

Aspergillus Niger QH27 in the Removal of Toxic Dyes: Biosorption of Eriochrome Black T under Various Experimental Conditions

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Water pollution by toxic dyes such as Eriochrome Black T (EBT) is an increasing environmental issue, and the use of biosorbents represents a sustainable alternative for their treatment. In this study, the ability of *Aspergillus niger* QH27 fungal biomass in removing EBT under various experimental conditions was investigated. The main objective was to evaluate biosorption efficiency and determine the optimal conditions for dye removal. The methodology consisted of performing experiments by varying pH (5, 7 and 9) and agitation speed (100, 150 and 200 rpm), and analysing dye removal efficiency and adsorption capacity. Results showed that EBT removal was most effective at low pH values and higher agitation speeds. At a pH of 5 and agitation of 200 rpm, the maximum removal efficiency (99.17 %) and adsorption capacity (1.24 mg/g) were achieved. Fourier transform infrared spectroscopy (FTIR) analyses were carried out to examine the changes in functional groups of fungal biomass before and after EBT biosorption. FTIR results suggested the active involvement of these groups in the dye removal process. In conclusion, *Aspergillus niger* QH27 biomass proved to be an efficient biosorbent for the removal of EBT dye in aqueous solutions. This study provides valuable information for the development of sustainable and low-cost strategies in treating wastewater contaminated with dyes and expands the knowledge of dye biosorption using fungal biomass.

1. Introduction

Synthetic dyes, derived from petrochemical compounds, offer various advantages such as a wide range of colors and stability, but they cause negative impacts when released into the environment (Slama et al., 2021). Dyes, even at low concentrations, cause environmental damage and, along with other toxic compounds, affect the penetration of sunlight, alter oxygen demand, hinder photosynthesis and plant growth, and are recalcitrant, bioaccumulative, carcinogenic and mutagenic (Ardila-Leal et al., 2021). The effective removal of these contaminants is crucial for preventing water resource pollution and protecting human health. Various countries have established regulations to ensure proper treatment of effluents before their release or reuse, using physical, chemical, and biological technologies, individually or combined, to protect the environment and human health (Al-Tohamy et al., 2022).

Conventional treatment of wastewater containing synthetic dyes involves methods such as adsorption, filtration, ion exchange, oxidation, coagulation, flocculation, photocatalytic and ozonation (Al-Tohamy et al., 2020; Dai et al., 2020; Vaiano et al., 2017). However, biological methods represent a promising and environmentally friendly approach. These methods are an economical alternative to the costly physicochemical techniques that generate

additional sludge, allowing for a more cost-effective and eco-friendly degradation process (Legerská et al., 2016). Biosorption, which employs biological materials to remove contaminants, emerges as a cost-effective and efficient alternative, utilizing resources such as natural waste, industrial by-products, and biomass of microorganisms or biosorbents (Danouche et al., 2021). The dye-binding process to biosorbents is due to the high attraction force present in the components of the cell wall, such as lipids and heteropolysaccharides, which possess various functional groups like carboxyl, hydroxyl, amino, phosphate, and other charged groups (Aragaw and Bogale, 2021).

Fungal biomasses offer economical, ecological solutions and do not require essential nutrients. Numerous fungal species have been successfully employed as candidates for removing various dyes present in effluents, such as *Rhizopus arrhizus*, *Trichoderma sp.*, *Penicillium simplicissimum* and even *Aspergillus sp.* (Ahmed and Ebrahim, 2020). *Aspergillus niger* is a filamentous fungus widely used in the production of enzymes and other compounds of commercial value. Moreover, it has been demonstrated to be an effective biosorbent agent in the removal of various contaminants, including synthetic dyes such as Acid Orange 56, Acid Blue 40, Methyl Blue, and Remazol Black B (Iscen et al., 2022; Li et al., 2019). The choice of the *A. niger* QH27 strain, isolated from hydrocarbon-contaminated soils, is based on its proven adaptability to toxic environments and its effectiveness in breaking down petroleum-derived compounds, as reported by Baldera et al. (2022). This study seeks to fill the existing gap in the scientific literature regarding the exploitation of its properties as a biosorbent, a potentially innovative and significant application in environmental remediation. Despite previous research focused on its adaptability and decomposition capabilities, the novelty of our study is in exploring its use as a biosorbent, an approach that has not been investigated until now.

Eriochrome Black T (EBT) is an azo dye that constitutes more than 50 % of the global dye production and is used in applications such as coloring silk, nylon and wool, as well as in research and laboratory teaching for determining water hardness (Regti et al., 2021). Due to its complex chemical structure, resistance to photodegradation, and light stability, it is difficult to biodegrade, making it a relevant target for removal and decontamination studies (Moeinpour et al., 2014). The present study aims to investigate the biosorption capacity of the *Aspergillus niger* QH27 strain in removing EBT dye from aqueous solutions. To this end, the influence of different experimental conditions, such as pH and agitation speed, on biosorption efficiency was evaluated.

2. Method

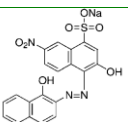
2.1 Preparation of *Aspergillus niger* biomass

The *Aspergillus niger* QH27 fungus was characterized and isolated from petroleum-contaminated soils at the Biotechnology and Genetic Engineering Laboratory of the Universidad Nacional de Trujillo, as described by Baldera et al. (2022). To prepare fungal pellets, a suspension of *A. niger* spores containing 4×10^8 CFU per mL in 1.5 % Tween 80 (Sigma Aldrich, USA) was used, previously cultivated for 7 d on Sabouraud medium (MS) agar and inoculated into 100 mL of MS broth (approximately 10^7 spores/mL) in a 250 mL conical flask. The inoculated flasks were incubated at 120 rpm at 28 ± 0.1 °C for 48 h. The pellets were then collected, washed three times with sterile distilled water, and stored at 5 °C for later use (Lu et al., 2017a).

2.2 Preparation of Eriochrome Black T solution

In this study, a dye with 99.9 % purity acquired from Sigma Aldrich was used. Mother solutions of EBT at 500 mg/L in distilled water were prepared, which were then diluted to achieve the initial concentration of 50 mg/L for the experiments. The properties of the dye are detailed in Table 1. The pH of the solutions was adjusted by adding 0.1 M HCl or 0.1 M NaOH.

Table 1: Chemical structure of Eriochrome Black T dye.

Dye	Structure	λ_{\max} (nm)	MW (g/mol)
Eriochrome® Black T		566	461.38

2.3 Biosorption experimental design

The effect of pH (5, 7 and 9) and agitation speed (100, 150 and 200 rpm) was evaluated using a multi-level factorial design. The matrix of factors and experimental levels comprised a total of 9 runs in triplicate, resulting in 27 tests. The statistical software Design Expert v13 was used to perform the experimental design. The trials were carried out in flasks with 50 mL EBT solution and 2 g of wet mycelium pellets at 30 °C for 24 h. All flask experiments were performed in triplicate (Patel and Suresh, 2008).

2.4 Analytical measurements

The samples were centrifuged at 6,000 rpm for 15 min to separate the solid phase from the liquid phase. The supernatant was collected to determine the dye concentration using a UV-VIS spectrophotometer (Analytik Jena, UVSTAR) at a wavelength of 530 nm. To calculate the percentage of dye removal (R %) and the biosorption capacity (Q_e) by the fungal biosorbent, Eq(1) and (2) were applied, respectively (Aly-Eldeen et al., 2018):

$$R (\%) = \left(\frac{C_i - C_f}{C_i} \right) \times 100 \quad (1)$$

$$Q_e = \frac{(C_i - C_f)}{m} \times V \quad (2)$$

where C_i and C_f are the initial and final concentrations of EBT dye (mg/L), respectively, V is the volume of the dye solution (L), and m is the fungal biomass used (g). A calibration curve of the dye was plotted against the absorbances obtained at different concentrations of EBT.

2.5 Characterization of *Aspergillus niger* biomass

To evaluate the structural characteristics of the biomass, a Fourier-transform infrared spectroscopy equipment from Thermo Scientific model Nicolet iS50 was used. The attenuated total reflectance (ATR) technique was applied in a range of 4,000-600 cm^{-1} with a resolution of 4 cm^{-1} (Bouras et al., 2021). The OriginPro 2018 software was used for signal processing, peak analysis and baseline correction.

2.6 Statistical Analysis

Data interpretation was performed using analysis of variance (ANOVA), and a p-value of <0.05 was considered significant. The Design Expert v13 statistical package was used (Mokhena et al., 2016).

3. Results and discussion

The results of this study show how different combinations of pH and agitation speed (rpm) affect the removal of EBT dye by a biosorbent based on *Aspergillus niger* QH27 biomass. The efficiency of dye removal ranged from 23.76 to 99.17 % (Figure 1a). It was observed that at a pH of 5 and an agitation of 200 rpm, the maximum removal of EBT dye was achieved (99.17 ± 0.24 %). The lowest dye removal (23.76 ± 2.41 %) was found at a pH of 9 and an agitation of 100 rpm. Overall, the results indicate that the removal of EBT dye is more effective at low pH and high agitation speed. It can be observed that the variability in the results, represented by the standard deviation, also differs depending on the experimental conditions. Figure 2b shows that the adsorption of the dye ranges between 0.2969 and 1.2397 mg/g, with the best result (1.24 ± 0.0030 mg/g) obtained at pH 5 and an agitation speed of 200 rpm. In contrast, the lowest absorption of the dye (0.2969 ± 0.03 mg/g) occurred at a pH of 9 and an agitation speed of 100 rpm. Therefore, it can be appreciated that the percentage of dye removal is directly related to the amount of EBT dye absorbed.

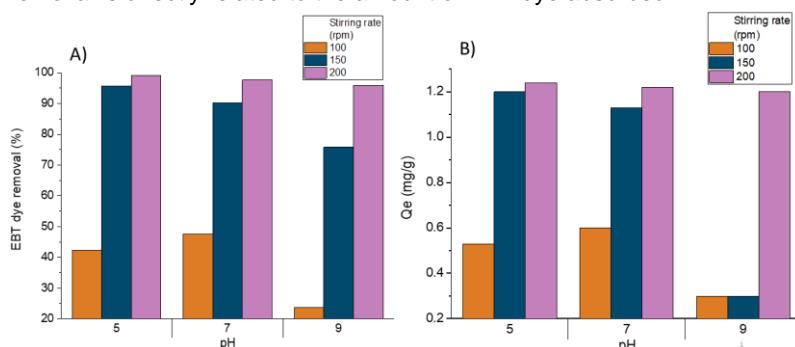


Figure 1. Percentage of EBT dye removal (A) and amount of EBT dye absorbed (B) as a function of pH and agitation speed.

The results outperform those reported by Hidayat et al. (2022) who achieved a maximum removal of 80.5 % using *Exserohilum rostratum* over 300 min. Similarly, Lu et al. (2017b) reported a removal rate of 96.4 % after 12 h using *Aspergillus niger*. *Aspergillus niger* has been reported to completely decolorize the DB 201 dye under conditions of 28-40 °C, pH 7 and agitation at 150 rpm after 96 h (Ekanayake and Manage, 2022). It was found that the Q_e of EBT by the fungal biomass QH27, at a concentration of 50 mg/L, yielded results within a range

similar to those reported by Kabbout and Taha (2014), who documented a Q_e fluctuating between 0.8 - 9 mg/g, utilizing concentrations of 2 - 24 mg/L of methylene blue with *Aspergillus sp.*, under conditions of a pH of 7 and ambient temperature. Although there are variations in the concentrations analyzed, the anionic chemical similarities between EBT and methylene blue indicate a comparable influence in their interaction with biosorbent substances. Fu and Viraraghavan (2001) reported a value that is approximately similar to the one obtained in our study, with 1.17 for 50 mg/L of Basic Blue 9 over a period of 2 days.

In Figure 2, different shades of the EBT dye can be observed, which are due to the pH changes and the chemical nature of the dye, which has azo bonds (-N=N-) that function as chromophores (Fajarwati et al., 2019). The fungal biomass is showed in the form of pellets, with an estimated diameter of 3.0 ± 1.0 mm. A decrease in the color intensity of the EBT solution was observed at pH 5 with 200 rpm, reaching a minimum absorbance of 0.08.

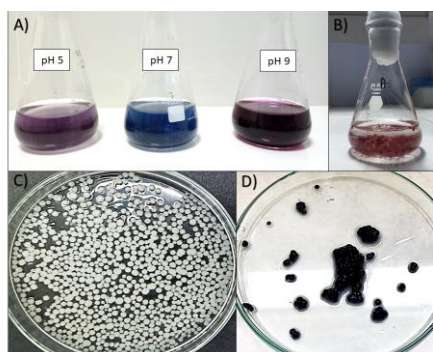


Figure 2. EBT dye solution at different pH (A), EBT dye biosorption by *Aspergillus niger* QH27 fungal biomass at pH 5 and 200 rpm (B), *A. niger* QH27 fungal biomass pellets without EBT (C) and with EBT (D).

Regarding individual factors, both pH and agitation speed have a significant effect on the removal of EBT dye and the absorbed amount (Q_e), with p-values < 0.0001 for both factors (Table 2). This suggests that both pH and agitation speed are important for the removal and absorption of EBT dye. The interaction between pH and agitation speed also has a significant effect on the removal of EBT dye and the absorbed amount (Q_e), with a p-value of 0.0011. This implies that the combination of these two factors influences the process of EBT dye removal. It was observed that both the percentage of removal and the amount of Q_e increased with decreasing pH. This behavior is due to the fact that pH influences the surface charges of the fungal biomass biosorbent. As the concentration of H^+ ions increase, the surface of the biosorbent acquires positive charges by absorbing H^+ , which favors a strong electrostatic attraction between the positively charged biosorbent surface and the negatively charged EBT molecules. This leads to maximum colorant absorption under low pH conditions (Bansal et al., 2020). Similar results were found by Rashidi et al. (2021), who achieved a maximum EBT absorption capacity (72.71 mg/g) using raw Montmorillonite by decreasing the pH to 3. Likewise, it was reported that increasing the agitation speed up to 200 rpm in the aqueous solution, increased the biosorption of EBT. This is because the agitation speed reduces the thickness of the boundary layer of the biosorbent, which in turn reduces the resistance to the transfer of EBT to the biosorbent surface (Altaher et al., 2014).

Table 2: Analysis of variance (ANOVA) for EBT dye removal and dye absorption (Q_e) as a function of pH and agitation speed.

Source	EBT dye removal (%)				Q_e (mg/g)			
	Sum of Squares	Mean Square	F-value	p-value	Sum of Squares	Mean Square	F-value	p-value
Block	81.64	40.82			0.0128	0.0064		
Model	19899.27	2487.41	163.25	< 0.0001	3.11	0.3887	163.25	< 0.0001
A-pH	1116.99	558.49	36.65	< 0.0001	0.1745	0.0873	36.65	< 0.0001
B-Stirring rate	18304.81	9152.4	600.66	< 0.0001	2.86	1.43	600.66	< 0.0001
AB	477.48	119.37	7.83	0.0011	0.0746	0.0187	7.83	0.0011
Residual	243.8	15.24			0.0381	0.0024		
Cor Total	20224.7				3.16			

Figure 3 shows the FTIR spectra of the EBT dye, as well as the fungal biomass before and after EBT biosorption. The EBT infrared showed characteristic peaks around 2926-2854 cm^{-1} , attributed to aromatic -C-H stretching, a peak at 1187 cm^{-1} corresponding to the asymmetry stretching vibration of the S-O group (SO_3-H) and its symmetry around 1045 cm^{-1} , and peaks at 1500 cm^{-1} attributed to N=N stretching vibration and 1334 cm^{-1}

assigned to -NO_2 stretching vibrations (Bartošová et al., 2017). The IR spectrum of the fungal biomass with EBT showed a peak around 3419 cm^{-1} attributed to the amino group (N-H), which differed from the fungal biomass without EBT, which showed a change in N-H intensity around 3355 cm^{-1} (Sule et al., 2019); a peak around 2925 cm^{-1} attributed to the hydroxyl (OH) group in the fungal biomass was observed, however, with EBT, there was variation and lower intensity at 2936 cm^{-1} (Lu et al., 2017b). These peaks indicate a possible participation of functional groups (OH and N-H) in the dye removal process. Even after biosorption, two new peaks at 1361 and 1147 cm^{-1} were detected, which could be associated with the $\text{SO}_3\text{-NH}_3$ complex (Haupa et al., 2015).

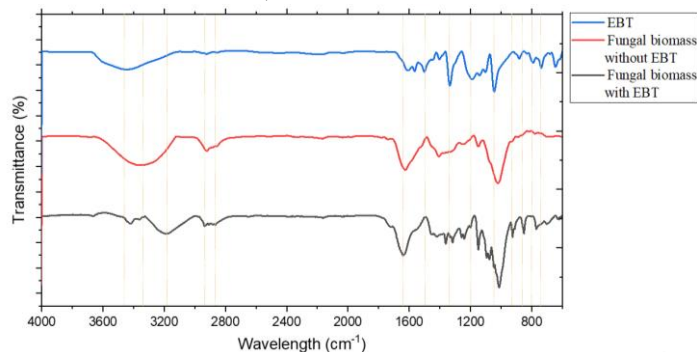


Figure 3: FTIR spectra of EBT, fungal biomass with EBT, and fungal biomass without EBT.

4. Conclusions

In conclusion, this study demonstrated that *Aspergillus niger* QH27 biomass is an efficient biosorbent for the removal of EBT dye in aqueous solutions. The maximum removal efficiency was achieved at pH 5 and an agitation speed of 200 rpm, reaching a 99.17 % removal and an absorption of 1.24 mg/g; this result proves to be competitive and even surpasses the results achieved in previous studies. It was observed that the removal of EBT dye is more effective at low pH and high agitation speed. The FTIR analyses revealed changes in the functional groups (-OH and NH-) of the fungal biomass before and after EBT biosorption, suggesting the active participation of these groups in the dye removal process. This study expands the knowledge of dye biosorption using fungal biomass and provides valuable information for the development of sustainable and low-cost strategies for the treatment of wastewater contaminated with dyes.

Acknowledgments

The authors acknowledge for the support provided by the PICFEDU Project 48852201101, carried out at the Universidad Nacional de Trujillo.

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