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Decolorization of Some Textile Dyes Using *Phormidium* sp. in Heterotrophic Culture Conditions

Tugba SENTURK*[1]

Abstract

Cyanobacteria have gained interest in recent decades as intriguing potential bioresources candidates due to their potential applications in biotechnology. Under heterotrophic circumstances, the decolorization of Dianix Blue CC, Benazol Black Zn, and Dianix Yellow Brown CC by the low-cost biosorbent *Phormidium* (Cyanobacteria) with three different initial dye concentrations of 25, 50, and 100 mg/L was examined. For the best dye decolorization, the carbon source, incubation period, temperature, pH, and agitation rate were 10 g/L glucose, 168 h, 40 0C, 8.5, and 60 rpm, respectively. *Phormidium* showed high dye uptake, with maximum efficiency ranging from 20% to 40% (5.47 to 40.04 mgg^{-1}) for Dianix Blue, 22% to 52% (5.95 to-52.32 mgg^{-1}) for Benazol Black ZN and 20% to 68% (13.18 to 20.78 mgg^{-1}) for Dianix Yellow Brown under heterotrophic conditions at all dye concentrations tested. The best color decolorization in terms of maximum efficiency was obtained 57% (57.76 mgg^{-1}) for Dianix Blue, 74% (74.04 mgg^{-1}) for Benazol Black at 100 mg/L and 77% (19.42 mgg^{-1}) for Dianix Yellow Brown at 25 mg/L dye concentrations. The study reveals that the decolorization of dye process using *Phormidium* offers an efficient, quit of charges and environmentally friendly biosorbent for the remediation of textile effluents.

Keywords: *Phormidium*, Decolorization, Heterotrophic culture, Bioremediation.

1. INTRODUCTION

Synthetic dyes and pigments have been widely used for various industries like dying textile fibers, plastic, leather, paper industries etc., to colour their final products [1]. The effluents of these industries are discharged into receiving waters cause damages to the ecological balance, affecting photosynthetic activity in aquatic food web because of curtained light refraction [2-6]. Many investigations focused on different techniques including almost all the known physical (membran flotation), chemical (coagulation, precipitation) and biological techniques (inactivated or activated biosorbents) for removal of dye from wastewaters [7]. Each process alone may not meet the requirements. Therefore, these processes are could be a combination like chemical-biological, biological-chemical, chemical-physical, chemical-chemical etc. Besides, it is necessary to develop a low-cost

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or cost-efficient sorbent or biosorbent that are easily accessible with lofty adsorption capacities. There are number of biosorbents for the treatment of various industrial effluents. Phytoremediation of wastewater from textile industry could be less expensive alternative techniques compared to the physical and chemical methods. Many microorganisms such as fungi, algae and bacteria etc., either in their living or dead cell, have been studied to decolorization methods from wastewaters [8-13]. However, there are few studies concerning the bioaccumulation of unwanted materials from wastewaters by living microalgae [14-16].

The potential synthetic dye uptake capability of a low-priced biosorbent *Phormidium* microalgae (cyanobacteria) is well recognized for its biosorption capacity [17]. *Phormidium* is prokaryotic blue green algae and its biomass can be easily and economically produced [18] and the results of this study supported that the algal biomass can be used as efficient biosorbent for treatment of textile wastewater. Therefore, utilization of living or dead biomass as a biosorbent could be one of the useful features for the regional communities. For this reason, *Phormidium* a cheap and low cost biosorbent has been chosen for this study.

The research objective was to determine decolorization potential of most widely used dyes Dianix Blue CC, Benazol Black Zn and Dianix Yellow Brown CC among textile producers by *Phormidium* cells grown in laboratory condition.

2. MATERIALS AND METHODS

2.1. Microorganism and growth conditions

Prokaryotic blue-green algae *Phormidium* (BDCC 002) cells, used in this study were obtained from Celal Bayar University Biology Department Culture Collection in Manisa, Turkey. The alga was grown in 250 mL erlenmeyer flasks containing 100 mL BG-11 medium, was like that described by Stanier et al. (1971) [19], and incubated under heterotrophic and autotrophic culture conditions.

The culture was incubated under 2.0-3.0 klux with measuring light meter continuous illumination using cool, white fluorescent lamps set on 16:8 h photoperiod. The carbon source, incubation time, temperature, pH and agitation rate were 10 g/L glucose (for heterotrophic culture), 168 h, 40 ^{o}C, 8.5 and 60 rpm, respectively, for the optimum dye decolorization. The pH of the culture medium was adjusted using concentrated (1 M) and dilute (0.01 M) sulfuric acid/sodium hydroxide solutions. The phenol-sulfuric acid method, a very widely used colorimetric method, was used to determine the glucose concentration [20].

Phormidium cells used in the experiment were harvested approximately on the 15th and 20th days of incubation. After completion of the logarithmic growth phase of algal cells, the biomass was settled by centrifugation (6000 rpm, 5 min, 25 °C) and dried in aluminum beakers at 70 °C for 24 hours, then homogenized and pulverized. The powdered biosorbent was passed through a sieve (0.5 mm) and used in biosorption experiments. Two types of culture of biosorbents were prepared and tested for removal of these dyes. One of them was heterotrophic culture (contain 10 g/L glucose) and the other one was autotrophic culture form (no glucose).

All measurements were repeated 3 times, and the results are reported as the average of these replicates.

2.2. Experimental procedures

The tests were performed by adding 0.1 g of biosorbent into 100 mL of dye solutions with the initial dye concentration ranging from 25 to 100 mg/L at pH 8.5 in incubatore (Biosan S 20/60) for 7 days. Initial pH of the dye solutions was adjusted with the addition of 0.1 M of NaOH or 0.1 M of HCl.

2.3. Dye uptake

The stock solution of Biological decolorization of Benazol Black, Dianix Blue and Dianix Yellow Brown, a reactive azo-type textile dyestuff, textile

dyes were obtained from the Ekoten Textile Factory in Turkey. The stock solution was prepared by dissolving of 0.1 g of dye in 100 mL deionized double distilled water.

The working solutions of dyes were prepared by diluting the stock solution to the desired concentrations (showed as 25-100 mg/L) in tables and figures) in each experiment. All of the dye removal experiments carried out in the laboratory were performed in Erlenmeyer flasks containing the desired amount of dye and 100 mL of working solution. Erlenmeyer flasks contained dye only and working solutions were used as controls. The Benazol Black, Dianix Blue and Dianix Yellow Brown concentrationswere determined at 590 nm, 624 nm and 550 nm, respectively using UV–Visible Spectrophotometer (Varian Carry).

The mixed dyes removal efficiency was demonstrated based on the following equation:

The amount of dyes uptake per unit weight of biosorbent (q_t, mg/g); were obtained by the following Eq. (1)

$$qt = \frac{(\mathrm{Co} - \mathrm{Ct}) * \mathrm{V}}{\mathrm{M}}$$

where, *Co* and *Ct* (mg L-1) represent at initial and at *t* time concentrations of dyes in the solution, respectively. *V* (L) is the volume of solution, *M* (g) is the mass of adsorbent.
The dyes removal efficiency was demonstrated based on the following equation: Eq. (2)

$$\text{Removal efficiency } \% = \frac{(\mathrm{C0} - \mathrm{Ce})}{\mathrm{C0}} X100$$

where Co, the concentration of the initial dyes, Ce, the final dyes concentration. All of the experiments were performed in triplicate [21].

3. RESULTS AND DISCUSSION

In this study carried out under laboratory conditions, the removal capacity of synthetic dyes such as azo and anthraquinone, one of the most used dyes in the textile industry, of *Phormidium* cells was investigated as a function of time (1,3,5 and 7d), initial dye concentration (25, 50 and 100 mg/L), and different growth culture conditions (heterotrophic and autotrophic).

Dianix Blue CC, Benazol Black ZN and Dianix Yellow Brown CC, which are widely preferred diazo type dyes, were used to determine the dye removal potential of *Phormidium*. The dye biosorption studies, published in the literature, were investigated the effect of dye concentration with different range of dye concentrations between 20 and 2500 mg/L [22-24]. The dye concentration between 25-100 mg/L was examined in order to reach the closest dye concentration of the real dye containing wastewater which was mentioned by Bilinska et al. (2016) [25].

It was determined that *Phormidium* cells grown in heterotrophic culture medium for 7 days removed the Dianix Blue dye at values ranging from 0.31 mgg^{-1} (min) to 57.76 mgg^{-1} (max) on average, and the determined values are shown in Figure 1. Cells grown in heterotrophic media performed dye removal at higher dye concentration (100 mg/L) rather than low dye concentration (25-50 mg/L). As the dye concentrations increased, the dye-absorbing capacity of *Phormidium* cells increased in direct proportion especially at 100 mg/L. At the end of the dye removal work carried out in a heterororthophic culture, 55% of the Dianix Blue textile dye was removed at 100 mg/L dye concentration at the end of 7 days of incubation.

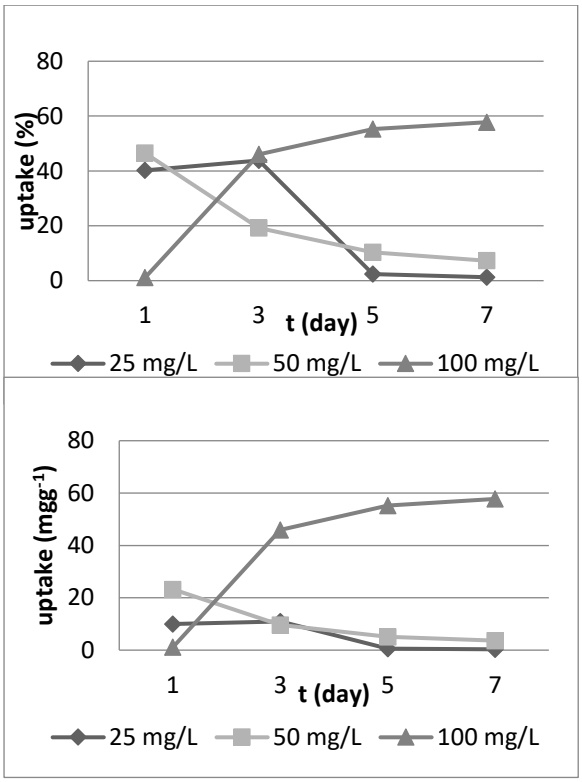

Figure 1 The effect of initial Dianix Blue CC dye concentration on the uptake yield (%, mgg^{-1}) of *Phormidium* sp. during the heterotrophic incubation period

In dye removal studies, the high initial dye concentration creates a significant driving force on the dye resistance between the aqueous and solid phases. As a result, dye uptake increases in parallel with higher concentration. The effect of the initial dye concentration on the biosorption capacity was found to be quite significant for the Dianix Blue CC azo dye used [26]. Ertugrul et al. (2008) [16] reported that, species of micro algae *Phormidium* are potential "biosorbent" for biological decontamination of Remazol Blue and Reactive Black B with maximum uptake yields ranging from 50% to 88% at all dye concentrations tested. In addition, Gul et al. (2019) [13] made research about the dye uptake of the dye acid red P-2BX by *Phormidium animale* (prokaryotic algae)and *Scenedesmus sp.* (eukaryotic algae). They reported that the maximum dye removal was performed by dried *P. animale* as 99.70±0.27% under thermophilic conditions in a batch system.

At high dye concentrations for the Benazol Black (100 mg/L, 1-3th days), uptake yield exceeded 70–75%. At the end of the dye removal study carried out in the laboratory condition, the dye uptake yield of *Phormidium* decreased especially at 25 and 50 dye concentrations; the highest dye removal was obtained in the samples having higher initial dye concentrations under the heterotrophic culture condition. At 100 mg/L dye concentration 74.04 mgg^{-1} of the initial dye was decolorized at the end of 3th day of incubation (Fig. 2).

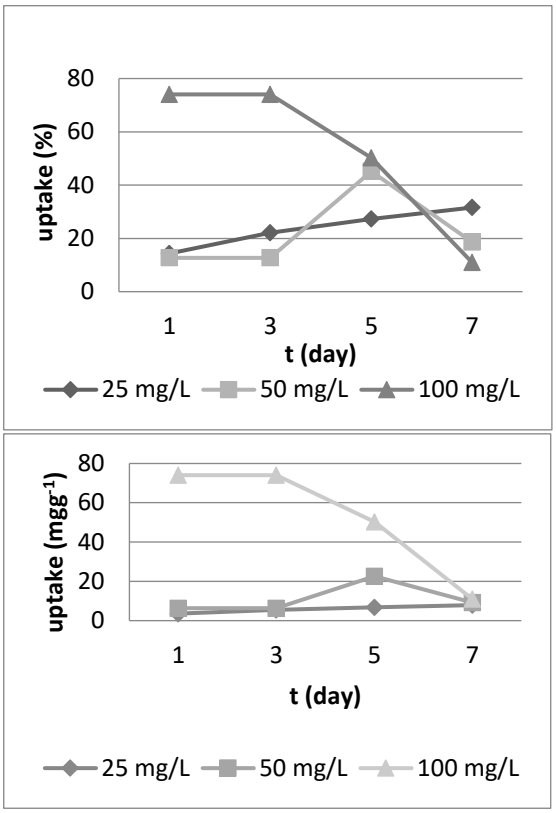

Figure 2 The effect of initial Benazol Black Zn dye concentration on the uptake yield(%, mgg^{-1}) of *Phormidium* sp. during the heterotrophic incubation period

In dye removal studies, the dye removal rate reaches maximum level (saturation) at the end of approximately 3 or 4 days. After that, the removal remains constant or the yield decreases.

In addition, the decrease in the dye removal concentration, especially after the 3rd day, may be due to the saturation (adsorption) of the surface

areas of the cells with the dye molecules and the lack of empty or unsaturated regions [27].

After mixing the microalgae with dye for 7 days at heterotrophic culture, the maximum uptake of Dianix Yellow dye was 77.69% and 69.10%, respectively, at about 25mg/L initial dye concentrations. At the end of the dye removal study, the maximum uptake level was obtained at lower initial dye concentrations at 5th and 7th days of incubation (Fig. 3).

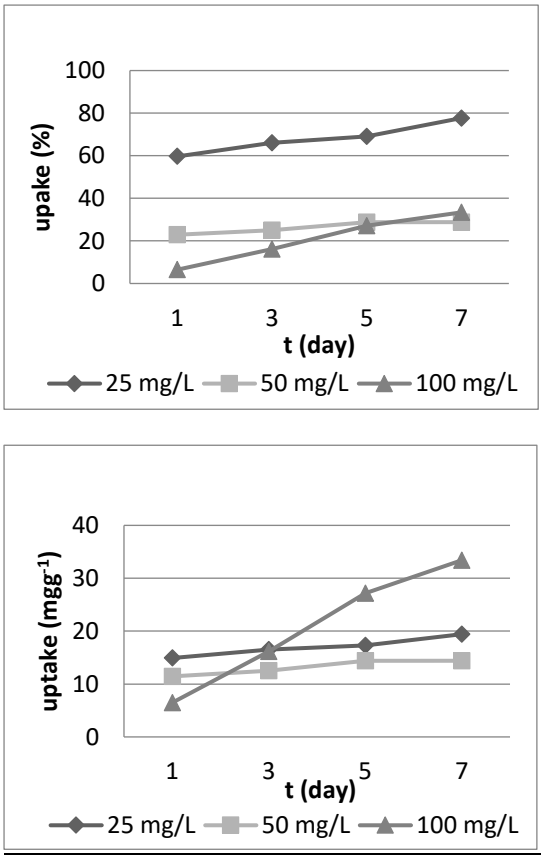

Figure 3 The effect of initial Dianix Yellow Brown CC dye concentration on the uptake yield (%, mgg^{-1}) of *Phormidium* sp. during the heterotrophic incubation period

In present study, *Phormidium* had average decolorization efficiency against both Dianix Blue and Benazol Black at 100 mg/L dye concentration. As the concentrations of the two textile dyes were decreased to 25 mg/L, the dye uptake capacity of cells was found to be decreased. Furthermore, *Phormidium* cells showed lower dye uptake efficiency against Dianix Yellow Brown at high dye concentration when it compared with Benazol Black and Dianix Blue. This may be due to changes in the chemical structures of substances. The decrease in dye removal rate may vary depending on its high molecular weight, structural complexity and the presence of inhibitor groups such as $-NO_2$ and $-SO_3Na$ in the dyes [28].

At the end of the 7 days dye removal, the mean dye uptake (%) value of *Phormidium* was calculated higher in the heterotrophic growth condition than autotrophic growth condition and the results are tabulated in Table 1.

Table 1 The comparison of the mean dye uptake (%) in the heterotrophic and autotrophic culture conditions of *Phormidium* sp.

mean uptake (%) under heterotrophic culture conditions				
	t (day)			
Dyes	1	3	5	7
Dianix Blue CC	29.26	36.33	22.68	22.09
Benazol Black Zn	33.68	36.28	40.89	76.08
Dianix Yellow Brown	29.68	35.72	41.67	46.64

mean uptake (%) under autotrophic culture conditions				
	t (day)			
Dyes	1	3	5	7
Dianix Blue CC	16.86	5.68	8.65	11.55
Benazol Black Zn	18.36	4.45	15.87	33.56
Dianix Yellow Brown	16.41	4.45	16.00	20.25

While the dye uptake concentrations were, in general, less than 34% in the effluent when no glucose was added, a significant increase in the dye uptake concentrations was observed in the heterotrophic growth media. Glucose or other carbon source which supplied an essential substrate for the production of enzyme and growth of the cell was previously shown to be crucial for dye decolorization studies [29].

Table 1 shows the dye uptake rates during the decolorization assays, including the condition where no glucose was added as the autotrophic culture assay. Zhang et al. (1999) [30] investigated that glucose concentration affected the dye uptake values and the most suitable concentration of glucose was about 5 g/L. Contrary to this, Mou et al. (1991) [31] studied the effects of glucose concentration on uptake of dyes by *Myrothecium verrucaria* and observed that the glucose concentration did not significantly influence the bio-dye uptake process.

Phormidium showed high dye decolorization, with maximum efficiency ranging from 20% to 40% (5.47 to 40.04 mgg^{-1}) for Dianix Blue, 22% to 52% (5.95 to-52.32 mgg^{-1}) for Benazol Black ZN and 20% to 68% (13.18 to-20.78 mgg^{-1}) for Dianix Yellow Brown under heterotrophic conditions at all dye concentrations tested. The best color decolorization in terms of maximum efficiency was obtained 57% (57.76 mgg^{-1}) for Dianix Blue, 74% (74.04 mgg^{-1}) for Benazol Black at 100 mg/L and 77% (19.42 mgg^{-1}) for Dianix Yellow Brown at 25 mg/L dye concentrations.

Results obtained in this study suggest that the biosorbent *Phormidium* a microalgae may provide a promising cost effective and environmentally friendly alternative to replace or supplement current treatment processes for the removal of very high concentrations of dyes in industrial wastewater effluents, as they are biodegradable and available for use and retainable.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

The first author contributed 100%.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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