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Biodegradation of Evercion Blue P-GR and Ostazin Black H-GRN in synthetic textile wastewater by membrane bioreactor system using *Trametes versicolor*

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Abstract. In this study, the decolorization of Evercion Blue P-GR (EBP) and Ostazin Black H-GRN (OBH) was investigated using white-rot fungi named as Trametes versicolor (T. versicolor) by Membrane Bioreactor (MBR) system. This study involved experiments employing synthetic textile wastewater in Membrane Bioreactor (MBR) system (170 ml), initially inoculated with a pure culture of fungi, but operated, other than controlling pH (4.5±0.2) and temperature (25±1℃), under non-sterile conditions. The effect of dye concentrations on fungal biodegradation was also investigated. The decolorization efficiencies were 98%, 90%, and 87% respectively, for EBP when the initial dye concentration of 50, 100, and 200 mg L^{-1} were used. However, the decolorization percentages for OBH dye were obtained 95% for 50 mg L^{-1} dye solution in 2 days and 66% for 100 mg L^{-1} dye solution in 5 days. Possible interactions between dye molecules and the fungal surface were confirmed by SEM, EDX, and FTIR analyses.

Keywords: biodegradation; textile dye; membrane bioreactor; wastewater treatment

1. Introduction

The wastewater of the textile industry includes complex pollutants having high concentrations of dyes and changing greatly characteristics (Akram *et al.* 2016). In the past decades, new techniques have been improved to apply an economic and efficient process for treatment of the textile wastewater such as physicochemical, biochemical, combined treatment processes, and other technologies (Tambunan *et al.* 2018). Recently, most of the studies have interested in microbial biodegradation of textile dyes in textile effluents (Ganapathy *et al.* 2018). Several microorganisms such as some bacteria, fungi, and algae can decolorize most of the dyes. A wide variety of fungal strains are known to degrade complex structures of dyes more successfully than bacteria (Fu and Viraraghavan 2001). White rot fungi are known to produce extracellular ligninolytic enzymes (lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase) which have roles in the

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decolorization process. Previously, the usage of white-rot fungi in the successful decolorization process was introduced in the literature (Blanquez *et al.* 2006). Membrane Bioreactor (MBR) systems are consist of membrane units and biological reactor systems, which responsible for the physical separation and biological degradation of wastes, respectively (Ö zan and Acikgoz 2015). MBR technology has proven to be highly effective in removing organic and inorganic contaminants as well as microorganisms from wastewater and has gained increasing popularity in recent years due to tight environmental regulations and increased water reuse initiatives (Ö zan and Acikgoz 2016). The MBR system with fungi is an ideal wastewater treatment approach including both the advantages of MBR and the inherent degradation ability of white-rot fungi. Some studies are working under sterile and/or non-sterile conditions in a continuous or semi-continuous mode in literature and these studies reported effective decolorization efficiencies. However, the working systems of bioreactors for decolorization published in the literature were smaller capacity than our MBR system (Acikgoz *et al.* 2016, Bharti *et al.* 2017).

In the current study, the white-rot fungus called *Trametes versicolor* was used to examine the decolorization of two reactive dyes in the submerged Membrane Bioreactor (MBR) system under non-sterile conditions. The Membrane Bioreactor (MBR) system was designed and presented in our previous study (Acikgoz *et al.* 2016). However, we investigated filamentous fungal strains for decolorization in this MBR system. To our best of knowledge, this is the first report that tests the MBR system having 170 L volume with white-rot fungi for decolorization of textile dye-containing water.

2. Materials and methods

2.1 Dye and chemicals

All chemicals were obtained from Merck. EBP (Evercion Blue P-GR) and OBH (Ostazin Black H-GRN) dye were supplied from the local textile factory. The molecular structures of the textile dyes used in the study were given in Fig. 1.

2.2 Microorganism

A fungal strain called *Trametes versicolor* was obtained from Ankara University Biology

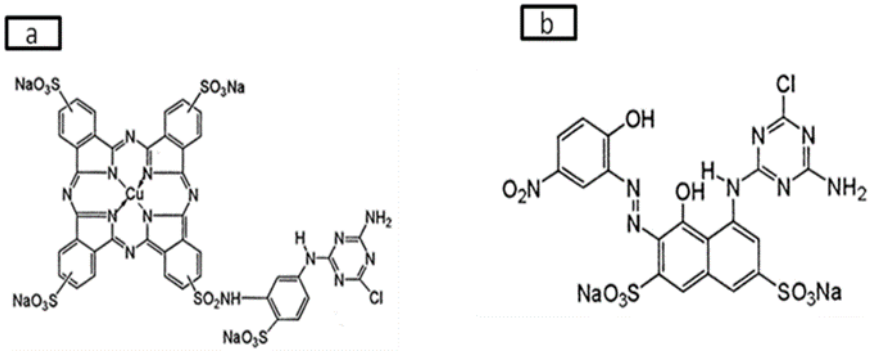

Fig. 1 Molecular structure of EBP (a) and OBH79 (b)

Table 1 The content of YPD medium

Ingredient	Amount (g L-1)
Yeast extract	10
Peptone	20
Dextrose (D-Glucose)	10

Department Biotechnology Laboratory Culture Collection. The pure cultures were kept at 4ºC and were transferred to PDA (Potato Dextrose Agar -PDA) media containing 39.0 g L^{-1} PDA every 3 months, immediately after they arrived at the laboratory.

2.3 Media cultures

The microorganism was cultivated in liquid media using the shake flask method. The ingredients of the growth medium yeast-peptone-dextrose (YPD) was given in Table 1. All chemicals were obtained from Merck. The pH of the medium was adjusted to 4 with dilute 0.1 M HCl and 0.1 M NaOH solutions before autoclaving. Once inoculated, flasks were incubated on an orbital shaker at 100 rpm for 5 days at 25ºC.

The effect of dye on fungal growth was also determined by a dried weight method. The fungus was inoculated into the 250 ml of flasks containing 100 ml of YPD with only EBP and only OBH dyes. The flasks without dye were used as a control. After 7 days of incubation, the fungal pellets were harvested from the medium, washed, and dried to determine dried weight.

2.4 Cultivation of the fungal mycelia pellets

The pre-culture of fungal strain (2 ml of inoculums) for the bioreactor system was inoculated into 100 ml of YPD medium. After 10 days of incubation, the inoculated culture volume (100 ml) was then distributed into ten 250 ml of flasks and was incubated at a shaking frequency of 100 rpm and a temperature of 25ºC on an orbital shaker. After 10 days of incubation, the inoculated 250 ml flasks transferred into ten 1 L shaking flask with the same conditions. After the pre-culturing, the 10 shake flasks were again pooled and 10 L of the pool were inoculated into the bioreactor with 170 L of synthetic wastewater. This process was repeated two times. At last, the starting fungal biomass inoculated at an 11.76% concentration of the total volume of the bioreactor medium for the decolorization process.

2.5 MBR system

The volume of the MBR system (Fig. 2) was 170 L, which can be assumed as a commercial-scale system. The detailed information about the MBR system used in this study was previously presented by Acikgoz *et al.* (2016) and also the schematic diagram of the MBR system was given by Ozan and Acikgoz 2018. The fungal pellets transferred to the membrane bioreactor system from flasks including medium. In each experimental run, 100 L of synthetic dye-containing water was fed into the aeration tank by the pump in the MBR system. The MBR system had a diffuser which supplied continuous air with an intensity of 9 L min^{-1} for complete mixed to supply dissolved oxygen to the fungus. The temperature of the system was controlled at 25 ± 1°C. Also, the pH and the dissolved oxygen (DO) concentration were in the range of 4.0-4.5 and 6-8 mg L^{-1} in the aeration tank.

Fig. 2 The photograph of the MBR system

2.6 Synthetic wastewater

The medium, included 1.5 g L^{-1} starch, 1 g L^{-1} glucose, 0.4 g L^{-1} urea, 0.099 g L^{-1} CaC12, 0.255 g L^{-1} NaCI, 0.17 g L^{-1} Na2CO3, 0.17 g L^{-1} NaHCO3, 1 ml L^{-1} trace elements and (5;10;20 g/L) dyestuff, was used for biodegradation process (Acikgoz *et al.* 2016). The trace element stock solution contained 0.125 g $CuSO_4.5H_2O$, 0.05 g H_2MoO_4, 0.061 g $MnSO_4.5H_20$, 0.043 g $ZnSO_4.7H_20$, and 0.082 g Fe2(SO4)3.14 H_20 in 1 L of Milli-Q water.

2.7 Analytical methods

The analytical measurements of dye-containing wastewater were done by using a spectrophotometer (JENWAY 7315 spectrophotometer). The absorbance peak was measured at 603 nm for EBP and 611 nm for OBH. Scanning was performed between 300 and 800 nm**.** The calibration curve of "absorbance vs. concentration" was used for the calculation of dye concentration and the reduction of dye concentration was used to calculate the rate of decolorization efficiency.

$$\text{Rate of decolorization (\%)} = (Co - Cf)/Co \times 100 \quad (1)$$

In this equation, Co and Cf represent the initial and final dye concentrations (mg L-1), respectively.

2.8 FTIR analysis

To explore the conformational changes in fungus caused by the addition of dyes using Fourier transform infrared spectroscopy (FTIR) techniques. Spectra of FTIR for the samples were obtained by an FTIR spectrophotometer (Agilent Technologies, Cary 630 FTIR). The spectra were acquired in the wavenumber range from 4.000 to 400 cm^{-1}.

2.9 SEM analysis

SEM was used to observe the 3-dimensional network structure of fungus and dye-loaded fungus samples. The surface morphology of the samples was observed with a scanning electron

microscope (SEM, Bruker, Germany) equipped with an energy dispersive spectrometer (EDX) attachment.

3. Results and discussion

EBP and OBH dye solutions by *Trametes versicolor* and MBR system were investigated using the different concentrations of dye solutions.

3.1 Decolorization assays

The decolorization of EBP and OBH dye solutions by *Trametes versicolor* and MBR system was investigated using the different concentrations of dye solutions. Fig. 3 shows the results obtained in a fungal decolorization process in the MBR system with an initial dye concentrations of 50, 100, and 200 mg L^{-1}. It is observed for the EBP that after 24 h of treatment the decolorization percentage was over 98%, 90%, and 87%, respectively. In the OBH decolorization study, the decolorization percentages were obtained 95% for 50 mg L^{-1} dye solution in 2 days and 66% for 100 mg L^{-1} dye solution in 5 days. Recently, Erdem and Cihangir (2017) reported that the decolorization of Reactive Blue dye by *Trametes versicolor* was 99% at 50 mg L^{-1} dye concentration after 6 days of incubation. In this study, the decolorization rate was 95% at the same

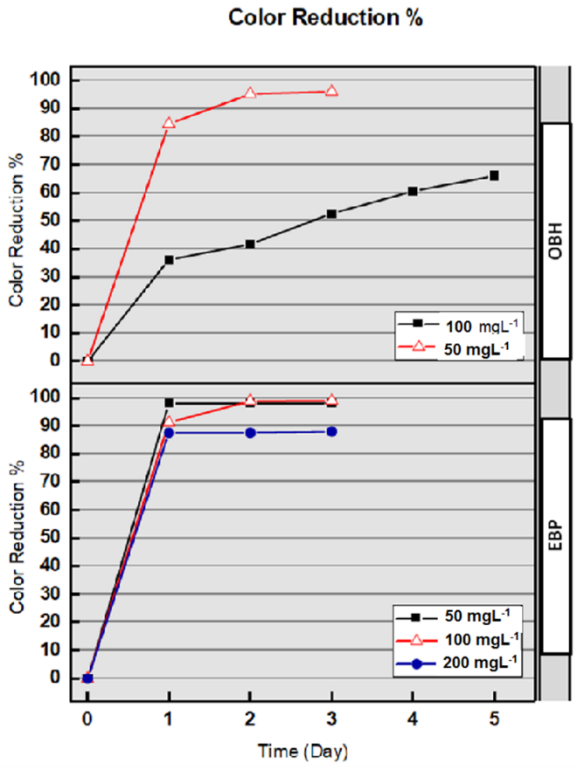

Fig. 3 Decolorization of EBP and OBH by fungi in the MBR system

Table 2 The dried weight of fungus in the medium with two dyes and without dye

	Fungal growth in the presence of EBP	Fungal growth in the presence of OBH	Fungal growth in the absence of dye
Dried weight (g L^{-1})	3.88	3.7	4.5

dye concentration after 2 days of incubation. The short operation time is important to save energy and provide cost-effectiveness. Also in literature, the decolorization experiments were conducted at low volumes such as 100 ml of dyeing solutions (Yang *et al.* 2017). In the current study, the operation volume of dying water was very high as 170 ml differently from the articles about decolorization in the literature.

As given in Fig. 3, the decolorization of EBP dye was reached the highest value after 1 day at all dye concentrations and the decolorization rate did not change after 1 day significantly. The decolorization was decreased while the dye concentration was increased for the two dyes. It was shown that higher dye concentration affected fungal growth negatively (Table 2), so the decolorization yield was decreased. The removal of the OBH dye at 100 mg L^{-1} dye concentration was gradually increased day by day (Fig. 3).

The effect of dyes on fungal growth was measured by using the dried weight method and the dried weights of fungi were given in Table 2. As shown in Table 2, the fungal growth was negatively affected by the presence of both dyes. Previously, it was reported that dyes had negative effects on fungal growth (Fu and Viraraghavan 2001). Also recently, Yang *et al.* (2017) showed that dyes have inhibited fungal growth due to their toxicity. Similarly, according to the results of this study both of the dyes influenced fungal growth negatively.

3.2 Comparison of the decolorization ability of MBR systems in the literature

The MBR systems were introduced as successful machines for the treatment of industrial wastewater. MBR system having gravity drain removed 58.7% of color from dyeing and printing wastewater (Zheng and Liu 2006). Lin *et al.* (2006) concluded that the combination of the MBR and ozonation treatment method decolorized 93% of dye-containing effluent. Kim *et al.* (2004) examined that the removal of reactive dye solutions (reactive blue 19, reactive blue 49, and reactive black 5) by the usage of MBR unit with the white-rot fungi and showed that two reactive blue dye solutions were removed with the 99% decolorization efficiency after 8 hours and the removal of reactive black 5 was relatively slow. Hai *et al.* (2008) studied the removal of an azo dye (Acid Orange II, 100 mg L^{-1}) in the membrane bioreactor (MBR) system with the pure culture of fungi and reported that the system decolorized 93% of dye-containing wastewater in operation time of 24 hours. Nilsson *et al.* (2006) studied the removal of textile dyes called Reactive Blue 4, Reactive Red 2 in batch and continuous reactor systems containing white-rot fungi and reported that The fungus called *Trametes versicolor* effectively removed both of the dyes and also decolorized real wastewater of textile industry in Tanzania. The detailed comparison of the results of this study with the other published studies about the decolorization by MBR units containing fungi was given in Table 3.

As seen in Table 3 there were some studies focused on the utilization of MBR units including different fungal strains. This study showed the MBR unit having a maximum working volume as 170 L of dye-contaminated water to reduce color and COD levels. Also, the results of this study

Table 3 The comparison of decolorization performances of MBR systems in the literature (*T. viride: Trichoderma viride; P. chrysosporium: Phanerochaete chrysosporium; Cerrena unicolor: C. unicolor*; *R. arrhizus + A. versicolor*: The mixed culture of *Rhizopus arrhizus* and *Aspergillus versicolor*; *T. versicolor: Trametes versicolor;* BV: Bioreactor Volume; DC: Initial Dye Concentration; T: Temperature; CR: Color Reduction; CT: Contact Time)

Fungal Strain	Dye	BV	DC (mg/L)	pH	T (oC)	CR (%)	CT (Day)	Reference
T. viride	Basic Red CLB		4 mg/L	-		97.00	6	Vinodha *et al.* (2013)
P. chrysosporium (ATCC 24725)	Poly R-478	1.5 L	-	4.5		58.00	9	Karthikeyan and Omprakash (2014)
C. unicolor	Acid Blue 62	50 mL	113.6 μm	5.3	30	98.30	4	Lewańczuk and Bryjak (2015)
T. versicolor KCTC 16781	Reactive black 5	1.7 L	100 mg/L^{-1}	4.5	28	98.80	2	Kim *et al.* (2004)
T. versicolor KCTC 16781	Reactive blue 19	1.7 L	100 mg/L^{-1}	4.5	28	76.20	2	Kim *et al.* (2004)
T. versicolor KCTC 16781	Reactive blue 49	1.7 L	100 mg/L^{-1}	4.5	28	96.80	2	Kim *et al.* (2004)
R. arrhizus + A. versicolor	Reactive blue	170 L	103.7 mg/L^{-1}	4.5	25	90.71	2	Caglayan *et al.* (2016)
R. arrhizus + A. versicolor	Reactive blue	170 L	238.15 mg/L^{-1}	4.5	25	80.84	2	Caglayan *et al.* (2016)
T. versicolor	Evercion Blue P-GR	170 L	50	4.5	25	98.00	2	In this study
T. versicolor	Evercion Blue P-GR	170 L	100	4.5	25	90.00	2	In this study
T. versicolor	Evercion Blue P-GR	170 L	200	4.5	25	87.00	2	In this study
T. versicolor	Ostazin Black H-GRN	170 L	50	4.5	25	95.00	5	In this study
T. versicolor	Ostazin Black H-GRN	170 L	100	4.5	25	66.00	5	In this study

emphasized that the removal of both dyes were very effective by the MBR unit with *T. versicolor* fungal strain. Also, it is reported that *T. versicolor* produces enzymes (laccase) that were involved in the biodegradation of dye molecules in the wastewater treatment system (Kim *et al.* 2004). The enzymatic systems in fungal strains provided highly decolorization performances by these microorganisms (Gül and Dönmez 2014). Similarly, the results of this study showed that the enzymatic system of *T. versicolor* performed successful decolorization activity in the MBR system.

3.3 FTIR and SEM analysis

To determine the existence of functional groups (i.e., carboxyl, amino, and phosphate) on the fungal biomass, the FTIR spectra of native fungal biomass, and two different dye-loaded fungal biomass sample was obtained in a range of 400-4000 cm^{-1}. The spectra of fungal biomass samples are given in Fig. 4.

The similar functional groups were observed in the FTIR spectra of all the three samples. In general, the FTIR spectra of all the fungal preparations have intense peaks at a frequency level of

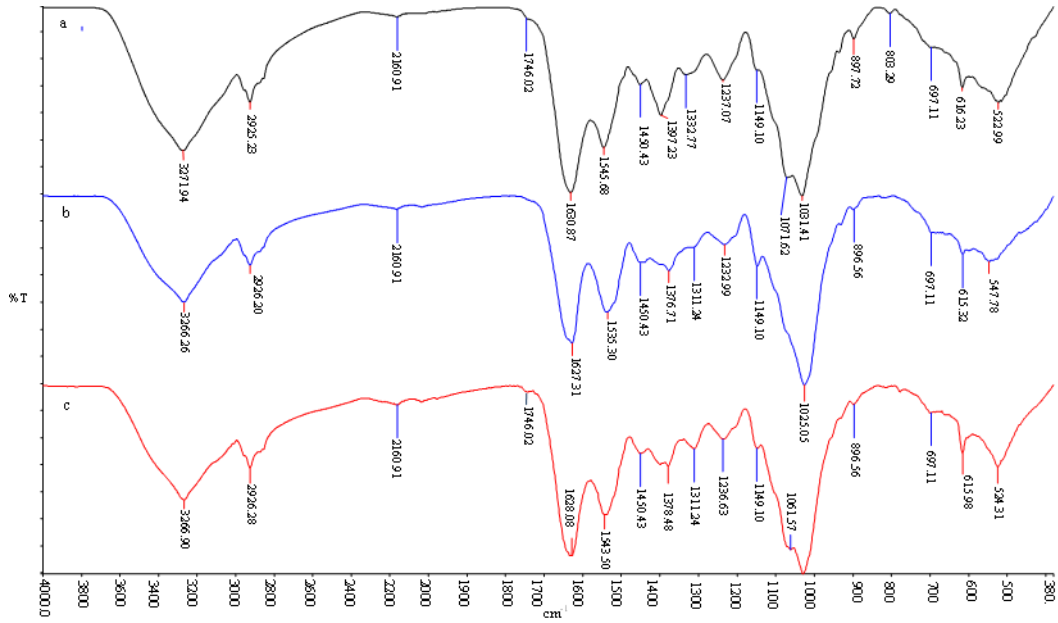

Fig. 4 FTIR spectra for (a) Fungus (T. versicolor), (b) EBP loaded fungus and (c) OBH loaded fungus

3400-3200 cm^{-1} and 1540 cm^{-1} amino groups stretching vibrations. A similar and strong absorption peak was seen for native fungal biomass and dye-loaded biomass samples at about 3270 cm^{-1} corresponding to the overlapping of -OH and -NH peaks. A peak at 2926 cm^{-1} represents the C-H group, the peak at around 1630,1400 and 1240 cm^{-1} are caused by C=0 stretching band of carbonyl groups (Bayramoğlu *et al.* 2006, Bayramoğlu and Arica 2007, Arica and Bayramoğlu 2007, Kumar and Min 2011). The carboxyl groups showed a characteristic absorption peak at 1746 cm^{-1} for fungus and the OBH dye loaded fungus samples. However, this peak disappeared in the FTIR spectrum of the EBP loaded fungus sample. The phosphate groups show some characteristic peaks around 1150-1078 cm^{-1} representing P=O and P-OH stretching, respectively (Tunali *et al.* 2009). On the other hand, the peaks of N–H stretching vibrations at around 1025 cm^{-1} are also masked with the broadband of C–O stretching and the peak at 615 and 524 cm^{-1} representing O–C–O scissoring and C=O bending vibrations are observed for the biomass samples. The new peaks and shifted peaks observed after dye loaded fungal biomass indicated that some of the functional groups involved in the decolorization process and some new interactions formed between the surface of the fungal biomass and the dye molecules.

The results were further validated by the SEM-EDX data obtained for all fungal biomass samples. The surface morphology and bulk structure of the fungus and dye-loaded fungal samples were observed by the scanning electron micrograph in Fig. 5.

As clearly seen in Fig. 5(a), the fungal sample has fibrous and porous surface structure. These surface properties could be thought of as a factor providing an increase in the total surface area. Fig. 5(b) and 5(c) show an SEM micrograph of dye-loaded fungal samples. The structure of the fungus changed upon sorbing the dyes molecules, and a tendency to form agglomerates. These findings confirm the coverage of the fungal surface by dye molecules. The energy-dispersive X-ray analysis (EDX) micrograph of samples shows the presence of Carbon (C), Oxygen (O),

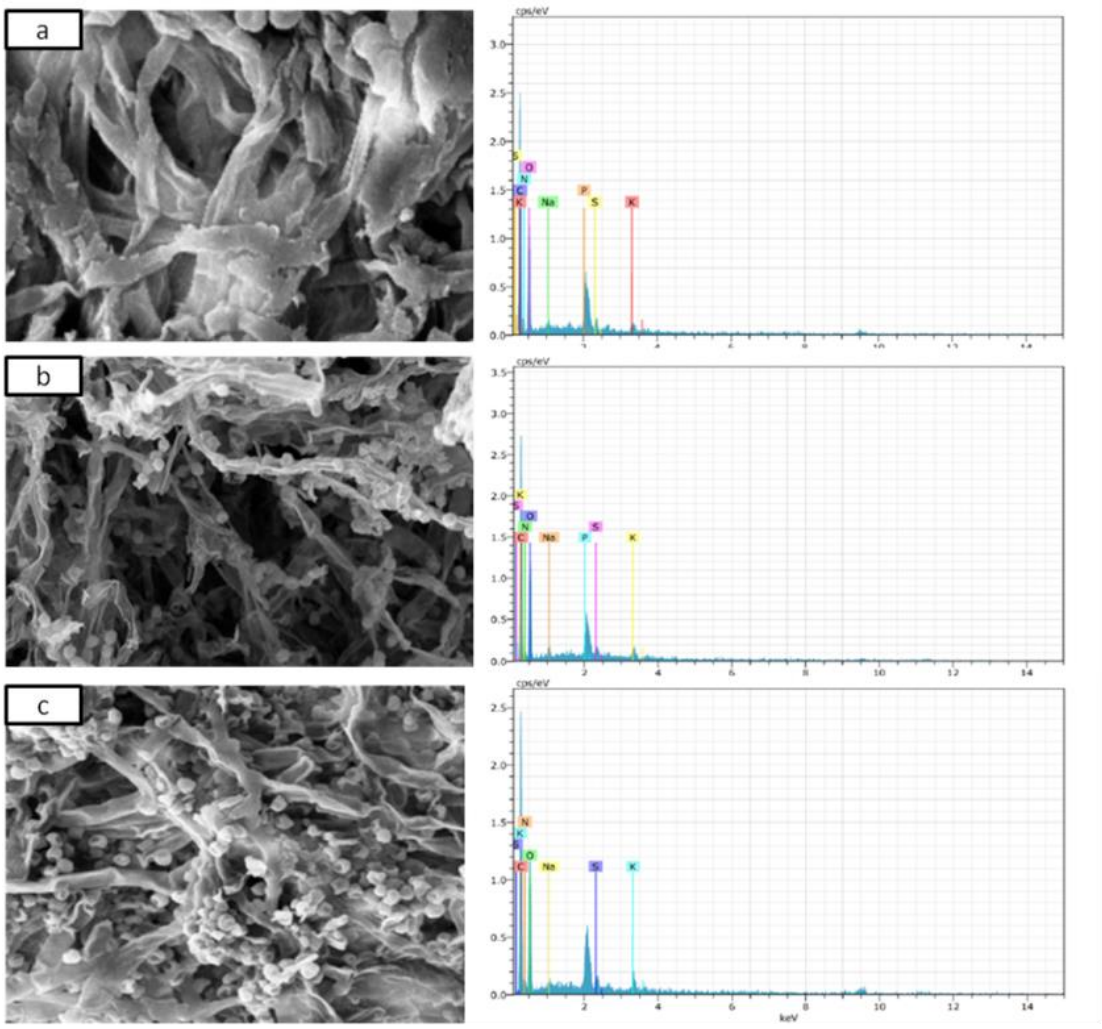

Fig. 5 SEM micrographs and EDX profiles for (a) Fungus (T. versicolor), (b) EBP loaded fungus and (c) OBH loaded fungus (10000 KX)

Nitrogen (N), Sulfur (S), Phosphorus (P), Potassium (K) and Sodium (Na) in the fungus structure and dye molecules.

4. Conclusions

Two reactive dyes were decolorized with an MBR system using a white-rot fungus called *Trametes versicolor*. Decolorization of dye solutions by the MBR unit containing white-rot fungus is a promising alternative to replace or supplement present treatment processes. The degradation of EBP using white-rot fungus *Trametes versicolor* in the MBR system was found to be faster than OBH. It can be concluded that textile dyes can be effectively decolorized by white-rot fungus *Trametes versicolor* in the MBR system.

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