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Extraction of Bio Polymers from Crustacean Shells and its Application in Refinery Wastewater Treatment

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Abstract

The fisheries sector is one of the most ancient and important sectors in the world and plays a significant role in providing the nutrition and socio-economic development of the country. The fish processing industry produces huge quantities of wastewater, encompassing significant amounts of contaminants in the form of soluble, colloidal, and particulate matters. The disposal of shellfish waste is a serious issue, and the effluents discharged from seafood-processing plants contain high amounts of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), dissolved solids, suspended solids, and turbidity. The existing waste management system lacks a cost-effective and environmentally friendly method. The current research focus on the extraction of a biopolymer chitosan from crab shells by ecofriendly methods and its application in refinery wastewater treatment. The chemical structure and crystallinity of the extracted chitosan was confirmed by Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD) analyses. Surface morphology and elemental composition were determined using Scanning Electron Microscopy (SEM), and Energy-Dispersive X-Ray Analysis (EDX). Thermal properties were detected using Thermo Gravimetric Analysis (TGA). The extracted chitosan was successfully employed in the batch treatment of refinery effluent by varying the experimental parameters (refinery effluent solution pH, contact time, dosage of chitosan, and stirring speed) and the optimizations of the processing conditions were established.

Keywords: Biochemical oxygen demand, Chitosan, Crab shell, Refinery effluent, Thermo gravimetric analysis

Introduction

Biopolymers are natural polymers derived from their natural sources, which assumed to be renewable and replenishable. Nearly 45 % of processed seafood consists of crustacean shells (crab shells, prawn shells etc.), of which 50 - 70 % of the weight of the raw material contains chitin, a protein. Chitin is the 2nd most abundant natural polysaccharide found in nature after cellulose [1,2] and exists in the shell wall of marine invertebrates, insects, certain fungi and algae [3,4]. The main sources of chitin are principally crabs and shrimps [5], from which the biopolymer chitosan is derived [6]. The attractive features of chitin and chitosan are their excellent biodegradability, biocompatibility, non-toxicity, antimicrobial and antioxidant activities, and wound healing capacities, which make them suitable in biomedical and pharmaceutical fields, drug delivery, cancer diagnosis, tissue engineering, wound dressing (artificial skin), and water treatment applications [7,8].

The processing stages of crustacean shells mainly include alkaline extraction in the deproteinization step, followed by acid treatment to dissolve calcium carbonate, which is present in high concentrations in shells [1,9]. Chitosan is a poly cationic polymer obtained by the deacetylation of chitin. The principal sources of production of chitin are crabs and shrimps. Chitosan is mainly applied in water treatment and purification because of its adsorption and chemical properties [11]. The consumption of crabs in Oman is on the higher side, and a considerable amount of crab shells are discarded into the environment as waste, which causes serious environmental problems. Hence, it is required to process the waste crab shells and convert them into value added products.

The coagulation, flocculation, cost-effective, and environmentally-friendly properties of chitosan have gained considerable attention in the area of wastewater treatment applications [12-18]. Themajority of the conventional approaches for the extraction of chitin and chitosan from crustaceans use chemical processes utilizing strong acids and bases for demineralization and deproteination steps. These processing steps generate huge environmental pressure for the treatment of wastewater, and also utilize huge quantities of fresh water for cleaning and washing steps. It has been reported that chemical purification of chitin is extremely hazardous, energy demanding, and damaging to the environment. In order to avoid these issues, the current research focuses on an environmentally-friendly and cost-effective method to extract chitin and chitosan from crab shell, to be used instead of the traditional extraction method employing harsh chemicals and acids.

Thus, the present study attempts to examine the green extraction of biopolymers from crab shells under mild experimental conditions, in order to reduce chemical usage, lower energy consumption, and protect the environment. The extracted chitosan was employed in the treatment of refinery wastewater in a safe and environmentally-friendly way.

Materials and methods

Raw materials

Crab shells were collected from a local fish processing plant located in Muscat. The shells were washed several times with fresh water, followed by drying in a hot air oven at 90 °C for 30 min. The dried samples were ground to fine powder using a ball bill, and the average particle size was measured by sieve analysis. Particles of size less than 75 µm were used for the extraction experiment. Citric acid, nitric acid, sodium hydroxide pellets, and acetone were purchased from Chemistry for Life Company, Oman. The refinery effluent samples were collected from the Occidental Company, Oman. The chemicals used for the experiment were of analytical grade and used without further purification. All experiments were repeated 3 times, and the averages of 3 values were considered as the final values.

The characterization techniques employed were Scanning Electron Microscopy (SEM JEOL JSM-7600F), X-Ray diffractometry (Rigaku, Mini Flex 600), Fourier Transform Infrared Spectroscopy (FTIR-Perkin Elmer Frontier), Thermo Gravimetric Analysis (TGA), and Energy Dispersive X-Ray Analysis (EDX). All samples used for EDX analysis were coated with gold to prevent the accumulation of static electric fields during imaging. Thermo gravimetric analysis was performed to determine the mass of sample over a range of 30 to 900 ºC.

Extraction of chitosan using organic acid

The extraction process was carried out by mixing powdered crab shell with 1.0 N citric acid. The crab shell powder and citric acid were mixed at a ratio of 1:1.5and theextraction process was carried out at room temperature for 1 h. The resulting mixture was washed with distilled water, followed by centrifugation at 5,000 rpm for 5 min, and was then dried at 100 ºC in a hot air oven. The dried samples were deproteinized by dissolving in 0.1 N sodium hydroxide solutions at 80 ºC for 6 h, followed by sequential washing with distilled water until pH reached 7.0, and was then filtered and dried to 60 ºC. The color of the powder was removed using acetone under continuous stirring for 24 h at a speed of 100 rpm. The sample after color removal was deacetylated to get chitosan. The acetyl groups were removed by dissolving 1.0 g of powdered sample in 10 % sodium hydroxide solution and stirring for 6 h at 85 ºC,

followed by washing with distilled water and drying in an oven at 60 ºC. The resulting samples were preserved for characterization and analysis using SEM, FTIR, XRD, TGA, and EDX, respectively.

Treatment of refinery effluent using chitosan

The extracted chitosan was employed in the batch treatment of refinery wastewater by varying the effluent solution pH, mixing time, agitation speed, and dosage of chitosan.

Effect of variation of refinery effluent solution pH

The influence of change in effluent solution pH on the removal of contaminants was studied by adjusting the pH of the refinery effluent from 2.0 to 7.0. The experiment was accomplished by mixing 0.2 g of chitosan powder with 150 mL of refinery effluent and stirring for 45 min with a stirring speed of 25 rpm. The resulting mixture was tested for TDS, TSS, COD and BOD, and turbidity.

Effect of variation of mixing time

Mixing time plays a significant role in pollutant removal. The effect of variation of mixing time in the reduction of parameters was studied by varying the contact time from 15 to 90 min. The pH of the effluent solution was maintained at 7.0 and a stirring speed of 25 rpm.

Effect of variation of dosage of chitosan

The effect of variation of dosage of chitosan on pollution removal was studied by changing the amount of chitosan from 0.1 to 0.5 g, with an optimized pH of the effluent and a mixing time.

Effect of variation of agitation speed

Effect of agitation speed of chitosan on contaminant removal efficiency was investigated by changing the stirring speed from 25 to 125 rpm, keeping all other parameters constant.

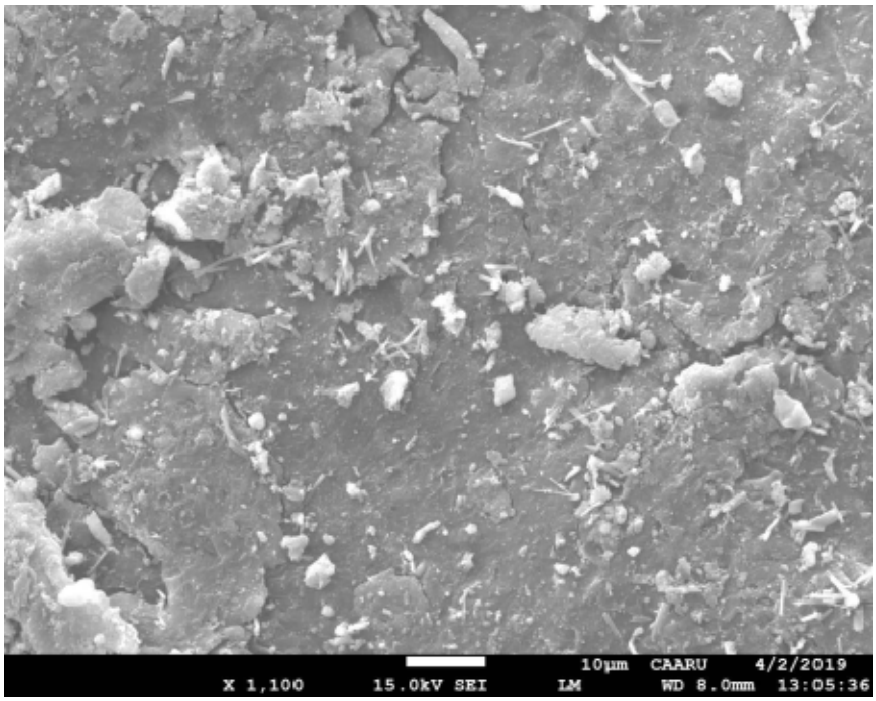

Figure 1 SEM image of chitosan at a magnification of 1,100 X.

Results and discussion

Surface characterization of chitosan

Scanning Electron Microscopy (SEM) analysis

The surface characterization of the extracted chitosan powder was performed using scanning electron microscopy, with the image is displayed in **Figure 1**. The SEM image indicates that the chitosan powder was distributed evenly without aggregation, which designates the successful extraction of chitosan. The morphological characterization of chitosan powder, illustrated in **Figure 1**, confirmed that the particles were scattered with non-homogeneous distribution, even though the size and shape of the particles were uneven. The samples exhibited rough and thick surface structure under scanning electron microscopic examination at a magnification of 1,100 X and an excitation voltage of 15.0 kV, as depicted in **Figure 1**.

SEM-EDX analysis

The elemental composition of the chitosan sample obtained from SEM-EDX analysis is shown in **Figure 2**. The elemental analysis indicated 42.8 % Carbon, 40.8 % Oxygen, 8.6 % Nitrogen, 4.2 % Sodium, 2.2 % Calcium, 0.9 % Magnesium, and 0.5 % Chlorine, respectively.

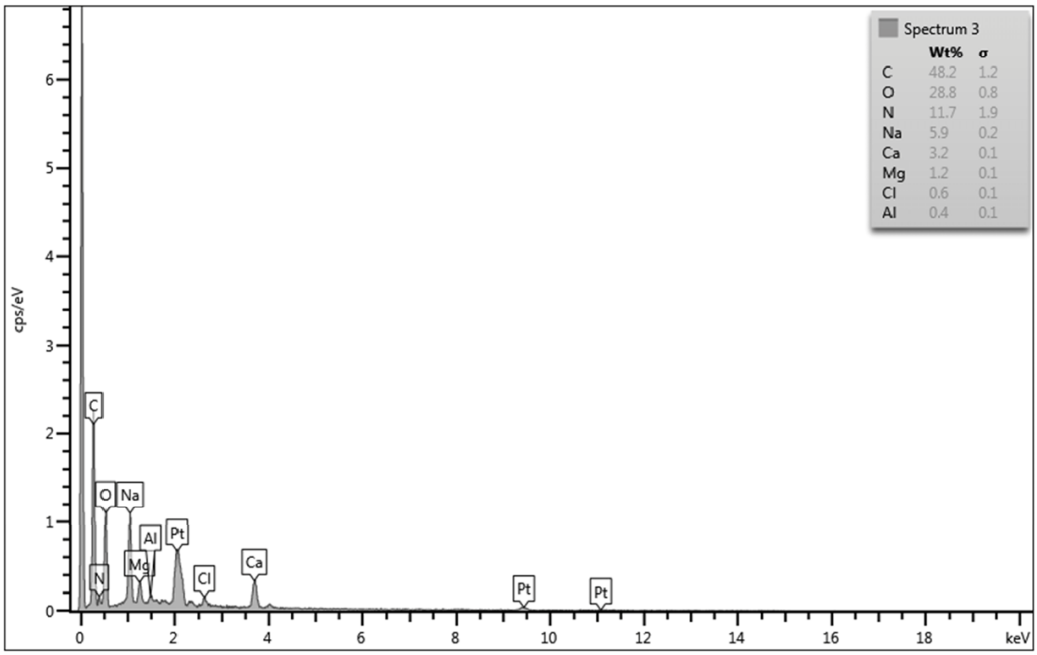

Figure 2 SEM-EDX elemental composition analysis of chitosan.

X-Ray diffraction analysis

The crystalline structures of the extracted chitosan powder are presented in **Figure 3**. The crystalline reflections were analyzed at a scan rate of 1°/min with scan angle ranges from 2 to 80º. The XRD pattern illustrates the crystalline nature of chitosan powder, and the diffraction peaks are observed at 2θ between 10 - 15º and 25 - 30º. The result was in agreement with the previous studies [19,20]. The extra peaks represent the nature of solvents used in the demineralization and deproteinization stages. The reflection peak indexed at 25º confirmed the presence of chitosan.

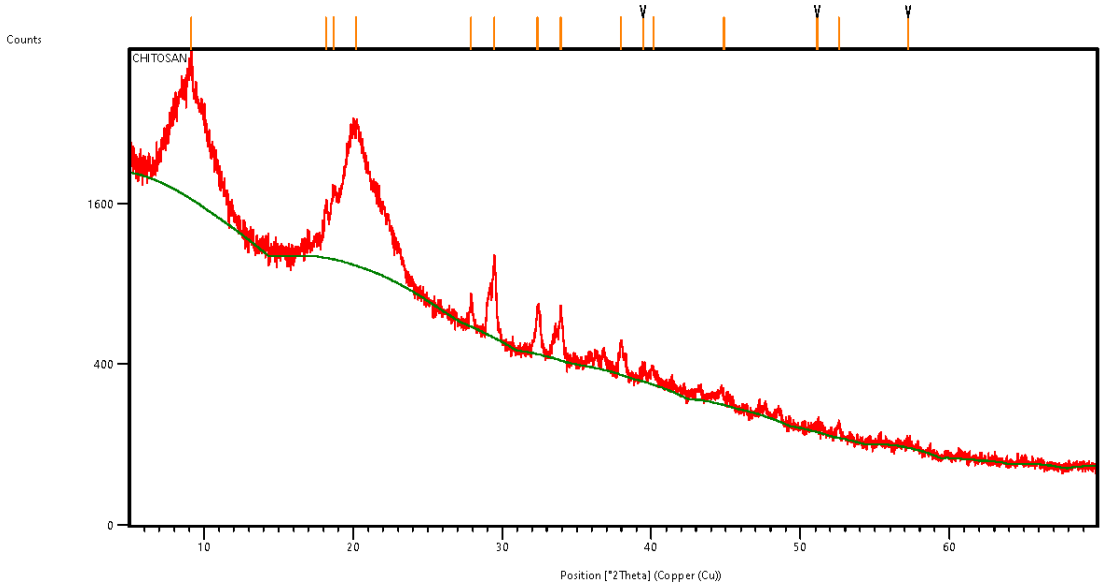

Figure 3 X-Ray Diffraction pattern of chitosan powder.

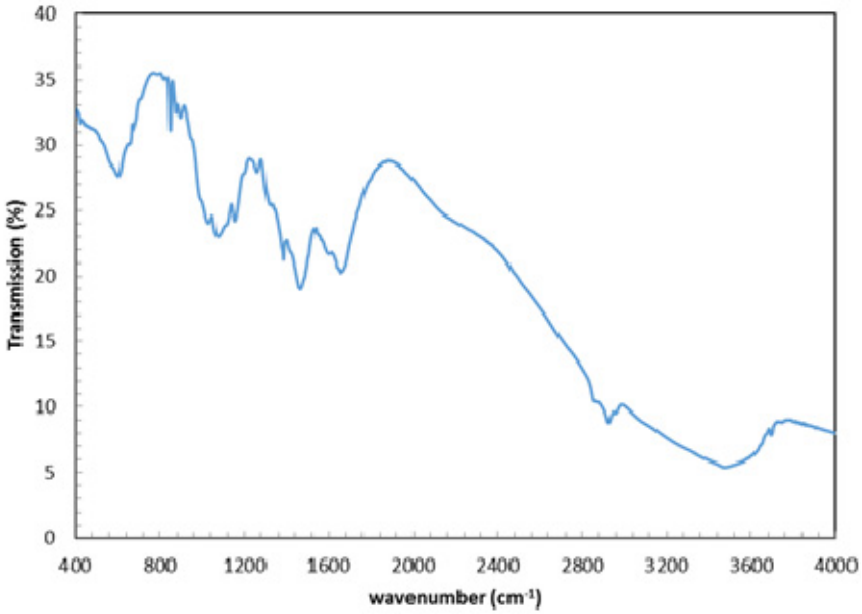

Figure 4 FTIR Spectra of chitosan powder.

Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR spectroscopic analysis of chitosan powder was obtained at a frequency range between 400 to 4,000 cm^{-1} and at a resolution of 4 cm^{-1}. The presence of functional groups and bond stretching present in the chitosan powder are indicated in **Figure 4**. The characteristic peaks of O-H, C-H, C=O, CH=H, CH_2OH, and N-H_2 stretching are seen at wave numbers corresponding to 3,400, 2,930, 1,659, 1,468,

1,380 and 1,680 cm^{-1}, respectively. The N-H and the O-H stretching bands of chitosan were observed between 800 to 1,600 cm^{-1}. The FTIR spectra shown in **Figure 4** match with the previous studies [21].

Thermo Gravimetric Analysis (TGA)

The thermal degradation temperature of extracted chitosan was examined using TGA with 2 major degradation steps, as shown in **Figure 5**. From **Figure 5**, it was observed that the 1st stage of degradation of chitosan occurred between 30 and 90 ºC, with nearly 4 % weight loss. The 2nd stage was between 190 and 270 ºC, with 60 % weight loss. This degradation process may have been due to the loss of moisture or water molecules from chitosan. The TGA curves shift toward higher temperatures along with growth in heating rate.

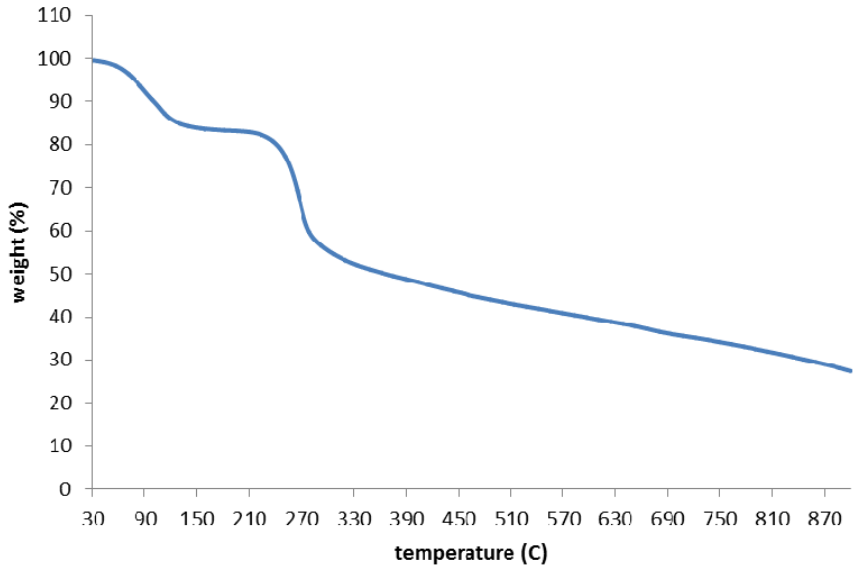

Figure 5 TGA Curves of thermal degradation of extracted chitosan powder.

Refinery wastewater treatment

Effect of refinery effluent solution pH

After the batch adsorption process, the resulting mixture was tested for TDS, TSS, COD, BOD, and Turbidity. The maximum reduction in parameters was obtained at an optimum solution pH corresponding to 6.0. The highest reductions in parameters were obtained due to the coagulation and flocculation properties of chitosan. The surface charge of chitosan was highest and, hence, the adsorption property also increased, resulting in the destabilization of pollutants in the wastewater. This could be achieved due to the adsorption of excess polymer on the surfaces. The charge transfer caused an electrostatic repulsion in the suspended solids, leading to reduction in the COD. Optimum pH was found to be 6.0, with a percentage reduction of COD as 78 %. **Figure 6** represents the effect of variation of refinery effluent solutionpH with parameter reductions.

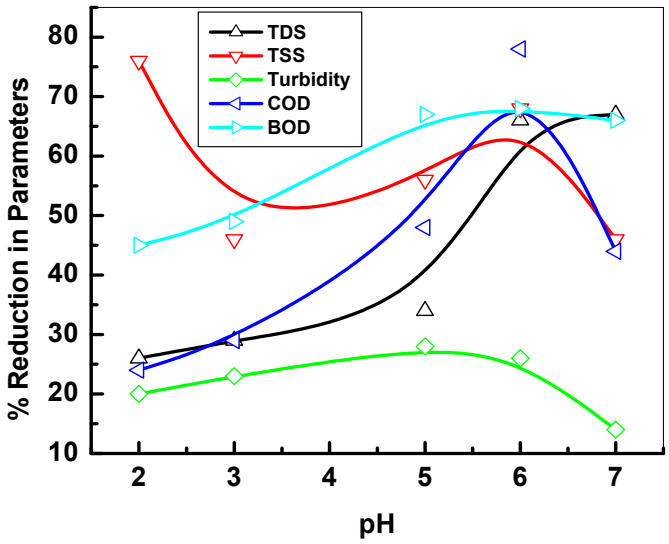

Figure 6 Effect of variation of refinery effluent solution pH with parameter reductions.

Effect of mixing time

As mixing time increased, the development of flocs increased and the flocculent started dispersing throughout the medium over a certain period of time. The extended mixing time enhanced the breakage of flocs into smaller ones, thereby retarding flocculation rate. The ideal contact time for the maximum reduction of COD was observed at 90 min, with a percentage reduction corresponding to 53 %. **Figure 7** illustrates the influence of contact time on parameter reductions. The percentage reduction in TDS was in an increasing order up to a contact time of 60 min, beyond which the percentage reduction diminishes.

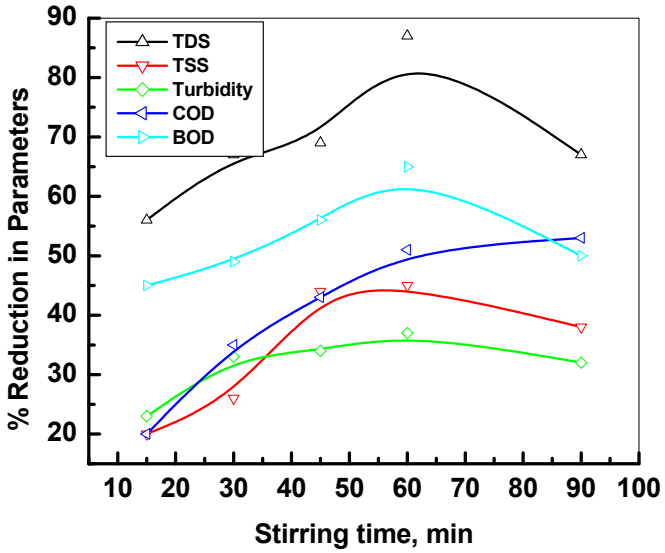

Figure 7 Effect of variation of mixing time with parameter reductions.

Effect of dosage of chitosan

The slow addition of chitosan powder into the effluent solution ensured an increase in the % reduction of TDS. The reduction in TDS was found to increase steadily due to the availability of more adsorption sites on the surface of chitosan, which enhanced the deposition tendency of pollutants. This may have been due to the strong molecular interaction between the amine groups present in chitosan and the pollutants present in the effluent. The % reduction in TSS showed a fluctuating behavior due to the formation of bigger flocs in the mixture upon changing the dosage of chitosan. As dosage increased, the turbidity reduction tended to decrease. The positive charge of chitosan with free amine groups electrostatically interacted with the negative charge of pollutants present in the wastewater. This lead to instability and to turbidity reduction. The % reduction in COD steadily increased up to 0.4 g of chitosan dosage and then decreased. This was due to the electrostatic repulsion between particles. The effects of variation of dosage of chitosan with pollutant removal efficiency are shown in **Figure 8**.

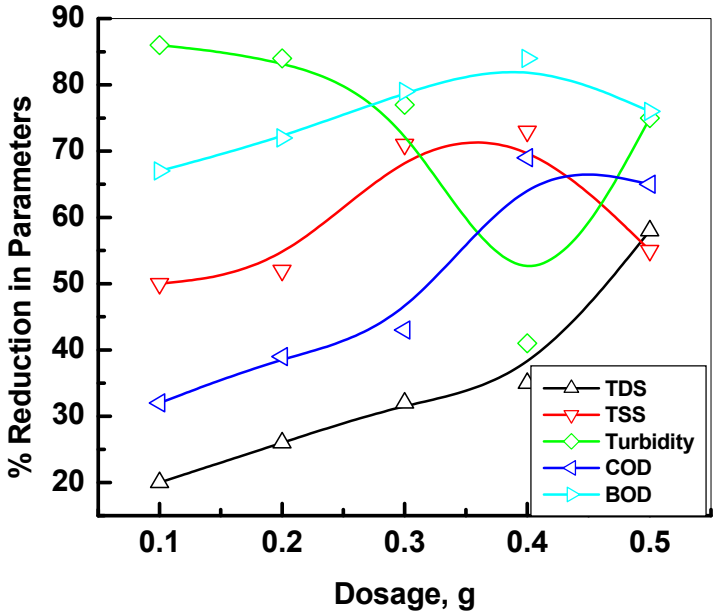

Figure 8 Effect of variation of dosage of chitosan with parameter reductions.

Effect of agitation speed

As the speed of agitation increased, the particle size tended to decrease, thereby creating an enriched surface area which allowed improved adsorption efficiency. The % reduction in TDS was found to increase with increase in stirring speed. The optimum reduction in TDS was obtained at a stirring speed of 100 rpm. The percentage reduction in COD increased with increase in stirring speed with the range of values studied, whereas the turbidity decreased with increase in stirring speed, as indicated in **Figure 9**. The percentage reduction in TSS increased with increasing speed up to 75 rpm, followed by a sudden decrease in removal efficiency.

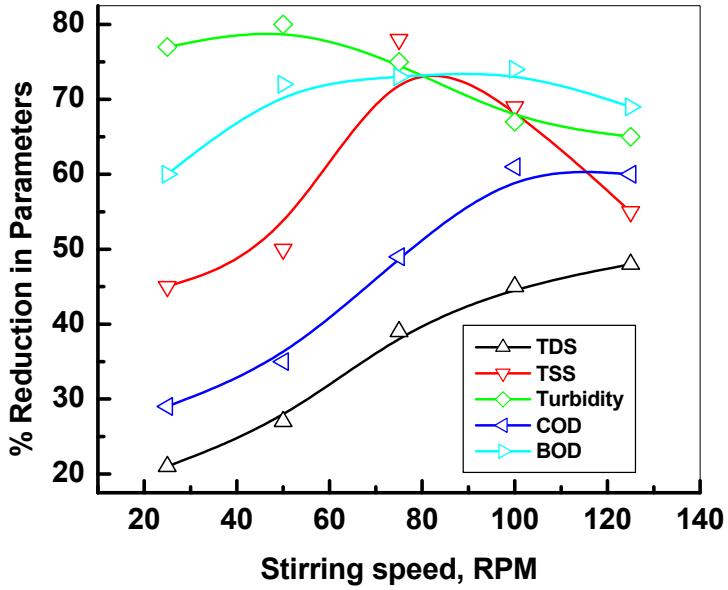

Figure 9 Effect of variation of stirring speed with parameter reductions.

Conclusions

In this research, a sustainable, ecofriendly, and green technique was employed for the extraction of chitosan from crab shell at elevated temperature. The surface morphology, structural characterization, composition analysis, and functional groups of the extracted chitosan were characterized using SEM, EDS, FTIR, XRD, and TGA. The X-Ray Diffraction analysis revealed a peak at 25 - 30 degrees, indicating the successful extraction of chitosan. The Fourier Transform Infrared (FTIR) analysis demonstrated the absence of acetyl groups and the breakage of glucosidic bonds. The extracted chitosan was successfully employed in the batch treatment of refinery wastewater by varying the processing conditions, and the optimum percentage reductions in parameters were determined. This research will serve as an incentive for fish processing industries, as it involves an environmentally-friendly approach for the extraction of chitosan and also minimizes marine pollution. Extraction of chitosan from waste crab shells would help to solve the environmental issues associated with fish processing industries, improve water quality, create green environments, and help reduce health hazards. It would also help to increase the country's per capita income, by exporting the extracted chitosan to suitable customers. In addition, this research will transform solid waste into a value-added and environmentally-friendly product. This environmentally-friendly approach for the green extraction of chitosan will be a better option for marine waste management without using harsh chemicals. Currently, the research team is exploring the possibility of extending the experimental work in column studies using textile mill effluent and dairy wastewater treatment to optimize the processing conditions and to compare the efficiency. This community engagement project will address 3 basic issues of human society- water, energy, and pollution- with a strong interdisciplinary lens ranging from engineering, ecology, and social work.

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