Efficient Chitosan-Based Biotermicide Prepared from Asian Tiger (Prawn) Shells for Anti-Termite Application

[1]Rimsha Shoukat, [2]Zulfiqar Ali*, [1]Riaz Hussain, [1,3]Mirza Arfan Yawer**, [4]Sumaira Naz and [5]Affifa Yawer
[1]*Department of Chemistry, University of Education, D.G Khan Campus, Punjab 32200, Pakistan.*
[2]*National Centre for Nanotechnology (NCN), Department of Metallurgy and Materials Engineering (DMME), Pakistan Institute of Engineering and Applied Sciences (PIEAS), Nilore, Islamabad 45650, Pakistan.*
[3]*Department of Chemistry, Division of Science and Technology, University of Education Lahore, Pakistan.*
[4]*Materials Division (MD), Directorate of Technology, Pakistan Institute of Nuclear Science and Technology (PINSTECH) Nilore, Islamabad 45650, Pakistan.*
[5]*RECETOX, Masaryk University, Faculty of Science Kamenice 753/5, building A29, Brno 62500, Czech Republic.*
*alizulfiqar161@gmail.com; **mirza.yawer@ue.edu.pk

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Summary: Termites have played havoc on common woodworks in houses especially at sub-continent regions. In this work, freshly prepared chitosan from Tiger prawn (*Paneous Monodon*) shells was studied for anti-termite growth on vulnerable Kail wood pieces. Prawn shells were systematically processed through deprotenization, demineralization, decolourization and deacetylation to get granulated chitosan powder. Freshly extracted chitin and chitosan were characterized by FTIR, SEM and XRD studies. Effect of different compositions of chitosan in acetic acid and contact time of termite with wood towards reduction in original weight were studied. Results show that 5 wt% chitosan-coated Kail wood pieces with 20 numbers of termite and contact time for 24 hours exhibit remarkable resistance against termite growth by showing just about 0.53 % weight loss. On the other hand, Kail wood without coating of chitosan shows about 52 % reduction in weight i.e. about half of the wooden material was eaten by termite under similar conditions. This work demonstrates the efficient termicidal effect of freshly prepared chitosan from prawn shells and presents a remarkable development of chitosan as a biotermicide material derived from natural resources.

Keywords: Biomaterial; Chitin; Chitosan; Tiger Prawn; Anti-termite; Wood Preservation.

Introduction

Chitosan is biopolymer, biocompatible, biodegradable, non-toxic and second most abundant polysaccharide material after cellulose [1]. Interestingly, chitosan is structurally similar to cellulose with the difference lying in amino group replacement with hydroxyl group at C2 position of glucose being the building block of cellulose. Chitosan is derived from chitin which constitutes the exoskeleton of crustacean shells i.e. crabs, shrimps, insects etc. and cell wall of fungi and mushrooms [2]. It is usually extracted from chitin through the process of demineralization, deprotienation, decoloration and deacetylation [3]. It has been used for many applications such as antimicrobial, drug delivery, agriculture, food industry, cosmetics, pharmaceuticals, metal chelation, dye removal, paper making and preservation [4-7]. For instance, Takkaki *et al* [8] have shown amidoxime -chitosan with cellulose hydrogels for efficient adsorption of copper and other metal ions. Amara *et al* [9] have demonstrated the use of chitosan beads blend with graphene oxide for selective copper adsorption. Interestingly, chitosan adsorption properties are even better than that of its counterpart material like carbon-based nanomaterials [10]. Chitosan with various degrees of deacetylation have been used by Matthew *et al* [11] into cation exchange membrane for improved desalination properties. Thus, it is reveled from literature that chitosan material has been used for diversified applications. Moreover, chitosan can be used in variety of physical forms such as powder, films, beads, and hydrogels depending upon the application of chitosan. In this nexus, chitosan nanocomposite beads and films have been used by Mehdi *et al* [12] and Amna *et al* [13] for drug delivery and antibacterial applications respectively.

***To whom all correspondence should be addressed.**

Among aforementioned applications of chitosan, the wood preservation studies are very rare in literature. However, paper preservation using chitosan was demonstrated by Muryeti et al [14] in which chitosan biotermicide effect has been reported. Since termites eat almost all cellulosic materials such as paper and wood. This insect has devastating effect on common woodwork in houses. Besides, termites damage the wood silently and uncontrollably. In order to preserve the woods from termite attack, the anti-termite chemicals are being frequently used which are potentially dangerous to environment and human beings. Therefore, there is dire need to develop environment friendly, cost effective and abundantly available natural material to address problem of termite attack on woodwork. Herein, we report the isolation of chitin from tiger prawn shells and its conversion into chitosan followed by anti-termite studies on common Kail wood pieces.

Experimental

Chemicals

Analytical reagent (AR) grade chemicals like; NaOH, HCl, $KMnO_4$ etc. were supplied by Merck Company. Prawn (Asian Tiger) seafood fish was purchased from local fish market.

Characterizations

Extracted chitin and its chitosan conversion were characterized by Fourier Transform Infrared (FTIR) spectrometer (Nicolet 6700), Secondary Electron Microscope (SEM) Model TESCAN at operating voltage of 20 kV and X-ray diffractometer Bruker D8 Discover with Cu Kα radiation.

Preparation of Chitin and Chitosan

Following is the detailed procedure adopted from previous study with some modifications [3]. Moreover, all the steps involved for the preparation of chitosan from prawn shells are pictorially shown at Fig-1.

Deprotienation

150 g prawn shells were washed with copious amount of water in order to remove dirt and residual flesh followed by drying in oven at 80-90ºC. Afterwards deprotienation was carried with 1M NaOH in 1:10 ratio for period of about 20 h at temperature 25-30ºC.

Demineralization

100 grams of deprotienated shells were added in a beaker containing about 600 ml 3M HCl with stirring for about 30 minutes followed by washing with distilled water till neutral pH.

Decolourization

After the demineralization step, the pinkish hue of the product was observed which was removed by treating the material with 1% $KMnO_4$ and oxalic acid. Finally, the ground off-white color powder was labeled as chitin.

De-acetylation

45 grams of chitin powder obtained from above step were reacted with 12 M NaOH solution at temperature 90-100ºC in heating mantle with periodic stirring. The process was allowed for about 3h followed by filtration, washing and drying at 60-70ºC in lab oven. Final product obtained from this step was labeled as chitosan.

Biotermicide Testing

Dip-coating of various Kail wood pieces of rectangular shape having weight in the range 6-8 grams were carried out for testing biotermidical effect of freshly prepared chitosan against termites. Typically, said pieces of wood were immersed into different concentrations of chitosan slurries in acetic for period of 8 h and dried at room temperature. Afterwards dried chitosan coated wood pieces were put in glass beakers along with 20 numbers of termites and allowed different contact times. Finally, weight loss of original pieces of wood was calculated as follows:

$$\text{Reduction weight (\%)} = \frac{W1-W2}{W1} \text{ X } 100$$

W1 = Weight of wood piece before termite attack
W2 = Weight of wood piece after termite attack

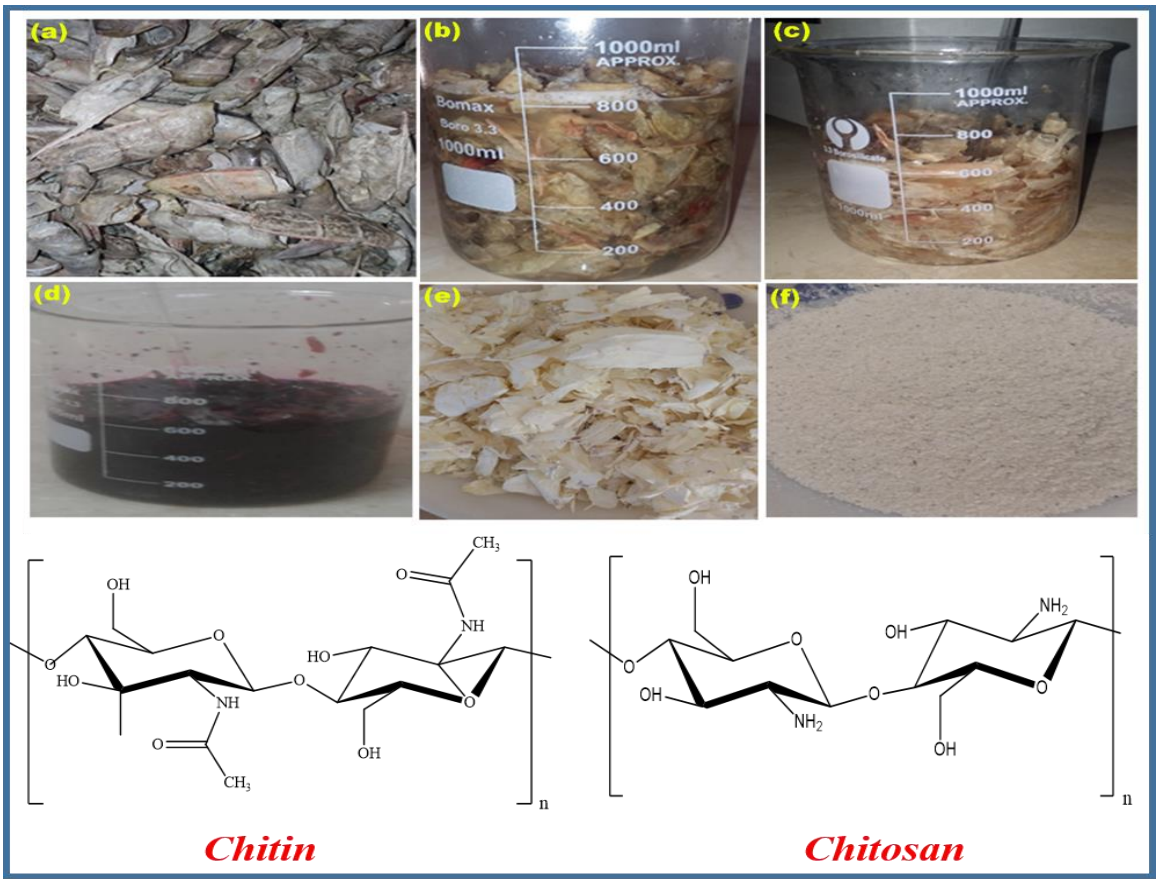

Fig. 1: Photographs for synthesis scheme of chitin from Tiger Prawn shells; (a) Water washing & drying, (b) Deprotenization, (c) Demineralization, (d) Decolourization by $KMNO_4$, (e) prawn shells before grinding and (f) prawn shells powder after grinding; chitin and chitosan structural formulae at the bottom.

Result and Discussion

Fig 2(a) shows the FTIR spectra of two extracted material samples from prawn shells labeled as chitin and chitosan respectively. IR peaks around 1636 cm^{-1} found in both the samples marked with dashed lines in Fig 2(a) are attributed to the stretching vibration of acetyl amide carbonyl bond ($O=C-NH_2$) and reduction in peak area intensity of these IR peaks is actually the measure of conversion of chitin into chitosan [15]. IR peaks around 1014 cm^{-1} and 1010 cm^{-1} observed for samples labelled chitosan and chitin respectively are overlapped with those of terminal bending vibration of hydroxyl group of carbon in glucosamine units at 1030 cm^{-1} and glycosidic linkage bond vibration (C-O-C) at 1006 cm^{-1} [15, 16] The IR region around 3200 cm^{-1} to 3600 cm^{-1} is generally associated with hydroxyl group (-OH) stretching vibration of water bound with chitin and chitosan samples [17]. Here, in IR spectra shown in Fig 2 at specified region 3000 cm^{-1} to 3600 cm^{-1} reflects that the chitosan sample is relatively more dried that of chitin. Fig 2(b) shows the typical XRD patterns exhibited by the samples synthesized from prawn shells labelled as chitin and chitosan from 2Θ angle 10º to 22º. Here, clear XRD peak at 19.23º revealed in sample labeled as chitosan can be seen which is typically known for it [18]. Moreover, XRD peaks in chitin sample are observed at 2Θ 19.51º and 19.92º which are characteristic of chitin material extracted from Mollusca shells [17]

Fig 3(a) & (b) show the typical SEM images of chitin samples with increasing order of magnifications. Here it can be seen that the surface of the sample is more compact which is transformed into rough and layered features upon deacetylation of the chitin samples i.e. chitosan shown at Fig 3(c) & (d). Similar morphologies of the chitin and chitosan samples have also been previously reported [19, 20].

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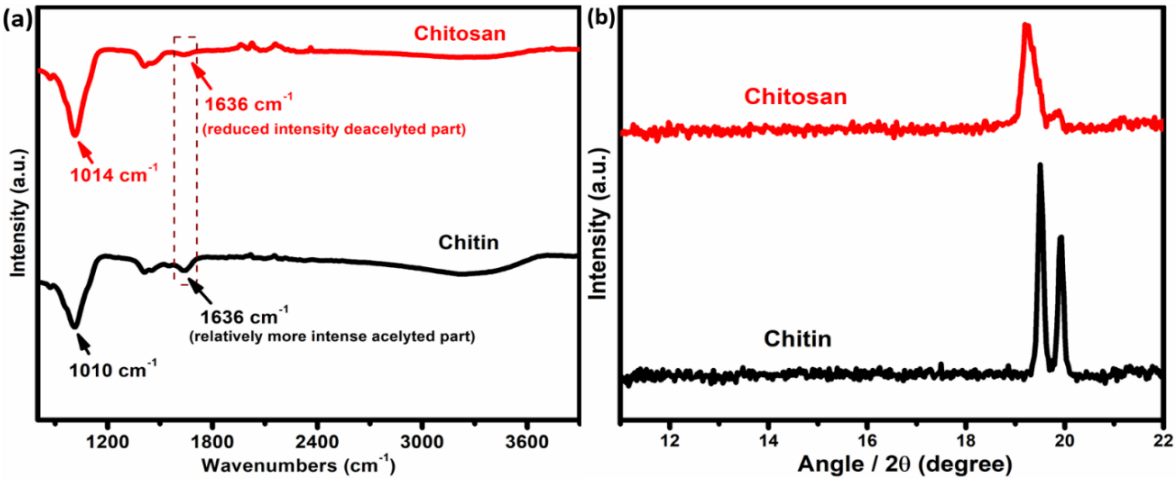

Fig. 2: FTIR spectra of freshly prepared (a) Chitin and (b) Chitosan.

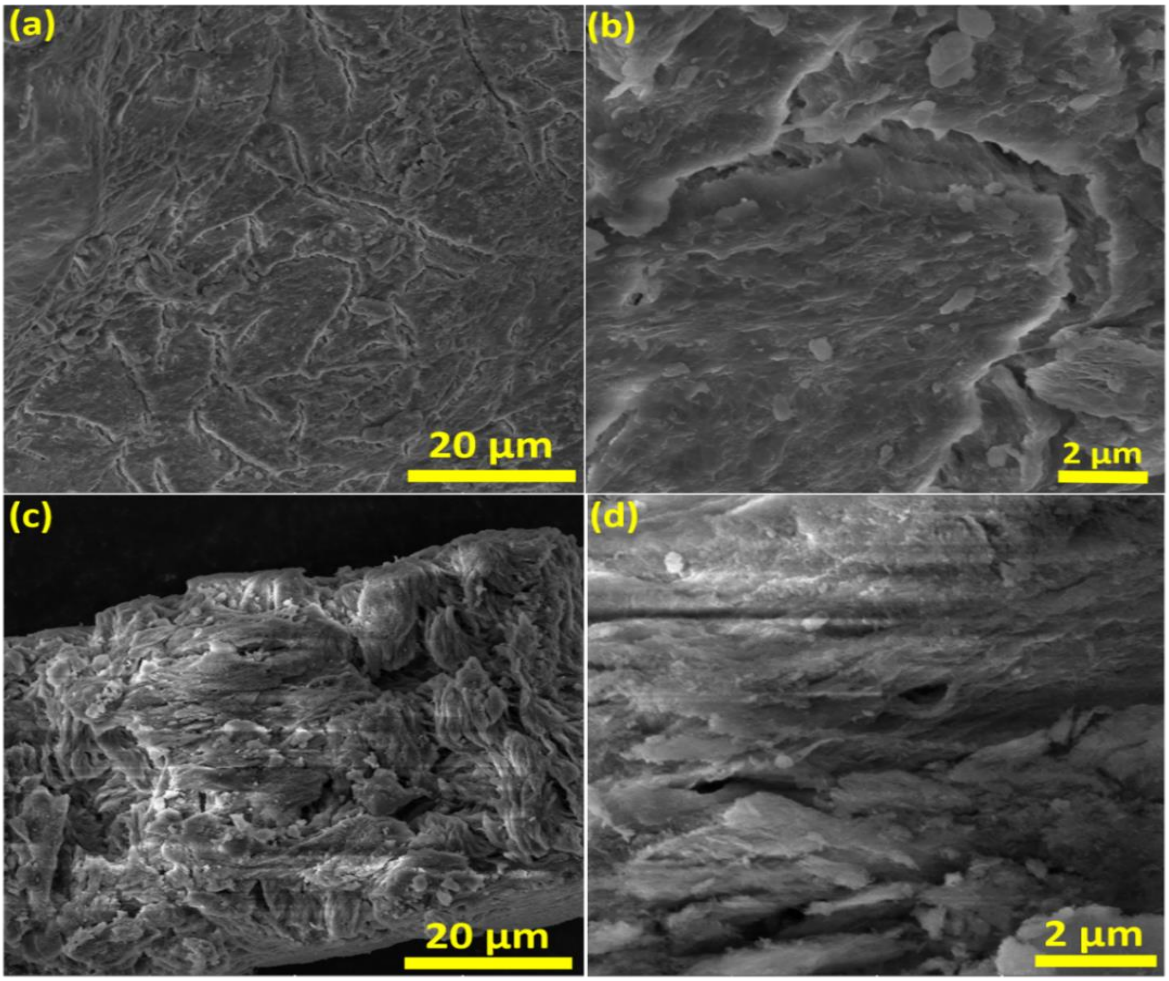

Fig. 3: SEM images (a) & (b of samples extracted Chitin from prawn shell and (c) & (d) deacetylated Chitin powder.

Fig-4(a) shows the effect of chitosan concentrations over the reduction of Kail wood affected by 20 number of termites with contact time of 24 hours. Here, it can be seen that the controlled sample i.e. the sample of Kail wood without the treatment/soaking of chitosan exhibits about 52 wt % reduction while under similar conditions the wood coated with chitosan slurries having concentrations in the range 1 to 5 wt% show the reduction in weight from about 7.66 to 0.53 wt % respectively. Chitosan composition in acetic acid greater than 3 wt % was found to have similar trend of weight reductions, therefore, the 5 wt % slurry was determined as an effective concentration of chitosan for anti-termite application in stated conditions. Biotermicide effect of chitosan is presumably attributed to the fact that when termite eats wood coated with chitosan, it kills the bacteria and single-cell protozoans live inside the gastrointestinal track of termite which otherwise are considered to be responsible for cellulosic digestion of termite [14]. Thus, termite growth and inhabitation was hindered on wood coated with chitosan. Fig-4(b) shows the effect of acetic acid concentration on resistance of wood towards termite attack. Since the weight reduction was found to be similar to that of controlled sample, it can be deduced that the wood pieces coated with only acetic acid at various concentrations do not exhibit any anti-termite properties. Fig 4(c) shows the effect of contact time of 20 numbers of termite with 5 wt % chitosan-coated wood pieces. It can be observed that weight reduction gradually increases and reaches the saturation level after 16 to 24 hours. It was noticed that the there was no substantial change in weight reduction of wood after 24 hours of contact time. Fig 4(d) shows the effect of prepared chitin powder slurries with concentration range from 1 to 5 wt %. Interestingly, results show that there is no any significant weight loss in Kail wood pieces coated with chitin slurries and values in reduction of weight are close to those of controlled samples. Moreover, concentration of chitin exceeding 4 wt % have similar values therefore further concentrations were not checked. This behaviour of chitin powder showing almost no response towards anti-termite properties compare to chitosan may be attributed to lack of functional amino group ($-NH_2$) presence in chitin material which otherwise seems to be responsible for anti-termite function of chitosan as discussed above. Thus, the data revealed in biotermicidal study that there is only the chitosan coated Kail wood pieces exhibit remarkable resistance against termite attack.

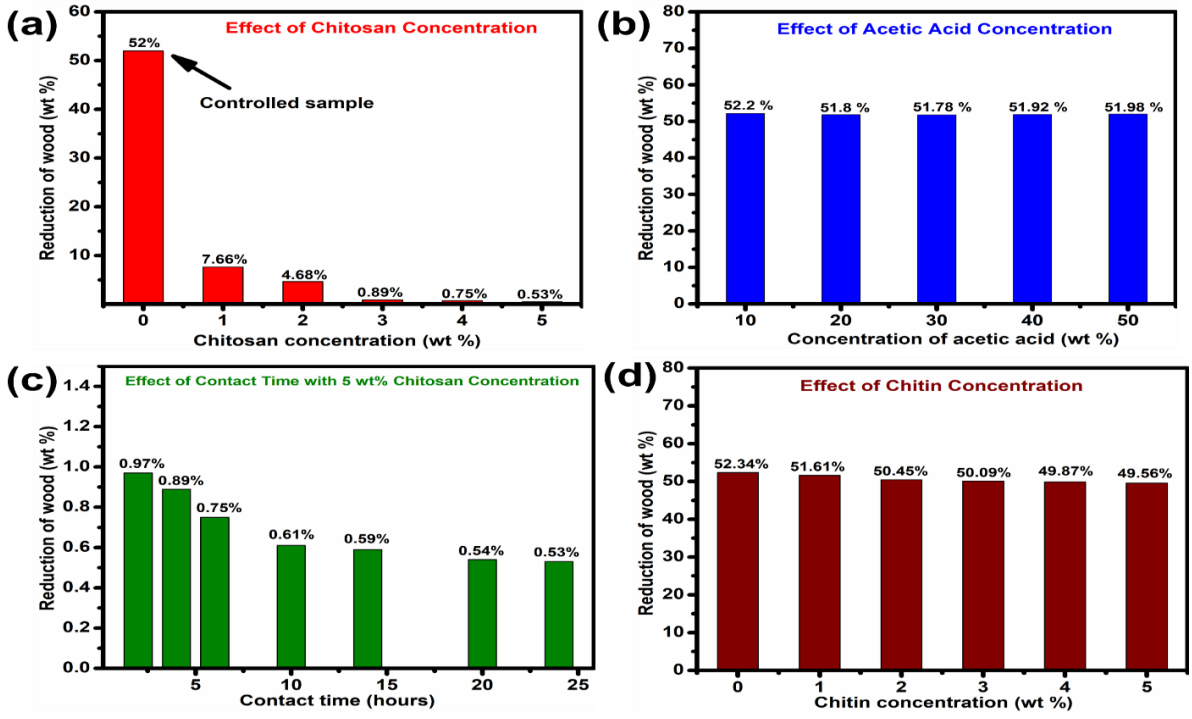

Fig. 4: (a) Effect of chitosan concentration, (b) Effect of acetic acid concentration, (c) Effect of contact time and (d) effect of chitin concentrations on reduction in weight of Kail wood pieces having 20 number of termites in test samples, respectively.

Conclusion

Chitin and chitosan were extracted from prawn shells by wet chemical methods. XRD and FTIR studies confirmed the presence of chitin and chitosan in samples. The most vulnerable Kail wood pieces towards termite attack exhibited remarkable resistance against termite when coated with 5 wt % chitosan slurries in acetic acid and exposed to 20 number of termites for 24 hours contact time. This study paves the way for the large scale application of chitosan based biotermicidal materials for wood preservation.

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