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 (C_0: 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*

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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

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effective technology using inactive and dead biomass to remove pollutants such as dyes from aqueous solutions (San & Dönmez 2012; Karatay Ertuğrul *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 & Karabayır 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 & Karabayır 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).

$$\text{Dye removal (D)} = (C_o - C_f)/C_o \times 100 \quad (1)$$

The uptake of dye by unit mass of biosorbent at any time (q_m: mg/g) was determined from

$$q_m = C_o - C_f/X_m \quad (2)$$

where C_o 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 wave length 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

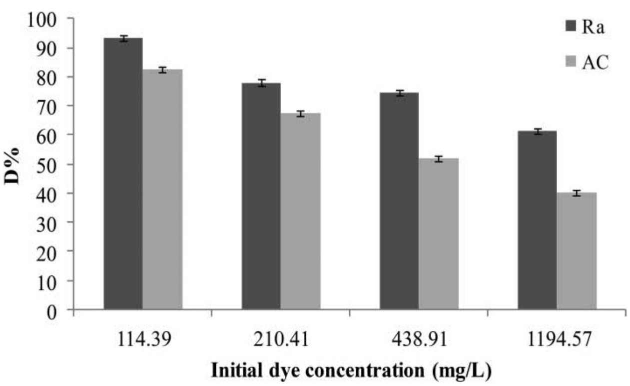

Figure 1 | Effect of initial dye concentration on EB dye removal (D%) by fungal biosorbent (Ra) and active carbon (AC).

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 (Armağan *et al.* 2003, 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

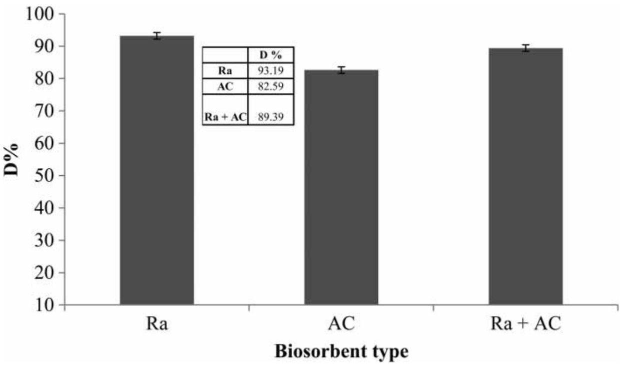

Figure 2 | The effect of adsorbent type on EB dye removal (D%) by different biosorbent types (Ra: *R. arrhizus*; AC: active carbon; Ra + AC: the mixture of fungus and active carbon; C_0: 114.39 mg/L; T: 25 °C; agitation rate: 150 rpm).

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

$$\frac{1}{q_e} = \frac{1}{q_{\max}} + \frac{1}{K_L\, q_{\max} C_e} \quad (3)$$

Table 1 | The effect of adsorbent dosage (g/L) and contact time on EB dye removal (D%) by fungal biosorbent and AC (Ra: *Rhizopus arrhizus*; AC: active carbon; C_0: 114.39 mg/L; pH: 2; T: 25 °C; agitation rate: 150 rpm)

	1 g/L		2 g/L		4 g/L	
Adsorbent	**D %**	**Time (hour)**	**D %**	**Time (hour)**	**D %**	**Time (hour)**
Ra	93.19	24	98.41	4	100.0	2
AC	82.59	24	95.41	24	99.52	4

$$\log q_e = \log K_{F+} 1/n \log C_e \quad (4)$$

In these equations, q_e is the amount of EB adsorbed per unit weight of adsorbent at equilibrium (mg/g), $q_{\max}$ 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

Table 2 | Langmuir and Freundlich constants for EB biosorption

	Langmuir			Freundlich		
Adsorbent	q_{max} **(mg/g)**	K_L **(L/mg)**	R^2	K_F	n	R^2
AC	344.82	0.01793	0.8978	21.87	2.125	0.9872
Ra	357.14	0.05234	0.8076	34.05	2.081	0.9462

Ra: *Rhizopus arrhizus*; AC: active carbon.

(a)

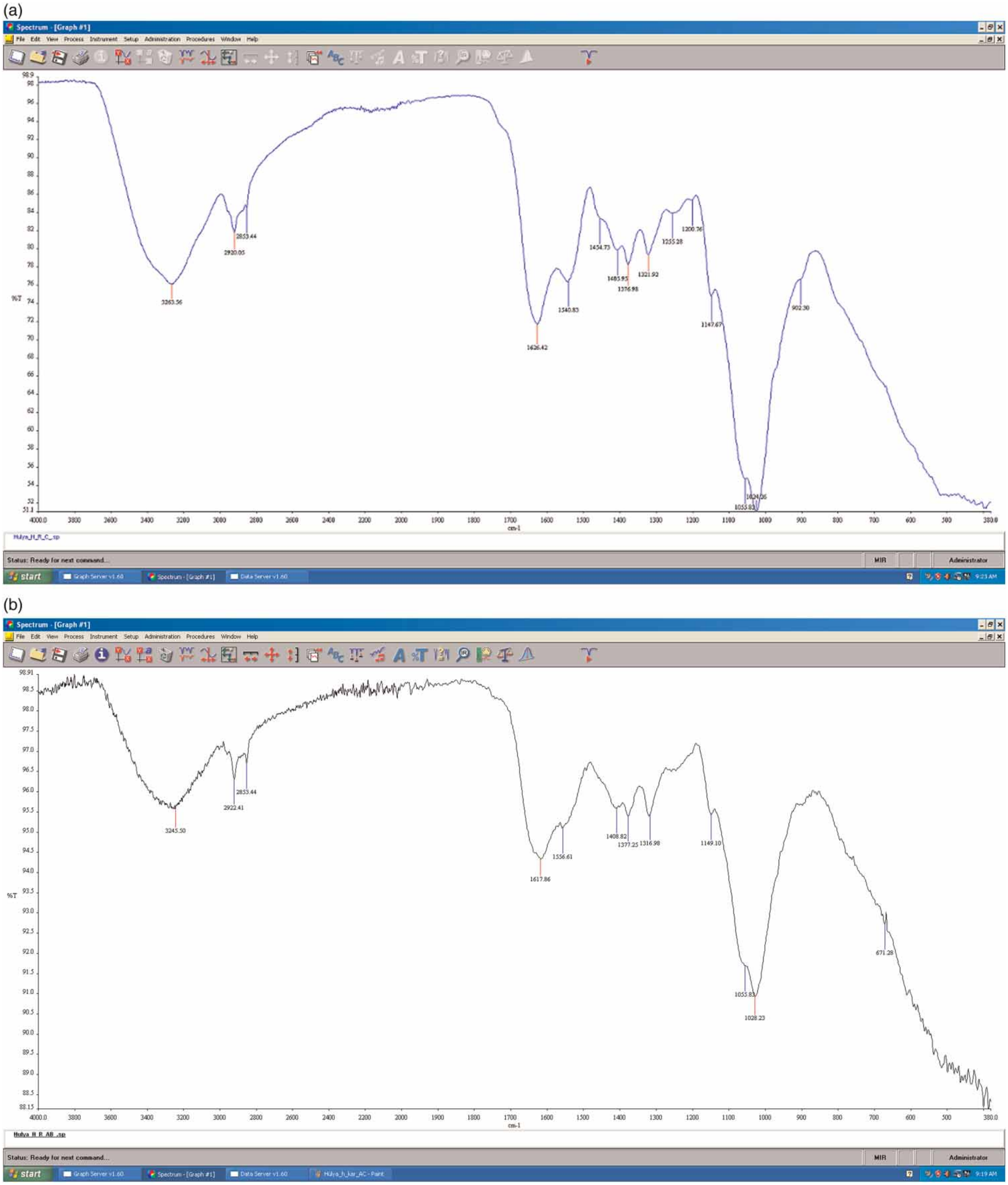

Figure 3 | Comparison and characterization of *R. arrhizus* biosorbent surface groups before (a) and after (b) biosorption.

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 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.

REFERENCES

Aksu, Z. 2005 Application of biosorption for the removal of organic pollutants: a review. *Process Biochemistry* **40** (3–4), 997–1026.

Aksu, Z. & Karabayır, G. 2008 Comparison of biosorption properties of different kinds of fungi for the removal of Gryfalan Black RL metal-complex dye. *Bioresource Technology* **99** (16), 7730–7741.

Armağan, B., Özdemir, O., Turan, M. & Celik, M. S. 2003 Adsorption of negatively charged azo dyes onto surfactant-modified sepiolite. *Journal of Environmental Engineering* **129** (8), 709–715.

Armağan, B., Turan, M. & Çelik, M. S. 2004 Equilibrium studies on the adsorption of reactive azo dyes into zeolite. *Desalination* **170** (1), 33–39.

Cardoso, N. F., Pinto, R. B., Lima, E. C., Calvete, T., Amavisca, C. V., Royer, B., Cunha, M. L., Fernandes, T. H. M. & Pinto, I. S. 2011 Removal of remazol black B textile dye from aqueous solution by adsorption. *Desalination* **269** (1–3), 92–103.

Couto, S. R. 2009 Dye removal by immobilized fungi. *Biotechnology Advances* **27** (3), 227–235.

Crini, G. 2006 Non-conventional low-cost adsorbents for dye removal: a review. *Bioresource Technology* **97** (9), 1061–1085.

Du, L., Li, G., Xu, F.-C., Pan, X., Wen, L.-N. & Wang, Y. 2014 Rapid decolorization of methyl orange by a novel *Aeromonas* sp. strain DH-6. *Water Science and Technology* **69** (10), 2004–2013.

Faria, P. C., Orfao, J. J. & Pereira, M. F. 2004 Adsorption of anionic and cationic dyes on active carbons with different surface chemistries. *Water Research* **38** (8), 2043–2052.

Feofilova, E. P. 2010 The fungal cell wall: modern concepts of its composition and biological function. *Microbiology* **79**, 711–720.

Fu, Y. & Viraraghavan, T. 2001 Fungal decolorization of dye wastewaters: a review. *Bioresource Technology* **79** (3), 251–262.

Ghaedi, M., Hekmati, J. A., Khodadoust, S., Sahraei, R., Daneshfar, A., Mihandoost, A. & Purkait, M. K. 2012 Cadmium telluride nanoparticles loaded on activated carbon as adsorbent for removal of sunset yellow. *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy* **90**, 22–27.

Gülnaz, O., Kaya, A. & Dincer, S. 2006 The reuse of dried active sludge for adsorption of reactive dye. *Journal of Hazardous Materials* **134** (1–3), 190–196.

Han, J. L., Is, N. G., Wang, Y., Zheng, X., Chen, W. M., Hsueh, C. C., Liu, S. Q. & Chen, B. Y. 2012 Exploring new strains of dye-decolorizing bacteria. *Journal of Bioscience and Bioengineering* **113** (4), 508–514.

Karatay Ertuğrul, S., Gül, Ü. D. & Dönmez, G. 2014 Determination of Methylene Blue biosorption by *Rhizopus arrhizus* in the presence of surfactants with different chemical structures. *Preparative Biochemistry and Biotechnology* **44** (7), 653–662.

Khataee, A. R., Denghan, G., Zarei, M., Ebadi, E. & Pourhassan, M. 2011 Neural network modeling of biotreatment of triphenylmethane dye solution by a green macroalgae. *Chemical Engineering Research and Design* **89** (2), 172–178.

Lin, Y., Yang, C., Xiu, R., Wang, J., Wei, Y. & Sun, Y. 2013 Decolorization of methyl orange by green rusts with hydrogen peroxide at neutral pH. *Water Science and Technology* **69** (2), 371–377.

Maas, R. & Chaudhari, S. 2005 Adsorption and biological decolourization of azo dye Reactive Red 2 in semicontinuous anaerobic reactors. *Process Biochemistry* **40** (2), 699–705.

Merzouk, B., Gourich, B., Madani, K., Vial, C. & Sekki, A. 2011 Removal of a disperse red dye from synthetic wastewater by chemical coagulation and continuous electrocoagulation. A comparative study. *Desalination* **272** (1–3), 246–253.

O'Mahony, T., Guibal, E. & Tobin, J. M. 2002 Reactive dye biosorption by *Rhizopus arrhizus* biomass. *Enzyme and Microbial Technology* **31** (4), 456–463.

Phugare, S., Patil, P., Govindwar, S. & Jadhav, J. 2010 Exploitation of yeast biomass generated as a waste product of distillery industry for remediation of textile industry effluent. *International Biodeterioration and Biodegradation* **64** (8), 716–726.

San, N. O. & Dönmez, G. 2012 Biosorption of chromium(VI), nickel(II) and Remazol blue by *Rhodotorula mucilginosa* biomass. *Water Science and Technology* **65** (3), 471–477.

Sathiya moorthi, P., Periyar selvam, S., Sasilkalaveni, A., Murugersan, K. & Kalaichevan, P. T. 2007 Decolorization of textile dyes and their effluents using white rot fungi. *African Journal of Biotechnology* **6** (4), 424–429.

Veglio, F. & Beolchini, F. 1997 Removal of metals by biosorption: A review. *Hydrometallurgy* **44** (3), 301–316.

Wang, B. E. & Hu, Y. Y. 2008 Bioaccumulation versus adsorption of reactive dye by immobilized growing *Aspergillus fumigates* beads. *Journal of Hazardous Materials* **157** (1), 1–7.

Wang, Y. F., Gao, B. Y., Yue, Q. Y., Wang, Y. & Yang, Z. L. 2012 Removal of acid and direct dye by epichlorohydrin-dimethylamine: flocculation performance and floc aggregation properties. *Bioresource Technology* **113**, 265–271.

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