

Investigation on Biosorption of Brilliant Green Dye from Aqueous Solutions by Newly Isolated Fungus *Aspergillus oryzae*: Rsm-Optimized Process Variables and *Daphnia magna* Bioassay

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Abstract

Biosorption is an effective process for the removal of dyes from wastewaters. In this study, a newly isolated fungus *Aspergillus oryzae* was used as a biosorbent for brilliant green (BG) dye biosorption from aqueous solutions. The effect of three independent factors such as biomass dosage (0.01–0.12 g), initial BG concentration (5–30 mg L⁻¹) and process time (10–120 min) was performed on BG biosorption by *A. oryzae* using batch system. The experimental design response surface methodology was aimed to determine the optimum levels of the process factors selected above. The optimum conditions for maximum biosorption removal were found 0.10 g, 17.25 mg L⁻¹, and 85 min for biomass dosage, initial BG concentration and process time, respectively. Under these optimized conditions, 67.32% removal of BG was achieved. A decrease in mortality of the treated BG dye solution was determined from *D. magna* bioassay. This indicated that a decreasing may have occurred in the toxic effect of the dye after biosorption process..

Keywords: Brilliant green, Biosorption, Response Surface Methodology, Mortality

Yeni İzole Edilmiş Mantar *Aspergillus oryzae* ile Brilliant Green Boyar Maddesinin Sulu Çözeltilerinden Biyosorpsiyonu Üzerine Araştırma: Rsm-Optimize Edilmiş Süreç Değişkenleri ve *Daphnia magna* Biyodeneyi

Öz

Biyosorpsiyon, boyaların atık sulardan uzaklaştırılması için etkili bir prosestir. Bu çalışmada, sulu çözeltilerden brilliant green (BG) boya biyosorpsiyonunda biyosorbent olarak yeni izole edilmiş bir mantar olan *Aspergillus oryzae* kullanıldı. Biyokütle dozu (0.01–0.12g), başlangıç BG konsantrasyonu (5–30 mg L⁻¹) ve işlem süresi (10–120 dk) gibi üç bağımsız faktörün etkisi, kesikli teknik kullanılarak *A. oryzae* tarafından BG biyosorpsiyonunda gerçekleştirilmiştir. Deneysel tasarım, yani tepki yüzeyi metodolojisi, yukarıda seçilen süreç faktörlerinin optimum seviyelerini belirlemeyi amaçlamıştır. Maksimum biyosorpsiyon giderimi için optimum koşullar biyokütle dozu, başlangıç BG konsantrasyonu ve işlem süresi için sırasıyla 0.10 g, 17.25 mg L⁻¹ ve 85 dakika olarak bulunmuştur. Bu optimize koşullar altında, BG'nin %67,32 oranında giderilmesi sağlandı. *D. magna* biyo-deneyi ile muamele edilmiş BG boya çözeltisinin ölüm oranındaki bir azalma belirlendi. Bu durum, biyosorpsiyon işlemi sonrasında boyanın toksik etkisinde bir azalma meydana gelmiş olabileceğini göstermiştir.

Anahtar Kelimeler: Brilliant green, Biyosorpsiyon, Yüzey yanıt metodu, Mortalite.

1. Introduction

Industrial dyes are commonly of the mainly used raw materials such as textile, paint, pulp, etc. in such countries (Salman and Ali, 2019). If the dyes are discharged into receiving environments without sufficient treatment methods, they stop the biological activities in the aquatic environment and discompose the equilibrium of the receiving bodies. Additionally, these materials combine with the dissolved oxygen in the system thereby consuming the dissolved oxygen required to sustain aquatic environment (Onu et al., 2020). The chemical components of most of the effluents make the aquatic livings toxic, mutagenic and carcinogenic and they also lead to dermatitis and even cancer. Moreover, the presence of industrial effluent dyes colorizes the receiving aquatic bodies making them esthetically unclear, while even deceptions them with problems for agricultural and domestic applications (Nwabanne et al., 2017). Consequently, one of the critical issues that effluents containing dyes are adequately treated the environment before discharging to these habitats. The reason for this is that dyes are anionic, resistant to oxidative agents, and therefore wastewater cannot be effectively removed by other complex and costly treatment processes (Kharat, 2015).

Recent years, biosorption method becoming the most suitable and capable technique, and so activated carbon method preferred adsorbent for sanitization of the dye waters (Pathania et al., 2017). At any rate, the application of biosorption method was restrained because of its expensive production and recovery cost. According to Oguanobi et al. (2019), biosorption method is ecofriendly, low cost and effective and also can give nearly full reduction of the effluents.

According to Milani Shirvan et al. (2017), method of the biosorption which can be used for complex reactions at the solid and liquid surface and is qualified on several environmental parameters such as temperature, acidity, alkalinity, sorbate ion concentration and shaking rate. The reason why the Response Surface Method (RSM) requires careful optimization before large-scale application to which it can be applied is because it uses a set of statistical and mathematical correlations that make it convenient to estimate the most advantageous values of certain process parameters. The benefit of this situation is to achieve the greatest processing speed and increase its repeatability. The effect of biosorption is not only related to biosorption, but also confirmed by many literature studies on the rate of heat and numerical optimization of heat transfer.

In recent years, microorganisms have been preferred more than the production of feed culture liners by biosorption method (Michalak et al., 2020). A wide range of fungi are suitable for decolorizing mostly used common dyes (Fu and Viraraghavan, 2001). Many genera of these microorganisms have been employed either in inactivated or living forms. There are various fungi types such as *Aspergillus* sp. (Fu and Viraraghavan, 2002), which can also biosorb and decolorize diverse dyes.

RSM is a combine of statistical and mathematical application adopted to foretell and lay out the operating parameters. In addition to minimizing the number of experimental trials, RSM

can also be widely used to evaluate the synergistic effect between operational quantities and responses (Ashish and Nayak, 2018). Central composite design (CCD) is seen as one of the most common and most preferred types of RSM, while keeping all the number of runs required to achieve full optimization of process quantities at a moderate level (Louhichi et al., 2018).

One of the essential components of RSM is CCD. CCD is a five-level experimental type that integrates factorial and axial points in the design of methodological applications, unlike the box-behnken models in RSM. Mohammad et al. (2014) demonstrated that, process optimization on CCD, was observed to be important in determining the values of factors for which the effect is at high levels. One of the advantages in determining the optimum experimental conditions is that only a few test runs are sufficient (Akash et al., 2019).

Daphnia are planktonic crustaceans that filter fresh water and play a role in improving water quality by consuming algae (Angelika et al., 2021). Water fleas show behavioral and physiological responses by being affected very quickly by environmental pollutants and chemical changes of their habitats, these features have caused them to be preferred as a model organism especially in toxicology studies (Barata et al., 2005, Ebert, D., 2005). The body size of *daphnia* provides a great advantage for laboratory studies, because it is possible to use many individuals at the same time in an experimental setup, and their high fertility characteristics and parthenogenetic type reproduction allow many water fleas to be tested in a short time (Koivisto, S., 1995). In experiments with water fleas, subjects such as lethality, reproductive, behavioral, physiological effects and change of biochemical properties of any chemical were generally examined. Among these parameters, determining the lethal effect is the most preferred studies (Angelika et al., 2021). Generally, the presence of various dyes and pigments emitted by wastewater from paint production, textile coloring, food coloring, cosmetics industry, paper industry and different industrial sources causes environmental problems. Since most of the dyes in industrial wastewater and wastewater from water sources are toxic, their removal has been of great importance in recent years. Pollution with paints negatively affects human health by reducing the quality of life and also poses a serious threat to the aquatic ecosystem. It can be shown among the undesirable properties of dyestuffs that they are resistant to natural degradation, carcinogenic and allergic. Brilliant green is a derivative of triarylmethane dyes commonly used to color silk and wool. It is a cationic dye and more toxic than anionic dyes. Brilliant green is defined as an irritant that causes skin and eye burns, nausea, vomiting, diarrhea and abdominal pain and is classified as very toxic and the possible lethal dose in humans is 50-500 mg/kg. In this study, brilliant green was chosen because of its toxic effects on humans and the environment.

The main purpose of this study is to investigate on batch biosorption of BG dye from aqueous solutions by dead biomass of newly isolated fungus *Aspergillus oryzae*. It is also aimed to reveal whether decolorization provides a reduction in toxicity with the *D. magna* bioassay.

2. Material and Method

2.1. Characteristics of the dye

The chemical formula of brilliant green is $C_{27}H_{33}N_2 \cdot HO_4S$ and its molecular weight is 462.65 g/mol. BG was obtained from Sigma-Aldrich. Chemical structure of BG is given in Figure 1.

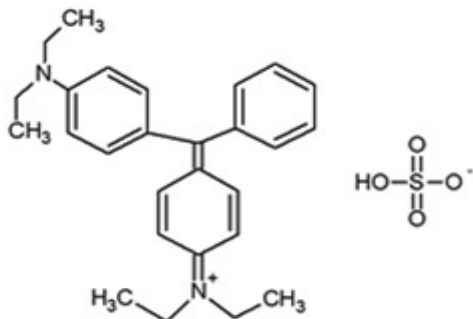


Fig. 1. Chemical structure of BG

2.2. Fungus Used in The Study

In summer 2020, soil sample taken from an agricultural area according to Association et al. (1912) in Edirne province of Turkey. These soils were taken to the laboratory of Munzur University, department of Environmental Engineering at +40°C. In the laboratory, the samples were diluted to 106 and taken to the malt extract agar plate media sterilized and prepared in autoclave and taken to 250C for finishing the logarithmic growing phase. For the identification process, when growing phase finished, the isolated and signed fungus at the malt extract agar plates were shipped to REFGEN Company in Ankara province of Turkey. The absorption abilities of fungi are higher than the composition of the surfactant-binding region compared to algae. For this reason, fungi were preferred instead of algae in the study.

For the DNA extraction and PCR amplification, the isolated fungus was chosen to identify the taxonomic patterns of fungal communities. Power Soil DNA Isolation Kit (REFGEN Laboratories, Ankara, Turkey) used for microbial DNA according to manufacturer's instructions. The fungal 18S RNA gene was expanded by PCR. PCR reactions were done three times with 20 μ L mixtures containing 4 μ L of 5x FastPfu Buffer, 2 μ L of 2.5 mM dNTPs, 0.8 μ L of each primer (5 μ M), 0.4 μ L of FastPfu Polymerase and finally 10 ng of template DNA. *Aspergillus oryzae* were identified with 18S RNA method by the PCR programs for fungal amplification setup on a Codon Code Alligner® version 9.0 software for DNA sequencing (Figure 2). At last level, the similarity rate for this fungus was found as 92%.

2.3. Preparation of dead fungal biomass

Newly isolated fungus, *A. oryzae* maintained by subculturing on savoroud dextrose broth. Inocula were taken from a six-day old broth culture. The 10 ml fungal culture was gently homogenized using a homogenizer. It was used to inoculate this 10 ml of homogenized medium in a 500 ml flask, and this flask was incubated on an orbital shaker (at 150 rpm) for 6 days at 27°C. The end of the incubation period, the biomass of the fungus was harvested by filtration from the growth medium and washed several times with distilled water to remove the medium. The fungal biomass was dried and finely ground for subsequent use.

2.4. Biosorption studies

The biosorption performance of *A. oryzae* biomass as a biosorbent was examined through a batch system in the bioremoval process of the BG. The system is a closed system and indicates that there is no interference or output from the outside. Erlenmeyer flasks were used in the study. For this objective, CCD was utilized to design the experiments under RSM. The BG (CAS Number: 633-03-4, molecular weight: 482.63 g mol⁻¹ and molecular formula:

Unassembled Samples: Traces for 1 sample

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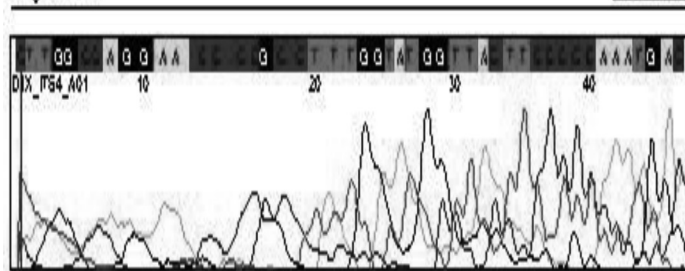


Fig. 2. Traces for *Aspergillus oryzae*

$C_{27}H_{34}N_2O_4S_n$) was provided from Sigma-Aldrich Company. The biosorption experiments were conducted in glass bottles (250 mL) containing 25 mL of BG solution with a known concentration at the natural pH of the solution at room temperature ($25 \pm 2^\circ\text{C}$) and at a constant stirring speed (250 rpm). After shaking, the suspension of each sample was centrifuged at 5,000 rpm for 10 min, and the residual concentration of BG in the solution was determined by UV visible spectrophotometer (Shimadzu, UV-1800, Japan) at maximum absorbance ($\lambda_{\text{max}} = 625 \text{ nm}$). The BG removal efficiency (R) and biosorption capacity of biomass (q_t) was calculated according to the following equations:

$$R\% = \frac{C_o - C_t}{C_o} \times 100 \quad (1)$$

$$q_t = \left(\frac{C_o - C_t}{m} \right) \times V \quad (2)$$

where C_o and C_t are the BG concentrations (mg L^{-1}) before and after biosorption, respectively, q_t is the biosorption capacity (mg g^{-1}), V is the total solution volume (mL), and m is the amount of the biosorbent (g).

2.5. Experimental design

Experimental design allows simultaneous optimization of factors and provides to minimize error with the minimum number of runs to and improve performance characteristics (Kousha et al., 2015). In present work, the experiments for BG biosorption were designed utilizing CCD with three factors (biomass dosage (A_1), initial BG concentration (A_2) and process time (A_3)) at five levels using Design-Expert (version 7, Stat-Ease, USA) with 20 runs. The operational ranges and coded of the three independent factors utilized in the matrix of experiments (-1 and +1) consisting 6 axial points, 6 central points and 8 factorial points showed in Table 1. In order to explain the behavior of the system was developed an empirical model based on second-order polynomial equation as given by Equation (3) (Sadhu Khan et al., 2016).

Table 1. Experimental range and levels in the CCD

Coded factor	Factor	Levels				
		-α	-1	0	+1	+α
A_1	Biomass dosage (g)	0.01	0.037	0.065	0.092	0.12
A_2	Initial BG concentration (mg L ⁻¹)	5	11.25	17.50	23.75	30
A_3	Process time (min)	10	37.5	65	92.50	120

$$y = b_0 + \sum_{i=1}^k b_i A_i + \sum_{i=1}^k b_{ii} A_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k b_{ij} A_i A_j + e \quad (3)$$

where y is the response, b_0 is the intercept, b_i are the linear coefficients, b_{ij} are the interaction terms, b_{ii} are the quadratic

2.6. Mortality assessment

The *D. magna* used in the mortality assays as a model organism. Water fleas were taken to the laboratory and then stocked in 120 L aquariums and optimum temperature conditions and photoperiod times were designed to adapt to laboratory conditions, they were regularly fed once a day with a mixture of dry spirulina powder and *Saccharomyces cerevisiae* as a baker's yeast.

For mortality assessment, three experimental groups were designed. For this purpose, approximately 400 mL of water taken from the environments of all application groups (treated, untreated and control) was added to polycarbonate containers and 10 daphnia units were added to these containers. Three replicates were done for each experimental group. Numbers of dead water fleas in each container were counted after one, two- and three-

coefficients, A_i or A_j are the independent factors and e is the random error.

days periods. At the end of the third day, mortality rates were calculated on each experimental group as percentages according to Babu et al (2015).

3. Results and Discussion

3.1. The RSM design for biosorption

3.1.1. CCD statistical analysis

In the present study, biomass dosage, initial BG concentration and process time were selected as independent factors, while the removal efficiency and the biosorption capacity were selected as the responses. The design matrix and response values were showed Table 2. The regression models that relate the responses and the independent factors were described in terms of the coded factors by the quadratic equations as given follows:

Table 2. Design matrix for BG biosorption factors and corresponding responses from the experiments

Run no	Actual factors			Removal efficiency (%)	Biosorption capacity (mg g ⁻¹)
	A_1	A_2	A_3		
1	0.03	24.93	32.30	41.90	8.70
2	0.10	24.93	97.70	65.33	4.07
3	0.03	10.07	97.70	61.27	5.14
4	0.07	17.50	65.00	64.23	4.01
5	0.07	17.50	65.00	64.23	4.01
6	0.10	24.93	32.30	63.31	3.94
7	0.07	17.50	65.00	64.23	4.01
8	0.10	10.07	97.70	65.25	1.64
9	0.07	5.00	65.00	61.89	1.10
10	0.03	10.07	32.30	47.74	4.00
11	0.07	17.50	10.00	55.96	3.49
12	0.10	10.07	32.30	61.93	1.55
13	0.12	17.50	65.00	67.25	2.45
14	0.07	17.50	120.00	63.34	3.95

15	0.03	24.93	97.70	54.17	11.25
16	0.07	17.50	65.00	64.23	4.01
17	0.07	17.50	65.00	64.23	4.01
18	0.07	30.00	65.00	58.43	6.26
19	0.01	17.50	65.00	40.83	17.86
20	0.07	17.50	65.00	64.23	4.01

$$\text{Removal efficiency (\%)} = + 64.23 + 6.97A_1 - 1.27A_2 + 3.19A_3 + 1.80A_1A_2 - 2.56A_1A_3 - 0.32A_2A_3 - 3.59A_1^2 - 1.43A_2^2 - 1.61A_3^2 \quad (4)$$

$$\text{Biosorption capacity (mg g}^{-1}\text{)} = + 4.04 - 3.21A_1 + 1.78A_2 + 0.34A_3 - 0.75A_1A_2 - 0.43A_1A_3 + 0.18A_2A_3 + 1.98A_1^2 - 0.31A_2^2 - 0.30A_3^2 \quad (5)$$

The analysis of variance (ANOVA) was used to evaluate the significance and statistical adequacy of the predicted models and the obtained results are given in Table 3. The p -value for the models was less than 0.05 ($p < 0.0001$ for removal efficiency and

$p < 0.0002$ for biosorption capacity), indicating that both models were statistically important.

Table 3. Analysis of variance (ANOVA) for removal efficiency and biosorption capacity

Source	Sum of squares	Degree of freedom	Mean square	F- Value	P-value Prob > F
Removal efficiency (%)					
Model	1125.59	9	125.07	70.70	< 0.0001
A_1	663.25	1	663.25	374.93	< 0.0001
A_2	21.91	1	21.91	12.39	0.0055
A_3	138.89	1	138.89	78.51	< 0.0001
$A_1 A_2$	25.92	1	25.92	14.65	0.0033
$A_1 A_3$	52.33	1	52.33	29.58	0.0003
$A_2 A_3$	0.82	1	0.82	0.46	0.5116
A_1^2	186.10	1	186.10	105.20	< 0.0001
A_2^2	29.46	1	29.46	16.65	0.0022
A_3^2	37.36	1	37.36	21.12	0.0010
Residual	17.69	10	1.77		
<i>Model statistics</i>					
R^2	0.9845				
Adjusted R^2	0.9706				
Adequate precision	25.149				
Biosorption capacity (mg g⁻¹)					
Model	254.68	9	28.30	12.75	0.0002
A_1	140.52	1	140.52	63.30	< 0.0001
A_2	43.27	1	43.27	19.49	0.0013
A_3	1.61	1	1.61	0.72	0.4149
$A_1 A_2$	4.49	1	4.49	2.02	0.1856
$A_1 A_3$	1.51	1	1.51	0.68	0.4295
$A_2 A_3$	0.26	1	0.26	0.12	0.7379

A_1^2	56.32	1	56.32	25.37	0.0005
A_2^2	1.41	1	1.41	0.63	0.4446
A_3^2	1.28	1	1.28	0.58	0.4649
Residual	22.20	10	2.22		

Model statistics

R^2	0.9198
Adjusted R^2	0.8477
Adequate precision	14.108

The regression coefficient (R^2) utilized to determine the relationship between predicted and experimental (actual) values was estimated as 0.9845 and 0.9198 for removal efficiency and biosorption capacity, respectively (Allouss et al., 2019). Similarly, the high adjusted R^2 values found (0.9706 for removal efficiency and 0.8477 for biosorption capacity) indicate a good agreement between predicted and experimental data. Values of Prob > F less than 0.05 indicate model terms are statistically significant (Dil et al., 2019). In this case, the important model terms for the removal efficiency are A_1 , A_2 , A_3 , $A_1 A_2$, $A_1 A_3$, $A_1 A_2 A_3$, A_1^2 , A_2^2 and A_3^2 , while the important model terms for the biosorption capacity are A_1 , A_2 and $A_1 A_2$. It is desirable that the adequate precision value,

which measures the signal-to-noise ratio, be greater than 4 (Arabpour et al., 2021). Here the values of adequate precision are 25.149 for removal efficiency and 14.108 for biosorption capacity indicating that the models can be used to navigate the design area.

3.1.2. Influence of factors via response surface

The response surface 3-dimensional (3D) plots were used to understand the combined effect of independent factors on the removal efficiency and biosorption capacity of BG (Figures 3 and 4).

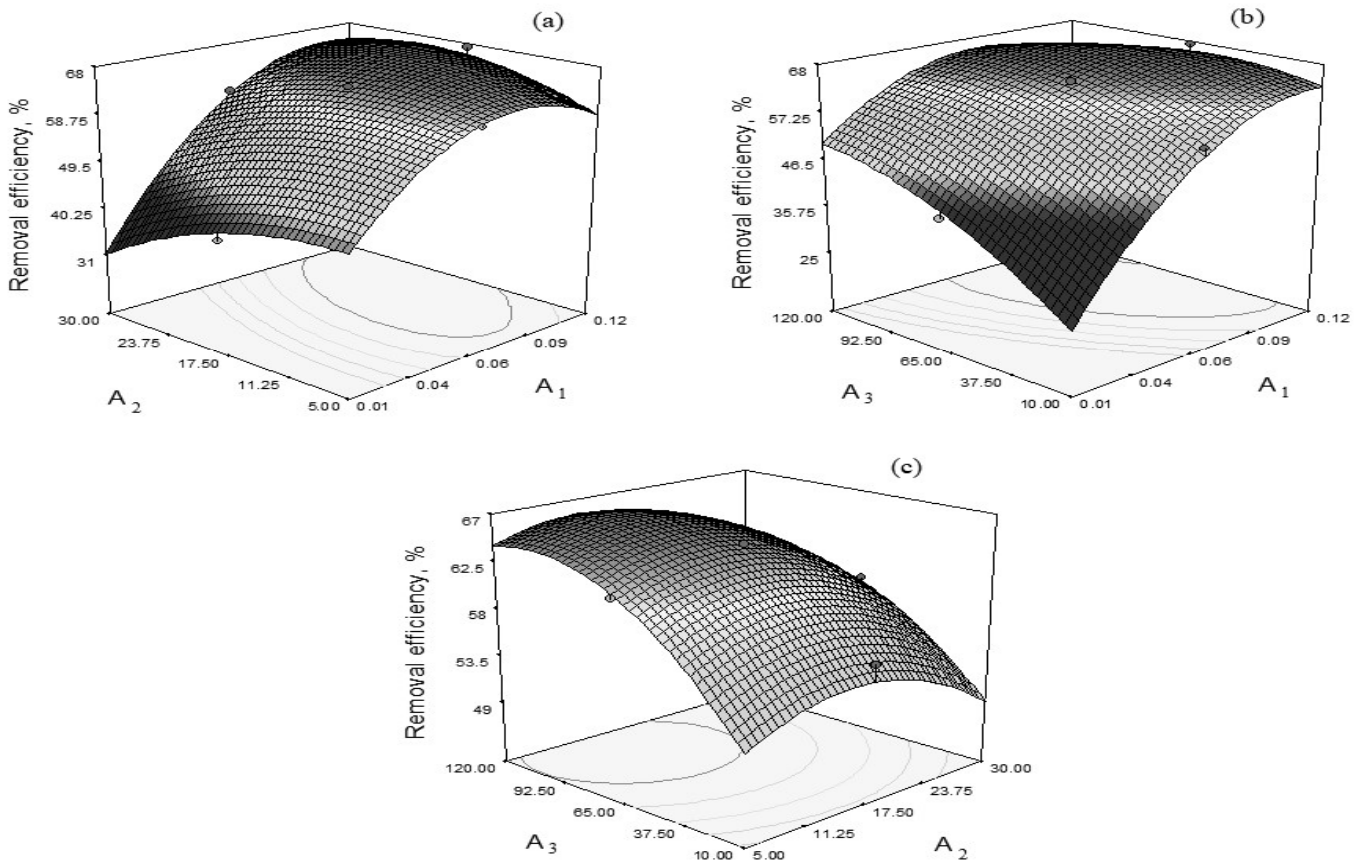


Fig. 3. 3D response surface plots for removal efficiency of BG onto *A. oryzae* (a) A_1 – A_2 , (b) A_1 – A_3 and (c) A_2 – A_3 .

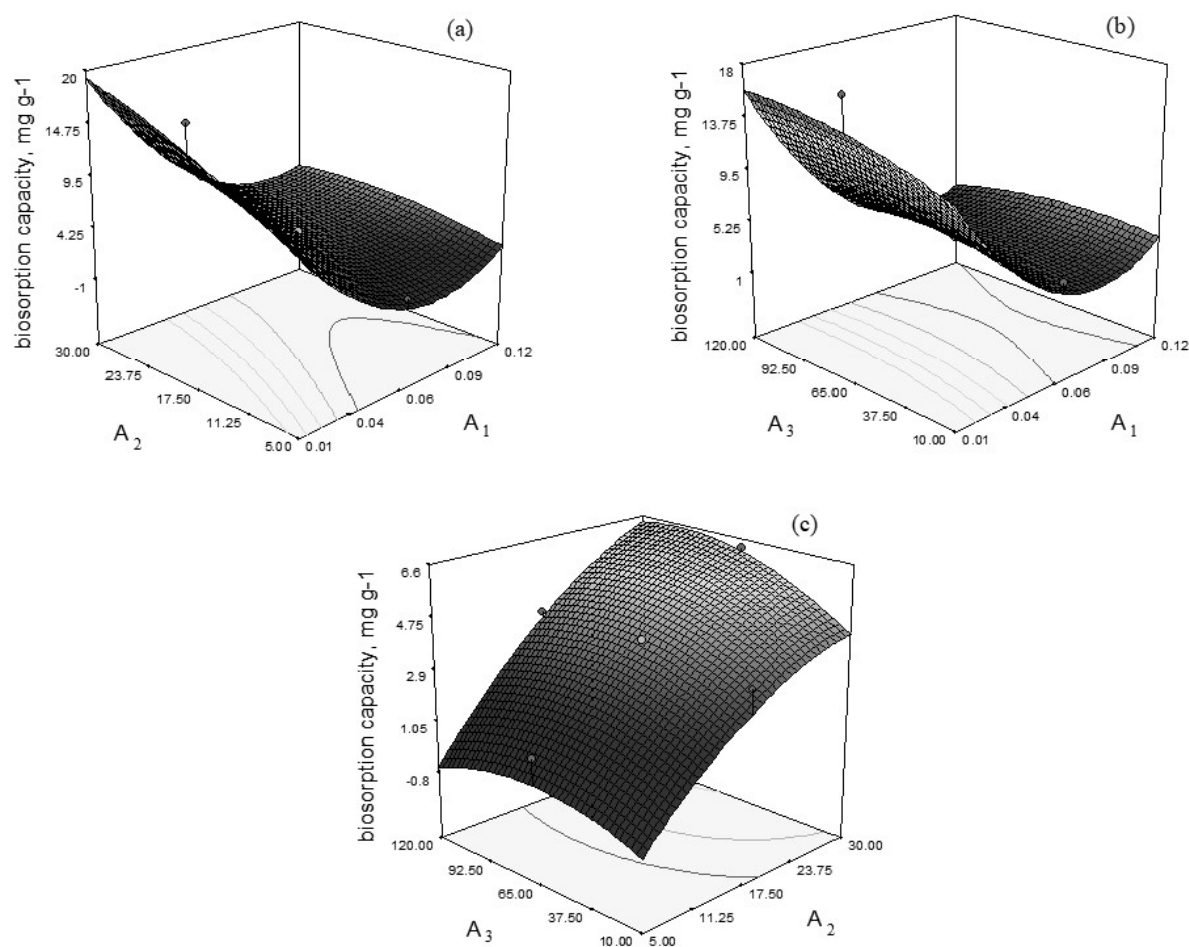


Fig. 4. 3D response surface plots for biosorption capacity of BG onto *A. oryzae* (a) A₁–A₂, (b) A₁–A₃ and (c) A₂–A₃.

Figure 3a displays the simultaneous effect of biomass dosage and initial brilliant green concentration on removal efficiency when the process time was kept at a constant value (65 min). The interactions demonstrated at lower concentrations of brilliant green the removal efficiency is higher and it decreases at a higher concentration of brilliant green. This is because at low dye concentrations, the contaminant is more likely to be biosorbed on the biomass, as the contaminant is more in contact with the biomass. Also, at higher initial concentrations of brilliant green, the biosorption percentage reduces in presence of large number of dye molecules in bulk solution as dye molecules in solution compete to reach the biomass (Pormazar and Dalvand, 2020). The effect of biomass dosage and process time on removal efficiency when the initial brilliant green concentration was maintained at a constant value (17.50 mg/L) is shown in Figure 3b. As the biomass dosage increased, the removal efficiency of brilliant green increased due to the better availability of the biomass surface area, which provides many accessible binding site for dye molecules (Ayazi et al., 2016). Figure 3c presents the interaction of initial brilliant green concentration with process time when the biomass dosage was kept at a constant value (0.07 g). At the first stage of the biosorption, the removal efficiency of brilliant green increased quickly, and afterward, it elevated increased slightly with extending the process time. The occurrence of this phenomenon is probably due to the vacant binding sites on the biomass surface for the brilliant green dye molecules that are initially enough. In the second stage of biosorption, the slower

removal efficiency achieved when the process time reaches 80 minutes may be due to reduced vacant binding sites in solution and slower diffusion of dye in biomass (Cheraghipour and Pakshir, 2021).

Figure 4a illustrated the main and interaction effects of biomass dosage and initial brilliant green concentration while maintaining process time at 65 min, Figure 4b illustrated the main and interaction effects of biomass dosage and process time while maintaining initial brilliant green concentration at 17.50 mg/L. The biosorbent capacity decreases with increasing amount of biomass up to about 0.07 g biosorbent dosage, then does not change significantly with increasing biosorbent dosage. This may be since the ratio of the quantity of brilliant green molecules in solution to the biosorbent free regions decreases with increasing biosorbent dosage. Thus, the possibility of interaction between the empty regions and brilliant molecules is reduced (Abe et al., 2019). Figure 4c is a 3D surface plot showing the simultaneous effect of process time and initial brilliant green concentration on the biosorption capacity of brilliant green at constant biomass dosage (0.07 g). It is obvious that the uptake capacity increases by increasing initial concentration of brilliant green. This is because higher dye concentrations provide an improved concentration gradient, a fundamental driving force that helps to resolve the mass transfer resistance of the dye molecules between the solid and liquid phases (Futalan et al., 2012).

For perform numerical optimization, the values of individual factors were stand the analyzed range, while the dependent factor (removal efficiency) was set to find maximum BG removal. For this purpose, by applying the desirability function, the removal efficiency of dye was found to be 67.32% at a biomass dosage of 0.10 g, initial BG concentration of 17.25 mg L⁻¹ and process time of 85 min with good desirability of 1.000. As a result of the

control experiments performed to determine the precision of the selected values, a removal efficiency of 68% was obtained, which shows the accuracy of the model in predicting dye removal. The reason for this is that the removal efficiency obtained in unit adsorbent amount is low and also the toxicity of the dyestuff is very high.

Table 4. Numerical value of the process factors for maximum BG removal efficiency (Desirability=1.000)

	Biomass dosage (g)	Initial BG concentration (mg L ⁻¹)	Process time (min)	Removal efficiency (%)
Optimum value	0.10	17.25	85	67.32

The biosorption efficiencies are related with many factors. Despite the optimization of the common factors that are most critical, the experimental research can be spread out. This situation can be explained with optimizing and discussing the role of temperature as a factor modifying desorption. Another aspect that may be useful is to focus on the properties of biosorption, where several microelements will be simultaneously absorbed into the biomass. Competition by ions for functional groups on the biomass surface is an interesting method to increase the scope

of such research. If the research is separate optimized research, it should be a pH and temperature dependent desorption. The scientists found that one of the other species of *Aspergillus* (*A. niger*) can remove dye materials from an aqueous solution and this is related with three major functional groups. These groups are carboxyl, amino and phosphate, and the lipid fraction in the biomass of *A. niger* play an important role in the biosorption of dye effluents.

3.2. Mortality bioassay

The mortality assessment of the treated and untreated mediums was analyzed using *D. magna* as a model organism. In this study, the mortality rate of *Daphnia* in treated medium were lower, 47,

77 and 90% while, the untreated medium showed 100, 100 and 100% after 24, 48 and 72 h, respectively. All *D. magna* died at untreated medium with an exposure time of 24 h. Additionally, no mortality has been determined in the natural living water (Figure 5).

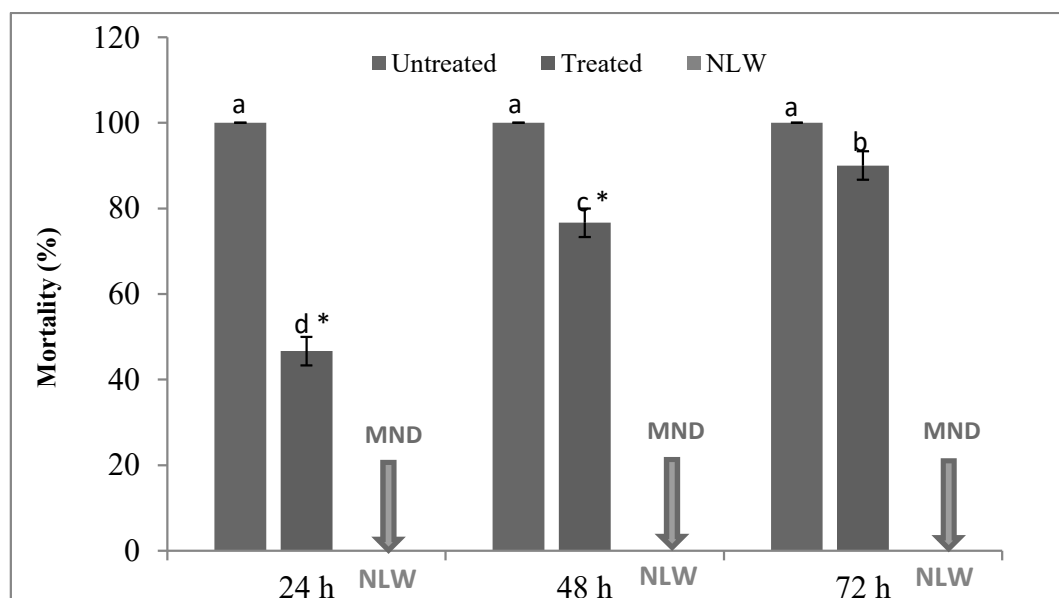


Fig. 5. The mortality rates of *D. magna* in treated and untreated mediums. MND: Mortality Not Determined, NLW: Natural Living Water

Although the decolorization rate under batch biosorption conditions by dead biomass of *A. oryzae* were achieved a significant value (~68%), *D. magna* mortality assessment demonstrated that the decolorization conditions was not sufficient to remove the toxicity of the dye.

In our study, the untreated BG dye has showed so toxic effect on *D. magna* with 100% mortality rate at 24h (Figure 5).

As a result of the treatment process by applying the biosorption method using *A. oryzae*, a significant decrease in the toxic effect (46% mortality) of the BG dye was determined. In our study, although approximately 68% decolorization was obtained, only 46% mortality decreasing was achieved.

Li et al. (2019) used *Aspergillus niger* fungal pellets for the removal of acidic anionic waste dyes with simultaneous growth

of *A. oryzae* pellets. The *A. oryzae* pellets removed nearly 98% of acid dye with a concentration of 200 mg/L.

Kumar et al. (2012) reported the effect of C/N ratio and initial concentration of dye effluents on the decolorization of BG by strains of *Aspergillus* sp. In addition, these scientists determined that the percentage of color removal increased with the increase in C/N ratio and decreased at high dye concentration. According to Deshmukh et al. (2016), the number of studies examining the biosorption properties of fungal spores for the removal of such contaminants is very few.

4. Conclusions and Recommendations

The use of RSM in biosorption studies for decolorization of dyes from aqueous solutions provides great benefits in terms of optimizing process variables. The originality of this study is that it is the first study ever to optimize process variables for decolorization of BG by dead biomass of *A. oryzae* and mortality assessment for *D. magna*. In recent years, the application of RSM among the most important factors has also contributed to reducing the number of necessary experiments, thus making it possible to determine the optimal parameters that affect the biosorption efficiency. At the same time, it would be a useful approach to evaluate the toxicity after decolorization of BG dye with *D. magna* bioassay. Further studies are required to reveal the reasons for the decrease in toxicity after decolorization.

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