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Decolorization of Reactive Black 5 by *Pseudomonas aeruginosa*

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The aim of this work is to evaluate decolorization of a diazo textile dye Reactive Black 5 by *Pseudomonas aeruginosa*, which was isolated from a non-dye contaminated activated sludge biomass. The effect of various physico-chemical parameters, initial dye concentration, temperature and yeast extract concentration as an organic source on decolorization were investigated. *P. aeruginosa* was able to decolorize 20 mg/L Reactive Black 5 completely within 144 h in the presence of 1 g/L yeast extract at 35 °C. Decolorizing of Reactive Black 5 increased with increasing yeast concentration from 0.1 to 1 g/L. Decolorization efficiencies of 20 mg/L Reactive Black 5 for 0.1, 0.5 and 1 g/L yeast extract were 48, 88 and 100 %, respectively. The temperature had a considerable effect on decolorization of Reactive Black 5 by *P. aeruginosa*.

Keywords: Decolorization, Reactive Black 5, *Pseudomonas aeruginosa*.

INTRODUCTION

Dyes usually have a synthetic origin and complex aromatic structures. These synthetic dyes are widely used in the textile, food, pharmaceutical, tanneries, cosmetics and electroplating factories [1]. These industrial processes can discharge dye containing wastewaters into water systems [2]. Presence of the dyes in water systems reduces light penetration into deeper layers, lowering the gas solubility, diminishing photosynthetic activity and deteriorating the water quality [3]. Reactive dyes represent the most widely used dyes in the textile industry due to their bright colors, colorfastness and simple and low cost application processes [4,5]. A large number of reactive dyes are azo compounds [6]. Azo dyes are aromatic compounds with one or more –N=N– groups [1]. There are several methods to treat dye containing wastewaters [7]. Extensively used coagulation/flocculation techniques produce large amounts of sludge, which requires safe disposal and uses more energy and chemicals than biological processes. Furthermore, coagulation/flocculation processes may be ineffective in removing highly soluble dyes. The adsorption of dyes by carbon is only successful in some types of dye within a certain pH range [8,9]. Photochemical and photocatalytic processes are the most studied advanced oxidation techniques for the removal of dyes from wastewater, but complicated procedures and high costs are disadvantages of these processes [10]. Conventional aerobic biological wastewater treatment systems usually cannot efficiently decolorize dye containing wastewaters due to the strong electron-withdrawing group [11]. Although dyes can be expected to be resistant to biological degradation because of their azoic structures, it has been reported that many bacteria can degrade numerous dyes under certain conditions [12-21].

Many studies have focused on the decolorization potential of dyes by acclimatized microorganisms, which isolate dye from contaminated areas such as soil and wastewater treatment plants [22-25]. The main objective of this study was to observe potential decolorization of Reactive Black 5 by non-acclimatized *P. aeruginosa*, which isolated from non-dye contaminated activated sludge.

EXPERIMENTAL

Reactive Black 5 dye obtained from the local textile industry in Turkey. The maximum absorbance wavelength of the dye is 597 nm. The chemical structure of Reactive Black 5 is given in Fig. 1.

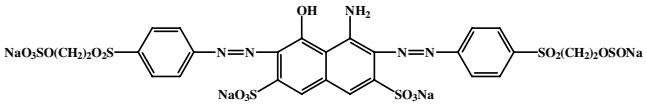

Fig. 1. Chemical structure of Reactive Black 5

Isolation of *Pseudomonas aeruginosa*: *P. aeruginosa* was isolated from an aeration basin of activated sludge at a soft drink factory located in Adana, Turkey. The isolation of *P. aeruginosa* was conducted in three main stages. Cetrimide

agar, which is a type of agar for selective isolation of *P. aeruginosa*, was used in the first stage of isolation. Cetrimide agar is selective for *P. aeruginosa*, however other bacterial species may develop [26]. In the second stage, a single colony growth on cetrimide agar at 42 °C was transferred to nutrient agar under sterile conditions. Thus a single species of bacterial culture was obtained. In the third stage, 16S rDNA gene was amplified using, PA-SS-F GGGGGATCTTCGGACCTCA and PA-SS-R TCCTTAGAGTGCCCACCCG primers for the single bacterial colony [27]. The pure culture of *P. aeruginosa* was transferred to a 500 mL Erlenmeyer flask containing 250 mL nutrient broth and incubated at 35 ± 2 °C.

Decolorization experiments: All decolorization experiments were conducted three times in static conditions and the average values were used for calculations. In order to determine the effect of initial dye and yeast extract concentration on decolorization, three different dye concentrations (20, 40 and 60 mg/L) were used with three different yeast extract concentrations (0.1, 0.5, 1 g/L) as the basal mineral medium. The experiments were performed at three different temperatures (15, 25, 35 °C) to examine the effect of temperature. The composition of the basal mineral medium was (g/L): K_2HPO_4 4.35, KH_2PO_4 1.7, NH_4Cl 2.1, $MgSO_4$ 0.2, $MnSO_4$ 0.05, $FeSO_4 \cdot 7H_2O$ 0.01, $CaCl_2 \cdot 2H_2O$ 0.03 [28]. The initial pH was adjusted to 7.0. Microbial culture at nutrient broth was centrifuged at 5000 rpm for 0.5 h, the supernatant was withdrawn and microbial culture was washed three times with sterile distilled water to remove the nutrient residues. Decolorization experiments were conducted in 250 mL sterile Erlenmeyer flasks. 1 mL microbial cultures were inoculated in 250 mL Erlenmeyer flasks including 120 mL basal mineral medium containing different dye and yeast extract concentrations. The amount of initial bacteria was approximately 5.5×10^6 CFU/mL in all experiments. Glass materials and solutions were autoclaved at 121 °C, Reactive Black 5 was made sterile by using a sterile filter with 0.45 μm pore size.

Dead culture autoclaved at 121 °C was used in the abiotic decolorization experiments. Thus biotic and abiotic decolorizations were compared. Abiotic decolorization experiments were conducted under the same conditions as biotic experiments.

Analytical methods: Decolorization was determined by measuring the absorbance of culture supernatant at 597 nm. Growth of microorganism was determined by the plate count agar (PCA).

RESULTS AND DISCUSSION

Effect of dye concentration on decolorization: Concentrations of azo dyes have an important effect on the decolorization process. High dye concentrations may negatively affect the decolorization efficiency due to the potential toxicity on microorganisms [29]. Decolorization efficiencies were found to be 100, 78 and 71 % for 20, 40 and 60 mg/L Reactive Black 5, respectively with 1 g/L yeast extract at 35 °C within 144 h. Although decolorization efficiencies decreased with increasing dye concentrations, the decolorization rate (mg/L/h) increased with increasing dye concentrations. Decolorization efficiencies and rates for initial dye concentrations are given in Table-1. There was no inhibitory effect caused by the studied dye concentrations. Effect of initial dye concentrations on decolorization efficiencies with 1 g/L yeast extract at 35 °C is given in Fig. 2. Abiotic decolorization of Reactive Black 5 by dead culture was found negligible for three different initial dye concentrations at three different temperatures. Biotic processes are the main decolorization mechanism of Reactive Black 5 by *P. aeruginosa*. The comparison of biotic and abiotic decolorization with 1 g/L yeast extract at 35 °C is given in Fig. 3.

TABLE-1
DECOLORIZATION EFFICIENCIES AND RATES FOR INITIAL DYE CONCENTRATIONS WITH 1 g/L YEAST EXTRACT AT 35 °C

Dye conc. (mg/L)	Decolorization efficiency (%)	Decolorization rate (mg/L/h)
20	100	0.129
40	78	0.216
60	71	0.301

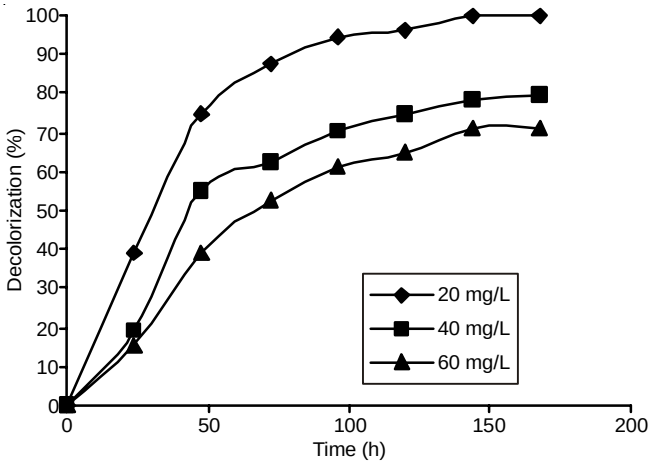

Fig. 2. Effect of initial dye concentrations on decolorization efficiencies with 1 g/L yeast extract at 35 °C

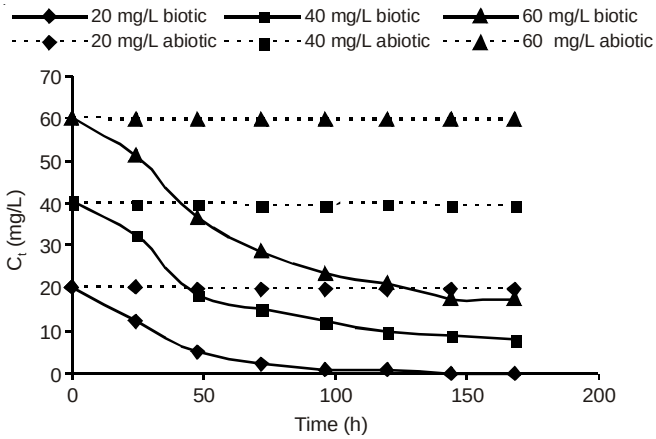

Fig. 3. Comparison of biotic and abiotic decolorization with 1 g/L yeast extract at 35 °C

Effect of yeast extract on decolorization: Decolorizing of Reactive Black 5 increased with increasing yeast concentration from 0.1 to 1 g/L. Decolorization of 20 mg/L Reactive Black 5 for 0.1, 0.5 and 1 g/L yeast extract were 48, 88 and 100 %, respectively at 35 °C within 144 h. Table-2 and Fig. 4 show the decolorization efficiencies at different yeast extract

TABLE-2
DECOLORIZATION EFFICIENCIES FOR INITIAL DYE CONCENTRATIONS WITH DIFFERENT YEAST EXTRACT CONCENTRATIONS AT 35 °C WITHIN 144 h

Dye conc. (mg/L)	Decolorization efficiency (%)		
	1 g/L yeast extract	0.5 g/L yeast extract	0.1 g/L yeast extract
20	100	88	48
40	78	65	31
60	71	54	28

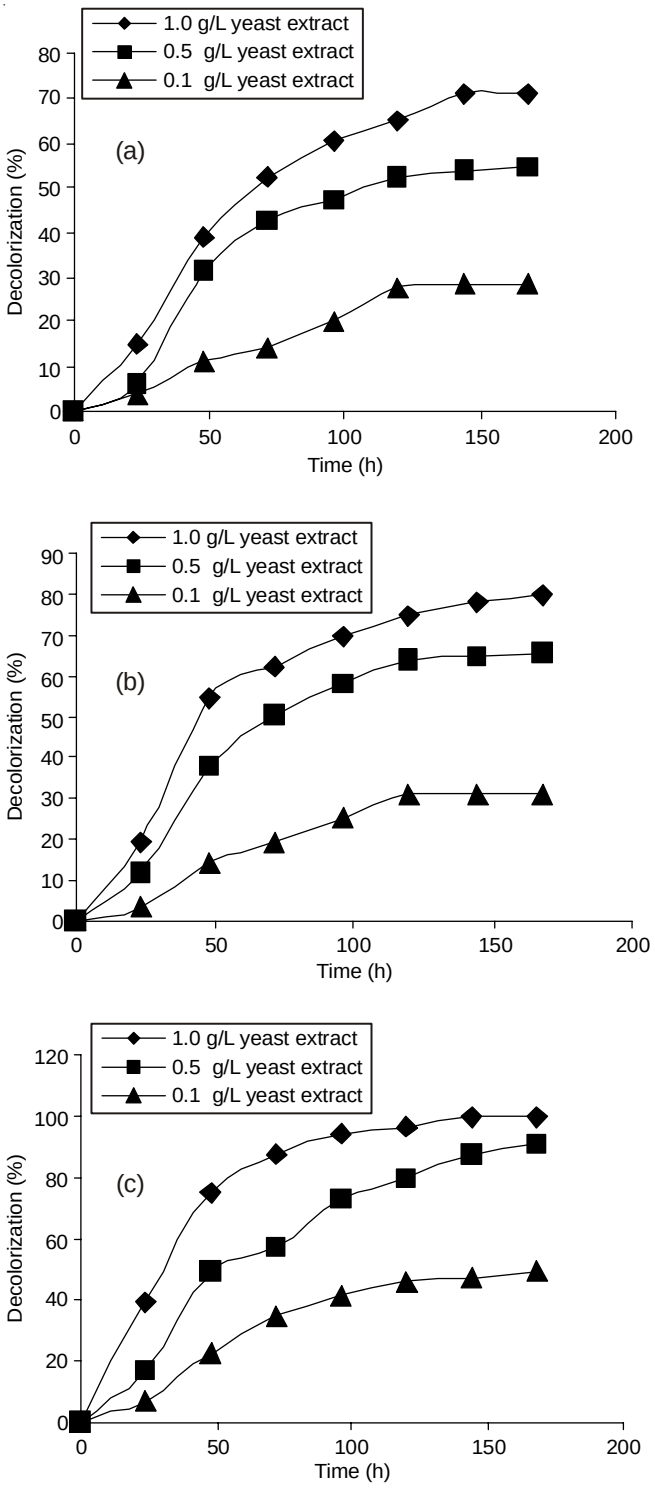

Fig. 4. Effect of yeast extract concentration on decolorization of 20 (a), 40 (b), 60 (c) mg/L Reactive Black 5 at 35 °C

concentrations. In many reports, several azo dyes were decolorized under static conditions by many bacterial strains with complex carbon and nitrogen sources such as peptone, yeast extract and glucose [11,30,31]. Some studies investigated the effect of different nitrogen sources on decolorization of azo dyes and reported that the best decolorization was achieved with the yeast extract. They also reported that the decolorization of azo dyes increased with the increase of yeast extract concentration [19,32-36]. The metabolism of yeast extract is thought to be important for the regeneration of NADH, which behaves as an electron donor during the reduction of azo bond [37]. In many studies, carbon and nitrogen sources were used at high concentrations such as nutrient broth containing 5 g/L peptone and 3 g/L meat extract for decolorization of azo dyes [22,38,39]. Thus they obtained high decolorization efficiencies in a shorter time than this study. However, these high nutrient concentrations may cause potential negative effects such as high COD in the possible application of microorganisms for the decolorization of dye containing wastewater.

During the logarithmic growth phase with 1 g/L yeast extract and 60 mg/L Reactive Black 5 a significant decolorization occurred. The dissolved oxygen also dropped to nearly 0.25 mg/L at the logarithmic growth phase. This result indicates that effective decolorization of Reactive Black 5 by *P. aeruginosa* occurs under low dissolved oxygen levels. Decolorization of azo dyes by bacterial cells is initiated by cleavage of azo bonds by azo reductase [40]. The presence of oxygen normally inhibits the activity of azoreductase. NADH acts as the electron donor for the reduction of azo bonds and aerobic respiration prevents the electron transfer from NADH to azo bonds [41,42]. Decolorization of different dye concentrations and cell growth at different dye and yeast concentrations are given in Fig. 5.

Effect of temperature on decolorization: Temperature of the environment directly establishes bacterial temperature.

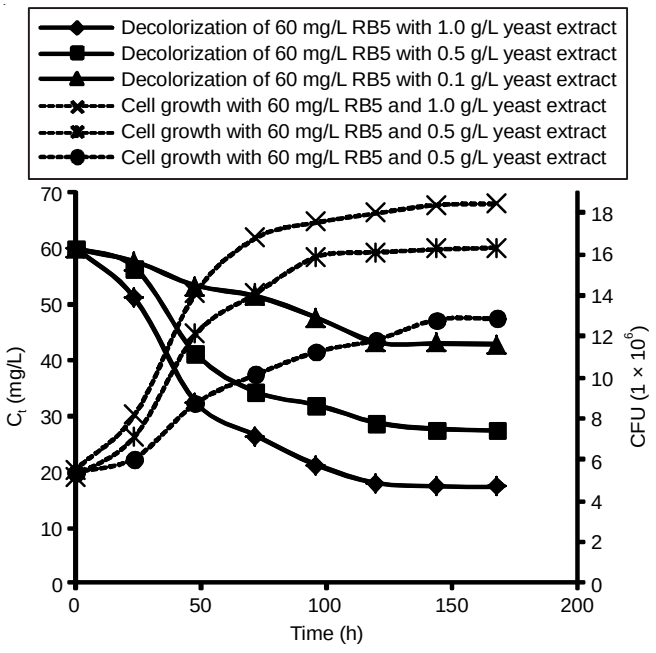

Fig. 5. Decolorization of 60 mg/L Reactive Black 5 and cell growth with 1, 0.5 and 0.1 g/L yeast extract at 35 °C

Microorganisms adapt to the temperature changes by biochemical or enzymatic mechanisms. Eventually, temperature has a considerable effect on microbial processes such as water remediation [43]. The effect of temperature on decolorization was evaluated. From Fig. 6, it can be seen that the temperature had a considerable effect on decolorization of Reactive Black 5 by *P. aeruginosa*. Decolorizing efficiency of Reactive Black 5 increased with increasing temperature. Decolorization of 20 mg/L Reactive Black 5 for 35, 25 and 15 °C were 100, 81 and 70 %, respectively with 1 g/L yeast extract within 144 h.

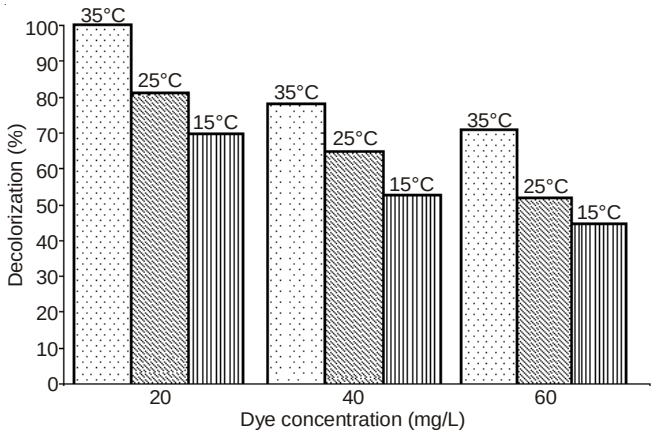

Fig. 6. Effect of temperature on decolorization of 20, 40, 60 mg/L Reactive Black 5 with 1 g/L yeast extract within 144 h

Conclusion

In this study, non-dye acclimatized *P. aeruginosa* was used for decolorization of a diazo textile dye Reactive Black 5, which is resistant to biodegradation. *P. aeruginosa* is able to decolorize Reactive Black 5 with 100 % decolorization efficiency under certain conditions. The results indicate the potential application *of P. aeruginosa* for decolorization of textile dye containing effluents. The culture requires yeast extract as the organic source for decolorization. Only 1 g/L yeast extract is sufficient for complete decolorization of 20 mg/L Reactive Black 5 within 144 h. Temperature has a considerable effect on decolorization. Higher decolorization efficiencies in shorter times may be obtained by using carbon and nitrogen organic sources with high concentrations. Nevertheless, high concentrations of organic sources may cause high chemical oxygen demand and high costs when used as potential applications for the decolorization of textile dye using pure cultures.

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