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The potentiality of cross-linked fungal chitosan to control water contamination through bioactive filtration

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ABSTRACT

Water contamination, with heavy metals and microbial pathogens, is among the most dangerous challenges that confront human health worldwide. Chitosan is a bioactive biopolymer that could be produced from fungal mycelia to be utilized in various applied fields. An attempt to apply fungal chitosan for heavy metals chelation and microbial pathogens inhibition, in contaminated water, was performed in current study. Chitosan was produced from the mycelia of *Aspergillus niger*, *Cunninghamella elegans*, *Mucor rouxii* and from shrimp shells, using unified production conditions. The FT-IR spectra of produced chitosans were closely comparable. *M. rouxii* chitosan had the highest deacetylation degree (91.3%) and the lowest molecular weight (33.2 kDa). All chitosan types had potent antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*; the most forceful type was *C. elegans* chitosan. Chitosan beads were cross-linked with glutaraldehyde (GLA) and ethylene-glycol-diglycidyl ether (EGDE); linked beads became insoluble in water, acidic and alkaline solutions and could effectively adsorb heavy metals ions, e.g. copper, lead and zinc, in aqueous solution. The bioactive filter, loaded with EGDE- *A. niger* chitosan beads, was able to reduce heavy metals' concentration with >68%, and microbial load with >81%, after 6 h of continuous water flow in the experimentally designed filter.

1. Introduction

Water is one from the most vital elements on the Earth because of its essential role in almost all biological functions in ecosystems. Threateningly, it is very difficult to attain a safe drinking water in many world parts due to its contamination by numerous causes, which is actually a life-threatening factors for people [1,2]. Large number of water pollutants, including waterborne microbial pathogens and more than 700 inorganic compounds, were designated as dangerous risk factors for human, due to their high pathogenicity, toxicity and carcinogenicity [3].

The control of pathogenic microorganisms and ensuring microbial safety of water are serious eco- toxicological challenges, for the protection of human health and prevention of bio- terrorism [4]. Unlike organic contaminants that can be eliminated or reduced by microbial activity and chemical oxidation techniques [5–7], heavy metals cannot be degraded because they are elements [8,9]. Therefore, once metals are introduced into the water, they will remain and transfer to all organisms that drink this water [10].

Chitosan is a linear long-chain polysaccharide with high molecular weight; depending on the processing conditions, the molecular weight of commercial chitosan is in the range between 10 kDa and 1000 kDa [6]. Chitosan has numerous reactive groups for chemical activation and cross-linking, which make it very appropriate for chelation, adsorption and complexing with further materials [11].

Commercially, chitosan is produced from crustacean shells (crabs, crayfishes or shrimp) by either biological or chemical processes and, promisingly, it was successfully produced from many fungal mycelia and proved to be as effective as commercial chitosan [12–16].

Unlike cellulose and chitin, chitosan is soluble in dilute aqueous solutions of organic acids such as acetic acid, formic acid and oxalic acid, and inorganic acids such as hydrochloric acid and nitric acid [17,18]. Therefore, chitosan can exist in three physical forms corresponding to solids, gels and solutions [19], and is more versatile than chitin and cellulose in terms of application.

The presence of amino and hydroxyl groups in chitosan is highly advantageous, since modification can be performed by their reaction yielding numerous useful materials for different fields of application [5,17]. Chitosan characteristics could be improved

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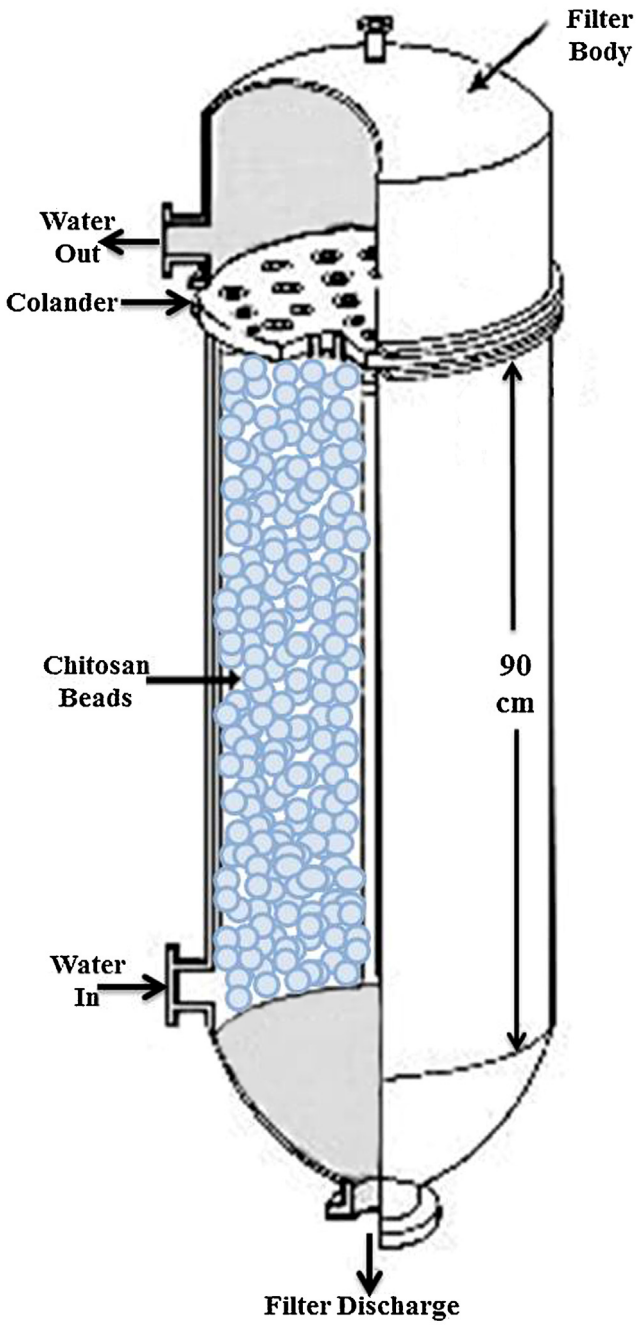

Fig. 1. A drawing illustrating the design of chitosan beads-based filter.

using physical modifications, e.g. preparation of fibers, membranes and gel beads, or using chemical reactions, e.g. grafting, hydrolysis, oxidation and cross-linking [20].

The cross-linking of chitosan was suggested to reinforce its resistance and chemical stability against other chemicals, alkali and acids. Also, cross-linking could improve crystalline nature of chitosan and increase its sorption capabilities [21,22].

However, the current study aimed to produce chitosan from various sources, enhance its characteristics through cross-linking and beads formation, and to apply cross-linked chitosan beads in experimentally designed bioactive filter for the elimination of water contamination with heavy metals and microbial pathogens.

2. Materials and methods

2.1. Chitosan sources

Chitosan was produced from different sources including fungal species and shrimp shells. The examined fungal species included *Aspergillus niger* (ATCC-9642), *Mucor rouxii* (DSM-1191) and *Cunninghamella elegans* (RCMB-012002). Shrimp shells were obtained from the research plant, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt.

2.2. Propagation of fungi

Fungi were grown on Czapek Dox agar (HiMedia Lab. Pvt. Ltd., Mumbai, India); spores of 7 days old cultures were scrapped and diluted in Tween 80 solution (20%, v/v) for preparation of the stock spores suspension. Spore suspension was inoculated into 500 ml from Czapek Dox broth, in 2 l Erlenmeyer flasks, to have a final concentration of $\sim 3 \times 10^4$ spores/ml, and the pH was adjusted to 4.5 ± 0.2 using 1 N hydrochloric acid. The flasks were then incubated at 25 °C for 96 h under shaking conditions at 75 × *g*.

2.3. Chitosan extraction and characterization

After the incubation of fungal cultures, mycelia were harvested through filtration using filter paper (Whatman No. 2), dried and weighted. The extraction of chitosan, from dried fungal mycelia, was implemented according to Tayel et al. [13]. The extraction method included fungal mycelia deproteinization through homogenization, at 90 °C for 2 h, with 1 M NaOH; centrifugation for separation of alkali-insoluble materials at 4000 × *g* for 15 min, repeated washing of the precipitate, with neutral water, and recentrifugation until reaching to a neutral pH. The resultant residues were further extracted, using acetic acid (10% v/v), at 60 °C for 6 h on a rotary shaking water bath and recentrifuged, then the acid insoluble materials were thrown out. Using concentrated NaOH solution (4 M), the pH of supernatant fluids was adjusted to 9.0, centrifuged and the chitosan precipitates were washed using deionized water, ethanol (95%) and acetone, respectively, and the chitosan was then dried at 50 °C for 24 h.

For the shrimp chitosan preparation, the same steps and procedures were applied using cleansed shrimp shell powder as a substrate.

Gel permeation chromatography (GPC- PN 3000, Post- Nova, Eresing, Germany) and a refractive index detector (PN-1000) were used for the determination of fungal chitosan molecular weight; pure pullulans were used for calibration (with standard molecular weights of 11.8, 47.3, 112, and 780 kDa). The following columns was employed: Gral 300 (GmbH, Mainz, Germany) and Nucleogel GFC 1000-8 (Macherey-Nagel, Düren, Germany).

The degree of deacetylation of produced chitosan (DA) was measured from their FTIR spectra (Biorad, model FTS 45, Germany) with absorbance ratio of A1655/A3450 [23].

2.4. Antimicrobial activity of chitosan

The antimicrobial activities of produced chitosan types were determined against two bacterial strains, i.e. *Escherichia coli* (*E. coli* ATCC-25922) and *Staphylococcus aureus* (ATCC-25923), using two different antimicrobial assays

2.4.1. Well diffusion assay

Chitosan was dissolved to prepare a final concentration of 1% (w/v), in acetic acid solution (1%), then the prepared solution was autoclaved. 25 μl from each chitosan suspension were pipetted into wells with 6 mm diameter in Nutrient agar media (NA) plates,