Comparison of color removal from reactive dye contaminated water by systems containing fungal biosorbent, active carbon and their mixture
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ABSTRACT
The adsorption of Everzol Black (EB) from synthetic aqueous solution onto active carbon (AC) and dried fungal biosorbent (Rhizopus arrhizus) was studied under the same experimental conditions. The effects of initial dye concentration, adsorbent dosage and contact time were examined at a batch-scale level. As an alternative to AC, fungus was investigated as a low-cost adsorbent for dye removal. The amount of EB adsorbed onto AC was lower compared with fungal biosorbent; dye adsorption capacity of AC and fungal biosorbent were 94.48 and 106.61 mg/g, respectively. The adsorbent dosage experiments showed that 4 g/L biosorbent removed 100% of EB (C₀: 114.39 mg/L) after 2 hours. The results obtained from this study showed that biosorbent effectively removed reactive dye from dye-containing water in a short time period. Langmuir and Freundlich adsorption isotherm models were used for mathematical description of the biosorption equilibrium data; the Freundlich model was found to exhibit good fits to the experimental data. According to the Freundlich isotherm, the maximum dye adsorption capacities of AC and biosorbent were calculated as 344.82 and 357.14 mg/g, respectively. The Fourier transform infrared spectroscopy spectral analysis showed the involvement of functional groups for dye bindings.

Key words | active carbon, decolorization, Everzol Black, Rhizopus arrhizus

INTRODUCTION
Dyestuffs are important environmental pollutants (Lin et al. 2013; Du et al. 2014). Dyes are synthetic and aromatic molecular structural compounds. According to their dissociation in an aqueous solution, dyes can be classified as acid, direct reactive dyes (anionic), basic dyes (cationic) and disperse dyes (nonionic) (Sathiya et al. 2007). Especially, synthetic dyes have increasingly been used in the textile and dyeing industries because of their ease and cost-effectiveness of synthesis; firmness; high stability to light, temperature, detergent and microbial attack; and variety in color compared with natural dyes (Couto 2009). It is estimated that 280.000 t of textile dyes are discharged every year worldwide (Maas & Chaudhari 2005).

Prevention of the negative effects of the dyestuffs requires reliable, low-cost and fast techniques for the removal of their remains in wastewater and soil. Various techniques have been employed for the removal of synthetic dyes, which include chemical and physical methods such as sedimentation, flocculation (Wang et al. 2012), filtration, adsorption (Ghaedi et al. 2012), coagulation (Merzouk et al. 2011), ion exchange and precipitation. Adsorption is the most promising option for the removal of pollutants from aqueous solutions, and active carbon (AC) is the most common adsorbent because of its effectiveness and versatility (Aksu 2005). ACs, which are usually supplied from materials with high carbon content, possess a great adsorption capacity due to their porous structure. Adsorption of reactive dyes on AC has been studied by a number of researchers (Faria et al. 2004; Crini 2006).

The biological treatment methods are increasingly used for the decolorization of dyes, including the usage of bacteria, yeast, algae, and fungi (Phugare et al. 2010; Khataee et al. 2011; Han et al. 2012). The biological techniques present some advantages in relation to many other techniques because they have good treatment efficiency, less waste sludge and no addition of extra chemicals. In addition, when compared to chemical techniques, the biological procedures are much more economical. Biosorption is an
effective technology using inactive and dead biomass to remove pollutants such as dyes from aqueous solutions (San & Dönmez 2012; Karatay Ertugrul et al. 2014). There are some advantages of biosorption such as high selectivity and efficiency, cost-effectiveness, good removal performance, possible regeneration at low cost, availability of known process equipment, sludge-free operation and recovery of the sorbate (Aksu & Karabay 2008). The binding of dye on to the fungal surface is related to the structure of dye molecule (size and ionic charge), surface properties of biosorbent and environmental conditions. Physical adsorption occurs between ionic dye molecules and the surface of fungal biomass by means of van der Waals forces, electrostatic interactions, ion-exchange reaction, complexation, surface precipitation, or their combinations (Veglio & Beolchini 1997). The filamentous fungus Rhizopus arrhizus has chitin and chitosan polymers in the structure of its cell wall (Cardoso et al. 2011). Chitin/chitosan is a component of fungal cell walls and the major site of sorption (Fu & Viraraghavan 2001).

There were some studies which showed the effective dye decolorization properties of dead R. arrhizus biomass (O’Mahony et al. 2002; Aksu & Karabay 2008) and AC (Faria et al. 2004; Crini 2006). A review of the literature revealed that no reports have been published comparing the reactive dye decolorization capacity of systems containing biosorbent (R. arrhizus) and adsorbent (AC). In this paper, the influence of dye concentration, adsorbent type and dosage in aqueous solution on the removal of Everzol Black (EB) by the systems containing biosorbent (R. arrhizus) and adsorbent (AC) have been studied. The aim of this study was to compare the color removal capacities of the systems containing biosorbent, AC and a mixture of them from water contaminated with reactive EB dye.

**METHODS**

**Microorganism and growth conditions**

The fungus R. arrhizus, obtained from the US Department of Agriculture Culture Collection, was used in the study. The composition of the growth medium was malt extract (17 g/L) and peptone (5.4 g/L) dissolved in deionized water. The initial pH of the medium was adjusted to 6.5 with 0.1 M HCl or NaOH. The pure cultures of R. arrhizus strain were incubated in 250 mL Erlenmeyer flasks containing 100 mL of growth medium. The fungal biomass was cultivated at 25 °C for 10 days.

**AC and biosorbent preparation**

The commercial AC used in this research was purchased from Sigma Aldrich (Darco type, 242233). The AC granules were smashed using a mortar and pestle. After 10 days of incubation, the fungal biomass was harvested and washed with distilled water, treated with 1% formaldehyde and dried at 60 °C for 24 hours. The dried biomass was smashed and used for biosorption studies. All experiment series were performed with the final solutions containing 1.0 g/L of biosorbent.

**Dye solution preparation**

Reactive EB dye was obtained from a textile factory. Stock solution was prepared as 1,000 mg/L in distilled water. The working solutions of EB were prepared by diluting the stock solution to the desired concentrations. Desired amount of dye solutions were added to the Erlenmeyer flasks at known initial pH value (pH 2) for the biosorption experiments.

**Dye removal studies at batch scale**

All of the dye removal experiments were performed in Erlenmeyer flasks containing 100 mL working solution with desired amounts of EB dye. The experiments were carried out at 100 rpm for 24 hours at 25 °C and pH 2. The effect of dye concentration (114.39–1194.57 mg/L) was examined with 1 g/L dried R. arrhizus biomass and AC. In order to compare adsorbent type, solutions containing 114.39 mg/L dye were prepared, and 1 g/L R. arrhizus biomass, 1 g/L AC or 1 g/L of a mixture of R. arrhizus (0.5 g/L) and AC (0.5 g/L) was added into Erlenmeyer flasks. The effect of adsorbent dosage was investigated in Erlenmeyer flasks containing 1, 2 and 4 g/L R. arrhizus biomass and AC with 114.39 mg/L dye concentration. For analysis, 2 mL of samples were taken at definite times from the working solution containing microorganism and dye. The samples were centrifuged (Hettich EBA12 model centrifuge) at 4000 rpm for 3 minutes and supernatant was used for dye analysis after appropriate dilutions. This experiment was also applied to investigate the isotherm studies by fitting the data with the two most commonly used isotherm models, namely Langmuir and Freundlich.

The percentage removal of dye was calculated from Equations (1) and (2).

\[
\text{Dye removal (D)} = \left(\frac{C_o - C_f}{C_o}\right) \times 100
\]
The uptake of dye by unit mass of biosorbent at any time \( (q_m: \text{mg/g}) \) was determined from

\[
q_m = \frac{C_0 - C_f}{X_m}
\]

(2)

where \( C_0 \) is the initial dye concentration (mg/L), \( C_f \) is the final dye concentration at any time (mg/L) and \( X_m \) is the sorbent concentration (g/L).

**Analytical methods**

EB dye concentration in the supernatant was determined spectrophotometrically (Labomed Inc. 22 model spectrophotometer). The concentration of EB was determined by measuring the absorbance at 584 nm, which was the wavelength for observing the maximum absorption peak for dye.

**Fourier transform infrared spectroscopy analysis**

The functional groups on the surface of \( R. \) arrhizus in the absence and presence of EB dye were identified using Fourier transform infrared spectroscopy (FTIR). Spectra were recorded using a Perkin Elmer (Spectrum 100) spectrophotometer.

**RESULTS AND DISCUSSION**

**Effect of initial dye concentration**

EB adsorption by \( R. \) arrhizus and AC were investigated at different initial dye concentrations of 114.39, 210.41, 438.91 and 1194.57 mg/L at pH 2. As shown in Figure 1, maximum dye adsorption yield was 93.19% for 114.39 mg/L EB dye concentration after 24 hours. Dye biosorption was also reported in previous studies performed with dried \( R. \) arrhizus strain (O’Mahony et al. 2002; Aksu & Karabayır 2008). Previous studies reported that the dye removal percentages were higher at low dye concentrations for dried \( R. \) arrhizus biosorbent because of availability of unoccupied binding sites on the fungal surface. Augmentation of initial dye concentration resulted in decreasing of dye removal percentages for both \( R. \) arrhizus biomass and AC (Figure 1).

Dye uptake capacities of fungal biosorbent and AC increased while the dye concentration was increasing. The dye uptake by adsorbent was enhanced with increasing dye concentration due to saturation at higher dye concentrations (Aksu & Karabayır 2008). The effect of dye concentration on Reactive Black 5 sorption by dried active sludge biosorbent was investigated by Gülnaz et al. (2006). Increasing dye concentration from 50 to 200 mg/L enhanced dye uptake by dried biosorbent from 34 to 104 mg/g (Gülnaz et al. 2006). Adsorption of EB by different kinds of adsorbents such as sepiolite and zeolite were studied previously (Armagan et al. 2005, 2004) and maximum dye removal capacities were 120.50 and 2.9 mg/g, respectively. Aksu & Karabayır (2008) examined the Gryfalan Black RL dye sorption capacity of dried \( A. \) niger, \( T. \) versicolor and \( R. \) arrhizus. Aksu & Karabayır (2008) showed that \( R. \) arrhizus was the most effective biosorbent performing a maximum dye uptake. In this study, dye removal capacities were 94.48 and 106.61 mg/g by AC and \( R. \) arrhizus biomass at 114.39 mg/L dye concentration. According to the Freundlich isotherm, the maximum dye adsorption capacities of AC and biosorbent (pH 2) were calculated as 344.82 and 357.14 mg/g, respectively.

**Effect of adsorbent type**

To examine the effect of adsorbent type on dye removal, dried \( R. \) arrhizus biomass, AC and a mixture of 1:1 biomass:AC were used as different adsorbent types. The Erlenmeyer flasks containing 114.39 mg/L dye solutions were prepared and 1 g/L \( R. \) arrhizus biomass, 1 g/L AC or a mixture of 0.5 g/L \( R. \) arrhizus and AC was added. Maximum dye removal (D%) occurred in the flasks containing only dried \( R. \) arrhizus biosorbent at 93.19% (Figure 2).

**Effect of adsorbent dosage and contact time**

To determine the effect of adsorbent dosage and contact time on EB removal (D%) by fungal biosorbent and AC, adsorbent dosage were varied as 1, 2 and 4 g/L for dried fungal biomass and AC in experimental series for
24 hours. Raising adsorbent dosage of dried *R. arrhizus* biomass from 1 to 4 g/L resulted in increased decolorization rates from 93.19 to 100% and reduced contact time from 24 to 2 hours (Table 1). Maximum dye removal percentages were observed as 99.52% and 100% for 4 and 2 hours by 4 g/L AC and *R. arrhizus* biosorbent (Table 1).

### Adsorption isotherms

The sorption data obtained for EB uptake were plotted using Langmuir and Freundlich equation given in Equations (3) and (4), respectively

\[
\frac{1}{q_e} = \frac{1}{q_{\text{max}}} + \frac{1}{K_L \cdot q_{\text{max}} C_e}
\]

(3)

\[
\log q_e = \log K_F + \frac{1}{n} \log C_e
\]

(4)

In these equations, \( q_e \) is the amount of EB adsorbed per unit weight of adsorbent at equilibrium (mg/g), \( q_{\text{max}} \) is the maximum EB uptake per unit mass of adsorbent (mg/g), \( K_L \) is the Langmuir constant (L/mg) related to energy of sorption, which quantitatively reflects the affinity between the sorbent and sorbate, \( C_e \) is the equilibrium concentration of adsorbate (mg/L), \( K_F \) (L/g) is the adsorptive uptake and \( n \) is the adsorption equilibrium constant indicative of the general shape of the isotherm. The values of \( n \) and \( K_F \) are calculated from the intercept and slope of the Freundlich plots, respectively.

The comparison of the \( R^2 \) values showed that the Freundlich model fits better with the experimental data than the Langmuir one (Table 2) for AC and biosorbent. The Freundlich isotherm model is usually adopted for heterogeneous adsorption. The \( n \) values were higher than 1 for *R. arrhizus* and AC, indicating a favorable adsorption process.

### FTIR analysis

The FTIR spectral analysis is important to identify the characteristic functional groups, which are responsible for biosorption of dye molecules. The FTIR spectra of *R. arrhizus* biomass before and after dye biosorption are shown in Figure 3. Results of FTIR spectra show that dried *R. arrhizus* has different functional groups mostly found in the cell wall. The cell wall of the microbial biomass (biosorbent) is the major site for biosorption of pollutants (Wang & Hu 2008). The fungal cell wall is 30% or more of the dry weight of fungus. The fungal cell wall consists of mostly polysaccharides (80%) and proteins (3–20%) (Feofilova 2010). FTIR results showed that dried *R. arrhizus* biomass has characteristic bands of proteins, lipids, polymeric compounds and carboxylic acid groups which are able to react with functional groups of dye molecules in aqueous solution. The analysis of the IR spectra shows the presence of numerous functional groups. After treatment with EB dye, peaks of
Figure 3 | Comparison and characterization of R. arrhizus biosorbent surface groups before (a) and after (b) biosorption.
biosorbent shifted their position, indicating involvement of functional groups on these peaks in dye bindings.

CONCLUSION

Laboratory-scale batch sorption studies showed that dried R. arrhizus biomass was an efficient alternative adsorbent to AC for the removal of reactive dyes from dye-contaminated water. Maximum dye capacity of AC and R. arrhizus biomass was 344.82 and 357.14 mg/g, respectively. The decolorization rate by the systems containing AC, mixture of AC and fungus, and only fungus was 82.59, 89.39 and 93.19%, respectively. The adsorbent dosage experiments showed that 4 g/L dried R. arrhizus biomass removed 100% of EB dye at 114.39 mg/L dye concentration after 2 hours. The Freundlich isotherm model well described the sorption of EB onto dried biosorbent and AC. FTIR results showed that waste dried R. arrhizus has different functional groups. These functional groups that are located on the surface of biomass are able to react with dye molecules in aqueous solution.

It can be concluded that dried R. arrhizus may be used as a low-cost material and alternative to more costly materials such as AC for the removal of reactive dyes.

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