EXTRACTION, CHARACTERIZATION AND DYE ADSORPTION ABILITY OF CHITIN FROM CRAB SHELL WASTE

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ABSTRACT

Chitin is the second most abundant natural polysaccharide after cellulose and is present in the crustacean exoskeleton like crab, shrimp, insects and fungi. It is the main structural component of the exoskeletons of the animals like insects and crustaceans. Crab, shrimp, squilla and fish scale waste is ideal raw material for chitin production. The present work is aimed at extraction,characterization and dye adsorption ability of chitin from crab shells. The methodology include acid hydrolysis, demineralization followed by deproteinization step. The chitin produced is analysed by FTIR based on the interpretation of the spectrogram of the two samples of chitin synthesized in the present work, it can be said that all functional groups expected are seen. The applications of the chitin are numerous but the study is focused on dye adsorption ability.

Key word: Chitin, Extraction, Characterization, Methylene blue

INTRODUCTION

The shell fish industry which is prominent in all costal countries generates about 60,000 to 80,000 tons of waste (Muzzarelli *et al*,1986). Even though the wastes are biodegradable, the dumping off large quantities makes degradation process slow resulting in accumulation of waste overtime which is a major environmental concern. A quick and effective solution to this is recycling of shell wastes and extraction of commercially viable substances like chitin from them. Chitin on its own has various applications. Chtin is a polysaccharide. This biopolymer is synthesized by enormous number of living organisms and it belongs to the most abundant natural polymers, after cellulose (Rinaudo *et al*,2006).

Expelling of dyestuff into water resource system causes major threat to the environment. Adsorption is the low cost and easy method to remove the dyes from the effluents. Effluents contain harmful agents, which have to be removed to maintain the quality of the environment. Paper, fabric, leather and dyestuff production are some of the industries that release harmful effluents (Lin S, Lin C*et al*,1993).The aim of the present study was to investigate the chitin adsorption capability on major industrial dye, Methylene Blue.

MATERIALS AND METHOD

Sample preparation- Crabs were collected from Dapodi fish market, Pune. Crabs inedible parts including head, body shells and tails were removed from the whole body for extraction of chitin. The crab shell were washed and air dried and used for extraction.

Extraction of chitin-

Figure 1.Flow chart of basic steps of Extraction of Chitin.

Process I-

10 grams of sun dried crab shell waste was demineralized by adding 1.5 N HCl at room temperature for 1 hour. Acid was discarded and the shells were washed with distilled water until the pH is neutral. The shells were then de-proteinized with 0.5% NaOH at 100^0C for 30 minutes. Protein solution was removed and washed thoroughly with distilled water and the pH was checked. The de-proteinization process was again repeated, for that 3% NaOH was added to the sample at 100^0C for 30 minutes. After draining the residual proteins along with the effluents, the sample once again washed and the pH was observed till it was approximately near to neutral. Hence the chitin slurry was obtained. The remaining water was removed. The alkali was drained off and washed thoroughly with distilled water until the pH is less than 7.5 and then dried at ambient temperature (30 ± 2°C)

Process II-

10 grams of crab shell waste were refluxed in 100ml of sodium hypochlorite (NaCLO)solution at 100^oc for 10 minutes. The NaCLO solution was decanted and the powder was washed with distilled water. The above step was repeated once more. The sample was again refluxed in 50ml of 1M HCL at 75^oC for 15 minutes. The solution was decanted and washed with distilled till it becomes neutral. The sample was then refluxed in 50ml of 1M NaoH(sodium hydroxide)solution at 100^oC for 2 minutes to remove any protein residues. The solution was decanted and remaining sample was washed with distilled water till it becomes neutral. They were filtered off and placed in an oven at 60^oC for a week.

Characterization of Chitin

Solubility Test - Chitin dissolves completely in 1% Acetic Acid. For the estimation of chitin produced the sample was taken out of the storage and weighed . Then the sample was put inside a clean beaker and 10 to 20 ml of 1% acetic acid was added to it. The solution was kept in shaker for 30 to 40 minutes. Then the sample was taken out and weighed, carefully(Abhrajyoti Tarafdar et al,2013)

FT-IR Spectroscopy: The samples were analysed by FT-IR spectroscopy in Istrumentation Centre Solapur university and the graph depicts wave number versus percent transmission.
(Pandharipande S *et al*,2016)

Dye adsorption by Chitin: Stock solution of the dye was prepared by taking 10mg of methylene blue powder and adding it to 1000ml of distilled water(Paula Szymczyk *et al*,2015).The pH of the dye solutions was adjusted using 1 N NaOH or 1 HCl.About 1g of extracted Chitin(adsorbent) was added to 100mL of dye solutions(adsorbate).A control was also maintained without addition of chitin. At specific time intervals, aliquots of 2-3 ml suspension were filtered and used to evaluate the adsorption of dye.The absorbance of the supernatant was subsequently measured using UV-Vis spectrophotometer. Concentration of dye adsorption was calculated by the absorbance value at 668nm.

Percentage of dye adsorption was estimated by the following formula:
% adsorption = 100 × [(C_O −C)/ C_0] Where: C_0 is the initial concentration of dye solution and C is the concentration of dye solution after Adsorption(S. Dhananasekaran *et al*,2015).

RESULTS AND DISCUSSION

Extraction of chitin from crab requires harsh chemical treatments. The crab shells even though contains majority of chitin, also has proteins and minerals. Proteins are removed by deproteinization and carbon and other salts are removed by demineralization(Badawy *et al*,2011).

Process I – Solubility test for sample 1

Initial weight of chitin was measured to be 0.40 gram. Final weight of chitin after reaction with 1% acetic acid was measured to be 0.22 grams and hence the total dissolved weight of chitin was calculated to be 0.18 grams.

Proecss II - Solubility test for sample 2

Initial weight of chitin produced was measured to be 0.40 grams .Final weight of shells after reaction with 1% acetic acid was measured to be 0.20 grams and hence the total dissolved weight of chitin was calculated to be 0.20 grams. Therefore, it was observed that chitin produced employing Process II was more readily soluble in 1% acetic acid solution then that produced through Process I.

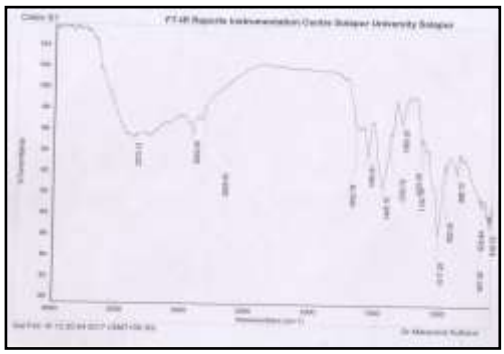
FTIR Analysis- The interpretation of FTIR analysis of the samples is done for the possible presence of functional groups and the details are given in Table 1(Dhananasekaran S *et al*,2016)

Table 1

Sr.no	Standard chitin wavelength in cm^{-1}	Crab chitin wavelength in cm^{-1}		Groups
		Sample-1	Sample-2	
1.	3300-3250	3373	3278	N-H
2.	2891	2952	2920,2826	C-H
3.	1680-1660	1653	1647	C=O
4.	1560-1530	1560	1568	Amide
5.	1072	1017	1024,1094	C-0-C
6.	952	952	901	Amide III
7.	750-650	667,625	685,617	N-H

The FT-IR spectra of chitin isolate from crab shell are given in Figure 2 and 3

Theoreitically,α-chitin is characterized by three characteristic amide bands appearing at 1650,1620, and 1550 cm^{-1}.In this study we observed FTIR bands at 1653 cm^{-1} and 1560 cm^{-1} for chitin sample 1 in figure 2.Here peak at 1653 cm^{-1} corresponds to symmetrical deformation to vibration of amide I band stretching C=O and 1560 cm^{-1} Coressponds to N-H deformation of amide II(Muhammed, R., et al,2010)



FT-IR spectra of chitin (Sample 1)

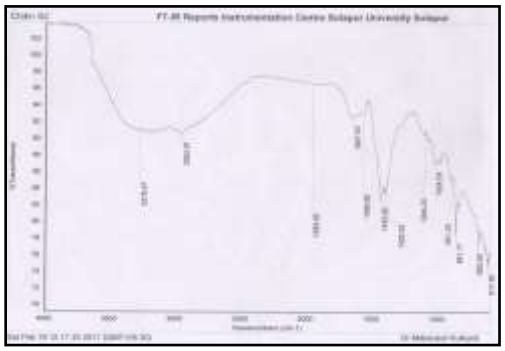
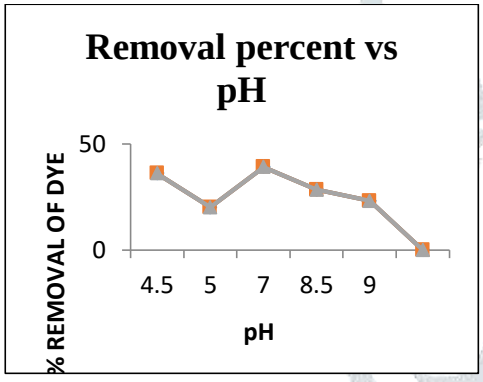


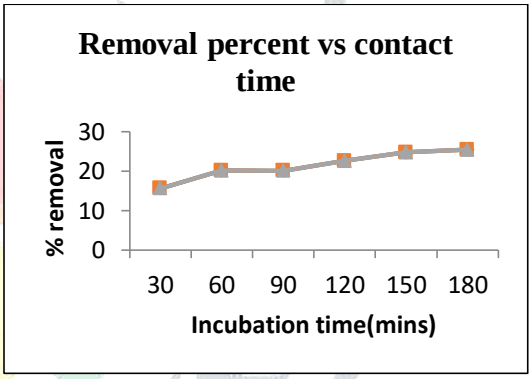
FIG 3: FT-IR spectra of chitin(Sample2)

FIG 2:

In sample 2,Figure 3 the peaks are observed at 1647 cm^{-1} and 1568 cm^{-1} which corresponds to symmetrical deformation to vibration of amide I band stretching C=O and N-H deformation of amide II respectively. From interpretation of FT IR it can be said that all functional groups which are during synthesis have been identified in the form of peaks that include amide,carbonyl and hydroxyl groups.This indicates the successive formation of chitin biopolymer(Muhammed, R., et al,2010)

Dye adsorption by Extracted Chitin





Graph 1:Effect of pH on removal of dye

Graph 2:Effect of contact time on removal of dye

Graph 1 shows the relationship between pH values and percentage removal of dye .The readings were taken having varying pH between 4-9 and between intervals of 30 minutes. A result shows that the effectiveness of dye adsorption onto chitin was decreasing along with the increasing pH value. Here, the effect of dye adsorption is found maximum at pH 7.

The dye removal percentage with contact time between dye and extracted chitin is shown in Graph 2. The range of observed contact time was 30 -180 minutes with the increment of 30 minutes. It is observed that with increase in incubation time the effectiveness of dye adsorption by chitin increases. The % removal was found to be maximum at 180 minutes at pH 7 such as after 3 hours compared to initial readings. The % removal was found to be 39% after 180 minutes.

CONCLUSION

Chitin is one of the most abundant biopolymers in nature and is a major component in the supporting tissues of organisms such as crustaceans, fungi, and insects. It has wide application in various fields. This study shows the production of chitin from crab shell. The FTIR and chemical characterization studies confirm the production of chitin. In this study removal of dyes by adsorption using crab

shell(chitin) was investigated. This study monitored the ability of chitin for removing dyes from aqueous solutions. Interaction between the chitin and dye were found to be strongly dependent on pH of the solution. The maximum percentage of dyes reduction was obtained at an optimum contact time 180 minutes and optimum pH of 7.Crab shell chitin has been found to be comparatively better adsorbent because it can remove almost 39 % of dyes within 3 hours. Finally, the result of adsorption study, it is concluded that chitin can be used as a coagulant of dyes because of its higher adsorptive capacity, cost effectiveness, environment friendly behavior and availability in nature.

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