Annals of Agri-Bio Research **28** (1): 12-17, 2023

Impact Score : 0.28 (Scopus)

Congo Red Removal by Self-Immobilized *Aspergillus terreus*

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(Received: July 5, 2022; Accepted: August 12, 2022)

ABSTRACT

As dyes are frequently discharged into wastewaters and dyes are extensively used in the textile industry so it's necessary to find out an efficient and eco-friendly methods for treating wastewaters resulting from industrial pollutions. Five fungal species were isolated from polluted soil two from them were able to absorb azo dyes and *Aspergillus terreus* was the most effective species to remove 90% of dye after 48 h. Dye removal was investigated in 500 mg/l concentration of Azo dyes at initial pH 5.5 and 2.0 g fresh wet of fungal pellets was kept in rotary shakers at 160 rpm/min at 25°C. Fourier transform infrared spectroscopy, the decolorization analysis of the biodegraded Congo red solution was achieved by the change in the absorption spectrum in the wavelength 495 nm and scanning electron microscopy analysis revealed that dye removal was dependent on hydroxyl and amine groups in the cell wall polymers. The cleavage of Congo red bond was confirmed through HPLC assay, the absorbance at the retention time of 1.97 min was recorded for the Congo red dye, where 100% concentration of the dye was eluted in aqueous solution after treatment showed no absorbance at the same retention time. *A. terreus* is highly capable of sorption azo dyes and could be employed to remove dyes in industrial wastewater.

Key words: Azo dye, filamentous fungi, immobilization

INTRODUCTION

The use of synthetic dyes in industrial processes has increased considerably due to the variety of available colours compared to natural dyes (Geed *et al.*, 2016). These dyes prevent the penetration of light and oxygen into the water and causing broad aquatic pollution (Edison *et al.*, 2016). Some of these dyes cause tumors and cancer in humans (Wang *et al.*, 2015). There are different methods for removal of these dyes from the effluents produced by the textile industry as physical, chemical and biological methods (Quan *et al.*, 2015; Zhao *et al.*, 2015). Physical and chemical methods have disadvantages compared to biological removal methods. The high cost of equipment and the production of large quantities of sludge have made biological methods of dye removal from textile effluents more appropriate than other methods due to their low cost from these biomaterials. Plants, fungi, bacteria and algae have demonstrated good dye removal capabilities (Aziz *et al.*, 2021). In the bio-absorption method, the dye in the effluent is attached to the functional groups present on the surface of the microorganism. Fungi, due to their extensive cell, are capable of removing dye by absorbing from textile effluents in wastewater (Lu *et al.*, 2017; Guo *et al.*, 2020). Many fungi can produce stable mycelial pellets that can be used as self-immobilizers (Espinosa-Ortiz *et al.*, 2016; Hamad *et al.*, 2021) other fungi like *Candida tropicalis* produces bio-surfactant as efficient in degradation different synthetic dyes in water, as well as exerting remarkable antibacterial and anti-biofilm activity against pathogenic bacteria (El-Shahed *et al.*, 2022) can be effective in the rate of adsorption or decomposition according to their ability to absorb azo dyes.

MATERIALS AND METHODS

Work was conducted in Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq. In this study, 10 soil samples were collected from areas near textile industries in Baghdad, Iraq at the coordinates 33°26'45.9540" East, 044°20'33.8575" North. These samples were completely homogenized and used to isolate fungi on PDA plates after growing fungal isolates were purified and identified depending on the morphological features. The culture medium consisted of the following compounds in grams per liter: glucose (20), Na_2HPO_4 (1), $FeSO_4.7H_2O$ (0.01), $MgSO_4.H_2O$ (0.5) and $CaCl_2$ (0.1). All chemicals

of BDH company adjusted at 5.5 pH were incubated at 25°C for seven days. Tetracycline antibiotic was added to the culture medium to prevent bacterial growth.

Fungal pellets formation of *Aspergillus terreus* (selected isolate) were inoculated into 100 ml media in a 250 ml conical flask (Spores suspension about 1×10^8 spores per 100 ml). During the inoculation period, the flasks were shaken at 160 r/min and 25°C for seven days.

Azo dye was used in this study. Congo red (Sigma Aldrich) was purchased from local scientific shops in Baghdad.

The dye concentration was 500 mg/l and the initial pH was 5.5 in sterile distilled water. Each flask (250 ml) containing 50 ml dye solution and 2.0 g fresh wet of fungal pellets was kept in rotary shakers at 160 rpm/min at 25°C for 72 h (Azin and Moghimi, 2020). The dye-removal studies were conducted at 5.5 pH values. All experiments were repeated in triplicate.

Each of the purified isolates of *A. terreus* was cultured in liquid culture medium containing 500 mg/l of dye in an incubator shaker at 160 rpm and 25°C. After one week, the culture medium was centrifuged at 4000 rpm for 5 min and the adsorption was examined using Jenway 3540 UV/VIS spectrophotometer at a wavelength of 500 nm. The following equation was used to calculate the amount of colour removal.

$$D = \frac{Ci - Ce}{Ct} \times 100$$

Where, D: dye removal percentage, Ci: initial absorbance and and Ct: absorbance after incubation time (Azin and Moghimi, 2018). The decolorization rate of the selected isolate was tested in different concentrations of dye (500, 1000) mg/l. The selected isolate was cultured in 500 and 1000 mg/l of dye and transferred to a shaker at 160 rpm and a temperature of 25°C. After 72 h, the amount of dye adsorption and removal was measured by centrifuge of the liquid and the amount of residual dye was measured using a spectrophotometer at a wavelength of 500 nm.

The Fourier transform infrared (FTIR) spectra of fungal pellets exposed to dyes (at concentrations 500 mg/l), exposure time (72 h) and those non-exposed to dyes were obtained using an FTIR spectrophotometer (FTIR Shimadzu, Japan). The samples were then dried in oven at 50 ^{0}C until constant weight, FTIR spectra were recorded at room temperature in the range of 400-4000/cm. Photomicrographs of fungal pellets were taken using a scanning electron microscope after preparation and fixation of the samples performed with glutaraldehyde (2.5%) at 4 °C for 1.5 h.

The experiments were carried out in 250 ml conical flasks containing 200 ml of normal saline to which 5 ml of dye from the stock solution was added to reach the concentration 500 mg/l. One g of fungal pellets was inoculated and incubated at 25°C. Three ml of aqueous solution from culture was taken after three days and decolourization study was performed. Three replicate flasks with the same dye concentration and fungal pellets were used for the study and the results were reported as an average of the three samples. The samples after four days of treatment were filtered through a 0.45 µm membrane filter prior to HPLC analysis. The dye solutions before and after the reaction were analyzed by the high performance liquid chromatography (HPLC) technique. HPLC was performed at (Waters model no 2690) C18 column having symmetry 250 × 4.6 mm using methanol as mobile phase with a flow rate of 1.0 ml/min for 12 min and UV detector at 250 nm (Wu *et al.*, 2020).

Statistical analysis was achieved by using one-way ANOVA followed by student t-test to find out the significance difference between different values of incubation conditions by SPSS version 16.0 software. Statistically significances were taken at $P \leq 0.05$.

RESULTS AND DISCUSSION

Five fungal isolates identified in morphological examinations of cotton-shaped colonies in slide culture medium were *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Penicillum* sp. and *Alternaria alternata*. *A. niger* and *A. terreus* formed pellets after one week (Table 1). Only *A. terreus* pellets were stable (Fig. 1). Dye biosorption was studied in wet and dry biomass of *A. sterreus* pellets at dye concentration of 500 mg /l.

The amount of dye absorption and its removal was measured by centrifuge and the amount of residual dye was measured using a

Table 1. Dye removal percentage and fungal pellets forming of isolated fungi

Fungi isolates	Pellet formation ability after 7 days	Mean±SD with 500 mg/l of dye	Mean±SD with 1000 mg/l of dye
Aspergillus niger	Able	66±1.7b	67±0.7b
Aspergillus terreus	Able	90±0.5a	92±1.4a
Aspergillus fumigatus	Non-able	67±0.3b	67±0.3b
Penicillum sp.	Non-able	67±0.3b	67±0.3b
Alternaria alternata	Non-able	67±0.3b	67±0.3b

Different letters represent significant difference at P≤0.05.

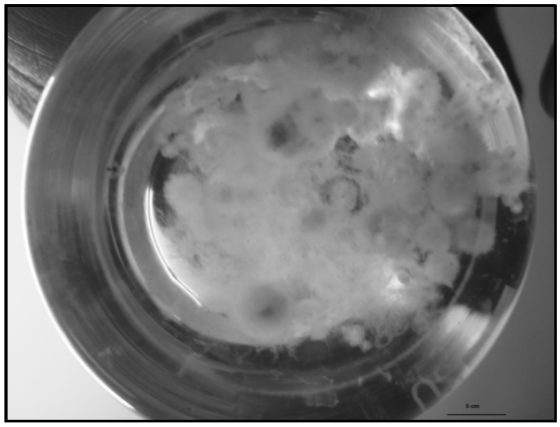

Fig. 1. Fungal pellets after seven days of incubation in rotary shakers at 160 rpm/min at 25°C.

spectrophotometer at a wavelength of 500 nm (Fig. 2). Experiments using wet and dry biomass showed that both types of biomass were able to absorb significant amounts of dye under incubation conditions similar to Asses *et al.* (2018). It was confirmed that *A. niger* removed above 97% of 200 mg/l of dye by adding 2 g mycelia and incubating at 5 pH, for six days at 28°C under 150 rpm shaking speed. Therefore, based on this observation, it can be assumed that the mechanism of dye uptake by the fungi was/is independent of fungal metabolism.

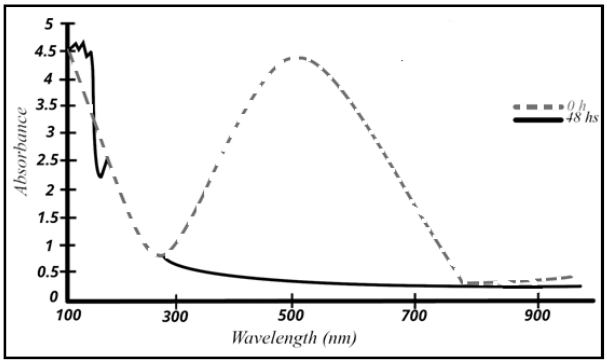

Fig. 2. UV-Vis spectra absorbance of Congo red at 0 and after 48 h of incubation of *A. terreus* pellets at 25°C in aqueous solution.

Previous studies found the ability of *Tricoderma harzianum* in decolorizing textile dyes with partial purified laccase on solid media. It was completely decolorization of Blue dye with concentrations of 50 and 100 and partially decolorizing with concentration of 150, 200 and 250 ppm. The fungi *A. terreus* produced lovastatin which showed thermal stability and storage time (Al-Sa'ady and Aziz, 2021). Azin and Moghimi (2018) found that *Mucor circinelloides* removed 94% Congo red at the concentration of 150 mg/l.

After three days the fungal pellets were centrifuged and the amount of dye removal from the effluent was investigated by a spectrophotometer (Figs. 3 and 4). To evaluate the amount of dye removal, the absorption of the tested effluent at wavelengths between 100 to 900 nm was read and compared with the absorption of the control samples. Measurement of decolorization of selected isolates studies on the amount and time of adsorption showed that this isolate had a high ability to absorb dye in less than 48 h. Based on the obtained results and statistical studies, different concentrations of dye did not have a significant effect on the growth rate of fungal isolates. *A. terreus* was selected because its ability to remove 90% of 500 mg/l of dye. Based on the results, other isolates also showed a removal rate of between 30-55%.

This study compared the dye biosorption by wet biomass and dry biomass, the adsorption mechanism was the same for both the types were capable of bio-dye adsorption. Based on the results obtained (Fig. 3), the continuation of experiments was performed on wet biomass. Investigation of fungal dye adsorption showed the ability of this fungus to remove dye from solutions.

Scanning electron microscope images (Fig. 4) showed biosorption of Congo red by *A. terrus* pellets taken before and after dye biosorption confirmed the attachment of dye molecules to

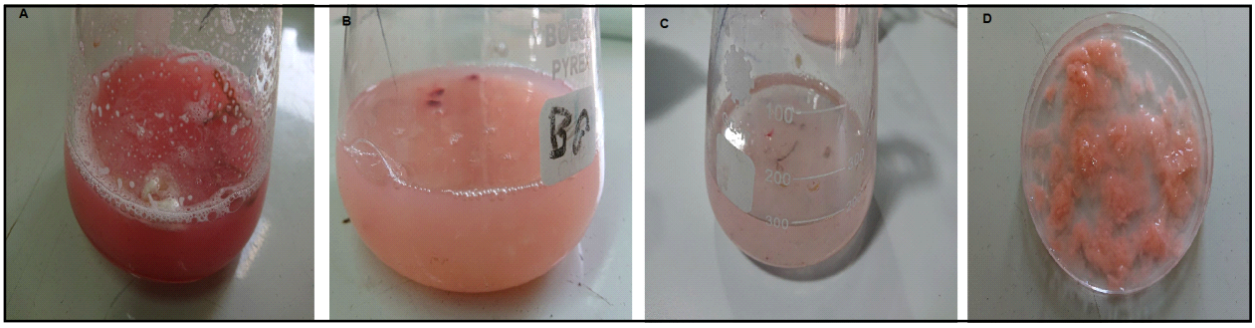

Fig. 3. A Congo red with 500 mg/l; B. wet biomass at 500 mg/l dye solution; C. dry biomass at 500 mg/l dye solution after 48 h from inoculation and D. fungal pellets after biosorpion.

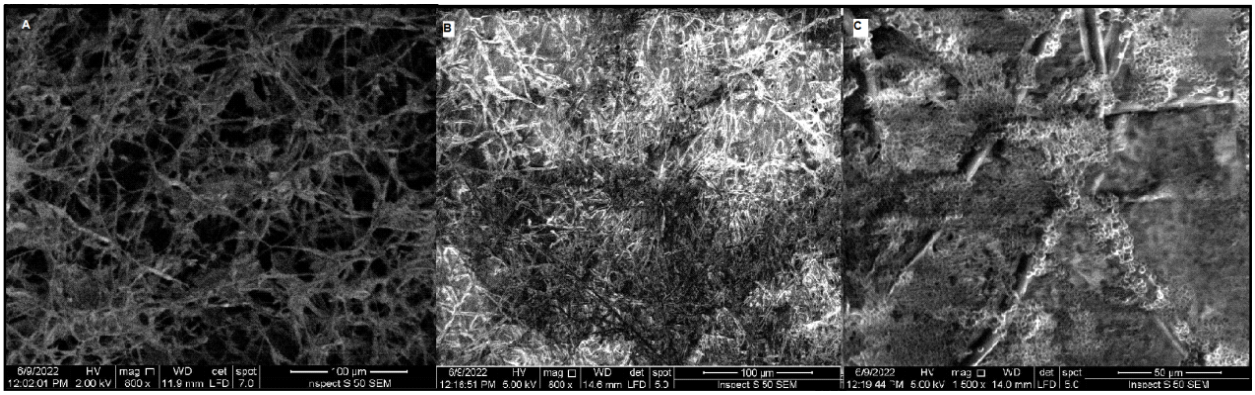

Fig. 4. Scanning electron microscopy of *A. terrus* after seven days of incubation: A. fungal pellets before dye sorption; B. fungal mycelia after dye sorption and C. mycelium surface coated with spores.

fungal mycelium. Scanning electron microscope (SEM) revealed the amorphous nature of the fungus and its affinity to Congo red dye and the surface morphologies of fungal pellets after absorbing Congo coated with dye while the surface of the fungus pellets was smooth in control , indicating that the dyes were adsorbed onto the fungus hyphae mainly by an electronic interaction force, Our results agreed with resent studies of Skanda *et al.* (2021) and Batana *et al.* (2022).

FTIR spectra for control and coloured biomass of the mycelial pellets before and after dye biosorption indicated the structures of surface functional groups and the results of FTIR analysis indicated considerable changes in biosorption peaks, including -OH and/or -NH stretching and NH bending of primary amines (Fig. 5). Therefore, it can be concluded that these functional groups may play a significant role in the adsorption of dye on the fungal mycelial pellet surface. In addition, the intensity of these peaks decreased after biosorption process. These data approved that there was interaction between hydroxyls and amines hydrogens of cell surface and aromatic rings of dye molecules. Results showed fungal

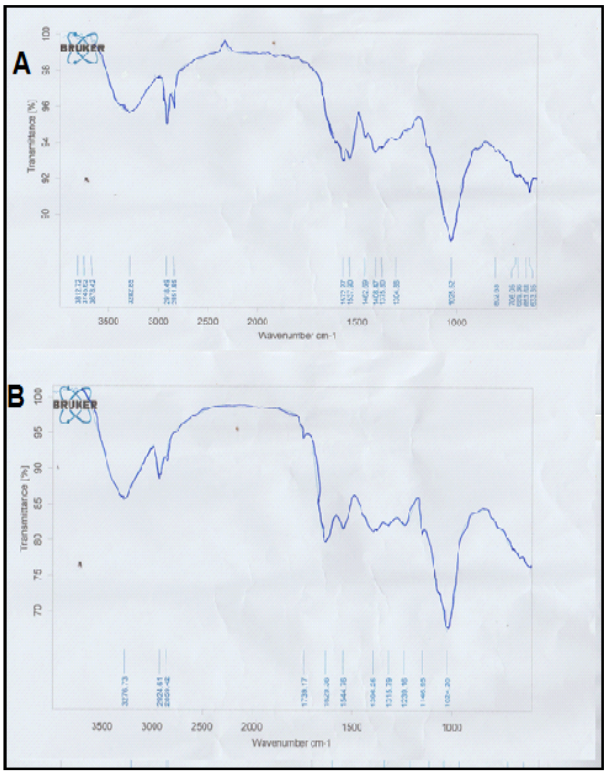

Fig. 5. FTIR spectra of *A. terrus* mycelial pellets: A. after Congo red adsorption and B. fungal pellets without dye.

mycelium had large number of hydroxyl and amine functional groups having a high ability to absorb various pollutants from effluents. The band at 1631/cm was due to the bending of N-H groups of chitin on the cell wall structure of fungal pellets (Batana *et al.*, 2022). Other study found *Phomabetae* produced laccase as an efficient biocatalyst for synthetic dye decolonization (Ali *et al.*, 2020). Biosorpion of Congo red on the fungal pellets induced an increase in some peaks intensity those around 3280, 2923, 1739, 1238 and 1071/cm. Appearance of new peaks at 3812, 3745.62, 2851, 1462 and 653.69/cm was due to introduction of new functionalities on the surface of biosorbent which confirmed the Congo red adsorption on fungal biomass. Similar FTIR results were observed for the azo dye biosorption on various fungus biomass (Asses *et al.*, 2018). The presence of high diversity of functional groups in the surface structure of fungal microorganisms is an important factor in the high ability of these organisms to absorb various compounds. Due to having a higher surface-to-volume ratio than bacteria, they showed a high ability to absorb pollutants. Previous study on fungi *Penicillium* sp. and *Aspergillus niger* observed 70% of dye at maximum of 300 ppm within 72 h (Rohini *et al.*, 2019). The amount of biomass produced in different concentrations of dye showed that high concentrations of dye on this fungus were not toxic and *A. terreus* was able to grow and absorb dye even at high concentrations (1000 mg/l). Based on the results obtained in this study, *A. terreus* can act a suitable bioactive agent and capable of absorbing high concentration of azo dyes from the effluents of textile factories. Recent studies (Thakor *et al.*, 2022) indicated mutualistic interaction between two different fungi *Penicillium oxalicum* (DS-2) and *A. tubingensis* for the rapid decolorization and degradation of Congo red dye.

The absorbance at the retention time of 1.97 min was recorded for the Congo red dye (Fig. 6a), where 100% concentration of the dye was eluted in aqueous solution after treatment showed no absorbance at the same retention time (Fig. 6b). The wavelength 250 nm was selected in the ultraviolet region because of the resulting solution, which became colourless after the treatment.

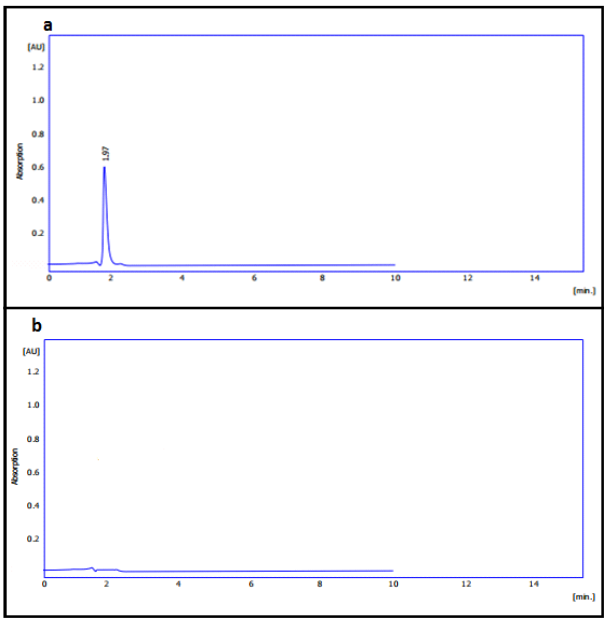

Fig. 6. The HPLC chromatogram of slandered Congo red dye: (a) the absorbance spectrum and (b) of solution resulting after treatment with fungal pellets (the absorbance spectrum detected at 250 nm).

In the current research, dicolorization confirmed through HPLC analysis that Congo red oxidized and completely disappeared after four days of incubation, confirming the complete degradation, compared with study of Wu *et al.* (2020). White-rot fungi *Irpex lacteus* converted Congo red into two compounds were newly formed during the decolorizing process. Due to the ability of this strain to absorb azo dyes, this isolate can be introduced as a valuable option for adsorption of dyes from textile industry effluents.

CONCLUSION

The removal of Congo red with high concentration from aqueous solution, the use of fungal adsorbents can be a suitable solution to remove contaminants from textile industry effluents. Fungi are very important due to their high adsorption level and bio-polymers with high adsorption capacity in their wall structure.

ACKNOWLEDGEMENT

The authors extend their gratitude to Biology Department, College of Sciences at Mustansiriyah University for their financial support.

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