Isolation of Chitin from Shrimp Shells and Its Use in Biosorption of Heavy Metals

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Abstract: ***In this research work natural bio polymer "chitosan" was extracted using locally available shrimp shells and absorption of copper by chitosan was studied. Synthesis of chitosan involved four main stages as preconditioning, demineralization, deproteinization and deacetylation. Chitosan was characterized using Fourier Transform Infrared Spectroscopy (FTIR). The affinity of chitosan for cupper was studied using anhydrous CuCl2 solution as the heavy metal solution containing Cu (II) ions.***

Keywords: biopolymer, chitosan, crustacean shells, chitin, heavy metals

1. Introduction

Chitin is the most widespread biopolymer in nature. Chitin and its derivatives have great economic value because of their biological activities and their industrial and biomedical applications. It can be extracted from three sources, namely crustaceans, insects and microorganisms. However, the main commercial sources of chitin are shells of crustaceans such as shrimps, crabs, lobsters and krill that are supplied in large quantities by the shellfish processing industries. Extraction of chitin involves two steps, demineralization and deproteinization, which can be conducted by two methods, chemical or biological. The chemical method requires the use of acids and bases, while the biological method involves microorganisms.

Enormous amounts of chitin can be found in the biosphere; it is the major component of cuticles of insects, fungal cell walls, yeast or green algae (1–3). Fungi provide the largest amount of chitin in the soil (6–12 % of chitin biomass, which is in the range of 500–5000 kg/ha) (4). Chitin is also widely present in crab and shrimp shells (5). A working estimate for the annual turnover is in the range of 1010–1011 tonnes (6, 7), making chitin one of the most abundant biopolymers. Chitin can be readily obtained by simple extraction (8). To date, the major source of industrial chitin comes from wastes of marine food production, mainly crustacean shells, e. g. shrimp, crab or krill shells (9–11). In the processing of shrimps for human consumption, between 40 and 50 % of the total mass is waste. About 40 % of the waste is chitin, incrusted with calcium carbonate. A small part of the waste is dried and used as chicken feed (11), while the rest is dumped into the sea, which is one of the main pollutants in coastal areas (12, 13). The utilization of shellfish waste has been proposed not only to solve environmental problems, but as a waste treatment alternative to the disposal of shellfish wastes (14). Crustacean shell waste consists mainly of 30–40 % protein, 30–50 % calcium carbonate, and 20–30 % chitin (15–18), with species and seasonal variations (19).

A good number of industries are responsible for the release of heavy metals into the environment through their wastewater (20). Some of these heavy metals are extremely toxic elements and can seriously affect many living species especially human; therefore, their removal from wastewater is important. Efficient removal of toxic metal ions from wastewater is an important and widely studied research area where a number of technologies have been developed over the years (20, 21, 22). However, these conventional technologies appear to be inadequate and expensive. In recent years' studies on polymers which bind metals ions have increased significantly (21, 23, 24). This approach is inherently attractive since only the toxic metals ions are removed while the harmless ions can be released into the environment. Some of the best chelation ion - exchange materials are natural biopolymers (24). Natural biopolymers are available in large quantities. Certain waste from agricultural operations may have potential to be used as low cost absorbents, as they represent unused resources, widely available and are environmentally friendly (21). Among the many other low cost absorbents identified, Chitosan has the highest sorption capacity for several metal ions because they possess a number of different functional groups such as hydroxyls and amines to which metal ions can bind either by chemical or by physical adsorption (21, 24, 25). Chitosan is a natural product derived from chitin (Figure 1), a polysaccharide foundation in the exoskeletons of shellfish like shrimps and crabs.

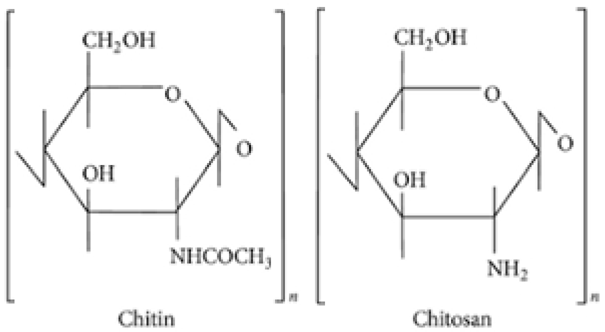

Figure 1: Structure of Chitin and Chitosan

2. Materials and Methods

2.1 Preconditioning

Shrimp shells were procured from local market. Shrimp shells were washed and dried in sun for two days. After sun drying the shrimp shells were crispy. Then the shells were grounded into powder. Dried powder shrimp shells were placed in opaque plastic bottles and stored at room temperature (27°C).

Figure 2: Shrimp shells

2.2 Isolation of Chitosan

Chitosan preparation is derived into three consecutive steps. Demineralization of shrimp shells, chitin processing (Deproteinization) and chitosan processing (Deacetylation).

2.2.1 Deproteinization

Depending upon the production sequence, the shrimp shells or demineralized shells was deproteinized by boiling with 1M NaOH solution for 1 hr with constant stirring at a solid to solvent ratio of 1: 10 (w/v) (26). This treatment was repeated several times. The number of bathes depends on clarity of the solution; the absence of protein was indicated by the absence of colour of the medium at the end of the last treatment. Washing with distilled water was then carried out up to neutrality after which the samples were dried.

2.2.2Demineralization

The deproteinized shells were demineralized with 1N HCl for 60 min at room temperature with a solid to solvent ratio of 1: 25 (w/v) (26), then filtered under vacuum. The filtrate was washed with tap water and oven - dried.

2.2.3 Deacetylation

It is the process to convert chitin to chitosan by removal of acetyl group. It is generally achieved by treatment with concentrated sodium or potassium hydroxide solution to remove some or all of the acetyl groups from the polymer (26). The N - acetyl groups cannot be removed by acidic reagents without hydrolysis of the polysaccharide, thus, alkaline methods must be employed for Ndeacetylation (27).

Figure 3: Demineralized shells.

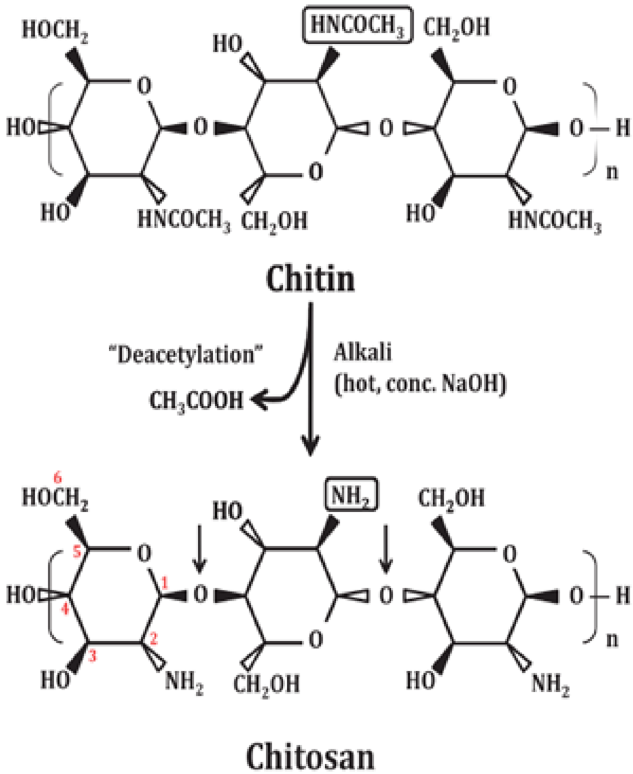

Figure 4: Chitosan

Mechanism of conversion of chitin into chitosan

2.2 Biosorption of cupric ions by chitosan

20mg/L cupper solution was prepared by dissolving 5.3 mg analytical grade CuCl2 anhydrous powder in distilled water. This solution was kept as stock solution and 3 mg/L solutions was prepared by diluting stock solution (28).50ml of 3mg/L CuCl2 solution was taken and 50mg of chitosan was added. Then the mixture was continuously stirred using magnetic stirrer for 2 hours at (30 ^{0}C). After that solution was filtered and the filtrate containing CuCl2 solution was analysed using UV - spectroscopy to determine amount of copper absorbed by chitosan at different time intervals. Finally, uptake of copper by amine groups on chitosan was investigated by using graph of absorption versus time interval.

Figure 5: Cupric solution with chitosan

3. Results and Discussions

In this research work natural bio polymer "chitosan" was synthesized using locally available shrimp shells adsorption of copper by chitosan was studied. Synthesize of chitosan involved four main stages as preconditioning, demineralization, deproteinisation and deacetylation.

Chitosan was characterized using Fourier Transform Infrared Spectroscopy (FTIR).

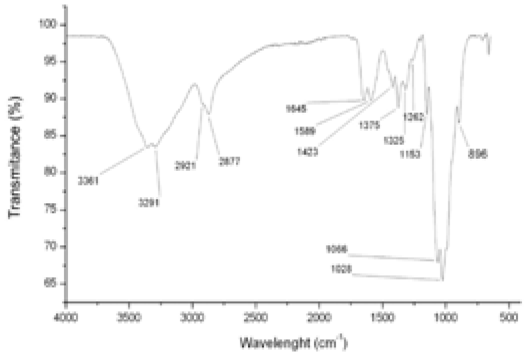

Figure 6: FTIR of standard chitosan

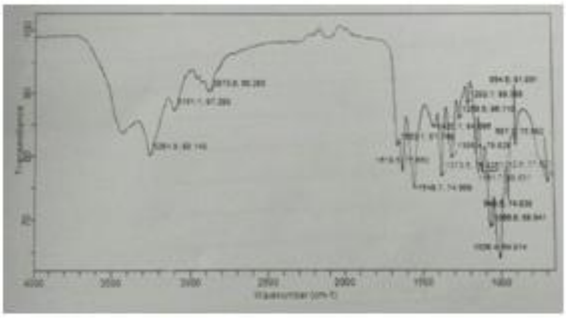

Figure 7: FTIR of chitosan extracted from shrimp shells

In FTIR, the major absorption band is observed between 1220 and 1020 cm - 1 which represents the free amino group (- NH2) at C2 position of glucosamine. Further the sample showed the absorption bands for the free amino group between 1026 and 1259 cm - 1. The peak at 1374 cm - 1 represents the –C - O stretching of primary alcoholic group (- CH2 - OH). The absorbance bands of 3268, 2930, 2878, 1563, and 1418 cm - 1 indicated the N - H stretching, Symmetric - CH3 stretching and asymmetric - CH2 stretching, - CH stretching, C=O stretching in secondary amide (amide I) and C–N– stretching in secondary amide (amide II), respectively. In the present study also the same absorbance bands were observed at 3283, 2921, 2865, 1643, 1552, 1421, 1022, 893and 752 cm $^{-1}$ which confirms the structure of chitosan.

The affinity of chitosan for cupper was studied using anhydrous CuCl2 solution as the heavy metal solution containing Cu (II) ions. Adsorption of cupper ions by chitosan was investigated under different conditions. Amount of copper absorbed was evaluated using UV spectroscopy.

A good number of industries are responsible for the release of heavy metals into the environment through their wastewater. Some of these heavy metals are extremely toxic elements and can seriously affect many living species especially human; therefore, their removal from wastewater is important. Efficient removal of toxic metal ions from wastewater is an important and widely studied research area where a number of technologies have been developed over the years (20, 21, 22). However, these conventional technologies appear to be inadequate and expensive.

Chitosan has the highest sorption capacity for several metal ions because they possess a number of different functional groups such as hydroxyls and amines to which metal ions can bind either by chemical or by physical sorption.

Table 1: Amount of Cupric ions absorbed by chitosan with time

Time in mins	Total amount of cupric ions absorbed in mg/l
20	2.862
30	2.864
60	2.736
90	2.702
120	2.682
150	2.682

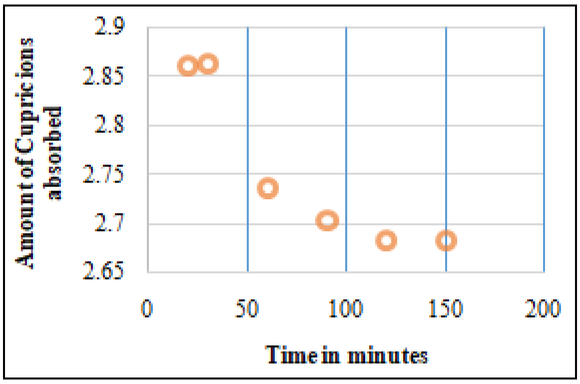

Figure 8: Graph of Cupric ions absorbed by chitosan at different time interval

From the above graph it indicates that amount of cupric ions absorbed by chitosan slightly increases in the beginning and then decreases with increase in time and finally remains constant after a particular time.

4. Conclusion

Chitin is the most widespread biopolymer in nature. Chitin and its derivatives have great economic value because of their biological activities and their industrial and biomedical applications. It can be extracted from the shells of crustaceans such as shrimps, crabs, lobsters and krill that are supplied in large quantities by the shellfish processing industries.

This study showed that chitosan absorbs heavy metals, in particular, copper ions, and hence can be used to remove heavy metals from aqueous effluents. Chitosan as a result of its bioavailability would be economically useful for the treatment of wastewater containing heavy metals.

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