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Degradation of Reactive Blue by the mixed culture of *Aspergillus versicolor* and *Rhizopus arrhizus* in membrane bioreactor (MBR) system

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ABSTRACT

The main purpose of this study was to investigate reactive dye removal capability of mixed fungal biomasses (*Rhizopus arrhizus* and *Aspergillus versicolor*) under non-sterile conditions with the inherent advantages of the submerged membrane bioreactor (MBR) system. Decolorization of Reactive Blue in synthetic textile wastewater by mixed filamentous fungi was carried out in the Erlenmeyer flasks (sterile conditions) and MBR system (non-sterile conditions). The Lab/Pilot Scale MBR system had a working volume of 170 L and was equipped with a coarse and fine air bubble creation mechanism for membrane and biological aeration, respectively. The dye and chemical oxygen demand (COD) concentration values were measured daily in influent as well as effluent to evaluate the removal efficiencies. Most of the analytical techniques used in this study were mentioned in the standard methods. The removal efficiency of color and COD were 90.71 and 90% in the MBR system, respectively. Using mixed fungal strains in the MBR system is a feasible technique to remove reactive dyes from the textile wastewater. To our knowledge, this is the first report showing the application of mixed filamentous fungal strains in the pilot scale (MBR) system.

Keywords: *Aspergillus versicolor*; Decolorization; Membrane bioreactor (MBR); *Rhizopus arrhizus*; Textile wastewater

1. Introduction

The textile industry is one of the most important industries in Turkey. Textile effluents can damage the environment as they contain dyes with complex and highly varied chemical structures. It is necessary to develop inexpensive wastewater treatment methods. Biological treatment methods have attracted much attention due to the relatively simple and ecologically friendly treatment processes as well as their relatively low fixed capital investment and operating costs [1,2]. Various types of micro-organisms, such as bacteria, yeasts, algae, and fungi, are able to remove different classes of dyes in biological wastewater treatment systems [3]. In these micro-organisms, fungal biomass can be produced cheaply using relatively simple and inexpensive techniques [4]. A variety of fungi can oxidize textile dyes under sterile conditions; however, an important consideration is that they could be used in treating wastewater containing textile dyes under non-sterile conditions [5–9]. There are some studies

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showing the effective dye removal properties of filamentous fungal strain such as *Rhizopus arrhizus* [10,11] and *Aspergillus versicolor* in sterile conditions [12,13]. Mixed culture has the advantage because of its synergistic effects on micro-organisms, therefore, it may quickly tolerate the toxic effects [14]. A large number of lab-scale studies have been conducted on decolorization of synthetic dye solutions through fungal biosorption/biodegradation in the literature [15]. However, their large-scale utilization has not been realized due to lack of an appropriate reactor system that can sustain stable performance in a non-sterile environment [16].

Recently, more attention has been paid to the membrane bioreactor (MBR) which is a combination of the conventional biological sludge process with a micro- or ultrafiltration membrane system, for wastewater treatment because of its higher efficiency of pollutant removal and excellent effluent quality [7,17,18].

This paper investigates reactive dye removal capability of mixed fungal biomasses (*R. arrhizus* and *A. versicolor*) under non-sterile conditions with the inherent advantages of the submerged MBR system designed and manufactured by our research team. This presented paper is the first experimental study carried out in our MBR system after pre-trail studies.

2. Materials and methods

2.1. Dye and chemicals

All chemicals were obtained from Merck. Reactive Blue dye was supplied from the local textile factory.

2.2. Micro-organisms

Fungal strains *A. versicolor* and *R. arrhizus* were obtained from Ankara University Biology Department Biotechnology Laboratory Culture Collection. The pure cultures were kept at 4°C and were transferred to Potato Dextrose Agar (PDA) media containing 39.0 g/L PDA every 3 months, immediately after their arrival at the laboratory.

2.3. Media cultures

The micro-organisms were cultivated together in liquid media using the shake flask method. The growth medium yeast–peptone–dextrose (YPD) consisted of yeast extract (10 g/L), peptone (20 g/L), and dextrose (20 g/L). The pH of the medium was adjusted to 6 with dilute HCl and NaOH solutions before autoclaving. Once inoculated, flasks were incubated on an orbital shaker at 100 rpm for 5 d at 25°C.

2.4. Cultivation of the fungal mycelia pellets

The mycelia inoculums (approximately 5 g wet mycelia in 100 mL media) were prepared and used as mycelia suspension. Erlenmeyer flasks (1,000 mL) containing 350 mL of YPD media were inoculated with 2 mL of the mycelia suspension and incubated at 25°C on a rotary shaker (100 rpm) for 10 d. The mycelia pellets were harvested after cultivation and transferred into MBR system (360 g wet weight), then used in the decolorization tests.

2.5. The sterile condition experiments

In order to examine dye removal activity of *R. arrhizus*, *A. versicolor*, and mixed culture, microorganisms were inoculated into 100 mL YPD medium (pH 6) containing 100 mg/L Reactive Blue on an orbital shaker (100 rpm) at 25°C in the sterile conditions. The experiments were performed in 250 mL Erlenmeyer flasks. The flasks were filled with 100 mL of YPD medium and autoclaved.

A 3 mL sample was taken daily from each flask and centrifuged at 10,000 rpm for 15 min to remove suspended biomass during the incubation period. The concentration of Reactive Blue was measured spectrophotometrically by reading absorbance at 616 nm. Cell-free medium was used as the blank.

2.6. Non-sterile condition experiments

2.6.1. Lab/pilot scale MBR system

A schematic diagram of the lab/pilot scale MBR setup is shown in Fig. 1. The MBR system had a working volume of 170 L and was equipped with a coarse and fine air bubble creation mechanism for membrane and biological aeration, respectively. Ultrafiltration membrane module, which had an area of 1.5 m^2, consists of six flat-sheet membranes (PVDF + PET) with the pore size of 0.08–0.3 μm and vertically placed in the aeration tank. The system was managed by an LCD display on the control panel.

The MBR system worked with synthetic textile wastewater is given in Section 2.6.2, and the mycelia pellets were harvested from medium after cultivation and transferred into MBR system. The system was first inoculated with 360 g (wet wt.) mixed fungal biomasses of *R. arrhizus* and *A. versicolor* aseptically grown for 10 d in Erlenmeyer flasks. Concentrated synthetic wastewater was prepared with tap water and then supplied into the reactor by pumps controlled by a water level controller. At the beginning of the each experimental period, 100 L synthetic

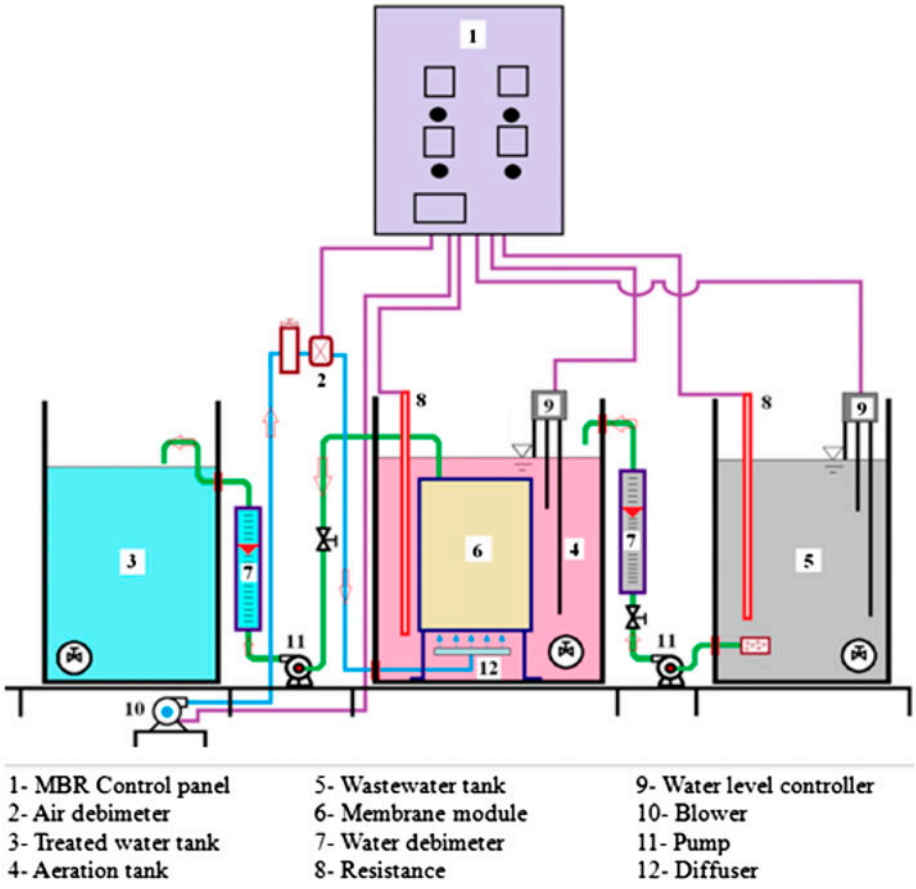

Fig. 1. A schematic diagram of the lab/pilot scale MBR setup.

wastewater was fed into the aeration tank at a flow rate fixed by the pump in the MBR system.

A diffuser supplied continuous air from the bottom of the reactor with an intensity of 9 L/min for complete mixing and supply of dissolved oxygen (DO) to the micro-organism. The temperature of the aeration tank was controlled at 25 ± 1 ℃, the pH value and the concentration of DO was kept, respectively, in the range of 4.0–4.5 and 6–8 mg/L in the aeration tank.

2.6.2. Synthetic wastewater

The media was made of 2 g/L starch, 1 g/L glucose, 0.4 g/L urea, 2 g/L KH_2PO_4, 0.099 g/L $CaC1_2$, 1.025 g/L $MgSO_4{\cdot}7H_2O$, 0.255 g/L NaCI, 0.17 g/L Na_2CO_3, 0.17 g/L $NaHCO_3$, 1 mL/L trace elements, and 10 g/L dyestuff. A stock trace element solution was prepared by dissolving 0.125 g $CuSO_4{\cdot}5H_2O$, 0.05 g H_2MoO_4, 0.061 g $MnSO_4{\cdot}5H_2O$, 0.043 g $ZnSO_4{\cdot}7H_2O$, and 0.082 g $Fe_2(SO_4)_3{\cdot}14\ H_2O$ in 1 L of Milli-Q water.

2.7. Analytical methods

Color measurements were carried out using a spectrophotometer (JENWAY 7315 spectrophotometer) to measure the absorbance of the sample at the peak wavelength (616 nm) of the dye used. The concentration of dyestuff was calculated from a calibration curve of "absorbance vs. concentration", and concentration values were used for calculations of decolorization efficiency. The percentage of color reduction was calculated by Eq. (1)

$$\text{Color reduction } (\%) = (C_0 - C_f)/C_0 \times 100 \quad (1)$$

In this equation, C_0 and C_f represent the initial and final dye concentrations (mg/L), respectively.

COD analyses were carried out by means of a spectrophotometric test (Spectroquant Merck) and later determined spectrophotometrically (Nova 60A spectroquant), at 528 nm. Samples (influent, effluent) were centrifuged (Nüve NF 400) for 5 min at 10,000 × g and diluted appropriately before each COD determination.

A DO meter (Hanna HI 9146) was used to detect the concentrations of DO in the reactor of the system.

The pH determinations were conducted with a pH meter (Thermo Scientific Orion 3 Stars).

Mixed liquor suspended solids (MLSS) were measured according to the analytical methods in standard methods [19].

Microscopic observations (Nikon Eclipse LV150) were conducted to observe the interactions between Reactive Blue and fungus (*A. versicolor*—Av and *R. arrhizus*—Ra) in sterile conditions, and mixed culture (Av + Ra) in the MBR system. Under bright field mode, 100 times magnified microscopic photograph was taken.

3. Results and discussion

3.1. The Sterile conditions experiment

In order to examine the decolorization of Reactive Blue by *R. arrhizus*, *A. versicolor*, and mixed culture in the sterile conditions, fungal strains were inoculated into YPD medium with 100 mg/L dye. As shown in Table 1, *R. arrhizus* and *A. versicolor* decolorized 26.98 and 100% of the reactive dye in 3 d, respectively. However, mixed culture of *R. arrhizus* and *A. versicolor* decolorized 100% of dye in 2 d. These results show the superiority of mixed culture over single strains. Mixed culture has the advantage due to its synergistic effects on micro-organisms to tolerate the toxic effects [20]. The previous study had showed that mixed fungal biomasses of *R. arrhizus* and *A. versicolor* have potentially removed 100% of Remazol Blue dye in molasses medium after 3 d of incubation [14]. In this study, the rich ingredients of medium enhanced decolorization rate in a short incubation period.

3.2. The non-sterile conditions experiment (MBR experiment)

The experimental works in the MBR system were divided in four run. In the first experimental study (Run 1), 15 d, a necessary period for biomass adaptation to new conditions was required in the MBR system. After inoculation of 360 g mixed culture to MBR system, process was started, and it was operated for 5 d achieving a batch fungal adaptation without synthetic wastewater in aeration tank. An additional 10 d was used for a batch fungal adaptation which also provided for the decolorization process in the MBR system with an initial dye concentration of 19.7 mg/L. The removal efficiency of color and COD results obtained in a batch fungal adaptation in the MBR system were given in Fig. 2. The greatest color reduction was obtained during start-up, where fungal biomass turned completely dark because of the initial dye adsorption. It was observed that after 3 d of treatment, the decolorization percentage reached to a maximum of 70%, but later, the biomass became discolored and the decolorization percentage decreased to 51% at the end of 6 d. However, the decolorization level increased to 73.75% at the end of the Run 1 (Fig. 2). It is considered that the increasing of decolorization rate again may depend on the increase of fungal growth.

The influent COD concentration was 1,500 mg/L, during the adaptation and also decolorization process in the MBR system. The aerobic treatment resulted in a COD abatement of 87–90% (Fig. 2).

In the second experimental study (Run 2), the concentration of Reactive Blue in synthetic textile wastewater was 103.7 mg/L. The results of Run 2 were given in Fig. 3. It was observed that after 1 and 2 d of treatment the decolorization percentages were over 87 and 90.71%, respectively. At the end of 3 d, the decolorization level was decreased to 87% (Fig. 3).

Table 1
Reactive Blue decolorization (%) by *R. arrhizus* (Ra), *A. versicolor* (Av) and mixed culture (Ra + Av) in liquid medium with 100 mg/L dye at 25 ± 1.0˚C, pH 6

Time (Day)	Ra (D%)	Av (D%)	Ra + Av (D%)
1	18.71	18.32	27.27
2	22.30	32.06	100
3	26.98	100	

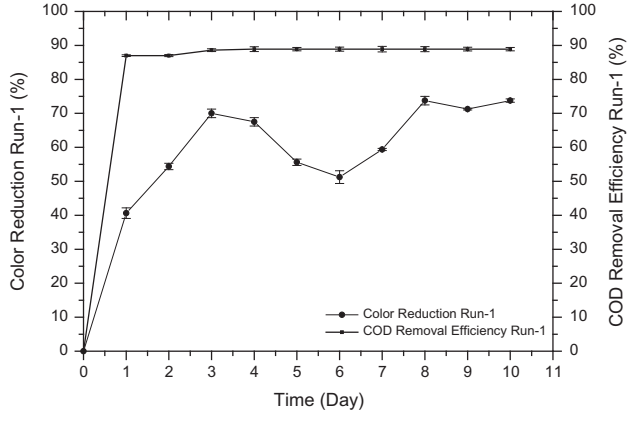

Fig. 2. Color removal (influent dye concentration = 19.7 mg/L, HRT = 10 d) and evolution of COD in the effluent (influent COD = 1,500 mg/L) as a function of time during the fungal adaptation to non-sterile condition and pre-treatment in the MBR.

In the third experimental study (Run 3), the concentration of Reactive Blue in synthetic textile wastewater was prepared as 111.23 mg /L. The results obtained in the third experimental study were shown in Fig. 3. It is observed that after 1 d of treatment the decolorization percentage was over 84%, and at the end of 2 d the decolorization level reached 88%. However, the decolorization level was not changed at the end of the 3 d (Fig. 3).

In the fourth experimental study (Run 4), the concentration of Reactive Blue in synthetic textile wastewater was prepared more concentrated. The dye concentration and COD concentration of the synthetic wastewater prepared for Run 4 were 238.15 and 2,200 mg/L, respectively. The removal efficiency of color and COD concentrations obtained from the Run 4 was given in Fig. 4. It is determined that after 1 d of treatment the decolorization percentage was approximately 84%, and at the end of the run the decolorization level decreased to 80.84% (Fig. 4).

Microscopic images of *A. versicolor*—Av (a) and *R. arrhizus*—Ra (b) in sterile conditions; mixed culture (Av + Ra) in MBR system with Reactive Blue were given in Fig. 5. Reactive Blue dye existed on the surface of *A. versicolor* strain and accumulated in the hyphae of *R. arrhizus* (Fig. 5) in sterile conditions. The images of mixed culture in non-sterile conditions were similar with the images of individual cultures in sterile conditions.

Some other researchers have also worked under sterile and non-sterile conditions in continuous or semi-continuous modes, achieving effective decolorization percentages. However, their experimental systems have smaller capacity than our MBR system. Blanquez et al. carried out the decolorization experiments of 150 mg/L Grey Lanaset G dye solution in the pilot-scale bioreactor (the total volume was 1.5 L) and showed that in both discontinuous and continuous treatment with an HRT of 2 d, the decolorization levels were higher than 90% [8]. Borchert and Libra performed the decolorization of Reactive Black five dye experiments in a sequencing batch process with *T. versicolor* under non-sterile conditions [21]. The experiment was maintained for 55 d (five run). The first four runs achieved high degrees of decolorization (91–98%), but in the fifth run the decolorization dropped to 72%, followed by an almost total loss of decolorization ability in the subsequent run due to the presence of bacteria in the culture. In our study, the dye removal rates were increased from Run one to three (73.75–90.71%) with an HRT of 2 d due to augmentation of microbial growth in bioreactor. The increasing in microbial growth was determined with MLSS concentration "unpublished results". Libra et al. examined dye removal by *T. versicolor* strain in Erlenmeyer flasks at non-sterile conditions and reported that high decolorization percentages were achieved by growing *T. versicolor* on sterilized grains as sole substrate and support material during two cycles, but in the third one, the decolorization percentage dropped to 55% due to negative effect of contamination [5]. Mixed cultures can easily tolerate negative environmental conditions [14]. In our study, decolorization activity of the mixed fungal cultures was not significantly affected by the bacterial contamination in non-sterile MBR conditions. Yang and Yu maintained the decolorization process of Red 533 dispersed dye in a biofilm bioreactor with *P. chrysosporium* immobilized on foam material for 11 d

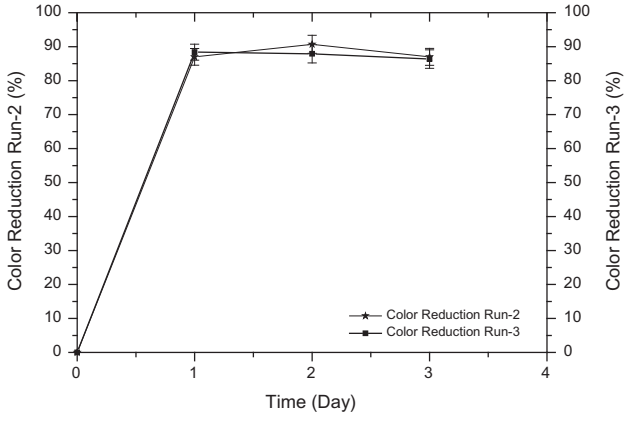

Fig. 3. Color removal during non-sterile operation of the MBR in sequencing batch mode (influent dye concentration Run 2 = 103.7 mg/L, influent dye concentration Run 3 = 111.23 mg/L, HRT = 3 d).

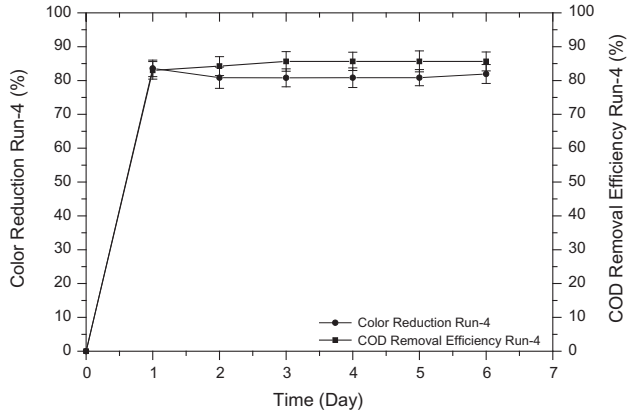

Fig. 4. Color removal during non-sterile operation of the MBR in sequencing batch mode (influent dye concentration = 238.15 mg/L, HRT = 6 d) and evolution of COD in the effluent as a function of time in the MBR (influent COD = 2,272 mg/L).

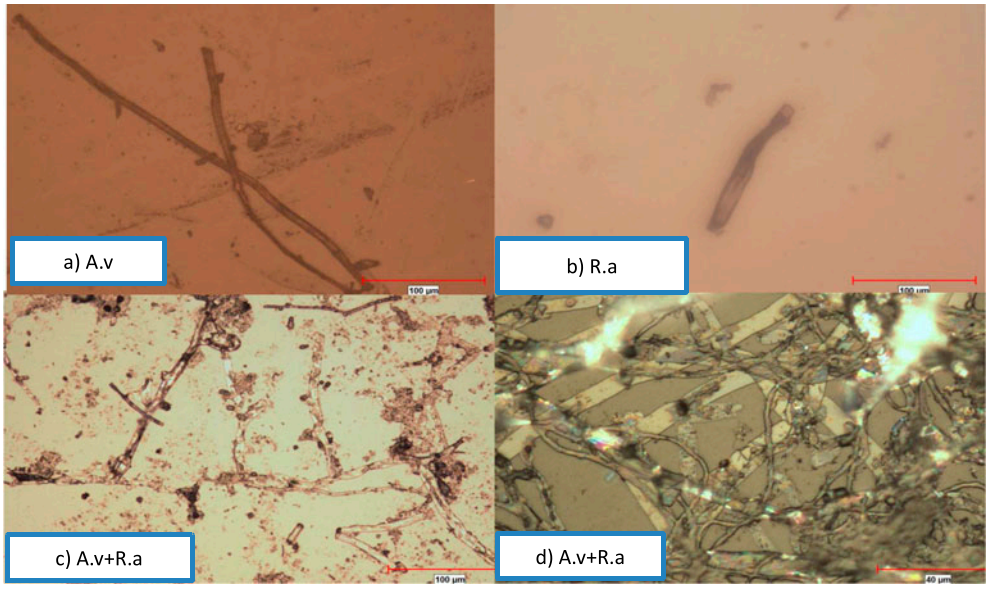

Fig. 5. Microscopic images of *A. versicolor*—(Av) (a) and *R. arrhizus*—(Ra) (b) in sterile conditions; mixed culture (Av + Ra) in MBR (c) and (d).

under non-sterile conditions [22]. The decolorization level was 100% during the first 8 d and dropped to 87% remaining stable for the rest of the experiment period. In our study, the decolorization rate was decreased (80.84%) in Run four, however the dye concentration was increased twofold (Fig. 4).

Organic removal efficiency was defined as the difference between influent COD and effluent COD. The influent COD ranged between 1,500 and 2,250 mg/L during experimental studies. Effluent COD concentration was frequently below 220 mg/L. The results indicated that the organic removal efficiency achieved was up to 91% with an HRT of 2 d by means of biological treatment, with the assistance of membrane filtration. This suggested that membrane filtration played a significant role in maintaining high and stable organic removal efficiency.

4. Conclusions

The decolorization rates were 90.71 and 80.84% for 103.7 and 238.15 mg/L dye concentrations, respectively, during non-sterile operation at an HRT of 2 d in the MBR system. The COD concentrations of synthetic textile wastewater were reduced from 1,500–2,272 to 198–220 mg/L, which corresponds to removal efficiencies between 87 and 90% in the experimental studies.

It can be concluded that textile dyes can be effectively decolorize by mixed fungal strains in the MBR system.

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