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# Decolorization of Remazol Brilliant Violet 5R and Brilliant Green dyes by *Hypnum cupressiforme* using response surface methodology-central composite design methodology: toxicity assessment studies by *Daphnia magna*

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**ABSTRACT**

This study is the first report that aims to optimize the removal of Remazol Brilliant Violet 5R (RBV 5R) and Brilliant Green (BG) from synthetic solutions using *Hypnum cupressiforme* samples from Abant Mountains of Bolu (Turkey) as biosorbent and to conduct toxicity evaluation studies of treated wastewater using *Daphnia magna*. Optimization was performed using central composite design under response surface methodology with the operational factors of dye concentration, *H. cupressiforme* dose, and process time. The optimal conditions for dye concentration, *H. cupressiforme* dose, and process time obtained by the numerical analysis were 19.02 mg/L, 0.18 g/50 mL and 110.59 min for RBV 5R, while these values were 18.57 mg/L, 0.18 g/50 mL and 99.45 min for BG, respectively. At the optimum values, maximum RBV 5R and BG removal were achieved as 99.39% and 99.22%, respectively. The *D. magna* mortality rates showed that treatment with adsorption were sufficient to remove the complete toxicity of dyes especially for RBV 5R.

*Keywords:* Decolorization; Response surface methodology; *Hypnum cupressiforme*; *Daphnia magna*; Dye

## 1. Introduction

Dye wastewater from the textile industry is one of the most dangerous reagents that can cause serious health problems in both humans and animals, as well as polluting water. The low biodegradability of these substances is due to their higher molecular weight, complex structural formulas and including metal ions [1,2]. Since these problems require serious attention, wastewater containing dyestuffs must be treated before being discharged into the receiving environment.

Physical, chemical, and biological methods are traditional methods for the treatment of dyes from wastewater [3]. Adsorption, which is one of the possible methods, is not only effective for removing organic dyes and dye pigments from wastewater, but it is also a simple and economical technique, and therefore it can be made suitable for use as drinking water in the community [4].

It was reported by Narayan et al. [5] that about 12% of most synthetic dyes such as methyl orange, methyl red, methylene blue, rhodamine B, remazol bright blue, Congo red, and about 12% of many other dyes were removed.

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In addition, it has been determined that approximately 20% of the total dyestuff produced in the world is removed in the dyeing process, and it is exposed to intense environmental pollution by leaving it to the receiving environments without undergoing any purification process. All these chemical pollutants have high toxicity, carcinogenicity and accompanying mutagenic effects.

Reactive dyes are widely used in industrial dyeing plants due to their highly soluble and photolytic stability and resistance to microbial degradation [6]. Remazol brilliant blue reactive (RBBR) dye is also a type of reactive dye that is resistant to degradation due to its anthraquinone structure [7]. This stands out as a positive factor, since the color of the produced textile article does not change during its use, and it only prevents dye removal from wastewater [8]. RBBR is a type of synthetic dye that provides a wide variety of bright and colorful tones and is mostly applied to cotton surfaces and is also widely used in the textile industry [9].

Reactive dyes are present in 45% of the textiles produced in the world. Remazol bright blue (RBB), remazol black (RB) and remazol bright purple (RBV) are examples of reactive dyes. They have positive features such as bright color, simple application techniques, low energy consumption in dyeing process and high solubility in water [10].

Brilliant Green (BG) is a type of cationic and synthetic dye widely used in the paper, textile, plastic, paint, and printing industries, and it has been reported in the literature to be mutagenic and carcinogenic for human health [11]. In addition, industrial wastes containing high levels of BG and having high solubility in water interfere with the photosynthesis cycle of aquatic plants, cause the oxygen levels in the water to decrease and the flora and fauna of the receiving aquatic environments to drown [12]. As a result, the effective, healthy, and economical removal of BG paint from wastewater is of primary importance to eliminate the eco-toxic hazards that this will create.

With mutagenic and carcinogenic effects affecting aquatic biota and humans, BG is a kind of triphenylmethane dye and is widely applied as an antiseptic. In addition, BG can cause permanent injuries to the eyes of animals and especially humans, and therefore eye burns [13]. Therefore, there was a need for effective methods for the disposal of BG from wastewater.

The focus of recent research has been on the development of environmentally friendly systems for the removal of pollutants from waste [14]. Green technology green technology used from plant-based sources is an environmentally friendly alternative method due to its biodegradability and non-toxicity [15]. The main features of this technology are its cheapness, reusability, and biodegradability [14]. Algae, bacterial strains, fungi, agricultural wastes, and their residues are the most important materials used as biosorbent.

Biosorption processes have been known for a long time and have become a necessity in recent years as it has emerged as a low-cost, environmentally friendly technology. Biosorption processes are the transfer of chemicals from aqueous solutions to biological materials through biological, physical, and chemical methods [16]. During this function, biomaterials will have to passively bind to non-living cells, take up and accumulate pollutants from wastewater by metabolically mediated or physico-chemical means in living cells [17]. Special mechanisms that provide metal uptake include extracellular and intracellular accumulation and cell surface sorption/precipitation [18]. Recently, several materials have been investigated for potential application of adsorbents [19]. In recent years, international research has focused on improving adsorption processes by changing and/or improving strong, efficient, and inexpensive adsorbents with high absorption capacity.

An effective tool for constructing the empirical model that can determine the useful static relationship between all variables in experimental design is the response surface methodology. Response surface methodology was used for this statistical analysis and estimation of optimum conditions was proposed. In the experimental stages, the input variables were changed to the analysis output response [20].

*Hypnum cupressiforme* is a plant belonging to Bryophyta division, Bryopsida class, Hypnales order, Hypnaceae family. It is one of the 16 taxa of the genus Hypnum in Turkey [21]. *H. cupressiforme* is a semi-neutral, semi-arid and shade-loving taxon that spreads on tree trunks, rock surfaces, dead logs, and soil surfaces [22,23]. *Hypnum cupressiforme* was chosen as an adsorbent to determine the treatment efficiency of water exposed to dyestuff pollution, due to its wide distribution on the earth and its widespread use in trace element removal studies [24].

The first aim of the study is to optimize the decolorization of Remazol Brilliant Violet 5R (RBV 5R) and BG dyes from aqueous solution with response surface methodology (RSM) using *H. cupressiforme*. The second-aim is to reveal out the toxic effect of the wastewater with *Daphnia magna* bioassays before and after treatment process with *H. cupressiforme*. As a result of the study, the effect of treated wastewater with *H. cupressiforme* to the model living *D. magna* is seemed to be clear, high significant and untoxic.

## 2. Materials and methods

### 2.1. Collecting Hypnum cupressiforme

*H. cupressiforme* samples were taken from the Abant Mountains in the Omerler region of Bolu, at an altitude of 865m, at the coordinates of N 40° 41′ 24.6″ E, 31° 26′ 35.5″ (Fig. 1). These samples were then taken to the Munzur University Environmental Engineering Department Laboratory. These samples were first dried and ground at room conditions and then passed through a 250 μm sieve.

### 2.2. RBV 5R and BG biosorption experiments

Remazol Brilliant Violet 5R (molecular formula $C_{20}H_{16}N_3Na_3O_{15}S_4$, $M_r$ = 735.58 g/mol) and Brilliant Green (molecular formula $C_{27}H_{34}N_2O_4S$, $M_r$ = 482.65 g/mol) were purchased from Sigma-Aldrich. Stock solutions of dyes were prepared using distilled water. Batch biosorption experiments were carried out under the conditions determined according to the central composite design (CCD) design matrix. The experiments were carried out in 250 mL Erlenmeyer flasks containing 50 mL of RBV 5R or BG solution of known concentration at 25°C, at the natural pH of the solutions, and using a stirring speed of 250 rpm. The samples taken at the specified time intervals were centrifuged at

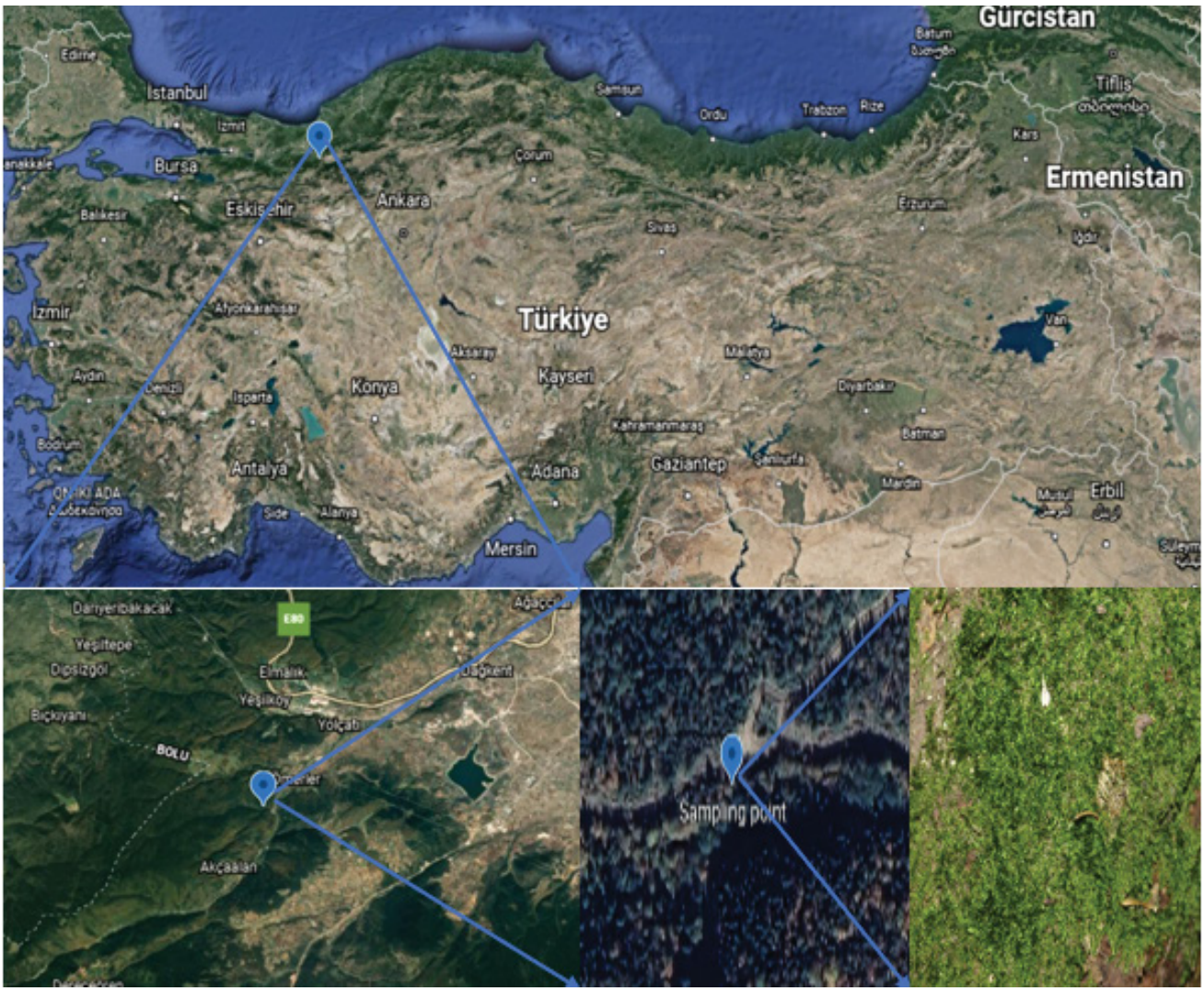


Fig. 1. Sampling point.

5,000 rpm for 10 min and the amount of dye remaining in the supernatant was determined using a UV-Vis spectrophotometer (Shimadzu, UV-1800, Japan) at 553 and 625 nm wavelengths for RBV 5R and BG, respectively. The dye removal yields were calculated using the equation below:

$$R\% = \frac{C_0 - C_t}{C_0} \times 100 \tag{1}$$

where $R$ is the removal yield of dye, $C_0$ and $Ct$ are the dye concentrations (mg/L) before and after biosorption, respectively.

### 2.3. Design of experiments with CCD

To determine the optimum conditions for the uptake of RBV 5R and BG dyes separately on *H. cupressiforme*, CCD under RSM was used as an appropriate design optimization approach. This model provides a reduction in the number of experimental trials required to examine the primary effect of each factor and the synergistic interactions of the factors. The Design-Expert 7.0 (Stat-Ease Inc., USA) software was used as a statistical tool for designing the experiments and performing statistical analysis. In this study, 3-factors ($X_1$, $X_2$ and $X_3$) and 5-levels ($-\alpha$, 1, 0, 1 and $+\alpha$) CCD were used with few experimental runs to evaluate and optimize removal efficiencies for both pollutants (RBV 5R and BG). The ranges of factors (input factors) were dye concentration ($X_1$, 10–50 mg/L), *H. cupressiforme* dose ($X_2$, 0.02–0.30 g/50 mL) and process time ($X_3$, 5–150 min). The response factor (output factor) is the removal yield of either RBV 5R or BG. The chosen factors and their levels are given in Table 1. The number of trial runs required for a total of three factors was determined as 20 (=$2n + 2n + 6$, where $n$ is the number of factors). In the optimization procedure, a quadratic model equation for response surface analysis when the response is quadratic can be given as follows:

$$Y = b_0 + \sum_{i=1}^{3} b_i A_i + \sum_{i=1}^{3} b_{ii} A_i^2 + \sum_{i=1}^{3-1} \sum_{j=i+1}^{3} b_{ij} A_i A_j \tag{2}$$

where $Y$ is the predicted response (% removal yield of RBV 5R or BG), $b0$, $bii$, $bi$, and $bij$ are the constant, quadratic, linear and interactive coefficients, respectively. $Xi$ ($i$ = 1–3) are the number of chosen independent factors.

### 2.4. D. magna mortality test

The reliability of the study was performed with mortality tests on *D. magna*. *D. magna* individuals were obtained from the current culture in our laboratory. The daily dark-light cycle was used for the photoperiod times of the *Daphnia* individuals. For bioassay experiments five groups were designed as shown in Table 2.

Then for transferring of 20 *Daphnia* individuals to in each polycarbonate containers, a filter (1.5 mm mesh) was used to ensure equal development levels or sizes of individuals for each application. Similar genetic strains were used, as the breeding conditions of the individuals were under constant surveillance. Number of dead *Daphnia* individuals in each container were counted after 24, 48 and 72 h. The mortality rates for each period were calculated as percentages on each experimental group. The death criteria adopted were lack of movement even after mild stimulation [25].

## 3. Results and discussion

### 3.1. Statistical analysis

20 experiments based on CCD with various combinations of factors were designed to examine the efficacy of *H. cupressiforme* on the RBV 5R and BG biosorption process, and the experimental (actual) and predicted responses from the CCD experiments are presented in Table 3. Eqs. (3) and (4) show the quadratic models obtained for RBV 5R and BG given with values coded according to the response:

$$\text{RBV 5R removal}(\%) = +95.29 - 3.06X_1 + 3.01X_2 + 2.37X_3 + 1.32X_1X_2 - 0.32X_1X_3 + 0.010X_2X_3 - 0.017X_1^2 - 5.14X_2^2 - 1.43X_3^2 \quad (3)$$

$$\text{BG removal}(\%) = +94.55 - 3.68X_1 + 5.63X_2 + 2.28X_3 + 0.53X_1X_2 - 1.59X_1X_3 + 0.70X_2X_3 - 0.065X_1^2 - 5.65X_2^2 - 1.53X_3^2 \quad (4)$$

Also, Fig. 2a and b show the comparison between the removal efficiencies estimated by Eqs. (3) and (4) obtained through experiments. There is good agreement between the estimated and experimental removal efficiency. Normal probability plots of residuals Fig. 3a and b are shown. As the figure shows, there are reasonable residuals, and the residuals are normally distributed, like a straight line.

In terms of coded factors, these equations can be used to find estimates of response for given levels of each factor. Each factor with a positive value of the coefficient has a positive effect on the response, while each factor with a negative value of the coefficient shows the inverse relationship between the response and the factor. The significantly and non-significantly of each factor is determined by its $p$-value and $F$-value. A high $F$-value and $p$-value less than 0.05 are considered significant, and those with a low $F$-value and $p$-values more than 0.05 are considered insignificant [26]. The data in Table 3 indicate that all factors (dye concentration ($X_1$), *H. cupressiforme* dose ($X_2$) and process time ($X_3$)) are important for both RBV 5R and BG removal.

Perturbation plots (Fig. 4a and b) also shows the comparative effects of the variables on RBV 5R and BG removal efficiency. In Fig. 4a and b, steep lines in dye concentration show that the response of RBV 5R and BG efficiency was very sensitive to this factor. The $p$-values for RBV 5R and BG for the final model showing the accuracy of the chosen

Table 1
Levels of factors for CCD experiments

| Independent factors | Coded and un-coded values | | | | |
|---|---|---|---|---|---|
| | $-\alpha$ | –1 | 0 | +1 | $+\alpha$ |
| Dye concentration (mg/L) ($X_1$) | 10 | 18.11 | 30 | 41.89 | 50 |
| *H. cupressiforme* dose (g/50 mL) ($X_2$) | 0.02 | 0.08 | 0.16 | 0.24 | 0.30 |
| Process time (min) ($X_3$) | 5 | 34.39 | 77.50 | 120.61 | 150 |

Table 2
Experimental set-up

| Groups | Bioassay mediums |
|---|---|
| RBV 5R untreated | 350 mL untreated RBV 5R solution + 20 *Daphnia* individuals in polycarbonate containers |
| RBV 5R treated | 350 mL treated RBV 5R solution + 20 *Daphnia* individuals in polycarbonate containers |
| BG untreated | 350 mL untreated BG solution + 20 *Daphnia* individuals in polycarbonate containers |
| BG treated | 350 mL treated BG solution + 20 *Daphnia* individuals in polycarbonate containers |
| Control | 350 mL natural living water + 20 *Daphnia* individuals in polycarbonate containers |

Table 3
CCD matrix of biosorption experiments with factor setup, actual and predicted response values

| Run | Factor 1 ($X_1$) | Factor 2 ($X_2$) | Factor 3 ($X_3$) | Response (% removal) | | | |
|---|---|---|---|---|---|---|---|
| | Dye concentration (mg/L) | *H. cupressiforme* dose (g/50 mL) | Process time (min) | RBV 5R | | BG | |
| | | | | Actual | Predicted | Actual | Predicted |
| 1 | 50.00 | 0.16 | 77.50 | 90.25 | 90.10 | 86.87 | 88.55 |
| 2 | 30.00 | 0.16 | 77.50 | 95.32 | 95.29 | 94.65 | 94.55 |
| 3 | 30.00 | 0.16 | 5.00 | 85.12 | 87.27 | 81.34 | 86.38 |
| 4 | 41.89 | 0.08 | 34.39 | 81.54 | 79.28 | 80.76 | 76.20 |
| 5 | 41.89 | 0.24 | 120.61 | 90.43 | 92.03 | 88.37 | 89.91 |
| 6 | 30.00 | 0.16 | 77.50 | 95.32 | 95.29 | 94.65 | 94.55 |
| 7 | 41.89 | 0.08 | 120.61 | 83.67 | 83.36 | 80.12 | 78.98 |
| 8 | 18.11 | 0.08 | 120.61 | 94.11 | 92.76 | 92.38 | 90.58 |
| 9 | 30.00 | 0.30 | 77.50 | 88.25 | 85.82 | 90.25 | 88.03 |
| 10 | 18.11 | 0.08 | 34.39 | 89.65 | 87.40 | 85.41 | 81.44 |
| 11 | 10.00 | 0.16 | 77.50 | 99.32 | 100.39 | 99.17 | 100.92 |
| 12 | 30.00 | 0.16 | 77.50 | 95.32 | 95.29 | 94.65 | 94.55 |
| 13 | 30.00 | 0.16 | 150.00 | 96.48 | 95.25 | 95.67 | 94.06 |
| 14 | 30.00 | 0.02 | 77.50 | 72.34 | 75.69 | 63.45 | 69.10 |
| 15 | 18.11 | 0.24 | 34.39 | 91.10 | 90.76 | 94.33 | 93.04 |
| 16 | 18.11 | 0.24 | 120.61 | 94.56 | 96.17 | 97.24 | 99.38 |
| 17 | 30.00 | 0.16 | 77.50 | 95.32 | 95.29 | 94.65 | 94.55 |
| 18 | 30.00 | 0.16 | 77.50 | 95.32 | 95.29 | 94.65 | 94.55 |
| 19 | 30.00 | 0.16 | 77.50 | 95.32 | 95.29 | 94.65 | 94.55 |
| 20 | 41.89 | 0.24 | 34.39 | 87.22 | 87.92 | 90.55 | 89.92 |

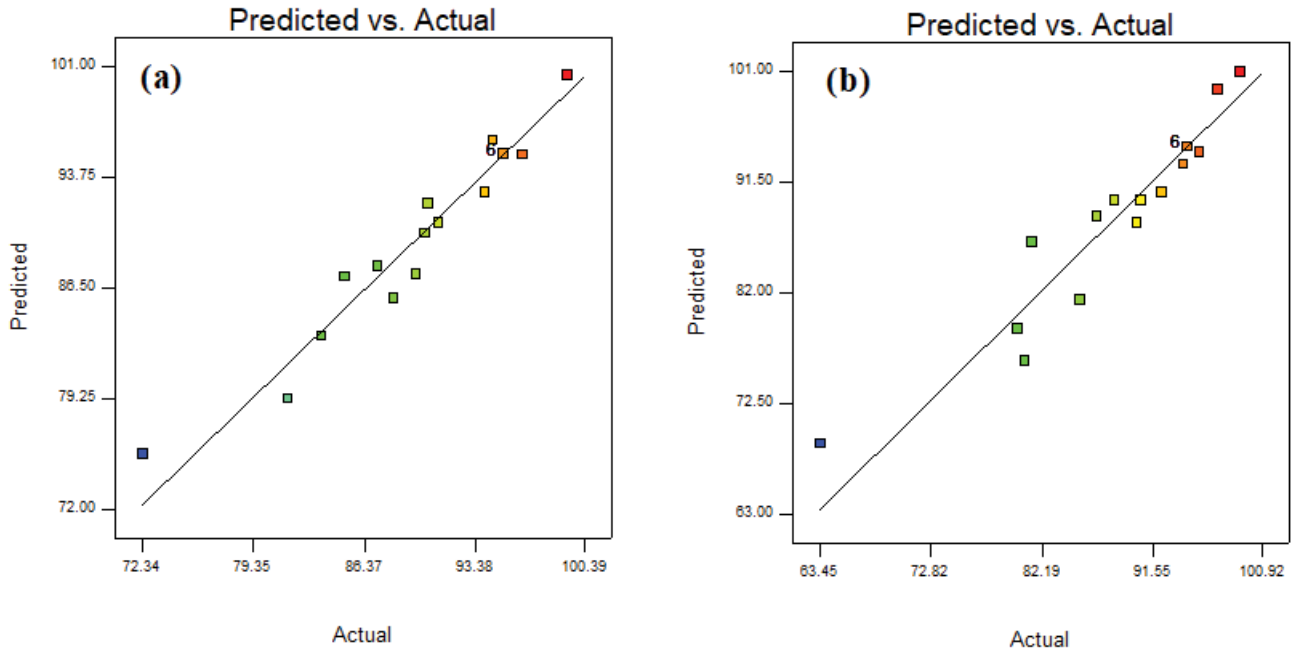


Fig. 2. Comparison plot between the experimental and model predicted RBV 5R removal (%) (a) and BG removal (%) (b).

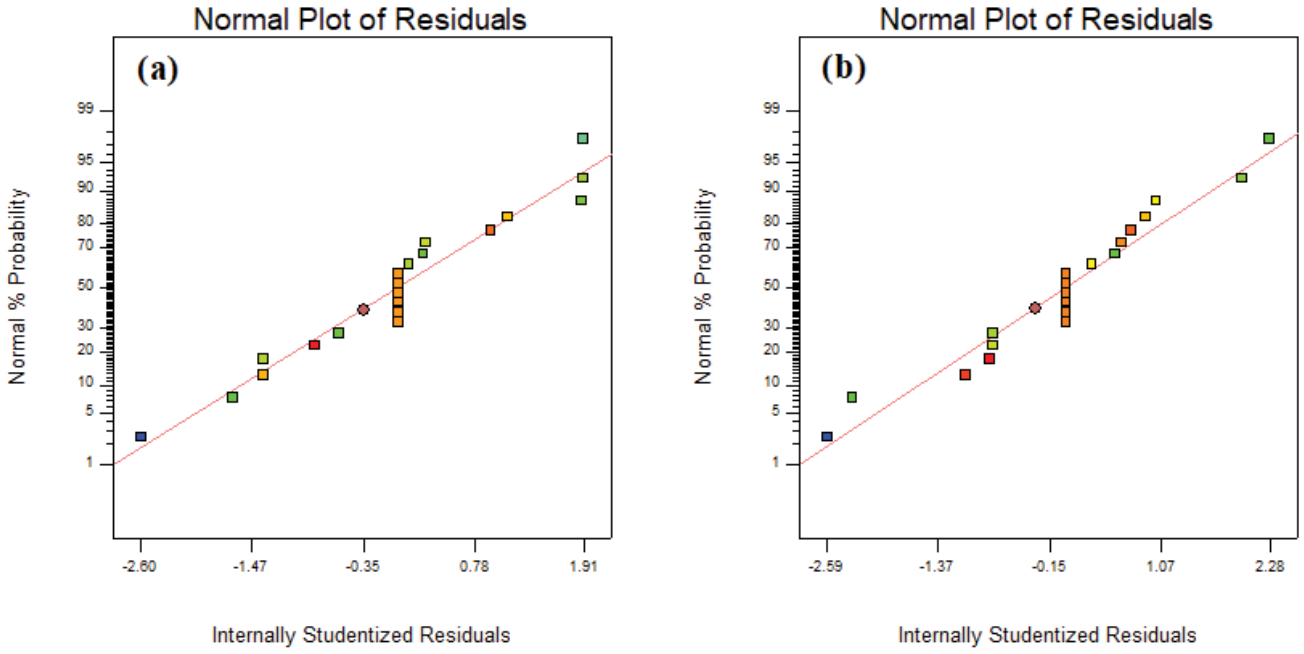


Fig. 3. Normal probability plots of residual for RBV 5R removal (%) (a) and BG removal (%) (b).

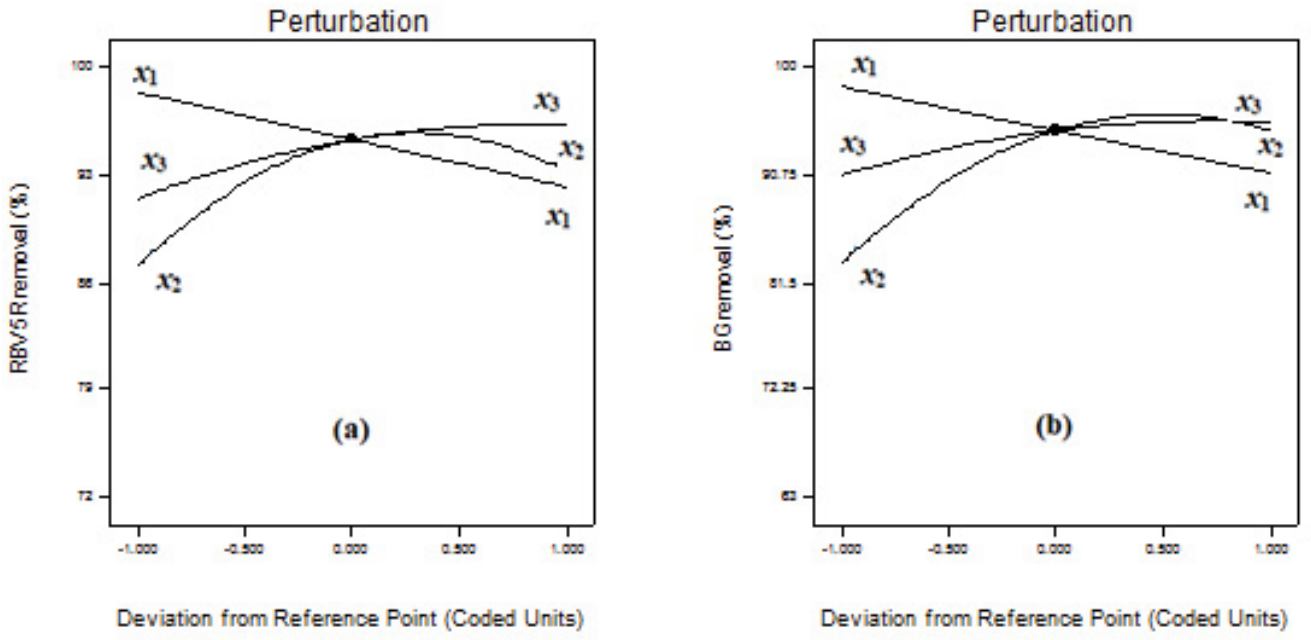


Fig. 4. Perturbation plots for RBV 5R (a) and BG (b) removal.

model were <0.0001 and 0.0004, respectively. Also, the statistical significance of the proposed model was evaluated with the parameters in Table 4 obtained from analysis of variance. The determination coefficient ($R^2$) indicates the accuracy of the designed models. In this study, $R^2$ values were obtained as 0.9459 for RBV 5R removal and 0.9083 for BG removal, confirming the good agreement of the data shown in Table 4. The $R^2$ values indicate that 94.59% of the variation in RBV 5R removal and 90.83% of the variation in BG removal can be identified by the proposed models. The adjusted $R^2$ ($R^2$-Adj) values showing the reliability of the mentioned model were 0.8973 for RBV 5R and 0.8258 for BG biosorption, and these values proved that the models were important [27]. Another parameter

Table 4
Analyzing the variance (ANOVA) and model summary statistic

| Source | Sum of squares | Degree of freedom | Mean square | *F*-value | *p*-value Prob. > *F* | |
|---|---|---|---|---|---|---|
| RBV 5R removal (%) | | | | | | |
| Model | 740.97 | 9 | 82.33 | 19.44 | <0.0001 | Significant |
| $X_1$ | 128.02 | 1 | 128.02 | 30.23 | 0.0003 | |
| $X_2$ | 123.67 | 1 | 123.67 | 29.21 | 0.0003 | |
| $X_3$ | 76.70 | 1 | 76.70 | 18.11 | 0.0017 | |
| $X_1X_2$ | 13.89 | 1 | 13.89 | 3.28 | 0.1002 | |
| $X_1X_3$ | 0.83 | 1 | 0.83 | 0.20 | 0.6670 | |
| $X_2X_3$ | 8.000E-004 | 1 | 8.000E-004 | 1.889E-004 | 0.9893 | |
| $X_1^2$ | 4.315E-003 | 1 | 4.315E-003 | 1.019E-003 | 0.9752 | |
| $X_2^2$ | 380.78 | 1 | 380.78 | 89.93 | <0.0001 | |
| $X_3^2$ | 29.31 | 1 | 29.31 | 6.92 | 0.0251 | |
| $R^2$ | 0.9459 | | | | | |
| $R^2$-Adj | 0.8973 | | | | | |
| CV % | 2.27 | | | | | |
| AP | 16.975 | | | | | |
| BG removal (%) | | | | | | |
| Model | 1,196.84 | 9 | 132.98 | 11.01 | 0.0004 | Significant |
| $X_1$ | 184.86 | 1 | 184.86 | 15.30 | 0.0029 | |
| $X_2$ | 432.92 | 1 | 432.92 | 35.83 | 0.0001 | |
| $X_3$ | 71.10 | 1 | 71.10 | 5.88 | 0.0357 | |
| $X_1X_2$ | 2.27 | 1 | 2.27 | 0.19 | 0.6740 | |
| $X_1X_3$ | 20.16 | 1 | 20.16 | 1.67 | 0.2255 | |
| $X_2X_3$ | 3.92 | 1 | 3.92 | 0.32 | 0.5815 | |
| $X_1^2$ | 0.060 | 1 | 0.060 | 4.980E-003 | 0.9451 | |
| $X_2^2$ | 460.43 | 1 | 460.43 | 38.11 | 0.0001 | |
| $X_3^2$ | 33.81 | 1 | 33.81 | 2.80 | 0.1253 | |
| $R^2$ | 0.9083 | | | | | |
| $R^2$-Adj | 0.8258 | | | | | |
| CV % | 3.88 | | | | | |
| AP | 12.949 | | | | | |

confirming the statistically significant and strength of the proposed models is the acceptable precision (AP) value, which indicates the amount of "signal-to-noise ratio". In this study, AP values for RBV 5R and BG was 16.975 and 12.949, respectively. More reliable models in CCD-defined space are models with AP higher than 4. Also, a low value of the coefficient of variation (CV) (2.27% for RBV 5R and 3.88% for BG) indicates the reliability and reproducibility of the predicted model [28].

### 3.2. Response surface designs and optimization

In this work, three-dimensional (3D) surface plots and contour plots were used to examine the effect of various levels of independent factors on the percentage of RBV 5R and BG removal. Figs. 5a, d and 6a, d represents the simultaneous effect of dye concentration and *H. cupressiforme* dose on RBV 5R and BG removal ($X_1X_2$) when the process time were maintained in the constant value (77.50 min). As can be seen from figures, the removal efficiency for both dyes decreased when the initial concentration of RBV 5R and BG increased from 10 to 50 mg/L. This may be due to the presence of multiple active sites on the surface of *H. cupressiforme* for dye biosorption at lower dye concentrations. According to Rehman et al. [29], the optimum parameters for adsorption were at; adsorbent dosage 10 g/L, agitation speed 200 rpm, contact duration 120 min, initial concentration 100 mg/L and temperature 298 K that resulted in 97% BG dye removal. However, with increasing dye concentration, the number of surfactant binding sites decreased due to saturation of the binding sites with dye molecules.

Figs. 5b, e and 6b, e represents the effect of dye concentration and process time on RBV 5R and BG removal by keeping *H. cupressiforme* dose constant at 0.16 g/50 mL. For both RBV 5R and BG, dye removal rate increased with increasing contact time. While the biosorption rate was high at the beginning, there was no significant increase in the percentage of dye removal afterwards. The reason for

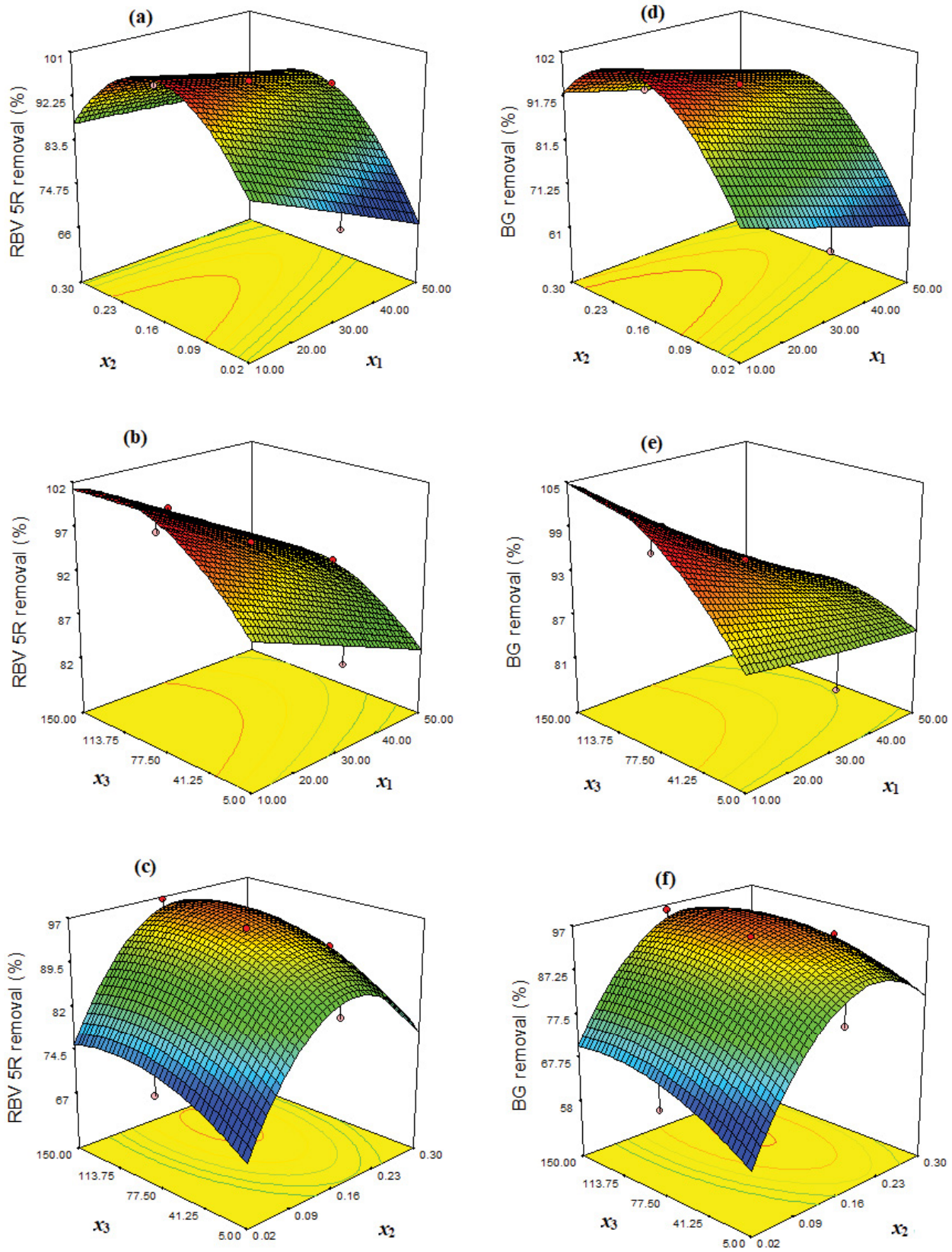


Fig. 5. 3D response surface plots for RBV 5R and BG removal ($X_1$: dye concentration (mg/L); $X_2$: *H. cupressiforme* dose (g/50 mL); $X_3$: process time (min)).

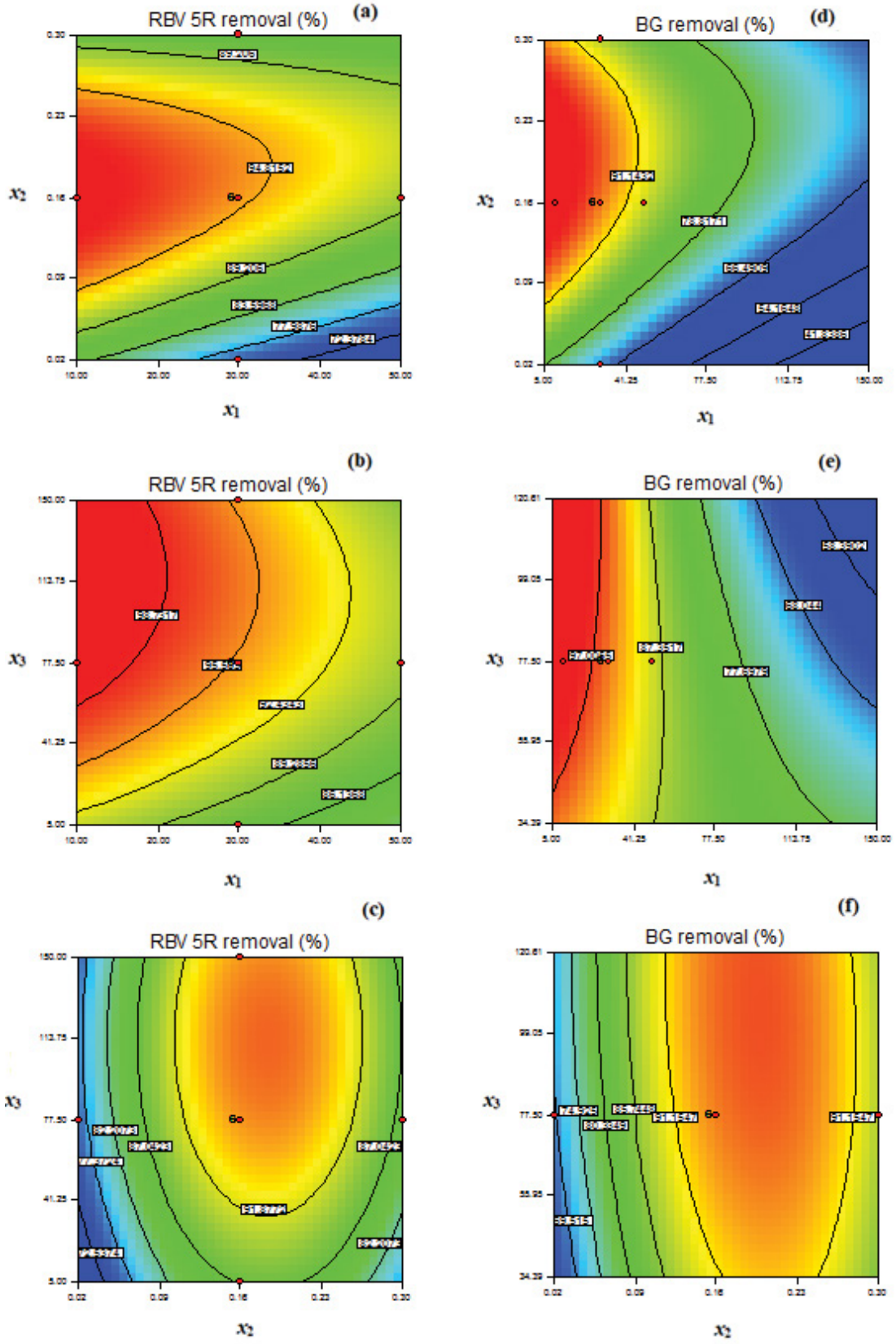


Fig. 6. Contour plots for RBV 5R and BG removal ($X_1$: dye concentration (mg/L); $X_2$: *H. cupressiforme* dose (g/50 mL); $X_3$: process time (min)).

this may be that the biosorption rate is higher since the initially soluble dye molecules are adsorbed on the external surfaces of the *H. cupressiforme* particles. After the biosorption in these pores reaches saturation, additional molecules cannot be biosorbed and the dye molecules can diffuse from the pores of the biosorbent to the inner surfaces of the particles. Since this phenomenon takes time, there would be an optimal biosorption time. Beyan et al. [30] revealed out that the predicted models were statistically optimized to maximize biochemical oxygen demand (BOD) and chemical oxygen demand (COD) removal. The optimal values were predicted to be 0.915 g/L and a reaction time of 98.91 min for maximized BOD and COD removal efficiencies of 0.0225 and 0.023 mg/L $min^{-1}$ for textile wastewater respectively.

Figs. 5c, f and 6c, f represent the effect of *H. cupressiforme* dose and process time on RBV 5R and BG removal by keeping initial dye concentration constant at 30 mg/L. As seen in Figs. 5c and 6c, RBV 5R removal efficiency increased with increasing biosorbent dose up to about 0.22 g, while it decreased at higher biosorbent amounts. Similarly, BG removal efficiency increased with increasing biosorbent dose up to about 0.24 g and decreased at higher biosorbent amounts. The reason for the decrease in biosorption removal efficiency is the decrease in biosorption capacity, considering that not all adsorption sites can participate in adsorption due to agglomeration or interaction [31].

In this work, the numerical optimization program in CCD was used to determine the optimum values. In the program, RBV 5R or BG removal efficiency, which is the response, was selected as maximum, while the level of each parameter is selected "in range". Optimum values and maximum biosorption efficiencies of the studied factors for RBV 5R and BG biosorption are presented in Table 5. These data are optimization results under selected conditions and have been verified by experiments performed under optimum conditions.

### 3.3. Mortality assessment

The results of the mortality assessment are presented as percent death of *D. magna* occurred in all application groups. The mortality rates of experimental groups for each period were determined using *D. magna*. In the RBV 5R dye application group, 100% mortality was detected after 24 h in the untreated medium, while the mortality rates were determined as 0%, 0% and 5% at 24, 48 and 72 h in the treated medium, respectively. In the BG dye application group, mortality rates were determined as 30%, 35% and 50% at 24, 48 and 72 h, respectively, in the treated medium, while 100% mortality was determined after 24 h in the untreated medium. No mortality was observed in all periods in natural living water medium.

When the treatment efficiencies of both dyes were compared in terms of mortality, it was concluded that the toxicity of RBV 5R was reduced more effectively. The mortality rates showed that treatment with adsorption were sufficient to remove the complete toxicity of dyes especially for RBV 5R (Fig. 7). Lade et al. [32] reported that acute toxicity test with *D. magna* revealed complete detoxification of azo dye degraded metabolites under sequential microaerophilic/

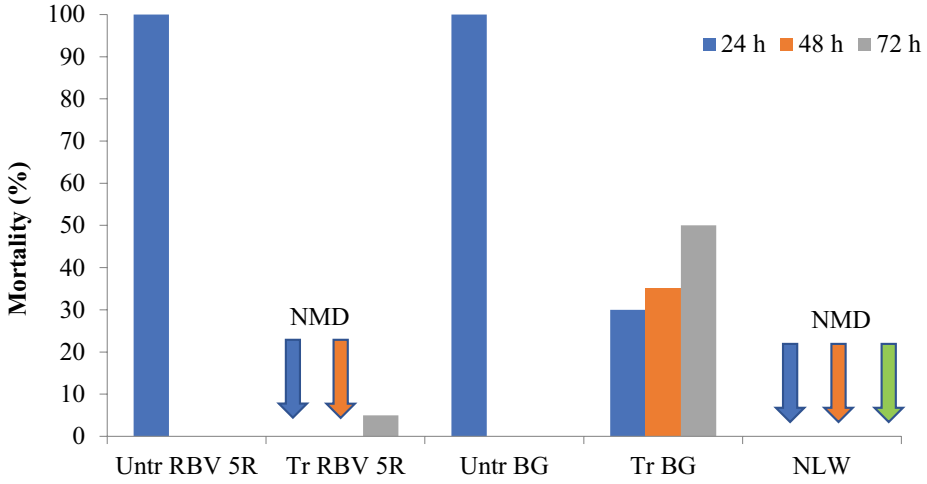


Fig. 7. Mortality rates (%), NMD; No mortality determined, NLW; Natural living water, Untr; Untreated, Tr; Treated.

Table 5
Results of numerical optimization (Desirability = 1.000)

| | Dye concentration (mg/L) | *H. cupressiforme* dose (g/50 mL) | Process time (min) | Removal, % |
|---|---|---|---|---|
| RBV 5R | 19.02 | 0.18 | 110.59 | 99.39 |
| BG | 18.57 | 0.18 | 99.45 | 99.22 |

aerobic processes. In their study, a bacterial consortium consisting of *Providencia rettgeri* strain HSL1 and *Pseudomonas* sp. SUK1 has been investigated for degradation and detoxification of structurally different azo dyes. The consortium showed 98%–99% decolorization of all the selected azo dyes viz. Reactive Black 5 (RB 5), Reactive Orange 16 (RO 16), Disperse Red 78 (DR 78) and Direct Red 81 (DR 81) within 12 to 30 h at 100 mg/L concentration at 30°C under microaerophilic, sequential aerobic/microaerophilic and microaerophilic/aerobic processes. In a study by Ghanbarian et al. [33] they found that mortality in the treated dye medium increased at a low level depending on the application time. In our study, a similar mortality increase was observed especially in the BG dye group. This increase was lower than in the untreated BG dye medium.

## 4. Conclusions

The analysis of variance (ANOVA) was performed for evaluating the significance of the proposed assumptions. This work investigated the interaction of dye concentration, *H. cupressiforme* dose and process time with *H. cupressiforme* for the biosorption of RBV 5R and BG dyes from aqueous solution using CCD. The regression models showed the validity of the quadratic equations for the biosorption of RBV 5R and BG using *H. cupressiforme*. The determination coefficient ($R^2$) obtained from the ANOVA for RBV 5R and BG are 0.9459 and 0.9083, respectively. The maximum removal yields of RBV 5R and BG were 99.39% and 99.22%, respectively, indicating that *H. cupressiforme* has a high removal capacity for RBV 5R and BG. *Daphnia* mortality rates showed that decolorization with *H. cupressiforme* significantly reduced toxicity for both dyes.

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