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Investigation of Decolorization of Reactive Violet 5R and Remazol Brillant Orange 3R by *Bacillus* sp. DT16

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Abstract

Dye pollution in water and soil is increasing rapidly depending on the industrialization. Synthetic dyes are mutagenic, toxic and resistant to degradation due to their complex chemical structures so their effluents cannot be directly discharged. The biological remediation of textile effluents has recently received an increasing attention, representing an attractive, cheap, environmentally friendly, and publicly acceptable alternative to the physico-chemical methods. Microorganisms play an important role in the decolorization and removal of dyes from polluted sites.

In this study, decolorization of RV-5R and RBO-3R by *Bacillu*s sp. which was isolated from textile effluent was investigated. The effect of environmental factors such as pH (5.5, 6.5, 7.5, 8.0, 9.0 and 10.0) temperature (20, 30, 37 and 42 °C), carbon (1 g/L: sucrose, glucose, starch and mannitol) and nitrogene sources (ammonium chloride, peptone and yeast extract) and initial dye concentration (10, 25, 50, 100, 200, 500 mg/L) on the microbial decolorization by *Bacillus* sp. was investigated.

The maximum dye removal was obtained at 500 mg/L initial dye concentration for RV-5R and 200 and 500 mg/L for RBO-3R by the bacterium. Bacterial decolorization of RV-5R was 54.54% (6h) in growth medium containing yeast extract (1g/L) and glucose (1g/L) at pH 10.0 and 37 °C. The same bacterium decolorized the RBO-3R dye at 96.15% (172 h) in growth medium containing peptone (1g/L) and sucrose (1g/L) at pH 10.0 and 30 °C.

Any report about the microbial decolorization of the RV-5R and RBO-3R dyes has not been seen in the literature. Therefore this study was the first report about the bacterial decolorization of these dyes.

Keywords: Bacillus, decolorization, optimization, RV-5R, RB0-3R

INTRODUCTION

Industrial effluents contain a significant amount of residual synthetic dyes. For example food, pharmaceutical, cosmetic, printing, leather and textile industries. Uncontrolled discharge of these dye effluent in aqueous ecosystems leads to the reduction in sunlight penetration which in turn decrease photosynthetic activity, dissolved oxygen concentration, biochemical oxygen demand (BOD), chemical oxygen demand (COD), water quality and are lethal to resident organisms [1,2].

In addition, many dyes are believed to be toxic carcinogenic or to be prepared from known carcinogens such as benzidine or other aromatic compounds that might be formed as a result of microbial metabolism [3,4]. Therefore, removal of such dyes before discharging them into natural water streams is essential. For this, appropriate treatment technologies are required. Traditional physical and chemical methods used for removal of dyes in wastewater are generally expensive; because of their limited applicability and the production activated sludge, the newer treatment technologies need to be investigated. But, bioremediation has been proved to be applicable alternative for detoxification and degradation of dye effluents due to the cheap, environmentally friendly, and publicly acceptable alternative [6] to the physico-chemical methods [5]. In this context, a wide variety of microorganisms such as bacteria, actinomycetes, algae and fungi found in soil and water are able to decolorize synthetic dyes [6-8]. Nevertheless, the effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms. Although it has been reported that various fungi could removed the synthetic dyes, bacteria are preferred due to their relatively short life cycle and faster decolorization process [9,10]. It has been reported that, many bacteria belonging to genera such as *Bacillus* [11], *Pseudomonas* [12], *Aeromonas* [13] and *Staphylococcus* [10], can be decolorized of synthetic dyes under aerobic and/or anaerobic conditions in natural characteristic, On the other hand, environmental conditions on dye removal such as pH [14,15], temperature [12,14], presence or absence of oxygen [16] and presence of additional C and N sources [17] is great of importance.

Denizli is one of the textile centers of Turkey. As a result of widespread textile industries in Denizli, there is a large amount of effluents loaded with dyes. In this background, the present study was designed to understand the decolorization ability of *Bacillus* sp. DT16 isolated from textile effluents from Denizli. We describe an optimization process and its behavior under different conditions of pH, temperature, initial dye concentration, and different C and N sources on the microbial decolorization of textile dyes Reactive Violet 5R (RV-5R) and Remazol Brillant Orange 3R (RBO-3R) by *Bacillus* sp. DT16.

MATERIALS AND METHODS

Dye stock

The industrial quality Reactive Violet 5R (RV-5R) and Remazol Brillant Orange 3R (RBO-3R) dyes stock solution was obtained from Dystar Textile Co., Turkey. The powdered dyestuff was dissolved in distilled water at 1000 mg/L (w/v) and sterilized by filter for the preparation of dye stock. Appropriate volumes of the stock dye were added to growth medium containing flasks.

Bacterial growth

The bacterial strain (*Bacillus* sp. DT16) used in the present work was obtained from the culture collection of the Pamukkale University, Bacteriology Laboratory that was isolated from textile effluent. The strain was inoculated to a 250 ml Erlenmeyer flask containing 100 ml Nutrient Broth (NB; g/l: Beef extract 1, peptone 5, yeast extract 2, NaCl 5) medium and the culture was aerobically incubated with constant shaking at 125 rpm; culture growth was monitored by measuring optical density (OD) at 600 nm.

Decolorization experiments

The experiments were performed in 250 ml Erlenmeyer flasks containing decolorization medium (NB). The dyes RV-5R and RBO-3R were added to the medium and 10% (w/v) bacterium (*Bacillus* sp. DT16) was inoculated into the medium. After incubation, the samples were withdrawn at different time intervals and analyzed for decolorization efficiency. The aliquot was centrifuged at 14000 rpm to separate the bacterial cell mass. The decolorization rate was monitored spectrophotometrically by reading the decrease in absorbance (595 nm) of the dye in culture supernatant. Decolorizing activity is expressed in terms of percentage decolorization.

The effect of environmental factors such as initial dye concentration (10, 25, 50, 100, 200 and 500 mg/L), carbon sources (1 g/L: sucrose, glucose, starch and mannitol), pH (5.5, 6.5, 7.5, 8.0, 9.0 and 10.0), temperature (20, 30, 37 and 42 °C) and nitrogen sources (ammonium chloride, peptone and yeast extract) on bacterial decolorization were investigated for the specify the optimum conditions. Nitrogen sources were added at different rates in the growth medium containing glucose. The rate of C/N was 1/1 and 1/0.5. Also, the growth of cells was routinely monitored by measuring optical density (OD) at 600 nm. The experiments were performed in duplicate and the mean values were taken into account.

Determination of decolorization efficiency

Decolorization extent was determined by measuring the absorbance of the culture supernatant at 560 nm for RV-5R and 494 nm for RBO-3R using a UV-Vis Lange DR5000 spectrophotometer. The decolorization efficiency was calculated using the following equation:

$$\text{Decolorization efficiency (\%)} = 100x\ (ODi - ODt) / ODi$$

Where ODi refers to the initial absorbance at 560 and 494 nm and ODt refers to the absorbance measured in the degradation. The percentage of decolorization was measured at different time intervals. All decolorization experiments were performed in duplicate. Abiotic controls (without microorganisms) were always included.

RESULTS

Effect of initial dye concentration

To determine the best decolorization ability of *Bacillus* sp. DT16 on RV-5R and RBO-3R, six different dye concentrations (10, 25, 50, 100, 200 and 500 mg/L) were used. Decolorization rate of RBO-3R and RV-5R by *Bacillus* sp. DT16 generally increase depending on the increase in the concentration of dye. The maximum decolorization time for 500 mg/L of RBO-3R and RV-5R dye was 107th (43.61%) and 83th (49.12%) hours respectively (Data not given).

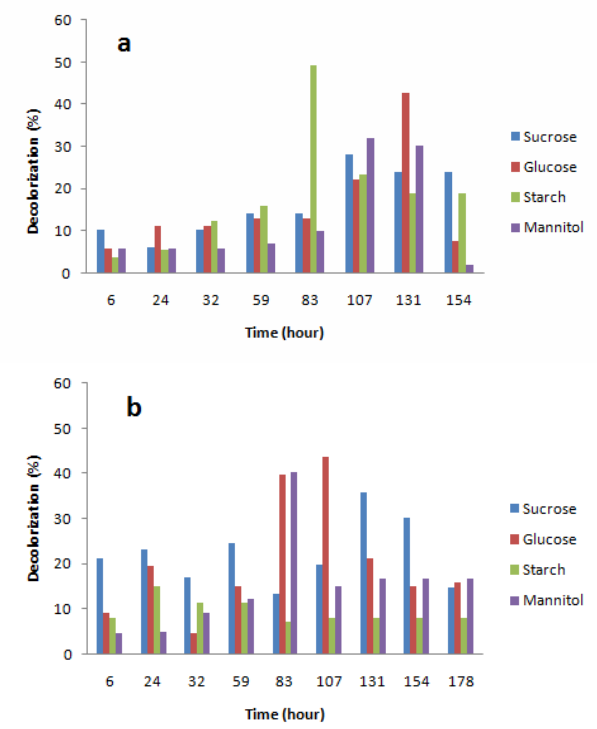

Figure 1. Effect of different carbon sources (1 g/L) on decolourization of RV-5R (a) and RBO-3R (b) with 500 mg/L dye concentrations in TSB media by *Bacillus* sp. DT16

Effect of carbon sources

The effects of carbon sources such as sucrose, glucose, starch and mannitol on decolorization by *Bacillus* sp. DT16 were tested; results are given in Fig. 1. Among the carbon sources used in the study, starch and glucose enhanced the decolorization rate of RV-5R (Fig. 1a). The maximum decolorization rate for 500 mg/L of RV-5R was 49.12% (83 h) and 42.59% (131 h). On the other hand, among the carbon sources used in the study, glucose and mannitol enhanced the decolorization rate of RBO-3R (Fig. 1b). But interestingly, other carbon sources (sucrose and starch) were not positive effect on decolorization of RBO-3R by DT16. At glucose and mannitol containing medium of 500 mg/L dye concentration, maximum color reduction was observed at 43.61% (107 h) and 40.35% (83 h) respectively.

Effect of pH

Fig. 2 shows the effect of the different pH values of the medium on the bacterial decolorization containing 500 mg/L initial dye concentration. To determine suitable pH value for the most efficient decolorization by DT16, pH 5.5, 6.5, 7.5, 8.0, 9.0 and 10.0 were tested. Bacterial decolorization of RV-

5R was 63.33% in growth medium containing glucose at pH 10.0 and 57.38% in growth medium containing starch at pH 8.0 (Fig. 2a). Also, bacterial decolorization of RBO-3R was 55.83% in growth medium containing glucose at pH 10.0 (Fig. 2b).

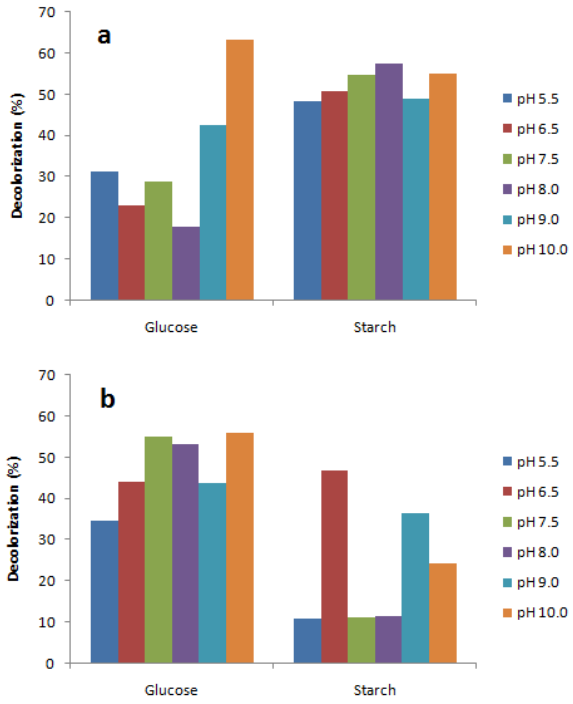

Figure 2. Effect of the different pH values on decolourization of RV-5R (a) and RBO-3R (b) with 500 mg/L dye concentrations in TSB media containing glucose and starch

Effect of temperature

To determine the effect of different temperature on decolorization, DT16 bacteria were inoculated into the NB medium containing 500 mg/L dye and 1g/L glucose at pH 10.0 and incubated at 20, 30, 37 and 42°C. And the maximum decolorization for RV-5R was observed at pH 10.0 and 37 ºC with the 63.33% of color reduction. On the other hand, the maximum decolorization of other dye (RBO-3R) was observed at pH 10.0 and 30°C with the 57.77% of color reduction (Fig. 3).

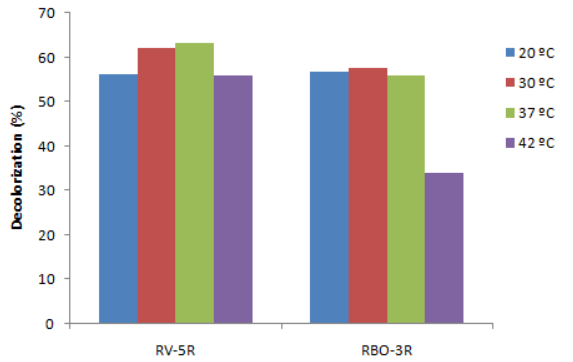

Figure 3. Effect of different temperature on decolorization of RV-5R and RBO-3R with 500 mg/L dye concentrations in TSB media containing glucose at pH 10.0

Effect of carbon/nitrogen rate

The effect of the carbon/nitrogen rate such as glucose/amonium chloride (1/0.5 and 1/1), glucose/peptone (1/0.5 and 1/1) and glucose/yeast extract (1/0.5 and 1/1) on decolorization by DT16 were tested; results are given in Fig. 4. Bacterial decolorization of RV-5R was 54.54% (6h) in growth medium containing glucose (1g/L) and yeast extract (1g/L) at pH 10.0 and 37°C. The same bacterium decolorized the RBO-3R dye at 96.15% (172h) in growth medium containing glucose (1g/L) and peptone (1g/L) at pH 10.0 and 30°C (Fig. 4).

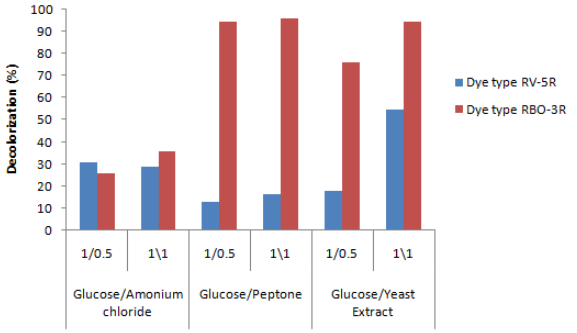

Figure 4. Effect of carbon/nitrogen rates on decolorization of RBO-3R and RV-5R with 500 mg/L dye concentrations at optimum conditions (37 ºC and pH 10.0 for RV-5R and 30 ºC and pH 10.0 for RBO-3R)

DISCUSSION

Environmental factors such as pH, dye concentration, carbon sources, and temperature is very important for bioremediation and detoxification of environmental contaminants. So, optimal environmental conditions should be determined to obtain efficient decolorization potential. This study revealed the potential of RV-5R and RBO-3R dyes removal by the bacterium *Bacillus* sp. DT16 and determined the effect of environmental factors on bacterial decolorization.

Many of bacterial strains require organic carbon sources, as they cannot utilize dye as the growth substrate [18]. *P. aeruginosa* was decolorized a commercial tannery and textile dye, Navitan Fast blue S5R in the presence of glucose under aerobic conditions [19]. There are only very few bacteria that are able to grow on azo compounds as the sole carbon source 20 [20]. Therefore the bacterial decolorization showed differences when the isolate DT16 was grown in the presence of various types of carbon sources with 500 mg/L dye (RV-5R and/or RBO-3R). The presence of starch and glucose in the culture medium resulted with high decolorization (49.12% and 42.59% respectively) according to mannitol and sucrose for RV-5R (Fig. 1a). On the other hand, the best decolorization was obtained for RBO-3R dye in the presence of glucose and mannitol (43.61% and 40.35% respectively) (Fig. 1b). Similarly, in another study, glucose, sucrose, and glycerol were found as effective electron donors while acetate, citrate, and lactate were unfavorable electron donors for AQDS (anthraquinone-2, 6-disulphonate, humus analog) reduction by *Planococcus* sp. MC01 humus-reducing facultative anaerobe strain. Also this bacterium showed high decolorizing activity of Orange I at the optimal glucose concentration [21].

The initial pH of the medium is an important effect on the efficiency of dye decolorization. In the present study, the effect of pH variation on decolorization at pH levels of 5.5, 6.5, 7.5, 8.0, 9.0 and 10.0 was carried out in the growth medium with 500 mg/L dye containing glucose and/or starch which were the best effective C sources. The decolorization potential of bacterial strain DT16 was strongly affected from pH change (Fig. 2). *Bacillus* sp. DT16 decolorized the RV-5R at the rate of 63.33%, at pH 10.0 in the growth medium containing glucose (Fig. 2a). However, at pH 6.0, 8.0 and 9.0 color removal rates of RV-5R dye was 31.42, 23.08, 28.85, 18.0 and 42.59% respectively. Also, maximum decolorization of RBO-3R dye was observed at pH 10.0 (55.83%) in the growth medium with glucose too (Fig. 2b).

Many studies have investigated the optimum pH values for bacterial reduction of synthetic dye. Cao et al. [22] reported that the influence of pH on the decolorization of dye mixture at pH 5.5-8.0. And they observed that a sharp decrease in decolorization rate when pH was decreased to below pH 6.0. *Shewanella oneidensis* MR-1 almost lost its decolorization ability at pH 5.5. In *Citrobacter* sp. CK3 optimum pH is 6.0 and 7.0 for decolorization of Reactive Red 180 and much lower decolorization was observed in strongly acidic (at pH 4.0) and strongly alkaline (at pH 12.0) conditions [23].

Fig. 3 shows the effect of temperature on decolorization of RV-5R and RBO-3R by DT16 bacterium in the growth medium with 500 mg/L dye containing glucose at pH 10.0 which was the best pH level. The decolorization potential was not very different at 20, 30, 37 and 42 ºC. However, the color removal rate at 37 ºC (63.33%) slightly higher than 30 ºC (62.26%) for RV-5R. On the other hand, the maximum rate of decolorization was 57.77% at 30 ºC for RBO-3R. It is necessary to determine the optimum temperature in different bacterial cultures in order to obtain maximum bacterial decolorization. For example *S. oneidensis* MR-1 exhibited the maximum decolorization efficiency for dye mixture at 35 ºC [22]. Wang et al. [23] reported that *Citrobacter* sp. CK3 showed strong decolorizing activity from 27 ºC to 37 ºC and at 42 ºC decolorization activity was decreased. Another bacteria *Pseudomonas* sp. RA20 efficiently decolorized the Reactive Black-5 at 25 ºC [24].

At optimal conditions for *Bacillus* sp. DT16 bacterium, the maximum reduction of 500 mg/L of RBO-3R occurred with the rate of 96.15%: Initial pH; 10.0, temperature; 30 °C, glucose/peptone rate; 1/1 (Fig. 4). Interestingly, the removal rate of other dye RV-5R was generally decreased in the presence of all three nitrogen sources.

CONCLUSION

The obtained results exhibited that *Bacillus* sp. strain isolated from textile effluent, effectively decolorized RBO-3R (96.15%) and RV-5R (63.33%) dyes under optimal environmental conditions (pH, temperature, dye concentration, carbon and nitrogen sources).

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