity indicate diminution of acetamide groups with a less ordered structure of the polymer. These observations are in agreement with those published (Crini et al., 2009 and Zhang et al., 2005), which explains the difference between the values of degree of deacetylation of commercial chitosan 95% and chitosan extracted 75%.

Characterization by scanning electron microscopy (SEM)

The image obtained by scanning electron microscope of chitosan extracted (Ex-Ch) is presented in Figure 6.

The Figure 6(a) shows that the Ex-Ch has a clear and lamellar surface with shrinkage and straps composed of several fibers bound together, the strength of their bond depends on the DDA (Callet, 2010), explaining the insolubility of chitosan.

Islam et al., 2011 reported that chitosan has a non homogenous and non smooth surface with straps and shrinkage. Figure 6(b) shows that the proposed chitosan has such trends on morphology with absence of crystal residues.

Application to coagulation-flocculation

(Zeng et al., 2008) found a novel composite chitosan flocculant that has been prepared according to the weight proportions of 1% chitosan: 2% Poly Aluminium Chloride (PAC): sodium silicate = 1:100–200:10–20 (w/w). Compared with the conventional flocculant such as PAC, the percentage of removing Chemical Oxygen Demand (COD), Suspended Solids (SS) and Aluminium ions (Al³⁺) in the treated water using this novel composite chitosan flocculant were enhanced by 1.8–23.7%, 50% and 61.2–85.5% respectively, where the running cost was reduced to 34%.

In this study we investigate the determination of coagulation capacity of chitosan towards sulfonic dyes (BG and MB as models), the chitosan was used as primary agent of coagulation and flocculation.

Effect of chitosan dosage and DDA on coagulation-flocculation mechanism

The coagulation-flocculation process consists of three distinct steps, to obtain a complete mixing and homogeneous dispersion of the coagulant in the solution, rapid and high intensity stirring was initiated, followed by slow stirring in order to increase the contact between the coagulated particles and to facilitate the development of large flocs. Finally, the mixing was allowed to settle (Szygula et al., 2008) The process is summarized in Table 3.

The concentration of chitosane was varied in order to
determine the best dosage of coagulant for each dye concentration. Two types of chitosan were used Ex-ch (Extracted chitosan from red shrimp) and Com-Ch (commercial chitosan Sigma) with different DDA 75% and 95% respectively. The coagulation flocculation results from various
mechanisms including electrostatic attraction, sorption (Protonated amine groups), and deriding (high molecular weight of polymer) (Roussy et al., 2005). The anionic dye, bearing sulfonic groups, is attracted electrostatically by protonated amine groups; it allows neutralization of dyes anionic charges, which can bind together and settle. Therefore, the efficiency of chitosan in the treatment of anionic dyes depends on its deacetylation degree, molecular weight and its concentration (Szygula et al., 2008).

Figure 7 and 8 shows the reduction rate of dyes as a function of chitosan concentration, when modifying the chitosan concentration, the residual dye concentration in the settled solution systematically reached a minimum before increasing again. It’s clearly seen that Com-Ch has a higher reduction rate than Ex-Ch due to its high DDA. The charge neutralization is the main mechanism while increasing the molecular weight, part of floculation effect increase due to bridging mechanism which usually requires highest dosage of coagulant floculant. For molecular weight between 200 and 50,000 g mol⁻¹, they define the concentration ranges in which the polyelectrolyte behaved as a floculant and above which it behaved as a dispersant.

High molecular weight of chitosan can be slightly less efficient due to possible interactions between the polymer chains, at least in the coagulation preparation, which contribute to decrease the mobility of polymer chains and the statistical probability for cationic sites present in the polymer chains to encounter anionic charges present in dyes the sulfonic groups of the dry bond into protonated amine groups of different glucosamine units. (Ashmore et al., 2001), observed that for chitosan of medium molecular weight increasing the degree of acetylation increased the rigidity of the chains which decrease interactions between polymer and charges.

Figure 9 shows the difference of efficiency of %R (elimination percentage) on B.G and M.B between Com-Ch and Ex-ch with function of its concentration.

The difference between used chitosans for M.B is in range of 2.5% for 1 to 4 g.L⁻¹ of chitosan while it is 1.5% to 0.5% for BG, this difference comes essentially from the DDA, which affects solubility, hydrophobicity, and electrostatic interactions between polyamions and protonated amino groups of chitosan (Shi et al., 2006 and Sorlier et al., 2001). Knowing that the Ex-Ch and Com-Ch have the DDA 75% and 95% respectively, secondly the structure of the dye has two sulfonic groups compared with M.B (one sulfonic gourp) which interact with protonated amine groups of glucosamine units on polymer chaine. The bridging mechanism involved in the floculation of colloids in particles assumed the sorption of one polymer molecule at the surface of two or more particles. A similar mechanism can be involved in the interaction of chitosan with dyes, one molecule of chitosan can interact with several dye molecules contributing to the formation of flocs (Szygula et al., 2008) due to the presence of stoichiometric reaction between the dye and chitosan (Guibal et al., 2007) so the use of chitosan with acceptable DDA (75%) for B.G elimination shows same rate with high DDA (95%) chitosan compared to M.B due to its structure which presents too sulfonic groups.

When increasing the amount of chitosan added to the solution the difference between the efficiency of two chitosans decrease because of the presence of an excess of protonated amine groups which provide the restabilization of the suspension and a decrease in the efficiency of the process.

**Effect of pH solution on coagulation-floculation**

The interaction between dye molecules and chitosan is basically the combined effect of the charges on dye molecules and the surface of biopolymer (Singh Maurya et al., 2006), both chitosan and used dyes are pH dependent. A dye is a complex aromatic organic compound with various functional groups; its net charge depends on the pH of solution. The pKa of chitosan is from 6 to 6.7 its overall charge vary with the pH of solution and the degree of neutralization of amine groups (Sorlier et al., 2001).

Fig.10 and 11 show for lower pH to 7 the removal of dye is significantly increasing, (Domb, 1999) confirms that at pH 4, 90% of the NH₂ functional groups of the surface of the chitosan are protonated, and gradually reduced to about 50% at pH 6. Consequently, the positive charges on the chitosan surface diminishes when the pH of the solution increases, as any other cationic polymers chitosan acts in a restricted range of pH (Ariffin Abu Hassan et al., 2007), so contributing to the chitosan charge neutralization to destabilize the particle becomes less important if the pH increases. The properties of chitosan, including its cationic behavior and the molecular weight may be used both for charge neutralization (coagulation for the anionic compounds), and the trapping of particles (floculation).

**Effect of dye concentration and stirring time**

The effect of initial dye concentration (1, 10, 20, 30, 40, 50) mg.L⁻¹ of B.G and M.B on the coagulation-floculation was
tested at initial pH of 4.

Figure 12 to 15 presents the coagulation-flocculation of dyes at different concentrations. It can be seen that lowest dyes doses are required to obtain a better color removal, (Guibal et al., 2007) confirmed the presence of stoichiometric reaction between the dye and chitosan by the interaction of various anionic groups (sulfonic) on the dye with different protonated amine groups (inter- and intra-chain associations) (Guibal et al., 2007), the obtained values exceed this stoichiometry reaction due to no accessibility of all amino groups, the dissolution of polymer breaks the hydrogenous bonds between polymer chains rendered the amino groups more available and more accessible why pH of 4 was used in experiments.

The studied dyes showed that low rate of elimination, it’s probably due to the purity of dyes which contain approximately 20% of additives, which may have influenced their aggregation properties.

According to (Walker and Weatherly, 2001), there existed a self-association of the dyes (even at low concentration) with a tendency towards the formation of dimmers and eventually further aggregates of higher molecular weight.

The stirring time has an important role on flocs formation and growth. During this period, the polymer was dispersed throughout the medium and is adsorbed on the surfaces of the colloidal particles by inter-particles bridging or charge neutralization. In addition, higher mixing time will lead to increase the floc formation to equilibrium beyond breaking of flocs is observed, therefore, decrease the rate elimination if the mixing time is too long, the chains tend to break and limit the size of formed flocs. On the other hand, if the mixing time is too short, collisions between flocculant and colloids are not effective to allow them to precipitate.

**Conclusion**

Nowadays chitin and chitosan were commercially prepared from crab and shells. In the present investigation chitin and chitosan were isolated from *Aristaeomorpha foliacea* and *Aristaeus antennatus*, red shrimps through chemical extraction by demineralization, deproteinization and deacetylation. The used conditions provided a chitosan with acceptable degree of deacetylation (75%) and an average molecular weight of 191.5 kDa. Qualitative analysis of extracted chitosan showed a semblance with the commercial chitosan (DDA: 95%).

Solubility of Chitosan acetic acid proved its efficiency for the coagulation-flocculation of Brilliant Green and Methylene Bleu. In acidic solutions, the charge neutralization was responsible for the dye removal; at pH 4 lower amounts of chitosan were required, probably due to the dye aggregation phenomenon. The optimum dosage correlated well with the initial concentration of the dye. The total color removal of the dyes could be obtained even for initial concentrations as high as 1 mg.L⁻¹.

At high concentrations, dye forms large flocs and settled out very quickly (15-20 min), but at low concentrations where small flocs were formed it required more time to settle out (30-40min).

As a result, the wastewater from textile industry can be treated by using chitosan via coagulation and flocculation processes which is more favorable in wastewater treatment due to its environment friendly characteristic.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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