

SINGLE AND BINARY BIOSORPTION OF HEAVY METAL IONS AND A REACTIVE DYE BY *ASPERGILLUS VERSICOLOR*

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ABSTRACT

Biosorption is becoming a promising heavy metal ions and dye removal process in wastewater treatment. Removal of Cr (VI), Cu (II), Ni (II) and Remazol Blue (RB) from aqueous solution by biosorption on dead fungus, *Aspergillus versicolor*, was investigated. The initial concentrations were 50-150 mg L⁻¹ for metal ions and 25-700 mg L⁻¹ for the dye in the removal tests for each of these pollutants. The highest single Cr (VI), Cu (II) and Ni (II) yields removed were 15.82 % (pH 6), 28.47 % (pH 5), and 14.59 % (pH 6) respectively, and RB yield was 91.89 % (pH 5), 73.20 % (pH 6) in optimum conditions. The initial heavy metal ions and dye concentrations were about 50 mg L⁻¹ and 100 mg L⁻¹ for binary effects of heavy metal ions and the dye, respectively. In the experiments for the binary biosorption of the metal ions and the dye, the maximum yields of Cr (VI), Cu (II), and Ni (II) were 17.76 %, 22.71 %, and 22.74 %, and the maximum RB yields were 21.23 % (pH 6), 61.66 % (pH 5) and 51.66 % (pH 6), respectively. This is the first report on biosorption of single and binary heavy metal ions and/or dye by non-living biomass of *A. versicolor*.

KEYWORDS: Biosorption; Fungus; heavy metal ions; Reactive dye; Wastewater treatment

INTRODUCTION

Aquatic pollution by heavy metal ions and dyes has been a major concern over the past decade due to increased industrial production and related discharges. A great number of industries such as textile, paper and pulp, printing, iron-steel, petroleum, paint, chemicals and pharmaceuticals, metal-cleaning, plating, and metal-processing have been releasing these highly toxic pollutants to the environment¹. Thus, removal of heavy metal ions such as Cr (VI), Cu (II) in metal complex dyes and dye has been the object of great attention in the last few years, not only because of the toxicity, but also due to the unpleasant appearance of polluted surface waters. The treatment methods that have been used to remove heavy metal ions and dyes in from wastewaters are coagulation, ozonation, oxidation, Fenton's reagent, NaOCl, chemical precipitation, coagulation, ion exchange, membrane filtration, adsorption and evaporation²⁻³. On the other hand, these technologies are not found economical and often just not feasible⁴.

As also mentioned in this article, the search for new economical and effective alternative treatment methods includes biosorption, which is focusing on the use of biological materials such as fungal, bacterial and algal biomass. Since biosorption is an economical and effective alternative for the removal of heavy metal ions and dyes

from wastewater, the studies on this treatment method is steadily increasing, as reviewed recently⁵.

Several microorganisms such as bacteria, fungi and algae have been tested for their biosorption ability to remove heavy metal ions and dyes⁶⁻²⁰. Many data in the literature confirmed the suitability of this method also for *Aspergillus* sp.^{6-7, 9, 12, 14-16, 19,20}.

The aim of this work was to study the biosorption capacity of non-living fungal strain *A. versicolor* for Cr (VI), Cu (II), Ni (II) and RB reactive textile dye by using both singly and binary combinations of these ions and the dye at batch scale level. *A. versicolor* was chosen as the test microorganism because of its different resistances against high concentrations of toxic substances and the presence of limited studies on this microorganism in the literature²⁴. Molasses as a main carbon source of growth media is widely used as a substrate in microbial fermentations, since it is also a valuable source of trace elements, vitamins and growth substances. Many of studies in line show that molasses stimulates bioaccumulation of microorganisms²¹⁻²³ also *Aspergillus versicolor*²⁴. To our knowledge, this is the first report correlating single and binary biosorption of heavy metal ions and dyes by non-living biomass of *A. versicolor*, which indicates its high efficiency in terms of bioremoval rate and capacity and the need for the further studies on this biosorption material.

MATERIALS AND METHODS

Microorganism and growth conditions

A. versicolor was isolated from Batman, Turkey, as described in our previous study²⁴. Fungal cells were cultivated at 30 ± 1 °C in 250 ml Erlenmeyer flasks containing 100 ml of sterile molasses medium, on a rotary shaker (New Brunswick Scientific Innova 4230) at 100 rpm constant shaking rate for 4 days²⁵. The pH of the medium was adjusted to 6 with dilute (0.01 M) or concentrated (1 M) H₂SO₄ and NaOH solutions.

Preparation of the microorganism for biosorption

After the growth period, the biomass was harvested from the medium and washed twice with distilled water, and then autoclaved for at least 15 min. at 121 °C, 0.99 bar. For the biosorption studies, a weighed amount of wet biomass was taken and measured by filtering an aliquot of the culture suspension through preweighed filters (Whatman No 1). At the beginning of biosorption, the wet cells that correspond to the dry weight of biomass as 6.62 g L⁻¹ were contacted with 100 ml of distilled water containing a known concentration of heavy metal ions and/or dye.

Heavy metal ions and dye solutions

Stock solutions of Cr (VI), Cu (II), and Ni (II) were prepared by dilution of potassium bichromate (Merck), copper sulfate (Merck), and nickel sulfate heptahydrate (Merck) to a final concentration of 10 g L⁻¹ of Cr (VI), Cu (II), and Ni

(II), respectively. RB is a dye commonly used in the cotton textile industry also in Turkey. It was obtained from Aytemizler Textile Co., Turkey, in pure form. The dye stock solution was prepared by dissolving the powdered dyestuff in distilled water to a final concentration of 2% w/v, appropriate volumes of the stock solutions were added to the media.

The aqueous media containing desired combinations of heavy metal ions and the dye were prepared by diluting stock solutions of heavy metal ions and dye, and mixing them in aqueous media. The initial pH value of each solution was adjusted to the required value with diluted or concentrated H₂SO₄ and NaOH solutions before contacting with the fungal biomass.

Batch biosorption studies

The experiments were conducted by the batch technique in 250 ml Erlenmeyer flasks containing 100 ml of distilled water at desired level of heavy metal ions and/or dye. The fungal cells were transferred into aqueous media and the flasks were agitated on a shaker at 100 rpm. During the biosorption period (15^o, 30^o, 1, 2, 4, 6 h), 3 ml sample was taken from each flask. Before analysis, the samples were centrifuged at 5000 rpm for 5 min using Hettich EBA12 model centrifuge and the supernatant fractions were analyzed for the remaining heavy metal ions and/or dye. All of the biosorption experiments were repeated twice to confirm the results.

To determine the single effect of initial heavy metal ions and dye concentrations, *A. versicolor* were transferred into aqueous media containing 50-150 mg L⁻¹ Cr (VI) (pH 6), Cu (II) (pH 5), Ni (II) (pH 6) and 25-750 mg L⁻¹ RB (pH 5 and 6). In order to determine the binary effects of initial heavy metal ions and dye concentrations, the cultures were transferred into aqueous media at pH 6 for Cr (VI) + RB and Ni (II) + RB, pH 5 for Cu (II) + RB containing 50 mg L⁻¹ heavy metal ions and 100 mg L⁻¹ dye.

Analytical Methods

The concentration of Cr (VI) in the supernatant was determined spectrophotometrically at 540 nm using diphenyl carbazide reagent in acid solution as the complexing agent. The concentrations of Cu (II) and Ni (II) in the supernatant were determined spectrophotometrically at 460 and 340 nm respectively, by using sodium diethyl dithiocarbamate as the complexing agent²⁶. RB concentration was determined by measuring the absorbance at 600 nm (Shimadzu UV 2001 Spectrophotometer)

The heavy metal ions and dye biosorption properties of *A. versicolor* were investigated in a batch system as a function of dye and heavy metal ions concentrations. The percentage removal of heavy metal ions or dye was calculated from Eq. (1):

$$\text{Removal (\%)} = (C_o - C_f) / C_o \times 100 \quad (1)$$

The biosorption capacity of heavy metal ions or dye is the concentration of heavy metal ions or dye in the biomass and can be calculated based on the mass balance principle from Eq. (2):

$$q_m = (C_o - C_f) / X_m \quad (2)$$

In these two equations, q_m (the maximum specific dye or heavy metal ions removal) represents the maximum amount of heavy metal ions or dye per unit dry weight of fungus (mg g^{-1}), X_m the maximum dried cell mass (g L^{-1}), and C_o and C_f the initial and final concentrations (mg L^{-1}) respectively.

RESULTS AND DISCUSSIONS

Single effects of heavy metal ions

Four heavy metal ion concentrations (about 50–150 mg L^{-1}) were studied in the aqueous media to investigate the effect of increasing heavy metal ion concentrations on the removal yield by *A. versicolor* at the optimum pH levels for each heavy metal²⁴ (Table 1). The removal yields of all three heavy metal ions decreased with increasing initial heavy metal ion concentrations up to 150 mg L^{-1} . The maximum Cr (VI) removal (15.82 %) was observed at 52.95 mg L^{-1} (Table 1). On the other hand, there was no significant difference between the Cr (VI) and the Ni (II) removal yields. For example, the highest Ni (II) removal yield was 14.59 % at 49.32 mg L^{-1} Ni (II) concentration and the lowest Ni (II) removal yield was 4.59 % at 109.77 mg L^{-1} Ni concentration, which was very near to the lowest Cr (VI) removal yield, 4.57 % at 148.95 mg L^{-1} Cr (VI) concentration at 1 hour (Table 1). The fungus was capable of removing Cu (II) in an efficient way. The maximum and the minimum biosorption yields for Cu (II) were 28.47 % at 35.12 mg L^{-1} and 17.24 % at 154.35 mg L^{-1} Cu (II) concentrations at 1 h, respectively.

Table 1. Removal of heavy metal ions by *A. versicolor* (T: 30 ± 1 °C; Stirring rate: 100 rpm; Biosorption time: 1 h).

pH	Heavy metal ions	C_o (mg L^{-1})	Y %
6	Cr (VI)	52.95	15.82 ± 1.14
		74.71	11.04 ± 1.15
		105.66	6.56 ± 1.30
		148.95	4.57 ± 0.36
5	Cu (II)	35.12	28.47 ± 0.84
		82.05	23.43 ± 1.26
		108.58	22.55 ± 0.61
		154.35	17.24 ± 1.34
6	Ni (II)	49.32	14.59 ± 0.53
		69.32	12.33 ± 1.75
		94.96	8.34 ± 0.73
		109.77	4.59 ± 0.15

The effect of increasing Cu (II) concentrations on biosorption by *A. flavus* in a liquid growth medium composed of the glucose and several components was reported in the literature¹², and it was found that the Cu (II) biosorption efficiency was 20.00 % at 100 mg L⁻¹ and 14.43 % at 150 mg L⁻¹ Cu (II) concentrations at pH 5.0 with equilibrium time of 2 h. Results showed that Cu (II) biosorption effects of *A. versicolor* and *A. flavus* was close to each other.

Single effects of the dye

A. versicolor was also tested for the ability to decolorize RB in the aqueous media without heavy metal ions at different initial dye concentrations. *A. versicolor* had much higher tolerance to dye molecules, compared to the heavy metal ions. The RB concentrations varying from 25.08 to 729.94 mg L⁻¹ and from 25.31 to 723.16 mg L⁻¹ were tested for pH 5 and 6, respectively and it was found that there was no significant difference between pH 5 and 6 for the dye removal yields. The highest biosorption capacity of RB was 91.89 % for pH 5 and 73.20 % for pH 6 in the presence of about 25 mg L⁻¹ RB while the lowest RB removal yield was 13.93 % for pH 5 and 14.37 % for pH 6 in the presence of about 750 mg L⁻¹ RB concentrations at 1 h "Fig. (1)". After 1 h, it was observed that the biosorption capacity did not change.

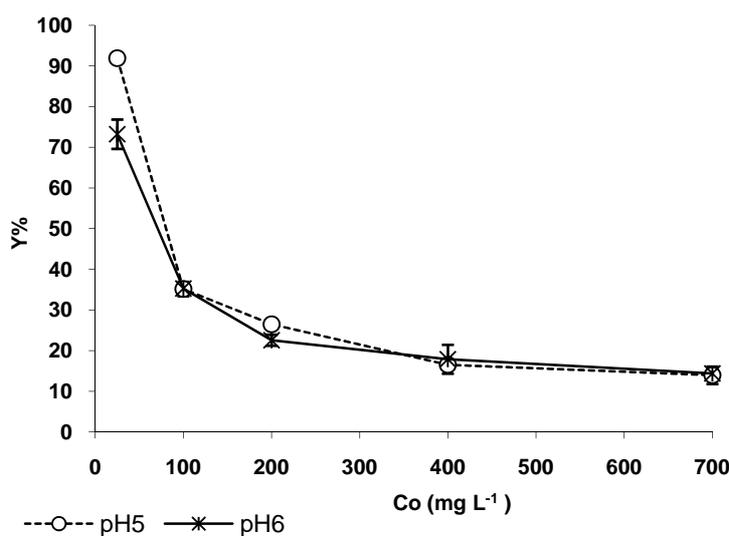


Figure 1. Removal of RB by *A. versicolor* (T: 30 ± 1 °C; Stirring rate: 100 rpm; Biosorption time: 1 h)

It was investigated the effect of biomass concentration on biosorption yield by Khalaf¹⁶ and tested the removal of dyes from textile wastewater containing 0.22 % Synazol Red HF6BN and 0.1 % Synazol Yellow HF2GR reactive dyes by *A. niger* and found a maximum dye removal of 84 % at biomass concentration of 8 g L⁻¹. In the present study, *A. versicolor* showed the maximum RB biosorption capacity (91.89 %) at 6.62 g L⁻¹ biomass concentration.

Binary effects of heavy metal ions and dye

For the binary removal studies of the heavy metal ions and the dye, the initial heavy metal ions concentration chosen was about 50 mg L⁻¹, which was the value at which the fungus could remove this pollutant efficiently. The selected metal concentration was 100 mg L⁻¹ for the dye removal studies due to the fact that the removal yield was very high at the lowest RB concentration (25 mg L⁻¹). In the biosorption medium of both Cr (VI) (45.04 mg L⁻¹) and RB (120.22 mg L⁻¹) at pH 6, the removal yield for Cr (VI) (17.76 %) was close to the yield of its single effect (15.82 %), while the removal yield for RB (21.23 %) was lower than the yield of its single effect (35.25 %) “Fig. (2)”. The statistical analysis indicated that although relatively high biosorption efficiency of *A. versicolor* was obtained for the single removal of Cr (VI) and dye, biosorption of both the components from binary mixture was affected negatively by the addition of other component at about 50 mg L⁻¹ due to inhibition caused by at this concentration rate.

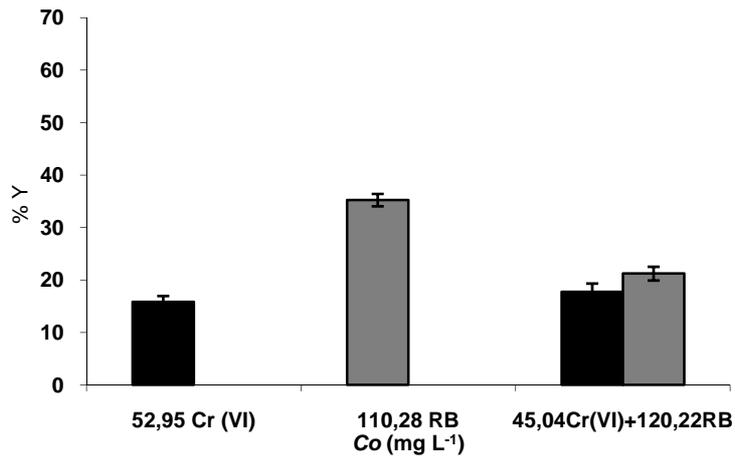


Figure 2. The single and binary effects of fixed Cr (VI) and RB concentrations (C_0) on the biosorption yield (Y %) of *A. versicolor* with about 50 mg L⁻¹ heavy metal ions and 100 mg L⁻¹ dye concentrations (pH: 6; T: 30 ± 1 °C; Stirring rate: 100 rpm; Biosorption time: 1 h).

The dye and Cu (II) removal yields were 61.66 % and 22.71 %, respectively in the biosorption medium containing 146.21 mg L^{-1} dye and 68.01 mg L^{-1} Cu (II) at pH 5. Lower removal yield for Cu (II) in the biosorption medium than that of only Cu (II) medium showed that the Cu (II) removal was not affected by the presence of the dye in the biosorption medium “Fig. (3)”.

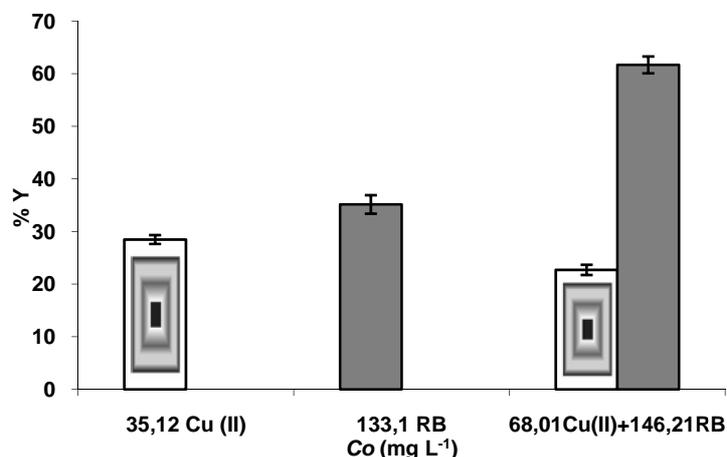


Figure 3. The single and binary effects of fixed Cu (II) and RB concentrations (C_0) on the biosorption yield (Y %) of *A. versicolor* with about 50 mg L^{-1} heavy metal ions and 100 mg L^{-1} dye concentrations (pH: 5; T: 30 ± 1 °C; Stirring rate: 100 rpm; Biosorption time: 1 h).

On the other hand, for Ni (II), the removal yield (22.74 %) in the biosorption medium with 126.77 mg L^{-1} dye was higher than that of only Ni (II) medium at pH 6 (14.59 %). Moreover, there was a significant difference for the dye removal in the presence of Cu (II) and Ni (II). The removal of the dye was about 30.00 % in the medium containing only dye, but it was 61.66 % in the medium containing both Cu (II) and the dye at pH 5 and 51.66 % in the medium containing both Ni (II) and the dye at pH6 “Fig. (4)”.

In contrast to binary biosorption of Cr (VI) and dye, *A. versicolor* was not affected by the presence of Cu (II) and Ni (II) in dye containing media. Some of the Cu (II) containing process was found to be very effective in the decolorization²⁷ and uptake of dyes due to the synergistic effects of Cu (II) and dye biosorption²⁸. In present study Ni (II) and dye biosorption indicated the same effects as Cu (II) and dye biosorption due to their same valance.

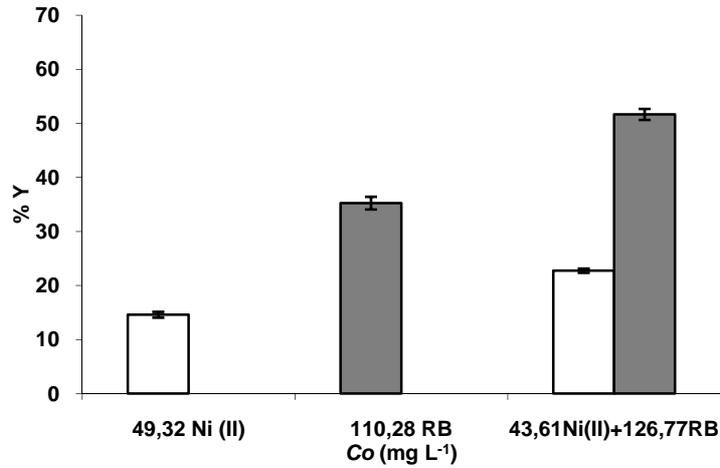


Figure 4. The single and binary effects of fixed Ni (II) and RB concentrations (Co) on the biosorption yield (Y %) of *A. versicolor* with about 50 mg L^{-1} heavy metal ions and 100 mg L^{-1} dye concentrations (pH: 6; T: 30 ± 1 °C; Stirring rate: 100 rpm; Biosorption time: 1 h).

The data given in Table 2 showed how the presence of both pollutants affected the capacity of the fungus in removing heavy metal ions and dye within the specified period. The maximum specific uptake value of the dye (5.87 mg g^{-1}) in the medium with Cr (VI) and the dye decreased when compared with the value in the medium without Cr (VI) (3.86 mg g^{-1}). On the other hand, there was no significant difference in the maximum specific uptake of Cr (VI) values between the samples with or without dye (Table 2).

Table 2. Single and binary removal of constant heavy metal ions and dye concentrations (Co) on the biosorption yield (Y %) and maximum specific uptake (q_m) of *A. versicolor* with about 50 mg L^{-1} heavy metal ions and 100 mg L^{-1} dye concentrations (pH: 6 Cr (VI), Ni (II) and RB; pH: 5 Cu (II) and RB; T: 30 ± 1 °C; Stirring rate: 100 rpm; Biosorption time: 1 h)

Pollution		Co (mg L ⁻¹)	Y %	q_m (mg g ⁻¹)
Cr (VI)		52.95	15.82 ± 1.14	1.26
RB		110.28	35.25 ± 1.18	5.87
Cr (VI) + RB	Cr (VI)	45.04	17.76 ± 1.58	1.21
	RB	120.22	21.23 ± 1.30	3.86
Cu (II)		35.12	28.47 ± 0.84	1.51
RB		133.10	35.14 ± 1.76	7.06
Cu (II) + RB	Cu (II)	68.01	22.71 ± 0.97	2.33
	RB	146.21	61.66 ± 1.60	13.61
Ni (II)		49.32	14.59 ± 0.53	1.08
RB		110.28	35.25 ± 1.18	5.87
Ni (II) + RB	Ni (II)	43.6	22.74 ± 0.39	1.50
	RB	126.77	51.66 ± 1.02	9.89

The highest removal yield and the maximum specific removal were observed for RB in the media with Cu (II) and RB (Table 2). When Cu (II) and dye were both present in the media, the maximum specific Cu (II) and RB removal increased (Table 2).

To date, most of the studies on the topic relating to the measure of single effects of heavy metal ions or dye biosorption have been observed^{7-8, 11, 13, 15, 19, 20} but, to our knowledge binary effects of heavy metal ions and dye biosorption of *Aspergillus* sp. have not been investigated in the literature. It was found that *A. versicolor* had bioremoval capacity of single and binary heavy metal ions and/or dyes²⁴. Biosorption was found to be the most effective with a yield capacity of 15.82 %, 28.47 % and 14.59 %, (1 h) compared with bioaccumulation with a yield capacity of 21.32 %, 4.52 %, and 8.01 %, (24 h) for Cr (VI), Cu (II), and Ni (II), respectively. A comparison of the yields of biosorption and bioaccumulation for RB was about 35.00 % (pH 5 and 6) for biosorption and 17.46 % (pH 5) and 22.07 % (pH 6) for bioaccumulation. Table 3 presents a comparison of bioaccumulation and biosorption yields of *A. versicolor* at their working conditions. The use of non-living biomass of *A. versicolor* in biosorption is more useful than bioaccumulation for water treatment, as the removal takes less time and continuous supply of nutrients is not required.

Table 3. Single and binary removal of constant heavy metal ions and dye concentrations (C_0) on the bioaccumulation²⁶ and biosorption yield (Y %) of *A. versicolor* during the incubation period with about 50 mg L⁻¹ heavy metal ions and 100 mg L⁻¹ dye concentrations (pH: 6 for Cr (VI), Ni (II) and RB; pH: 5 for Cu (II) and RB; T: 30 ± 1 °C; stirring rate: 100 rpm; biosorption time: 1 h; bioaccumulation time: 24 h; biosorption media: aqueous media; bioaccumulation media: molasses media).

Pollutant		Biosorption	Bioaccumulation
		Y %	Y %
Cr (VI) + RB	Cr (VI)	22.56 ± 3.21	17.76 ± 1.58
	RB	4.15 ± 3.30	21.23 ± 1.30
Cu (II) + RB	Cu (II)	4.66 ± 3.00	22.71 ± 0.97
	RB	18.63 ± 4.00	61.66 ± 1.60
Ni (II) + RB	Ni (II)	6.36 ± 1.78	22.74 ± 0.39
	RB	7.80 ± 1.30	51.66 ± 1.02
Cr (VI)		21.32 ± 1.25	15.82 ± 1.14
Cu (II)		4.52 ± 3.10	28.47 ± 0.84
Ni (II)		8.01 ± 1.53	14.59 ± 0.53
RB pH 5		17.46 ± 3.50	35.14 ± 1.76
RB pH 6		22.07 ± 2.00	35.25 ± 1.18

CONCLUSIONS

Biosorption offers an economically feasible technology for efficient removal of pollutants from aqueous solutions; it will be particularly useful for the treatment of industrial effluents, carrying heavy metal ions and dyes. The following conclusions can be drawn from the present study:

- Results obtained from this work showed that biomass production of *A. versicolor* is simple and economical.
- The biosorption process is affected by increasing heavy metal ions and dye concentrations as expected.
- The experimental evidence shows the strong effect of the high biosorption rate on the performance of non-living biomass of *A. versicolor* for single and binary effects of heavy metal ions and/or dye.

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ÖZET: Biyosorpsiyon atksu arıtımında kullanılan, gelecek vaat eden bir metottur. Çalışmada, ölü *Aspergillus versicolor* fungusu ile sucul ortamdan Cr (VI), Cu (II), Ni (II) ve Remazol Blue (RB) boyasının tekli ve ikili giderimleri araştırılmıştır. Ağır metal iyonları için başlangıç konsantrasyonları 50-150 mg L⁻¹, RB için ise 25-700 mg L⁻¹ olarak belirlenmiştir. En yüksek Cr (VI), Cu (II) ve Ni (II) giderim verimleri sırasıyla; 15.82 % (pH 6), 28.47 % (pH 5) ve 14.59 % (pH 6) olarak, RB giderim verimleri ise 91.89 % (pH 5) ve 73.20 % (pH 6) olarak kaydedilmiştir. İkili etki çalışmaları için başlangıç ağır metal iyon ve boya konsantrasyonları sırasıyla yaklaşık 50 mg L⁻¹ ve 100 mg L⁻¹ olarak seçilmiştir. İkili etki çalışma sonuçlarına göre en yüksek Cr (VI), Cu (II) ve Ni (II) giderimleri sırasıyla; 17.76 %, 22.71 %, and 22.74 % ve RB verimleri 21.23 % (pH 6), 61.66 % (pH 5) ve 51.66 % (pH 6) olarak kaydedilmiştir. Çalışma literatürde *A. versicolor* ile ağır metal iyonları ve boyanın tekli ve ikili biyosorpsiyon etkilerinin araştırıldığı ilk çalışmadır.

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