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DECOLORIZATION OF TEXTILE DYE BY CHITOSAN EXTRACTED FROM *Aspergillus niger*

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Abstract

In this present study, chitosan was extracted from *Aspergillus niger* and was applied in the decolourization of the textile dye. Chitosan was extracted from mycelia of *Aspergillus niger* which was allowed to grow in the Potato dextrose agar medium. Then, the fungal isolate was transferred to Yeast peptone glucose (YPG) broth for the highest production of chitosan which was then confirmed through FTIR analysis. Chitosan is a polysaccharides which was found primarily as the main component of the fungal cell wall. It has excellent properties such as non-toxicity and high anionic dye binding capacity. Chitosan has a massive range of application in textile industries particularly decolourization of textile dyes. This present research was aimed to study the decolourization of the textile azo dye Malachite green by Chitosan which was extracted from the fungal mold *Aspergillus niger* isolated from spoiled coconut.

Key words: Chitosan, Yeast peptone glucose medium, *Aspergillus niger*, FTIR, Malachite green and Dye Decolourization

1. Introduction

Chitosan is a cationic biocompatible polysaccharide composed by repeated units of N-acetyl-D-glucosamine and D-glucosamine, derived from the partial deacetylation of chitin, a natural polysaccharide extracted from the crustacean shells. Chitosan was also found in some microorganisms in yeasts and fungi (Reema Mohammed Abed Aloubaidy, 2013). Chitosan is a cationic polymer which was considered as the second most polymers used in industries after cellulose.

Biodegradable properties of chitosan have recently led to increasing interest in the development of slow-release formulations for gastro-retentive drug delivery (Senthil Kumar *et al*., 2015). Nowadays, fungal isolates are widely used in the bioremediation of waste water and toxic textile azo dyes colour removal. Among the various fungal species, *Aspergillus niger* is one of the well known fungi which was responsible for textile dye degradation. In environmental point of view, *Aspergillus niger* can be a useful bioadsorbent to detoxify and decolorize the wastewater polluting our water bodies. In an industrial and pharmaceutical applications, fungal bioremediation can also be used as an inexpensive method for recycling wastes and desirable source for commercial production of some expensive and useful biopolymers such as chitin and chitosan (Mohsen Shahlaeia and Alireza Pourhossein, 2013).

The wastewater released from textile and tanning industries contain high amount of exhausted dyes and pigments. Previously, it has been estimated that, about 10,000 dyes and pigments were produced and about 7×10^5 tons were used in different industries such as textile,

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rubber, paper, cosmetic, etc. Among these various industries, textile grades first in the usage of various kinds of dyes for coloration of fibers. These dyes are mutagenic and carcinogenic and cannot be completely removed by conventional wastewater treatment systems. Moreover, fungi mediated decolourization of different textile dyes have been found to be affected and influenced by different physico - chemical conditions like pH and temperature.

Decolourization of dyes involves either adsorption on biomass or biodegradation by microbial cells. Biosorption process employing biopolymers (sawdust, wood chips, chitin/ chitosan, starch, cyclodextrin and cross linked chitosan/cyclodextrin) and microbial biomass (fungi, algae and bacteria) has emerged as one of the most powerful and attractive option. Since, it is an inexpensive, effective and simple to operate and also possess good mechanical properties against abrasion. Biosorption is becoming a promising alternative to replace or supplement the present dye removal processes from dye waste waters. It involves binding of pollutants to the surface of cell membranes and cell walls through physical adsorption, electrostatic interaction, ion exchange, chelation and chemical precipitation (Agnes Mariya Dorthy, 2012). The objective of this present study was to determine the effects of extractable chitosan obtained from *Aspergillus niger* by Cell cultivation technique. The extracted Chitosan was confirmed by FTIR analysis and Biochemical methods. To estimate the capacity of extracted chitosan, the different concentration of textile dye malachite green was treated with chitosan.

2. MATERIALS AND METHODS

Collection of Rotten Coconut

Aspergillus niger was isolated from spoiled coconut and the strain was confirmed by performing Lactophenol Cotton Blue Staining (LPCB) and observed under the microscope.

Isolation of *Aspergillus niger*

The microorganisms were isolated by serial dilution technique on Potato Dextrose Agar (PDA). In this technique, a sample suspension was prepared by adding 1.0 g collected soil to 10 ml distilled water and mixed well for 15 min. Each suspension was serially diluted 10^{-1} to 10^{-6}. From this, 1 ml was pipetted onto plates with PDA media, spread with a L-rod and incubated at 37 °C for 7 days and fungal observation was made (Waksman, 1927; Nazir, 2007).

Identification of *Aspergillus niger*

The identification of molds is based on the shape, method of production and arrangement of spores (conidial ontogeny). Lactophenol Cotton Blue solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of *Aspergillus niger*.

Aspergillus niger Cultivation

For the determination of optimum condition of isolated fungi is transferred to the liquid media using sterile inoculation loop, fungal cultivation media (Sucrose 150 g/L, K_2PO_4 - 0.26 g/L, $MgsSO_4.7H_2O$ - 0.3 g/L, Ammonium nitrate - 1.14 g/L, $FeSO_4$ - 0.1 mg/L, $ZnSO_4.7H_2O$ 0.25 g/L) was used for the cultivation of *Aspergillus niger*. The media was adjusted to pH 4 and autoclaved in 120 ^{0}C for 20 min. For optimization of the incubation period, the cultivation bottle were incubated at 28 °C for 4 to 7 days in fungal mate growth (Bhattacharyya and Jha, 2011; Azzaz *et al*., 2012). After growth, the fungal mate was collected using sterile forceps.

Cell Cultivation and Harvest

Complex medium was used to screen the best biomass producing fungi. Then, the media (1.0 % yeast extract, 0.3 % peptone, 0.4 % dextrose and 0.01 % antifoam agent (vegetable oils) per liter of distilled water adjusted to pH 4.5. The fermentation medium was sterilized at 121 ^{0}C for 15 minutes. After sterilization the fermentation broth was centrifuged in 5000 rpm for 10 min, for mycelia suspension. The moisture mycelium was dried in 100 ^{0}C for 2 hours.

Chitosan extraction

The chitosan was extracted in following four steps, (i) Deproteinization, (ii) Demineralization, (iii) Decoloration and Deacetylation.

Deproteinization

The dry cell fermentation broth is usually ground and treated with dilute sodium hydroxide solution (1:40 %) at elevated temperature (65 – 100 ºC) to dissolve the proteins present. Prolonged alkaline treatment under severe conditions causes depolymerization and deacetylation. To obtain uniformity in reaction, NaOH solution for 30 min at 65 ºC with constant stirring with homogenizer and a solid to solvent ratio of 1:40 (w/v) during the deproteinization process, foam formation can occur, but the foam was not as brisk and intense as that produced during demineralization. After cooling, the suspension was filtered through a Buchner funnel using Whatman no. 1 filter paper. The filtrate was discarded and extracted cell material was move in demineralization process.

Decoloration

Acid and alkali treatments alone produce a colored Chitosan product. For commercial acceptability, the chitosan produced from *Aspergillus niger* sources, needs to be decolorized to extracted cell material was washed three times with 95 % ethanol and three times with distilled water. Extraction, distilled water for more than 2 to 3 times washing was needed to obtain a commercially acceptable white product.

Demineralization

Demineralization is usually accomplished by extraction with dilute hydrochloric acid (up to 10 %) at room temperature with agitation to dissolve lyophilized cell wall material the use of hydrochloric acid. The cell wall-to-acid ratio was 1:40, and samples were heated to 95 °C in a round-bottom flask fitted with a condenser. After centrifugation of the extracted slurry in a refrigerated centrifuge at 5,000 rpm for 10 min. Pellet was discarded and supernatant was collected and the presence of chitosan in it was checked by the formation of white precipitate upon neutralization with 1 N NaOH.

Deacetylation

Deacetylation is the process to convert chitin to chitosan by removal of acetyl group. The ideal purpose of deacetylation is to prepare a chitosan that is not degraded and is soluble in dilute acetic acid in minimal time. The acid-soluble supernatant was adjusted to pH 8.5 with sodium hydroxide, and a base-insoluble precipitate (chitosan) was collected by centrifugation. The chitosan precipitate was washed four times with distilled water and lyophilized. The white precipitate obtained after recovery was centrifuged at 5000 rpm for 15 minutes. It was washed twice with distilled water (pH 7). Then precipitate was resuspended in 0.5 ml of distilled water (pH 7) and this suspension was taken in watch glass. It was allowed to dry at 55 °C for 2 – 4 hours. The dried precipitate was used for the confirmatory test.

Chitosan Confirmatory Test

Bio-chemical Test

On the dried precipitate (Chitosan), 2 - 3 drops of iodine/potassium iodide solution were added and mixed and the mixture was acidified with 2 - 3 drops of 1 % H_2SO_4. Then, the solution becomes colorless and on addition of sulfuric acid the dark brown colour formed indicates the presence of chitosan. Cell wall material was treated with Lugol's stain, which was prepared by adding 4.0 g of I_2 and 6.0 g of KI to a final volume of 100 ml of distilled water. Mycelial walls appeared pink to violet, cytoplasmic debris appeared bright yellow, and intact mycelia appeared reddish brown.

FTIR analysis

FTIR characterization of samples was performed with a Perkin Elmer-Spectrum RX1 instrument. For the preparation, 2 % w/v chitosan was dissolved in 2 % acetic acid solution, poured on a petridish, and finally dried at 60 °C for 16 hours under vacuum. Then, the sample was mixed with (Potassium bromide) to make a 13 mm

diameter pellet. The spectra of chitosan samples were obtained within a frequency range of $\lambda = 500 - 4000\ cm^{-1}$.

Degradation of textile dye using Chitosan

Malachite green which is a textile dye was used for the degradation using isolated Chitosan from *Aspergillus niger*. Various concentrations such as 0.05 %, 0.1 % and 0.15 % were used as trials and the degradation was sharply noted for a period of 7 days by centrifuging the solution at 5000 rpm for 10 min, the supernatant was used for the estimation of degradation using UV-vis Spectrophotometer. The readings were taken at 500 nm.

3. Results and Discussion

Isolation and confirmation of *Aspergillus niger*

Figure – 1: Spoiled coconut with *Aspergillus niger*

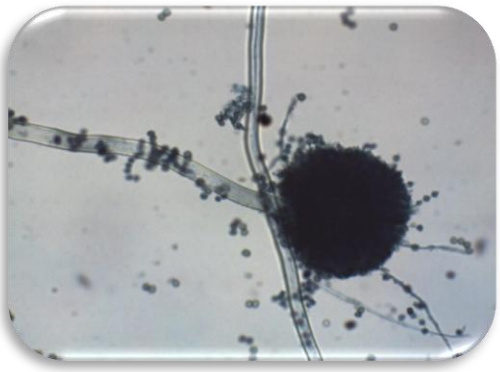

Figure – 2: Microscopic examination of *Aspergillus niger* under LPCB Staining

The fungi *Aspergillus niger* was isolated from the rotten coconut. *Aspergillus niger* morphological characteristics were observed under microscopic view. It possesses globus shape, a stalk and very rough irregular surface. The growth of *Aspergillus niger* was investigated at different time intervals and the results were furnished in Table – 1. Initially the growth of Aspergillus niger was low at 20 minutes and then the growth was increased till 120 minutes and then the fungal growth was decreased at 140 minutes.

Table – 1: Growth curve of *Aspergillus niger*

Time (min)	Absorbance
20	0.002
40	0.0310
60	0.0714
80	0.1529
100	0.2107
120	0.2111
140	0.1974

Treatment of Chitosan

The acid-soluble supernatant was adjusted to pH 8.5 with sodium hydroxide, and a base-insoluble precipitate (chitosan).

Drying of Chitosan

The white precipitate was collected and centrifuged and then allowed to dry to isolate dry Chitosan.

Confirmatory Test

On addition of sulfuric acid, the purple colour was formed. This indicates the presence of Chitosan (Figure – 3).

Figure – 3: Chitosan produced by *Aspergillus niger*

FTIR analysis

FTIR spectrum analysis was done for synthesized Chitosan. The FTIR spectra identified functional groups of the product. The x-axis expressed wavelength in cm^{-1} and the y-axis represents the light transmittance (%) through the sample. Most of the important functional groups are summarized in Table – 2 and Figure - 4, showing good accommodation between synthesized chitosan.

Table – 2: FTIR functional group

FTIR Absorption Produced Chitosan	Functional groups
3460	OH stretching
2885	C–H symmetrical stretching
1644	C=O in amide groups (amide I band)
1414	NH_2 bending vibration in amino group ($-NH_2$)
848	C–O group (-C–O) in amide group

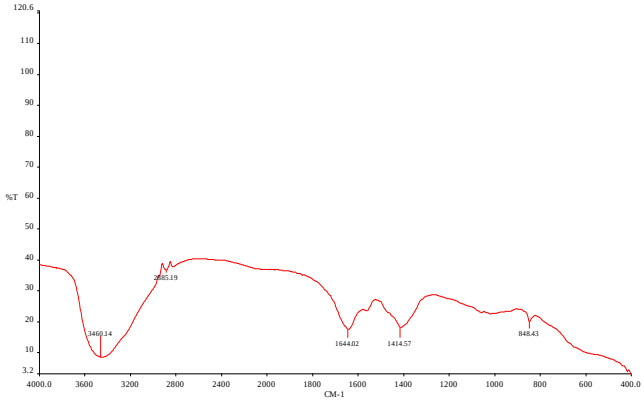

Figure - 4: FTIR Analysis of Chitosan

Degradation of textile dye using Chitosan

Degradation of textile dye Malachite green was studied at various concentrations and the findings were presented in Table – 3 to Table – 5. At 0.05 % of Chitosan, an effective degradation of Malachite green - 0.05 % was achieved on 12th day with absorbance of 0.0045 OD at 600 nm using extracted Chitosan (Table – 3). At 0.1 % of Chitosan, an effective degradation of Malachite green - 0.05 % was achieved on 12th day with absorbance of 0.2651 OD at 600 nm using extracted Chitosan (Table – 4). At 0.15 % of Chitosan, an effective degradation of Malachite green - 0.05 % was achieved on 12th day with absorbance of 0.5521 OD at 600 nm using extracted Chitosan (Table – 5).

Table – 3: Degradation of textile dye using Chitosan at 0.05%

Days	Control	Absorbance
1	0.8871	0.7672
2		0.6988
3		0.5212
4		0.5192
5		0.4665
6		0.4010
7		0.2756
8		0.1121
9		0.0467
10		0.0231
11		0.0078
12		0.0045

Table – 4: Degradation of textile dye using Chitosan at 0.1%

Days	Control	Absorbance
1	0.8871	0.9718
2		0.9514
3		0.8816
4		0.7901
5		0.7641
6		0.5616
7		0.4511
8		0.4088
9		0.3610
10		0.2951
11		0.2720
12		0.2651

Table – 5: Degradation of textile dye using Chitosan at 0.15%

Days	Control	Absorbance
1	0.8871	0.7523
2		0.7506
3		0.7498
4		0.7426
5		0.7335
6		0.7308
7		0.7221
8		0.6776
9		0.6007
10		0.5910
11		0.5787
12		0.5521

4. Conclusion

Aspergillus niger was isolated from spoiled coconut and Chitosan was extracted from *Aspergillus niger* by Fermentation. The relative content of chitosan from *Aspergillus niger* was determined. The chitosan obtained from cultivation media was dried. Chitosan was confirmed by a Biochemical test by the formation of purple color. Further, the chitosan was confirmed by employing FTIR spectroscopy and all the functional groups in chitosan macromolecules are elucidated. In the experimentally prepared chitosan, the bands were more pronounced than in the standard one, which proves the higher degree of morphological arrangement (higher degree of crystalline order) in the former. Later, from the isolated Chitosan, degradation experiment was done using the textile dye Malachite green which was highly dangerous when discarded out from the Textile industries that has the possibility to mix in the lakes or nearby ponds. In order to eliminate the dyes, it is possible to degrade them by using Chitosan which has the capacity to degrade the dyes in the former stage itself at lower concentrations. As the concentration of dye increases, the degradation capacity found to be slow which can be avoided and treat the water immediately in the first stage itself. It is completely environmental friendly and no side effects rather usage of chemical treatment causes serious side effects.

5. References

1) Agnes Mariya Dorthy, Rajeshwari Sivaraj and R. Venckatesh. 2012. Decolorization efficiencies of dyes and effluent by free and immobilized fungal isolates. *International Journal of Environmental Sciences and Research,* 1 (4): 109 - 113.
2) Angham G. Hadi. 2013. Dye Removal from colored textile waste water using synthesized Chitosan. *International Journal Science And Technology,* 2 (4): 278 - 289.
3) George Z. Kyzan, Margaritis Kostoglou, Nikolaos K. Lazaridis and Dimitrios N. Bikiaris. 2013. Decolorization Of Dyeing Waste Water Using Polymeric Absorbents – An Review. In Tech Open Science And Open Minds.
4) Mohsen Shahlaei and Alireza Pourhossein. 2013. Biomass of *Aspergillus niger*: Uses and Applications. *Journal of Reports in Pharmaceutical Sciences,*2 (1): 67 - 73.
5) Senthilkumar, V., P. Yosodha and B. Krishnaveni. 2015. Extraction of Chitosan from *Aspergillus terreus* and study of its antimicrobial and dye degradation ability. *Journal of Phytopharmacology*, 6 (4): 196 - 202.