



BIOCOMPATIBLE NANO-COMPOSITE FROM CRAB SHELL WASTE: PREPARATION, CHARACTERIZATION AND THEIR POSSIBLE APPLICATION

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ABSTRACT

Chitosan which is known to have multiple functional properties has created significant interest among the researchers due to their biological activities and applications in the food, pharmaceutical and agricultural industries. Chitin and chitosan are the second most available biopolymer after cellulose. The present study was designed to evaluate the alternative uses for seafood wastes that are economically feasible. In the present work, extraction of crude chitosan using strong acids and one-step extraction of chitosan by co-fermentation with *Lactobacillus sps.* from red crab shell waste were tried. To achieve demineralization of crab shell waste by chemical and biological treatments, hydrochloric acid and lactic acid bacterium were applied. The pH decreased from 6.17 to 5.49 and from 6.12 to 5.29 at day 7 respectively. The study also deals with, the preparation, characterization and dye adsorption properties of novel biocompatible composite (Chitosan-zinc oxide nanoparticle) (CS/n-ZnO) were investigated. Two textile dyes, Coomassie Brilliant Blue and Acid Blue, were used as model compounds. The effect of CS/n-ZnO doses, initial dye concentration, and pH were elucidated. The results showed that the CS/n-ZnO being a biocompatible, eco-friendly and low-cost adsorbent that can be used for the adsorption of dye from colored solution.

KEYWORDS: Chitosan-zinc oxide nanoparticle, preparation, characterization, Dye adsorption properties



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INTRODUCTION

Biopolymers are abundantly available from its natural sources of extraction. These natural sources are said to be renewable with its ability to be replenished through plantations or growth of animals. Typically, it is categorized by its biological compositions into a few major groups, including polysaccharides, proteins, lipids, polyphenols and others [1, 2]. Among them, polysaccharides like cellulose, chitin and chitosan are commonly applied. Chitin and chitosan are the second most available biopolymer after cellulose. They are sourced mainly from the exoskeleton of crustaceans such as shrimp, but are also available from other sources such as fungi and some insect's wing [3]. In some studies, chitosan was reported to be developed from squilla (mantis shrimp), crab [4, 5] and silkworm chrysalides [6], etc. Chitin or poly- β (1:4)-2-acetamido-2-deoxy-D-glucose is a chiral natural biopolymer with a helical structure similar to that of cellulose [7, 8]. In nature chitin occurs in the exoskeleton of invertebrates and in the cell walls of fungi. Chitosan is the deacetylated form of chitin. It is composed primarily of glucosamine, 2-amino-2-deoxy- β -D-glucose. Chitosan is a natural nontoxic biopolymer, a major component of the shells of crustaceans such as crab, shrimp, and crawfish. Chitosan has different types of reactive functional groups. By modification of these groups various materials for different fields of application can be achieved. These modifications can be done chemically or enzymatically using enzymes such as chitosanases and lysozymes [9]. Potential and usual applications of chitin, chitosan and their derivatives are estimated to be more than 200 [10]. The duo is the second-most abundant high molecular-weight biopolymers and is recognized as a versatile, environmentally friendly raw material [11]. Chitosan as well as chitosan oligomers have been shown to inhibit growth of several fungi and bacteria, especially pathogens [12, 13, and 14] This property of chitosan as an antimicrobial agent could be useful for a number of applications in the food and pharmaceutical industry.

MATERIALS AND METHODS

1. Crab shell waste

Crab waste from *Charybdis cruciata*, was collected from the fish market in Chennai, India. The shell and meat portions were separated and cleaned. The shell material was collected and dried at 50°C in oven for 24 hours. The dried material was homogenized in a laboratory mixer. The moisture content was determined by weighing before and after being dried. The specimens were then crushed and preserved in air tight containers.

2. Demineralization with hydrochloric acid and NaOH

Crab shell waste (Dry weight, DW 20 g) was thoroughly mixed and soaked with 3% HCl. This treatment was carried out at room temperature. Crab shell waste (DW 20 g) was immersed in 40% NaOH solution, adjusted to pH 7.5, at room temperature for 2hrs. After deacetylation, the chitosan is then washed thoroughly with water. The resulting chitosan was then dried..

3. Demineralization with lactic acid bacterium fermentation

Microorganisms and Culture Media

Three species of *Lactobacillus* named *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* were provided from Microbial Type Culture Collection (MTCC). They were sub-cultured in sterile deMan Rogosa Sharpe (MRS) agar.

Flask Fermentation

Crab shell waste (DW 20 g) was thoroughly mixed with 50 mL of 20% glucose and inoculated with 5% of the inoculums. Each fermentation was carried out at 30°C with rotary shaking (180 rpm) for 7days.

Chitosan-zinc oxide nanoparticle

2.5 g of Chitosan flake was dissolved in a solution of 300 mL (0.1 M) acetic acid and 40 mL (0.2 M) NaCl. The viscous solution was stirred continuously for 12 h to fully dissolve the

Chitosan flake. Then, 1.25 g of n-ZnO powders was added into the solution. Subsequently, another 50 mL of (0.1 M) CH₃COOH was added. The slurry was stirred continuously for 24 h to obtain the final transparent solution. [15]

Physicochemical characterization of CS/n-ZnO adsorbent

The morphological structure of the CS/n-ZnO was examined by Transmission electron microscopy (TEM).

Adsorption procedure

Dye adsorption was carried out using two different commonly used anionic textile dyes namely, Coomassie Brilliant Blue and Acid Blue. Adsorption experiments were carried out at different dye concentrations (2.5, 5.0, 7.5 and 10 mg/L) using optimum amount of CS-ZnO (0.5 g/L) at pH 2, agitation speed of 200 rpm and 4 °C for 30 min. The changes on the absorbance of the samples were determined at regular time intervals (0, 30 min, 24hrs) during the adsorption process.

Screening for Antimicrobial activity

The Chitosan–zinc oxide nanoparticle (1:0.1) was tested for their antimicrobial activity by the agar well diffusion method. The microbial strains *Aspegillus niger*, *Candida albicans* and *Salmonella typhi* were used for this analysis. This culture suspension was seeded in agar plates by the pour plate technique. Three cavities were made using a cork borer (10 mm diameter) at an equal distance and were filled with the chitosan-zinc oxide nanoparticle solution and then incubated at room temperature for 7 days. The formation of a clear zone (restricted microbial growth) around the cavity is an indication of antimicrobial activity.

RESULTS AND DISCUSSION

Chitosan was extracted from marine crab *Charybdis cruciata* through chemical, microbial/biological and fermentation/bioprocess methods using microorganisms namely *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* along with *Bacillus subtilis*.



Figure 1
Chemically extracted chitosan from crab shells

Effect of pH

The pH of the culture broth increased from the 1st to 2nd day and later decreased gradually due to the production of organic acid (Table: 1). The acidification of crab shell waste plays an important role in suppressing the growth of spoilage organisms. The production of organic acids by the lactic acid bacterium decreased the pH and made the environment selective against spoilage microorganisms.

Table 1
Variation of pH

Day	pH	
	Chemical	Microbial
1	6.17	6.12
3	6.06	5.81
5	5.98	5.68
7	5.49	5.29

Effect of dye concentration

Adsorption is mass transfer processes that can generally be defined as the accumulation of material at the interface between two phases [16]. The adsorption efficiencies of CS-ZnO were evaluated by determining the percentage decrease of the absorbance at 630 nm. The influence of varying the initial dye concentration of two dyes was assessed.

Comassie Brilliant Blue

Table 2
Absorbance of Chitosan alone before incubation at 630 nm

Sample	25 mg/l	50 mg/l	75 mg/l	100 mg/l
Chitosan produced by chemical method	0.674	1.113	1.237	1.203
Chitosan produced by biological method	0.732	1.080	1.267	2.122

Table 3
Absorbance of Chitosan alone after 20 hrs of incubation at 630 nm

Sample	25 mg/l	50 mg/l	75 mg/l	100 mg/l
Chitosan produced by chemical method	1.504	1.208	1.209	1.206
Chitosan produced by biological method	1.053	0.781	0.843	0.863

Table 4
Absorbance of Chitosan+ ZnO before mixing at 630 nm

Sample	25 mg/l	50 mg/l	75 mg/l	100 mg/l
Chitosan produced by chemical method	1.192	0.505	0.516	0.614
Chitosan produced by biological method	0.893	0.388	0.385	0.453

Table 5
Absorbance of Chitosan+ ZnO after mixing for 1 hr at 630 nm

Sample	25 mg/l	50 mg/l	75 mg/l	100 mg/l
Chitosan produced by chemical method	1.216	1.779	1.448	1.457
Chitosan produced by biological method	1.631	0.804	0.965	1.009

Acid Blue

Table 6
Absorbance of Chitosan only before incubation at 630 nm

Sample	25 mg/l	50 mg/l	75 mg/l	100 mg/l
Chitosan produced by chemical method	1.896	1.010	1.284	1.234
Chitosan produced by biological method	1.598	0.644	0.831	0.843

Table 7
Absorbance of Chitosan alone after 20 hrs incubation at 630 nm

Sample	25 mg/l	50 mg/l	75 mg/l	100 mg/l
Chitosan produced by chemical method	0.349	0.438	0.468	0.404
Chitosan produced by biological method	0.327	0.514	0.463	0.504

Table 8
Absorbance of Chitosan+ ZnO before mixing at 630 nm

Sample	25 mg/l	50 mg/l	75 mg/l	100 mg/l
Chitosan produced by chemical method	0.249	0.433	0.471	0.314
Chitosan produced by biological method	0.199	0.505	0.524	0.423

Table 9
Absorbance of Chitosan+ ZnO after mixing at 630 nm

Sample	25 mg/l	50 mg/l	75 mg/l	100 mg/l
Chitosan produced by chemical method	1.674	0.939	1.037	0.939
Chitosan produced by biological method	1.382	0.623	0.656	0.636

Antimicrobial activity

The effect of chitosan obtained from crab- shell was evaluated against *Salmonella typhi*, *Candida albicans*, *Aspergillus niger* and the results were represented in Table 10. Chitosan-ZnO nanoparticles show efficient antimicrobial property due to their extremely large surface area, which provides better contact with microorganisms.[17]

Table 10
Antimicrobial activities

Method of chitosan production	Zone of inhibition					
	<i>S. typhi</i>		<i>C. albicans</i>		<i>A. niger</i>	
	48 hrs	72hrs	48 hrs	72hrs	48 hrs	72hrs
	Biological	1 cm clear zone	1 cm clear zone	1.1 cm clear zone	1.6 cm clear zone	1.7 cm partial zone
Chemical	1.6 cm clear zone	1.6 cm clear zone	1.6 cm clear zone	1.6 cm clear zone	1.2 cm clear zone	1.3 cm clear zone
Bioprocess	0.8 cm clear zone	0.8 cm clear zone	0.9 cm clear zone	1.1 cm clear zone	0.9 cm clear zone	0.9 cm clear zone

TEM Analysis

Transmittance electron microscope was used for the determination of the particle size and the morphological structure of the prepared chitosan-zinc oxide nanoparticles. It was found that CS/n ZnO has an average particle size in the range of 35-50 nm.

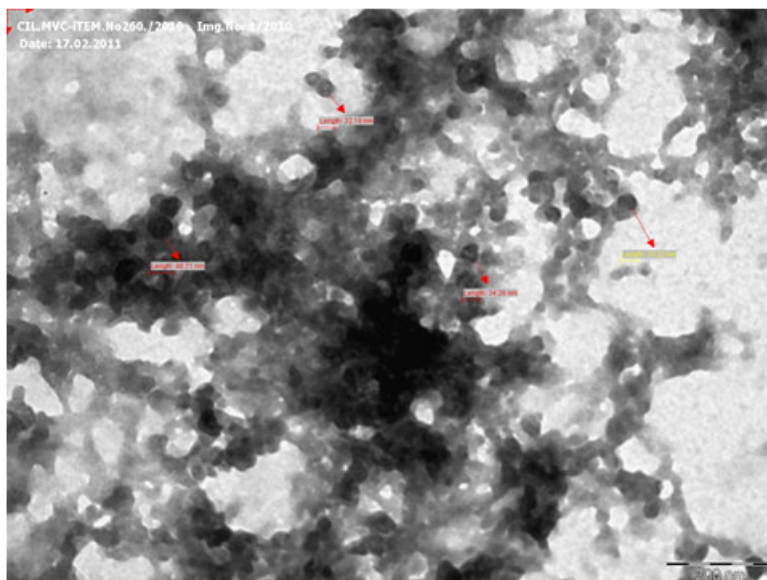


Figure 2
Structure of ZnO coated Chitosan nanoparticles produced by chemical method -particle size is about 32-46 nm.

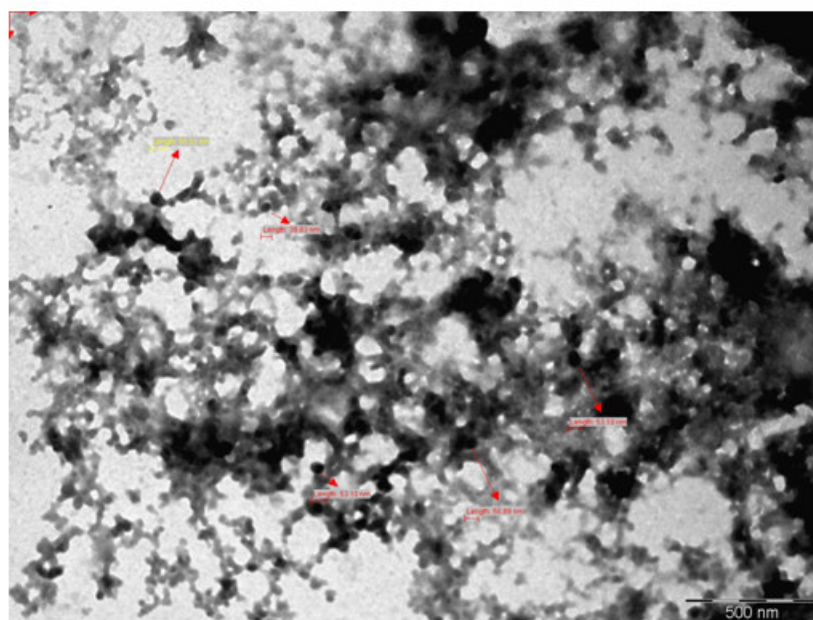


Figure 3
Structure of ZnO coated Chitosan nanoparticles produced by microbial method -particle size is about 39-55 nm.

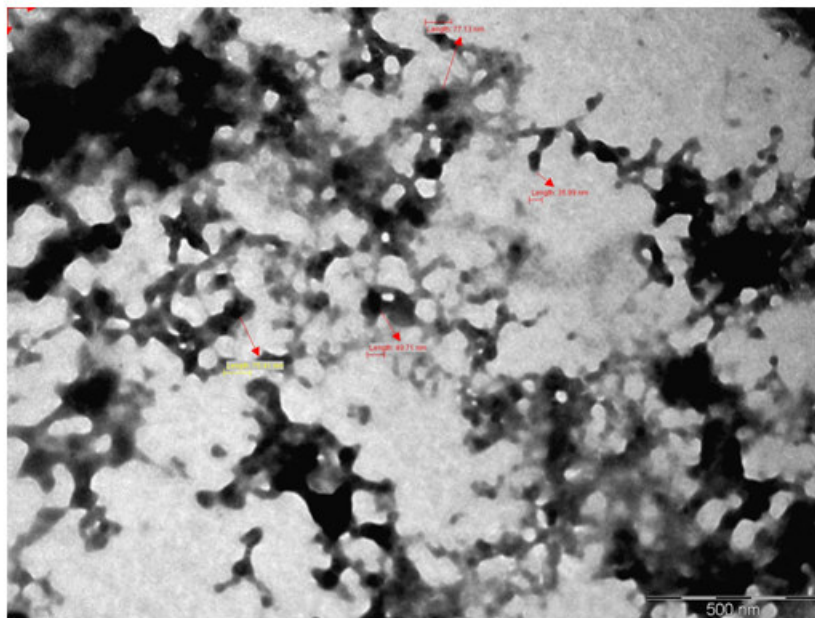
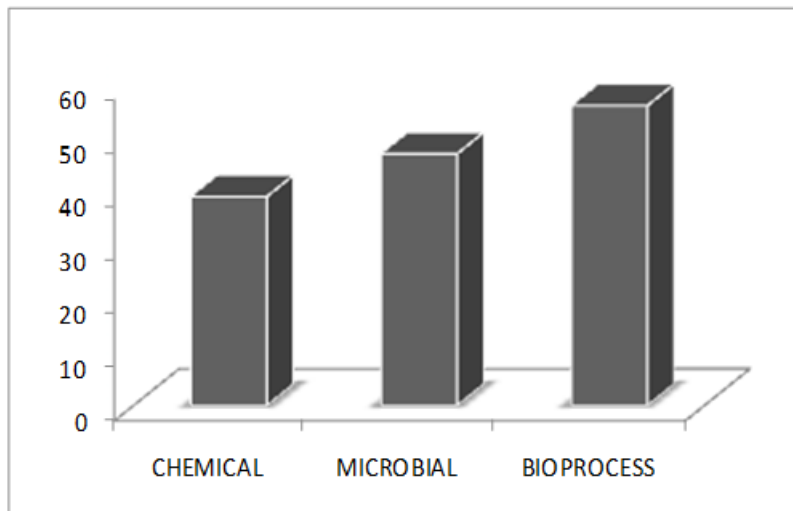


Figure 4
Structure of ZnO coated Chitosan nanoparticles produced by bioprocess method -particle size is about 35-77 nm.

Table 11
Mean values of the size of ZnO coated Chitosan nanoparticles



To improve the extraction of chitin from crab shell waste, fermentation was conducted using both lactic acid bacterium and *Bacillus subtilis*. In the earlier study, co-fermentation together with *L.paracasei* and *S. marcescens* was exploited to improve the extraction efficiency of chitin from crab shell waste [18]. The results warrant the necessity of other protocols in order to improve the bio-deproteinization of

crab shells. Thus, the successive fermentation was tried with a combination of the 1st step with *L. paracasei* and the 2nd step with *S. marcescens*, and vice versa, as a candidate protocol for the biological extraction of chitin from crab shells. Traditional method involving the use of strong acids makes this process ecologically aggressive and a source of pollution [19]. It also reduces a certain degree

of depolymerization and thus chitin and chitosan quality [20 and 21]. Use of organic acids such as HCl for the demineralization of chitin, results in detrimental effects on the molecular weight and the degree of acetylating that negatively affects the intrinsic properties of

the purified chitin.[22] Biotechnologically produced chitosan is at present not commercially available, but offers new perspectives for the production of high viscosity chitosan and therefore, application in biomedicine and pharmacy.

CONCLUSION

Throughout the literature on chitosan, the main emphasis is on its functional and physicochemical properties which vary widely with crustacean species and preparation methods. Upon this emphasis, this research study was attempted to prove or dispute these views by conducting similar studies and monitoring the modification of processing protocols of the chitosan production using crab shell waste, and to determine whether such modifications had any effect on the various physicochemical and functional properties of chitosan. From our results, we found that specific physicochemical and functional properties of chitosan have affected by process protocol alteration/modification.

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