Chapter 3

Chitosan from Marine Crustaceans: Production, Characterization and Applications

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65258

Abstract

Chitosan is a very useful marine polysaccharide that forms structural components in the exoskeleton of crustaceans. In this chapter, the production of chitosan (CH) and chitosan reticulated micro/nanoparticles (CHM) is described. Three case studies corresponding to different effective applications of chitosan are discussed: (i) the performance of CH to destabilize oil/water emulsion waste for water clarification. It was observed that as long as colloidal charge was maintained around zero, turbidity also showed low values and water clarification was achieved. However, when the applied doses were higher than the optimum, colloidal charge and turbidity both increased, showing emulsion restabilization. Emulsions treated with the optimum chitosan doses were clarified in very short periods; (ii) CH and CHM were used as effective antibacterial agents against three different pathogenic microorganisms that are problematic for aquaculture: *Vibrio alginolyticus* and *parahaemolyticus*, and *Lactococcus garvieae* and the minimum bactericidal concentrations were determined; and (iii) the removal of hexavalent chromium and the comparative performance of CH versus CHM. Results showed that at pH < 2, the adsorption capacity of CHM was higher because CH is unstable. Additionally, Cr(VI) was adsorbed on CH without further reduction; in contrast, Cr(VI) adsorbed on CHM was reduced to nontoxic Cr(III).

Keywords: chitosan, coagulation, flocculation, antimicrobial properties, aquaculture, hexavalent chromium removal, micro and nanoparticles

1. Introduction

The largest group of marine arthropods is the class crustacean, made up of approximately 30,000 species. Marine crustaceans include animals as shrimp, crabs, lobsters, etc. The

crustaceans have particular biological characteristics; they have an exoskeleton made of the polysaccharide chitin and calcium. This external shell, in addition to being protective, gives rigid support for the attachment of the muscles. When crustaceans grow, their outer shell, the exoskeleton, does not grow with them, so they must regularly shed these shells in order to increase in size. This process is known as molting; it occurs periodically whenever the body is ready to increase in height and weight, and involves the detachment of the exoskeleton [1]. Chitin, poly β-(1-4)-N-acetyl-D-glucosamine, is a natural polysaccharide synthesized by an enormous number of living organisms. Considering the amount of chitin produced annually in the world, it is the most abundant polymer after cellulose. Chitin occurs in nature as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods, like crustaceans, or in the cell walls of fungi and yeasts and in the pens of squids [2]. Crustaceans are of great direct and indirect importance to humans. The larger crustaceans (shrimps, lobsters and crabs) are used as food throughout the world and are therefore important to human economies. However, seafood processing industry discards large amounts of crustacean shellfish wastes; exoskeletons are converted in a solid residue, which accumulate in landfills becoming an environmental pollutant. The crustacean processing industries over the world turn out more than 60,000 ton of waste every year [3]. The exoskeleton of the crustaceans represents approximately 50–60% of the total weight in crabs and between 35 and 50% in shrimps. These crustacean wastes contain about 10–25% of chitin on dry weight basis, depending on the species. The proper utilization of these shell wastes not only solves the problem of their disposal but also forms the basis for many potential products used in different fields such as textiles, photography, medicine, agriculture, food processing, etc. Despite the widespread occurrence of chitin, up to now the main commercial sources of chitin have been crab and shrimp shells [4]. Chitosan (poly β-(1-4)-D-glucosamine) is a cationic linear polysaccharide obtained by partial deacetylation of chitin (**Figure 1**). It is composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The cationic nature of chitosan is owed to the free amino groups left by partial removal of acetyl groups of chitin. Chitosan is a nontoxic, biocompatible and biodegradable polysaccharide with many biomedical, chemical, agriculture and wastewater treatment applications [3], and represents an attractive alternative to other biomaterials because of its physico-chemical characteristics, chemical stability, high reactivity and excellent chelation behavior. Chemical and physical modifications of chitosan have been used to increase the stability of the polymer and to improve its functionality. In the last years, the production of chitosan nanoparticles (CHM) was investigated in different scientific areas as carriers of drugs, antifungal and antibacterial agents and metal bioadsorbents [5–7].

The major global producers of chitosan are Japan and the US as well as China, India, Australia, Poland and Norway [8].

In the present chapter, chitosan obtained from shrimp exoskeletons and chitosan micro/nanoparticles were tested in different applications. Three case studies are described: destabilization and clarification of emulsified wastewater, antimicrobial activity in aquaculture and removal of heavy metals from residual water.

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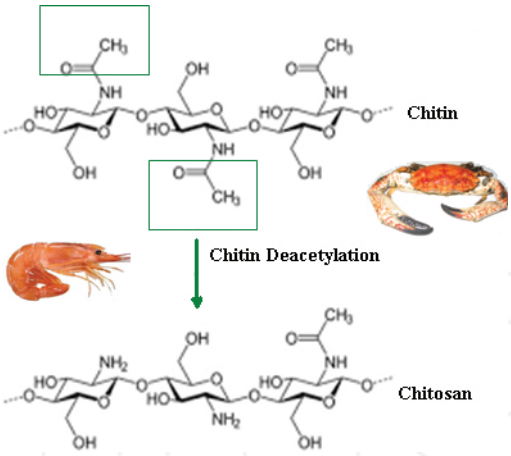

Figure 1. Chitin and chitosan.

2. Chitin and chitosan production and characterization

Shrimps shells (*Pleoticus muelleri*) were used for the extraction of chitin and chitosan. The shells were provided by the industry from Puerto Madryn, Patagonia, Argentina. Crude shrimp chitin was purified using acid and alkaline treatments, according to Dima et al. [9]. The exoskeleton powder was treated with HCl solutions to remove minerals and then treated with aqueous sodium hydroxide solution to remove proteins. The solid was washed with deionized water to reach neutral pH. Finally, the wet mass was dried at 65°C for 24 h; the product obtained was designated as chitin. Chitin was treated with concentrated sodium hydroxide solution at 120°C. After filtration, particles were washed with deionized water to neutral pH and dried at 65°C for 24 h, obtaining chitosan particles.

The yield of purified chitin was 24.8%, and this value was into the range reported by different authors [10, 11]. After N-deacetylation, the yield of shrimp chitosan represented the 76.9% of the initial crude chitin. The degree of N-deacetylation and the molecular weight were measured to characterize the obtained chitosan.

The N-deacetylation degree (DD%) of shrimp chitosan samples was determined using the potentiometric technique and Fourier transform infrared spectra (FTIR). The DD% by the potentiometric technique was determined according to Broussignac [12]. A sample of chitosan was mixed with 0.3 mol/L HCl. Potentiometric evaluation was carried out with a 0.1 mol/L NaOH solution, using a pH meter. The potentiometric curves were obtained by measuring the change in pH after each 2 mL addition of base solution. The titration curve shows two inflection points (**Figure 2a**); the difference between these points corresponds to the amount of acid required to protonate the amino groups of chitosan. The percentage of N-deacetylation was determined according to the following expression:

$$DD\ (\%) = \left(\frac{203 M_{eq}}{1 + 42 M_{eq}} \right) \quad (1)$$

where $M_{eq} = (N\Delta V)/w$; ΔV is the volume difference between both inflection points, N is the normality of NaOH solution, w is the chitosan mass used; 203 is the molar mass of glucosamine and 42 is the molar mass of the acetyl group.

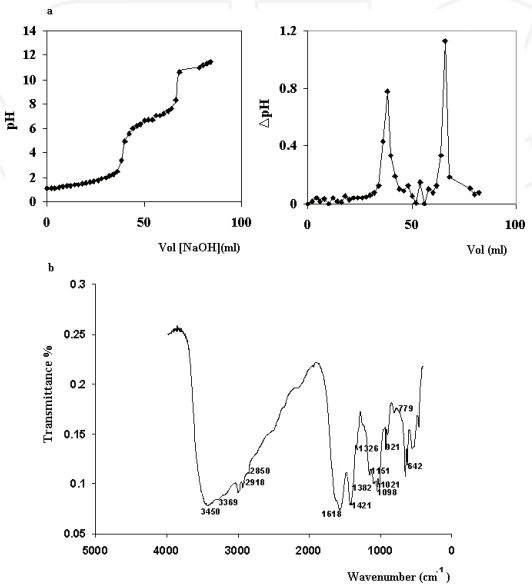

Figure 2. (a) Potentiometric titration curve for shrimp chitosan. Peaks correspond to the inflection points of the titration curve. (b) FTIR spectrum of CH obtained from shrimp.

For the determination of the degree of N-deacetylation by FTIR, chitosan was mixed with potassium bromide and compressed into pellets. The absorbances at 1320 and 1420 cm^{-1} were used to calculate the DD% according to the following equation proposed by Brugnerotto et al. [13]:

$$DD\ (\%) = 100 - \left(31.92 \times \frac{A_{1320\ cm^{-1}}}{A_{1420\ cm^{-1}}} \right) - 12.20 \quad (2)$$

Figure 2b shows the FTIR spectrum of shrimp chitosan; different characteristic bands can be observed: at 3450 cm^{-1} (N-H and O-H stretching vibrations), 2918 and 2852 cm^{-1} (tension group C-H), 1721 cm^{-1} (C=O carbonyl group), 1618 cm^{-1} (amine group), 1326 cm^{-1} (amide III), 1382 cm^{-1} (—CH_3 symmetrical deformation mode (scissoring) in amide group). The N-

deacetylation degree calculated by the potentiometric technique was 90.2% and by FTIR was 86.3%; these values fall into the range of commercial chitosan. The degree of deacetylation necessary to obtain a chitosan product must be 65% or higher [14, 15].

The molecular weight of chitosan was determined by the viscometric method. The intrinsic viscosity [η] of shrimp chitosan was measured with an Ostwald capillary viscometer. The chitosan sample was dissolved in 0.3 M acetic acid, 0.2 M sodium acetate buffer. The viscosity average molecular weight of chitosan (Mv) was calculated by measuring the intrinsic viscosity [η] according to Mark-Houwink-Kuhn-Sakurada (MHKS) equation:

$$[\eta] = k\ Mv^{a} \tag{3}$$

where k and a are viscometric coefficients, which depend on the polymer, the solvent used and the temperature; the parameters proposed by Rinaudo et al. [4] were adopted. In the case of chitosan with 12% degree of acetylation (deacetylation degree DD = 88%) in a solution 0.3 M acetic acid, 0.2 M sodium acetate buffer (at 25°C), the recommended values are k = 0.074 ml/g and a = 0.80 obtaining an average molecular weight of 1.46×10^5–1.52×10^5 Da; this value falls into the range reported by other authors, for different chitosan sources (1×10^5–5×10^5 Da) [4, 14, 15].

3. Chitosan as destabilizing agent of oil/water emulsion wastes for water clarification

Food processing plants generally discharge large volumes of wastewater. Emulsified oil in wastewater constitutes a severe problem in the different treatment stages. Oil in wastewaters has to be removed in order to: (1) prevent interference in water treatment units; (2) reduce fouling in process equipment; (3) avoid problems in the biological treatment stages; and (4) comply with water discharge requirements. Cationic polyelectrolytes such as chitosan can be used to coagulate and flocculate colloidal systems [16].

Chitosan is a natural linear polyelectrolyte at acidic pH; it has a high charge density, one charge per each glucosamine unit. The chain structure has positively charged amine functional groups which are responsible for the polyelectrolyte behavior. Chitosan can coagulate negatively charged material with its positively charged functional groups to give electric neutrality [17, 18].

The performance of chitosan as a destabilizing agent for emulsion wastes in order to clarify residual water was tested in the laboratory. Experiments were carried out on a model waste system consisted of sunflower oil/water emulsions with variable ionic strength (NaCl concentrations ranging between 10^{-3} and 10^{-1} mol/L). A biodegradable ionic surfactant (sodium dodecyl sulfate, SDS, molecular weight = 288.36) was added to each sample to stabilize the emulsions inhibiting the coalescence of the oil droplets. The presence of SDS produced a negatively charged emulsion. The emulsions were prepared in a colloidal mill at maximum

speed with a stirring time of 15 min. Stability of the emulsions was verified by micrographs obtained by light microscopy over three days storage time. Sizes of the emulsion drops were measured by image analysis software. For comparison purposes, simultaneous experiments using another polyelectrolyte, a cationic polyacrylamide of high molecular weight (MW = 4.106), were carried out. Chitosan solutions (5 g/L) were prepared by dissolving chitosan in 1% (v/v) acetic acid solution during continuous agitation for several hours [16]. Cationic polyacrylamide (1 g/L) was prepared by dissolving the polyacrylamide in distilled water. The performance of both destabilizers was tested in terms of the doses and the time necessary to reach minimum turbidity in the system. The pH of the system changed from 4 to 8 by adding either NaOH or HCl. Flocculation experiments were carried out by adding the desired amount of the tested destabilizers to the emulsion with continuous agitation by a magnetic stirrer. To analyze the flocculation process, different techniques were used: electrophoretic mobility, colloid titration, jar test, turbidimetric method and light microscopy observation. Colloid titration was used to determine the colloidal charge and the isoelectric point of the system. A known excess amount of methyl glycol chitosan (MGC) was added in each test. MGC is a cationic polysaccharide which acts as a positively charged titrant over the entire pH range. The oppositely charged colloids react almost stoichiometrically, neutralizing the charge of the system. The remaining excess of MGC was back-titrated by potassium polyvinyl alcohol sulfate (PVSK), a negative colloid using toluidine blue (TB) as an indicator [16]. Electrophoretic mobility and colloidal titration methods gave equivalent information about the dose of polyelectrolyte to reach zero colloidal charge [18]. Microscopy observations were done on the emulsions and on the flocs. Flocculation assays were performed using the jar test with six stirrers having a maximum speed of 250 rpm. Different amounts of polyelectrolyte were added to 500 ml aliquots. Samples were stirred at high speed for 3 min and then at 50 rpm for 10 min. In the polyelectrolyte treatments, as long as colloidal charge was maintained around zero, turbidity showed the lowest values. However, when the applied doses were higher than the optimum, colloidal charge and turbidity both increased showing emulsion restabilization (**Figure 3**).

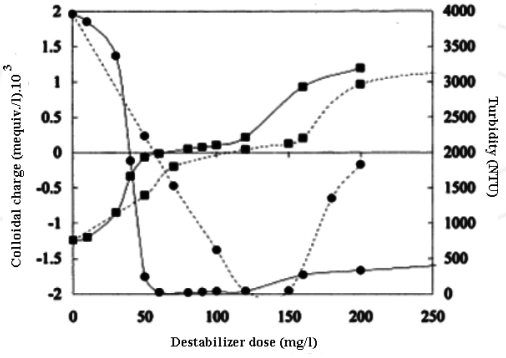

Figure 3. Colloidal charge (■) and turbidity (•) as a function of destabilizer dose: chitosan (—) and cationic polyacrylamide (----) for emulsions formulated with 5 g oil/L, 0.2 g SDS/L and 10^{-3} mol NaCl/L.

Nonsignificant differences between the doses to reach zero colloidal charge and those to reach minimum turbidity (optimum dose) were observed for both polyelectrolytes. Doses to reverse colloidal charge were lower for chitosan than for polyacrylamide; these results can be explained based on the higher charge density of chitosan, requiring lower doses to destabilize the emulsion. Differences in charge density between chitosan and polyacrylamide were detected by the titration of their electrical charge with potassium polyvinyl sulfate; results showed that charge density ratio, between chitosan and polyacrylamide, was 1.64 [16].

When NaCl concentration increased (higher ionic strength), the dose of destabilizer necessary to reach zero colloidal charge decreased. An increase in neutral salt concentration decreases the thickness of the double layer, and the electric potential falls markedly with distance. The repulsive forces diminish and the distance between colloidal particles is reduced, producing coagulation.

In the case of chitosan treatment, flocculation occurred immediately after its addition, requiring a shorter agitation time compared to that of polyacrylamide; pH values ranging between 4 and 8 had no significant effect ($p < 0.05$) on the optimum chitosan dose to destabilize the emulsion.

A linear relationship between initial colloidal charge and the chitosan dose necessary to reach zero colloidal charge was found and this result allows to determine the optimum dose of chitosan, knowing the initial physico-chemical variables of the colloidal system.

4. Synthesis and characterization of chitosan reticulated micro/nanoparticles

Chemical and physical cross-linking techniques are usually employed to modify chitosan. Chitosan chains can be chemically cross-linked with glutaraldehyde leading to quite stable matrixes; however, this reagent is toxic and a strong irritant. On the other hand, chitosan hydrogels can be obtained by ionic gelation, where micro- or nanoparticles are formed by electrostatic interactions between the positively charged chitosan chains and polyanions employed as physical cross-linkers. Chitosan reticulated microparticles (CHM) were prepared by ionic gelation of chitosan with a nontoxic reagent (tripolyphosphate), according to Calvo et al. [19] and modified by Dima et al. [9]. To obtain CHM, different concentrations and relative proportions of chitosan-TPP solutions were mixed under magnetic stirring; in each case, particle size was evaluated. When an opalescent suspension was detected, the presence of microparticles was observed (**Figure 4**). Microparticles were collected by centrifugation and were observed by SEM. Size distribution and zeta potential of CHM were determined by dynamic light scattering. For a concentration range of 1.00–1.50 g/L of TPP and 1.00–1.25 g/L chitosan, the obtained CHM size ranged between 88 and 140 nm (polydispersity <1), coinciding with that observed by SEM (**Figure 5**); pH has a marked effect on the electrokinetic behavior (Z-potential) and on particle size distribution. A high absolute value of Z-potential denotes stability of particles in suspension; Z-potential decreased with the amount of TPP added. The

effect of pH and the amount of TPP added on particle size distribution and Z-potential are shown in **Figure 6**.

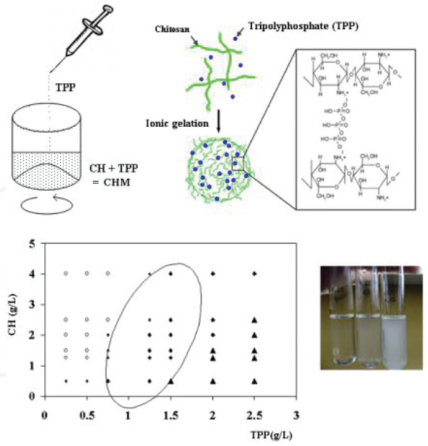

Figure 4. (a) Schematic procedure for the synthesis of micro/nanoparticles (CHM). (b) Effect of the relative concentrations of chitosan and tripolyphosphate on the formation of CHM. The photograph shows the aspect of the different zones in the graph: (♦) aggregates, (▲) clear solution, (○) opalescent suspension.

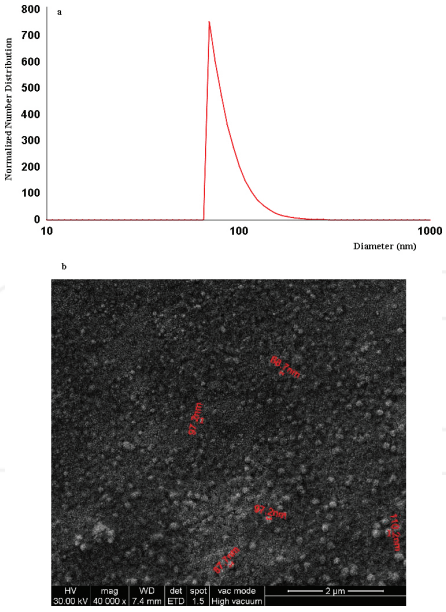

Figure 5. Particle size distribution determined by the Beckman Coulter equipment and SEM micrograph of chitosan micro- and nanoparticles for a ratio of CH:TPP of 1.25:1.50 (g/L).

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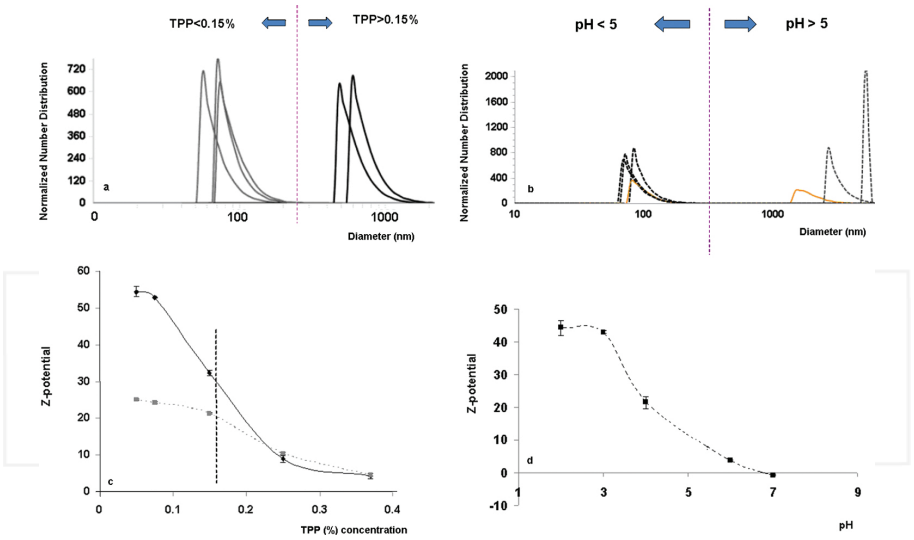

Figure 6. Characterization of chitosan-TPP micro/nanoparticles. Effect of TPP concentration on: (a) particle size distribution; (c) Z-potential (pH = 4): CHM in the original suspension (full line), CHM resuspended in water (dotted line). Effect of pH on: (b) particle size distribution; (d) Z-potential [Ratio of CH:TPP of 1.25:1.50 (g/L)].

5. Applications of chitosan and chitosan micro- and nanoparticles

5.1. Chitosan as antimicrobial agent in aquaculture

Aquaculture is the farming of aquatic organisms such as fish, crustaceans, mollusks and aquatic plants. It is one of the fastest growing food-producing sectors. This accelerated growth of finfish aquaculture has resulted in a series of developments detrimental to the environment and human health [20]. The use of a wide variety of antibiotics in large amounts, including nonbiodegradable antibiotics useful in human medicine, ensures that they remain in the aquatic environment, for long periods of time. This process has resulted in the emergence of antibiotic-resistant bacteria in aquaculture environments, in the increase of antibiotic resistance in fish pathogens, in the transfer of this resistance to bacteria of land animals and to human pathogens, and in alterations of the bacterial flora both in sediments and in water [20]. Chitosan as an antimicrobial agent can be considered an alternative to the use of these antibiotics. Chitosan and its derivatives have attracted considerable interest due to their antimicrobial and antifungal activity. It has been documented that chitosan itself has antimicrobial activity due to its cationic properties that cause a membrane-disrupting effect [20–23]. The antibacterial activity of chitosan is influenced by a number of factors that include the degree of chitosan polymerization and some of its other physicochemical properties. Chitosan exhibits higher antibacterial activity against Gram-positive than Gram-negative bacteria [23]. A number of chitosan derivatives with different modifications have been prepared to improve its antibac-

terial activity; in this way, chitosan micro/nanoparticles display unique physical and chemical features [6]. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus- and sulfur-containing compounds such as DNA and protein [21, 22].

Experiments were performed using chitosan and reticulated chitosan microparticles as antibacterial agents against different microorganisms that are problematic for aquaculture: *Lactococcus garvieae* (Gram +), *Vibrio parahaemolyticus* and *Vibrio alginolyticus*, both Gram −. These microorganisms have been implicated as the main bacterial pathogens of mariculture industry and are responsible of important economic losses in cultured fish and seafood worldwide [24].

The minimal bactericidal concentration (MBC) values were determined by a standard broth dilution method. For the analysis, a number of sterile test tubes containing 5 mL of TS broth (Tryptic Soy) with NaCl for vibrios and MRS broth for *L. garvieae* were used. The initial concentration of stock solution was 1% (m/v) for chitosan in acetic acid solution (pH 4.8) and 0.8% (m/v) for chitosan reticulated microparticles in distilled water. Serial twofold dilutions of stock solutions were performed for each culture broth. The tubes were inoculated under aseptic conditions with 50 μL of the freshly prepared bacteria suspension (*Vibrio alginolyticus*, 9.6×10^7 UFC/mL; *V. parahaemolyticus*, 1×10^8 UFC/mL and *L. garvieae*, 5×10^7 UFC/mL). MBC was determined transferring an aliquot of contents of each tube after 24 h to TS or MRS agar plates and incubated at 30°C for 24 h. The lowest concentration showing no revival strain on agar plates was considered as the MBC. For the selected pathogens, the MBC for reticulated chitosan was 0.20%, while for chitosan was 0.25% for *V. alginolyticus* and 0.125% for *V. parahaemolyticu* and *L. garvieae*. The antibacterial activity of chitosan particles and microparticles can be attributed to the disruption of cell membranes and the leakage of cytoplasm [21, 22, 25].

5.2. Removal of heavy metals from water using chitosan and chitosan reticulated micro/nanoparticles

Chitosan has been broadly used for the sorption of heavy metals; it combines with metal ions by three forms: ion exchange, adsorption and chelation [7]. This biopolymer has been shown to effectively remove metals such as chromium, copper, mercury and lead [7, 9, 25, 26] from aqueous solutions. In recent years, research has been performed on novel adsorbents to maximize their adsorptive capacity [27, 28]; chemical and physical modifications of chitosan have been used to increase the stability of the polymer and to improve its functionality. According to Schmuhl et al. [26], chitosan forms chelates with metal ions by releasing hydrogen ions, and hence, the adsorption of a metal ion on chitosan depends strongly on the pH of the solution. Chitosan is soluble in most dilute mineral acids (except in sulfuric acid solutions) and in dilute organic acids, such as acetic, propionic, formic and lactic acids [28]. Consequently, its chemical stability needs to be reinforced through treatments using cross-linking agents for its application in acidic media. These treatments induce new linkages between the chitosan chains allowing the polymer to be highly resistant to dissolution, even in solutions, such as hydrochloric acid [28, 29].

Cr(VI) is a toxic metal and must be removed from wastewater before it can be discharged. Cr(III) and Cr(VI) are the stable oxidation states for chromium in nature. Cr(III) is stable and

less toxic or nontoxic and is considered an essential element for the good health and nutrition of many organisms. Cr(VI) is 500 times more toxic, mutagenic and carcinogenic than Cr(III). The United States Environmental Protection Agency has laid down the maximum contaminant level for Cr(VI) into inland surface waters as 0.1 mg/L and in domestic water supplies to be 0.05 mg/L [9, 26, 30].

The performance of chitosan and reticulated chitosan micro/nanoparticles in the adsorption process of Cr(VI) was analyzed in the laboratory [9]. Adsorption experiments were performed using different initial concentrations of Cr(VI) (50–400 ppm), contact times (30 min–2 h) and pH values (2–6). All the adsorption experiments of Cr(VI) ions onto CH or CHM were carried out in batch at 25°C, under constant stirring. Final concentrations of Cr(VI) were determined by flame atomic absorption spectrometry. The equilibrium adsorption capacity of Cr(VI) onto chitosan (Q_e) was calculated according to the following equation:

$$Q_e = \frac{(C_i - C_{eq})}{w} V \tag{4}$$

where Q_e (mg/g) is the amount of metal ions adsorbed by the CH or CHM, C_i and C_{eq} are the metal concentrations (mg/L) in the solution initially (time zero) and after equilibrium, respectively, V(L) is the volume of the solution and w is the mass (g) of adsorbent used.

The percentage of Cr(VI) removal was calculated according to:

$$(\%)\ \text{Removal} = \left(\frac{C_i - C_{eq}}{C_i} \right) \times 100 \tag{5}$$

The pH and the initial chromium concentration have a marked effect on the adsorption process. The optimum pH value for the adsorption of Cr(VI) was 4 for CH and 2 for CHM. The highest value of Q_e (equilibrium adsorption capacity) was 127.1 mg/g for CH (pH = 4), and in the case of CHM, a higher value of Q_e = 135.2 mg/g was observed at pH = 2. At very low pH, the adsorption capacities were higher for the CHM because chitosan is unstable at pH < 2.5, and thus cross-linking with TPP improved the adsorption performance of Cr(VI). The adsorption capacity increased from 31.4 to 230.2 mg/g for CH and from 35.5 to 71.4 mg/g for CHM, when the initial Cr(VI) concentration varied from 50 to 400 mg/L, at pH = 4. At pH = 2, the adsorption capacity of CHM increased and adsorption capacity of CH decreased. **Figure 7a**, **b** shows the simultaneous effect of initial Cr(VI) concentration and pH on the percentage of Cr(VI) removal [Eq. (5)] for CH and CHM, respectively. Contact time is an important parameter because this factor determines the adsorption kinetics of an adsorbent at a given initial concentration of the adsorbate. The adsorption kinetic curves of Cr(VI) (initial concentration of 100 mg/L, pH = 4) onto CH and CHM are shown in **Figure 7**. The curves show that equilibrium was reached after approximately 1 h for CH (**Figure 7c**) and after 2 h for CHM (**Figure 7d**). The maximum amounts of adsorbed chromium were produced after 3 h contact time obtaining equilibrium

values of 66.9 mg/g for CH and 38.8 mg/g for CHM. After these periods, both systems remained almost unchanged until the end of the experiment.

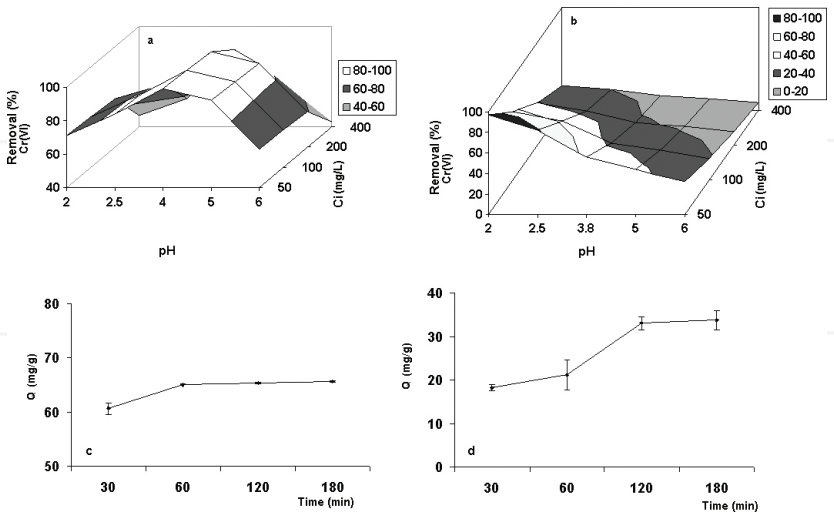

Figure 7. Effect of contact time on the adsorption capacity (Q) of chromium (VI) on: (a) CH; (b) CHM. Simultaneous effect of chromium (VI) initial concentration and pH on the percentage of Cr(VI) removal using; (c) CH; (d) CHM.

Other important observation was that the chemical analysis to determine the oxidation state of the adsorbed Cr showed that Cr(VI) was adsorbed on CH particles without further reduction; in contrast, Cr(VI) removed from the solution was reduced and bound to the CHM as Cr(III). The reduction in toxic Cr(VI) to the less or nontoxic Cr(III) by the reticulated chitosan micro/nanoparticles can be considered a very efficient detoxification technique for the treatment of Cr(VI) contaminated water [9].

Langmuir, Freundlich and Temkin equilibrium sorption isotherms [30] were used for the mathematical description of the adsorption equilibrium of Cr(VI) ions on CH or CHM adsorbents. The operating parameters were: T = 25°C, pH = 4, time = 3 h. Adsorption was evaluated by the determination of Cr(VI) concentration in the solution under equilibrium conditions reached at the end of the experiments (3 h, pH = 4). The Langmuir isotherm is given by the equation:

$$\frac{C_{eq}}{Q_e} = \frac{C_{eq}}{Q_m} + \frac{1}{K_L Q_m} \tag{6}$$

where Q_e is the amount adsorbed per unit weight of adsorbent at equilibrium (mg/g); C_{eq} is the equilibrium concentration of adsorbate in solution after adsorption (mg/L). K_L is the

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Langmuir constant (g/L) related to the affinity of the binding sites; Q_m is the maximum monolayer adsorption capacity (mg/g).

The Freundlich isotherm is an empirical equation that assumes that the adsorption process takes place on heterogeneous surfaces and adsorption capacity is related to the concentration of Cr(VI) at equilibrium. It is defined as follows:

$$\ln Q_e = \ln K_f + \left(\frac{1}{n}\right)\ln C_{eq} \tag{7}$$

where K_f is the Freundlich constant or capacity factor (mg/g) and 1/n is the Freundlich exponent; n is the heterogeneity factor related to adsorption intensity.

The Temkin isotherm in its linear form is given by the equation:

$$Q_e = B_t \ln(K_t) + B_t \ln(C_{eq}) \tag{8}$$

The obtained parameters from Freundlich, Langmuir and Temkin adsorption equations and the corresponding R^2 values (coefficients of determination) are shown in **Table 1**. The regression values indicate that the adsorption data for Cr(VI) removal fitted well to Langmuir isotherm. This equation is representative of monolayer adsorption occurring on an energetically uniform surface on which the adsorbed molecules are not interactive. For CHM, adsorption at pH = 2 was more effective than at pH = 4.

	Langmuir			**Freundlich**			**Temkin**		
	Qm (mg/g)	**K_L (l/mg)**	**R^2**	**1/n**	**K_f (mg/g)**	**R^2**	**B_t**	**K_t (l/mg)**	**R^2**
Chitosan (CH) pH = 4	250	0.018	0.999	0.43	44.70	0.941	45.01	2.76	0.997
Reticulated microparticles (CHM) pH = 4	68.9	0.014	0.990	0.36	7.02	0.983	13.95	1.76	0.989
Reticulated micro/nanoparticles (CHM) pH = 2	124	0.086	0.990	0.12	60.42	0.977	22.55	1.46	0.939

Table 1. Parameters of the equilibrium isotherms for Cr(VI) adsorption upon chitosan and chitosan microparticles.

The kinetic data were analyzed using the pseudo-first-order, pseudo-second-order kinetic models and Elovich equation [31]. Kinetic analysis is required to get an insight into the rate of adsorption and the limiting step of the transport mechanism, which are primarily used in the modeling and design of the process [32]. The pseudo-first-order kinetic model of Lagergren has been widely used to predict the metal adsorption kinetics and is given by:

$$\frac{dQ}{dt} = k_1(Q_e - Q) \tag{9}$$

where Q is the amount of metal adsorbed at any time (mg/g), Q_e is the amount of metal adsorbed at equilibrium time (mg/g) and k_1 is the pseudo-first-order rate constant (min^{-1}). Integrating Eq. (9) becomes:

$$\ln\left(\frac{Q_e}{Q_e - Q}\right) = k_1 t \tag{10}$$

The adsorption kinetic data can be further analyzed using the pseudo-second-order kinetics, which is represented by:

$$\frac{dQ}{dt} = k_2 (Q_e - Q)^2 \tag{11}$$

where k_2 is the pseudo-second-order rate constant. Integrating Eq. (11) gives:

$$\frac{t}{Q} = \frac{1}{k_2 Q_e^{\,2}} + \frac{1}{Q_e} t \tag{12}$$

The Elovich or Roginsky-Zeldovich equation is satisfied in chemical adsorption processes and is suitable for systems with heterogeneous adsorbing surfaces. This equation has been widely used in adsorption kinetics, which describes chemical adsorption mechanism. It is generally expressed as follows:

$$\frac{dQ}{dt} = \alpha \exp(-\beta Q) \tag{13}$$

where Q is the amount of metal adsorbed by chitosan at a time t, α is the initial adsorption rate (mg g^{-1} h^{-1}), and β is the Elovich constant. To simplify the Elovich equation, Chien and Clayton [33] assumed $\alpha\beta t \gg 1$, and on applying the initial condition $Q = 0$ at $t = 0$, the equation becomes:

$$Q = \beta \ln(\alpha\beta) + \beta \ln t \tag{14}$$

	Pseudo-first order		**Pseudo-second order**		**Elovich**		
	k_1(h^{-1})	**R^2**	**k_2 (g/mgh)**	**R^2**	**β (g/mg)**	**α (mg/gh)**	**R^2**
Chitosan (CH)	2.71	0.871	0.76	0.999	0.37	6.0×10^{10}	0.750
Chitosan micro/nanoparticles (CHM)	1.94	0.932	0.078	0.946	0.10	116.8	0.877

Table 2. Kinetic parameters for Cr(VI) adsorption on chitosan flakes (CH) and chitosan reticulated microparticles (CHM).

http://dx.doi.org/10.5772/65258

The parameters obtained by the different tested kinetic equations with the corresponding correlation coefficients are given in **Table 2**.

For CH, the kinetic studies indicated a rapid removal of chromium from aqueous solutions. The large value of α in Elovich equation for chitosan particles indicates a very high initial adsorption rate in comparison with the microparticles. The kinetic analysis of chromium adsorption showed that pseudo-second-order kinetic model fitted successfully the experimental data.

6. Conclusions

Chitosan is a marine polysaccharide with multiple applications in different fields. Chitin and chitosan were obtained from shrimps shells (*Pleoticus muelleri*) from Patagonia, Argentina. The degree of N-deacetylation and the molecular weight were measured to characterize the obtained chitosan. The N-deacetylation degree (DD%) of shrimp CH samples was determined using the potentiometric technique and Fourier transform infrared spectra. The molecular weight of CH was determined by the viscometric method measuring the intrinsic viscosity and using the Mark-Houwink-Kuhn-Sakurada equation.

Chitosan is a cationic polyelectrolyte that was applied to coagulate and flocculate colloidal systems; oil/water emulsion wastes were destabilized for water clarification. Results showed that as long as colloidal charge was maintained around zero, turbidity showed the lowest values and water clarification was achieved. However, when the applied doses were higher than the optimum, colloidal charge and turbidity both increased showing emulsion restabilization. Emulsions treated with the optimum CH doses were clarified in very short periods.

Chitosan reticulated micro/nanoparticles were prepared by ionic gelation of chitosan with a nontoxic reagent (tripolyphosphate, TPP). Microparticles were observed by SEM; size distribution and zeta potential were determined by dynamic light scattering. Chitosan and reticulated microparticles were used as effective antibacterial agents against different pathogenic microorganisms that are problematic for aquaculture: *Vibrio alginolyticus* and *parahaemolyticus*, and *L. garvieae* and the minimum bactericidal concentrations were determined.

Finally, the performance of CH and reticulated chitosan micro/nanoparticles in the adsorption process of Cr(VI), a very toxic metal, was analyzed. At very low pH, the adsorption capacity was higher for the microparticles because CH is unstable at pH < 2.5. Chitosan cross linking with TPP improved the adsorption performance of hexavalent chromium at low pH. In addition, Cr(VI) was reduced and bound to the microparticles as Cr(III). The reduction of toxic and carcinogenic Cr(VI) to the less soluble and less toxic Cr(III) by the reticulated chitosan microparticles can be considered a very efficient detoxification technique. Therefore, the use of marine polysaccharides such as chitosan is a potential tool for innovative technologies to improve environmental quality and sustainability.

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