Application of Box–Behnken Design for the Optimization of Crystal Violet Removal from Aqueous Solutions Using Acorn Waste from Quercus ilex

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Abstract. This study explores the optimization of Crystal Violet (CV) dye removal from aqueous solutions using *Quercus ilex* acorn waste (AW), a natural and low-cost adsorbent. The Box–Behnken Design (BBD), a response surface methodology (RSM) approach, was employed to assess the effects of three primary operational variables: adsorbent mass, initial CV concentration, and temperature. The main objective was to maximize removal efficiency while minimizing operational costs. Seventeen experiments were conducted, and the results were modeled using a second-order polynomial equation. Analysis of variance (ANOVA) confirmed the statistical significance of the model (**p** < 0.0001), with a high coefficient of determination (**R²** = 0.99), demonstrating strong predictive capability. Adsorbent mass and initial dye concentration positively influenced removal efficiency, whereas temperature had a negative influence. The optimal conditions for maximum removal were an initial CV concentration of 100 mg/L, an adsorbent dosage of 0.5 g/L, and a pH of 10. This study highlights the potential of AW as an effective and sustainable adsorbent for textile dye remediation and demonstrates the value of BBD in optimizing adsorption processes for environmental applications.

Keywords: Box–Behnken design; crystal violet (CV); adsorption; *Quercus ilex* acorn waste (AW); dye removal.

1 Introduction

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The discharge of synthetic dyes into aquatic ecosystems has become a pressing environmental concern, largely driven by their extensive application in industries such as textiles, paper, plastics, and pharmaceuticals. Among these dyes, Crystal Violet (CV) is commonly used for its strong coloring properties. However, this dye presents severe environmental and health risks, as it is known to be toxic, mutagenic, and resistant to natural degradation. Its persistence in water bodies can lead to long-term contamination, causing detrimental effects on aquatic life and human health due to its bioaccumulation and carcinogenic potential. Upon release into water systems, these dyes not only cause aesthetic pollution but also hinder the penetration of light and oxygen, disrupting aquatic ecosystems [1].

Various physical, chemical, and biological methods have been employed to remove dyes from wastewater, including coagulation, membrane filtration, and oxidation processes. However, many of these techniques are costly, energy-intensive, or produce secondary pollutants. Adsorption has gained prominence as an effective and economically viable method for dye removal due to its simplicity, low cost, and high efficiency—particularly when using natural or waste-derived adsorbents [2].

Among natural adsorbents, agricultural wastes have shown great potential due to their abundance, low cost, and eco-friendly nature. Acorn waste from *Quercus ilex* is a promising candidate for dye adsorption, given its high lignocellulosic content and surface properties that enhance dye removal.

This study aims to evaluate the effectiveness of *Quercus ilex* acorn waste as an adsorbent for the removal of CV from aqueous solutions, examining both kinetic and equilibrium parameters to assess the adsorption process.

To further investigate the influence of key operational parameters including initial dye concentration, adsorbent dosage, and solution pH on the adsorption of Crystal Violet (CV), response surface methodology (RSM) was applied using the experimental design method (EDM). RSM was selected as a powerful statistical and mathematical tool to evaluate the efficiency of an experimental system. This approach allows for the simultaneous assessment of multiple parameters while minimizing the number of experiments required. Consequently, using RSM helps lower costs, reduce process variability, and save time compared to the conventional one-factor-at-a-time approach [3].

The main objective of this study is, first, to conduct a statistical analysis of experimental data from a previous study to assess the effects of three independent variables (temperature, initial dye concentration, and adsorbent dosage) [4] on the adsorption of CV dye onto a biosorbent derived from acorn waste. The second objective is to optimize the factors influencing adsorption using RSM. Additionally, the interactions between independent variables will be examined. Finally, the experimental data analysis will facilitate the development of a mathematical model to describe the adsorption process.

2 Materials and methods

2.1 Preparation and characterization of the adsorbents

2.1.1 Biosorbent Preparation

Quercus ilex Acorn waste (AW) was collected from the Fez-Meknes region, Morocco. The biomass was first washed with tap water to remove impurities, then dried in an oven at

100 °C for 24 hours to eliminate all moisture. After drying, the material was ground and sieved to obtain particles in the size range of 200–500 µm.

2.1.2 Adsorbat

Crystal Violet (CV), with the chemical formula $C_{25}H_{30}ClN_3$, was selected as a model dye pollutant for wastewater treatment studies. The pH of the reaction medium was adjusted to the desired values using hydrochloric acid (HCl) and sodium hydroxide (NaOH), both supplied by Alpha Chemicals.

2.2 Batch Adsorption Studies

The adsorption study was conducted at 25 °C and a pH of 10 using a batch mode process. Initially, 0.5 g of acorn waste was added to a flask containing 100 mL of Crystal Violet (CV) solution at an initial concentration (C_o) of 100 mg/L. The mixture was then placed in a thermostatic water bath to maintain a constant temperature.

At predetermined contact times, the mixture was centrifuged at 3000 rpm for 10 minutes. The solid and liquid phases were separated by filtration using a microporous membrane. The resulting filtrate was analysed by UV-Visible spectrophotometry (Shimadzu UV-1800) at a wavelength of 570 nm, specific to CV. Dye concentrations at each contact time were obtained from a calibration curve based on standard CV solutions.

The CV removal efficiency (Rt, %) and the adsorption capacity of the acorn waste (qt, mg/g) were calculated using the following equations:

$$Q_t = (C_0 - C_e) \times \frac{V}{M} \tag{1}$$

$$R_t\,(\%) = \left(\frac{C_0 - C_e}{C_o}\right) \times 100 \tag{2}$$

Where Qt represents the adsorption capacity (mg/g), C_0 denotes the initial concentration of CV (mg/L), Ce (mg/L) signifies the equilibrium concentration of the CV solutions, m represents the mass of the adsorbent (g), and V indicates the volume of the CV solutions (L).

2.3 Box-Behnken Design

The Box-Behnken Design (BBD) is a widely used statistical method in response surface methodology (RSM) that is designed to optimize processes with multiple variables. It is an experimental design that is particularly efficient when it comes to modeling quadratic relationships, and it is ideal for situations where the goal is to optimize a response while considering the interaction between factors. One of the major advantages of the BBD is that it requires fewer experimental runs compared to other designs, such as the central composite design, making it a cost-effective approach. Additionally, the BBD does not require extreme factor levels, which helps minimize the risk of experimental failures that might occur due to harsh conditions [5].

This design is especially advantageous for processes where interaction effects between factors are significant, allowing for a more detailed understanding of these relationships without the need for excessive experimental effort [6].

In this study, BBD was used to optimize the removal efficiency of Crystal Violet by varying three key parameters: mass, temperature, and concentration. These parameters were selected because they significantly influence the adsorption process and may have complex interactions that affect the overall efficiency. Table 1 presents the experimental factors and their corresponding ranges. The mass of the adsorbent was varied between 10 mg and 100 mg, with a mean value of 55 mg.

This factor was chosen because the amount of adsorbent plays a critical role in determining the surface area available for Crystal Violet adsorption. Temperature was tested across a range of 30°C to 60°C, with a mean value of 45°C, as it is known to affect the rate and equilibrium of adsorption reactions. Finally, concentration of CV was varied between 3 mg/L and 30 mg/L, with a mean value of 16.5 mg/L, as it influences the driving force for the adsorption process due to the concentration gradient.

By exploring these factors within their specified ranges, BBD allowed for the identification of optimal conditions for contaminant removal while also revealing the potential interactions between these parameters. This experimental design provided a comprehensive and efficient way to study the effects of these factors on the adsorption process [7].

Table 1 : Experimental Factors and Their Ranges

Factor	Name	Units	Minimum	Maximum	Mean
A	Mass	Mg	10.00	100.00	55.00
B	Temperature	°C	30.00	60.00	45.00
C	Concentration	mg/L	3.00	30.00	16.50

3 Results and discussion

3.1 Experimental results

The diffractograms Table 2 shows the experimental runs and their corresponding results. It includes the values of mass, temperature, and concentration used in each run, along with the experimental removal percentage and the predicted removal percentage. The table 2 provides a comprehensive view of how different combinations of these parameters affect the removal efficiency of the Crystal Violet. For example, run 2, with a mass of 100 mg, temperature of 30°C, and concentration of 16.5 mg/L, resulted in an experimental removal of 95.46%, which is very close to the predicted removal of 95.82%. This alignment between experimental and predicted values suggests that the model is a reliable representation of the process, demonstrating the effectiveness of the Box-Behnken design in capturing the relationships between the factors [8].

After performing the Box-Behnken Design (BBD) and obtaining the experimental data, the next step in analyzing the results is to assess the model's adequacy and understand the influence of the different factors on the response. One effective way to evaluate the model is through Analysis of Variance (ANOVA) [9]. ANOVA helps determine whether the model terms are statistically significant and whether the factors involved are influencing the response variable. A key indicator of the model's fit is the R^2 value. The R^2 value, or coefficient of determination, measures the proportion of the variance in the response variable that is explained by the model. It ranges from 0 to 1, where a value closer to 1 indicates a better fit of the model to the experimental data [10]. If R^2 is high, it suggests that most of the variability in the response can be explained by the factors and their interactions considered in the model. A low R^2, on the other hand, would indicate that the model does not adequately explain the variability and may need adjustments or additional terms. In addition to R^2, the Adjusted R^2 is also important to consider, as it accounts for the number of terms in the model. The Adjusted R^2 is especially useful in models with multiple predictors, as it adjusts for the complexity of the model and can prevent overfitting [11]. ANOVA also provides other useful statistics, such as the p-value, which helps determine whether the factors have a significant effect on the response . A p-value less than 0.05 typically indicates that the corresponding factor or interaction is statistically significant . Factors or interactions with higher p-values suggest they may not be contributing significantly to the response and can potentially be removed from the model. The F-statistic is another crucial ANOVA output that compares the model's explained variance to the unexplained variance. A higher F-statistic value generally indicates that the model is statistically significant, meaning the factors and their interactions are important in predicting the response variable [12]. The Eq. (1) shows the relationship between the dependent variable (Yi) and the independent variables (Xi).

$$Y_i = \beta_0 + \sum_{i=1}^{n}(\beta_i X_i) + \sum_{i=1}^{n}(\beta_{ii} X_i^2) + \sum_{i=1}^{n-1}\sum_{j=1}^{n}(\beta_{ij} X_i X_j) \quad (3)$$

In this model, Yi indicates the response measured for the i experiment, β is the constant regression coefficient, and Xi and Xj represent the coded independent variables or factors.

Table 2 : Experimental and Predicted Removal Percentages of the Crystal Violet Adsorption under Various Conditions

Run	Mass	Temperature (°C)	Concentration (mg/L)	Removel Experimental (%)	Removel(%) Pedit
1	10	30	16.5	26.0363	25.01
2	100	30	16.5	95.4602	95.82
3	10	60	16.5	58.0333	57.67
4	100	60	16.5	89.1302	90.16
5	10	45	3	37.716	37.64
6	100	45	3	87.9685	86.51
7	10	45	30	13.3972	14.86
8	100	45	30	69.2023	69.28
9	55	30	3	83.0845	84.19
10	55	60	3	89.255	89.69
11	55	30	30	56.6272	56.19
12	55	60	30	78.7863	77.68
13	55	45	16.5	90.4581	89.95
14	55	45	16.5	88.1262	89.95
15	55	45	16.5	87.7031	89.95
16	55	45	16.5	92.6934	89.95
17	55	45	16.5	90.7785	89.95

3.2 Analysis of variance (ANOVA)

The ANOVA (Analysis of Variance) results for the Box-Behnken Design (BBD) demonstrate the statistical significance of each factor and their interactions in the removal efficiency of Crystal Violet, as detailed in Table 3. The Model itself has a significant F-value of 305.21 with a p-value < 0.0001, indicating that the over all model is statistically significant and provides a good fit for the experimental data. This suggests that the factors considered in the model significantly influence the response variable. Looking at the individual factors, the main effects of mass (A), temperature (B), and concentration (C) all show p-values < 0.0001, which means that they each have a significant impact on the CV removal. In particular, the factor mass (A) has the largest contribution, with a sum of squares of 5334.32 and a high F-value of 1419.49, demonstrating its dominant effect on the response. The interaction terms also provide valuable insights into how combinations of factors affect the removal efficiency. The interaction between mass and temperature (AB) is significant, with a p-value < 0.0001 and a substantial F-value of 97.72, indicating that these two factors interact to influence the response. Similarly, the interaction between temperature and concentration (BC) is significant with a p-value of 0.0044 and an F-value of 17.01, suggesting a notable combined effect. However, the interaction between mass and concentration (AC) has a p-value of 0.1952, indicating that it is not statistically significant at the 0.05 level and may not significantly affect the response [13].

The quadratic terms also demonstrate significant effects. The quadratic effect of mass (A^2) is highly significant, with a p-value < 0.0001 and an F-value of 636.11, while the quadratic effect of concentration (C^2) also shows a significant influence with a p-value < 0.0001 and an F-value of 221.30. On the other hand, the quadratic term for temperature (B^2) has a p-value of 0.3073, suggesting that it does not significantly impact the response. Regarding the residuals, the value is 26.31, with a mean square of 3.76, and the Lack of Fit has a p-value of 0.5772, indicating that the lack of fit is not significant, and the model is adequately describing the experimental data. The pure error term shows a sum of squares of 16.85 with a mean square of 4.21, which is relatively small, further suggesting a well-fitted model.

Table 3. Analysis of Variance (ANOVA) for the Removal Percentage Model of Crystal Violet

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	10322.48	9	1146.94	305.21	< 0.0001	significant
A-Mass	5334.32	1	5334.32	1419.49	< 0.0001	
B-Temperature	364.45	1	364.45	96.98	< 0.0001	
C-Concentration	800.22	1	800.22	212.94	< 0.0001	
AB	367.24	1	367.24	97.72	< 0.0001	
AC	7.71	1	7.71	2.05	0.1952	
BC	63.91	1	63.91	17.01	0,0044	
A^2	2390.44	1	2390.44	636.11	< 0.0001	
B^2	4.56	1	4.56	1.21	0.3073	
C^2	831.62	1	831.62	221.30	< 0.0001	
Residual	26.31	7	3.76			
Lack of Fit	9.46	3	3.15	0.7487	0.5772	not significant
Pure Error	16.85	4	4.21			
Cor Total	10348.79	16				

The ANOVA results also provide important statistics for assessing the model's quality, as shown in Table 4. The standard deviation (Std. Dev.) of the residuals is 1.94, which is relatively low, suggesting that the predicted values are close to the observed values and that the model fits the data well [14].The R^2 value of 0.9975 indicates that the model explains 99.75% of the variance in the response variable, demonstrating an excellent fit between the experimental data and the model. The Adjusted R^2 of 0.9942 further supports the model's robustness, accounting for the number of terms and ensuring that the model does not overfit.

The Predicted R^2 value of 0.9828 is also high, suggesting that the model has good predictive capability and can be used reliably for future predictions. The C.V. % (Coefficient of Variation) is 2.67%, which is quite low, indicating that the variability in the response is small relative to the mean, and thus the model is precise[15].Finally, the Adeq Precision value of 54.4532 is well above the desired threshold of 4, which means the model has an adequate signal-to-noise ratio, further confirming that the experimental design and model are both reliable for making predictions and optimizing the process.

Table 4 : Statistical Parameters for the Removal Percentage Model of Crystal Violet

Std. Dev.	1.94		R^2	0.9975
Mean	72.62		**Adjusted R^2**	0.9942
C.V. %	2.67		**Predicted R^2**	0.9828
			Adeq Precision	54.4532

The regression model for the Crystal Violet removal efficiency (R%) based on the Box-Behnken Design (BBD) provides important insights into how mass, temperature, and concentration influence the removal process. Eq. (2) , shows that mass (A) has the most significant positive effect on removal efficiency, with a large coefficient of 25.8223, meaning more mass leads to higher removal efficiency. Temperature (B) also contributes positively, but to a lesser extent (6.74957). In contrast, concentration (C) has a negative effect (-10.0014), indicating that higher concentrations reduce efficiency, likely due to the saturation of adsorption sites. The interaction terms between the factors such as AB, AC, and BC show both positive and negative effects, with AB having a negative influence and the others enhancing removal efficiency. Additionally, the quadratic terms reveal that increasing mass (A^2) and concentration (C^2) beyond certain points reduces the removal efficiency, while the temperature squared (B^2) has a slight positive effect.

$$R\ (\%)\ removal = 89.9519 + 25.8223\,A + 6.74957B - 10.0014C - 9.58176\,AB + 1.38815\,AC + 3.99715\,BC - 23.8271A^2 + 1.0402\,B^2 - 14.0538\,C^2 \qquad (4)$$

3.3 validity and accuracy of the model

The diagnostic plots presented in Fig.1(a-d) confirm the robustness and reliability of the developed model. In Fig. 1(a), the Normal Plot of Residuals shows that the residuals align closely with the straight line, indicating that they follow a normal distribution and satisfying the assumption of normality. In Fig. 1(b), the Residuals vs. Predicted plot demonstrates a random scatter of residuals around the horizontal line at zero, confirming homoscedasticity (constant variance) and the absence of bias in the model. In Fig. 1(c), the Residuals vs. Run plot reveals that the residuals fluctuate randomly without any discernible pattern or trend, suggesting no systematic errors occurred during the experimental runs and that the residuals are independent. Finally, in Fig. 1(d), the Predicted vs. Actual plot shows that the data points align closely with the diagonal line, indicating strong agreement between the predicted and observed values and demonstrating the high accuracy of the model in capturing the experimental response. These results collectively validate the model's assumptions and its predictive capability.

3.4 Response surface and contour analysis

Fig2. (a) and (b): Interaction of Adsorbent Mass and Temperature

The 3D plot (Fig.2(a)) demonstrates the combined influence of adsorbent mass and temperature on the CV removal percentage (R). As the adsorbent mass increases, the removal efficiency improves significantly, attributed to the increased availability of active adsorption sites. Additionally, a slight enhancement in CV Removal is observed with rising temperatures, which may indicate the adsorption process is endothermic or that higher temperatures improve molecular diffusion or interaction between adsorbate and adsorbent. The response surface reveals that adsorbent mass exerts a more substantial effect compared to temperature, as seen in the steep incline of CV Removal with increasing mass, while temperature changes yield a more gradual effect. The corresponding contour plot ((Fig.2(b)) highlights these trends, showing a wide region of high CV Removal (above 90%) at higher adsorbent masses, even at moderate temperatures. Conversely, at low masses and lower temperatures, the removal efficiency declines sharply, emphasizing the critical role of sufficient adsorbent mass in achieving effective adsorption.

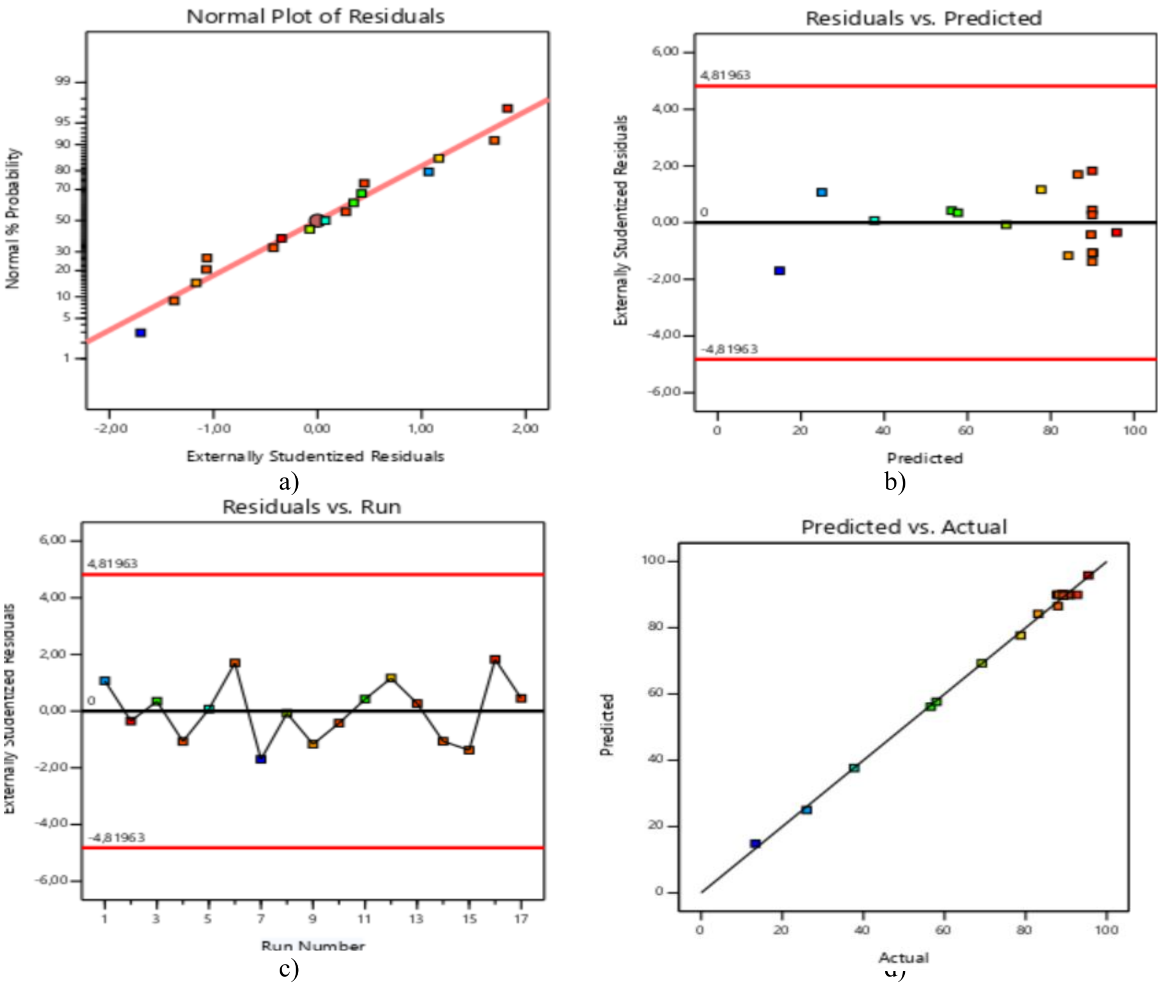
Fig. 2(c) and (d): Interaction of Adsorbent Mass and Initial Concentration

The 3D plot ((Fig.2(c)) illustrates the interaction between adsorbent mass and initial concentration, with CV Removal increasing as the adsorbent mass rises, consistent with the trend of more active sites being available. However, CV Removal decreases notably with increasing initial concentration, likely due to the higher demand for adsorption sites, which may become saturated at lower adsorbent masses. The response surface reveals the negative impact of concentration as dominant, with a strong tilt reflecting reduced efficiency at higher concentrations unless adsorbent mass is sufficiently increased.

The contour plot ((Fig.2(c)) further confirms these observations, where high CV Removal (above 90%) are achieved at low concentrations and higher adsorbent masses. In contrast, low mass combined with high initial concentration results in poor performance, demonstrating that maintaining a high adsorbent mass is essential to counteract the adverse effects of increased initial concentration.

Fig. 2(e) and (f): Interaction of Initial Concentration and Temperature

The 3D plot ((Fig.2(e)) examines the interplay between initial concentration and temperature on CV Removal. The results reveal a significant decrease in R with increasing concentration, as higher concentrations saturate the available adsorption sites, leaving fewer active sites unoccupied. Temperature shows a mild positive effect, particularly at lower concentrations, possibly due to enhanced adsorption kinetics or the endothermic nature of the adsorption process.

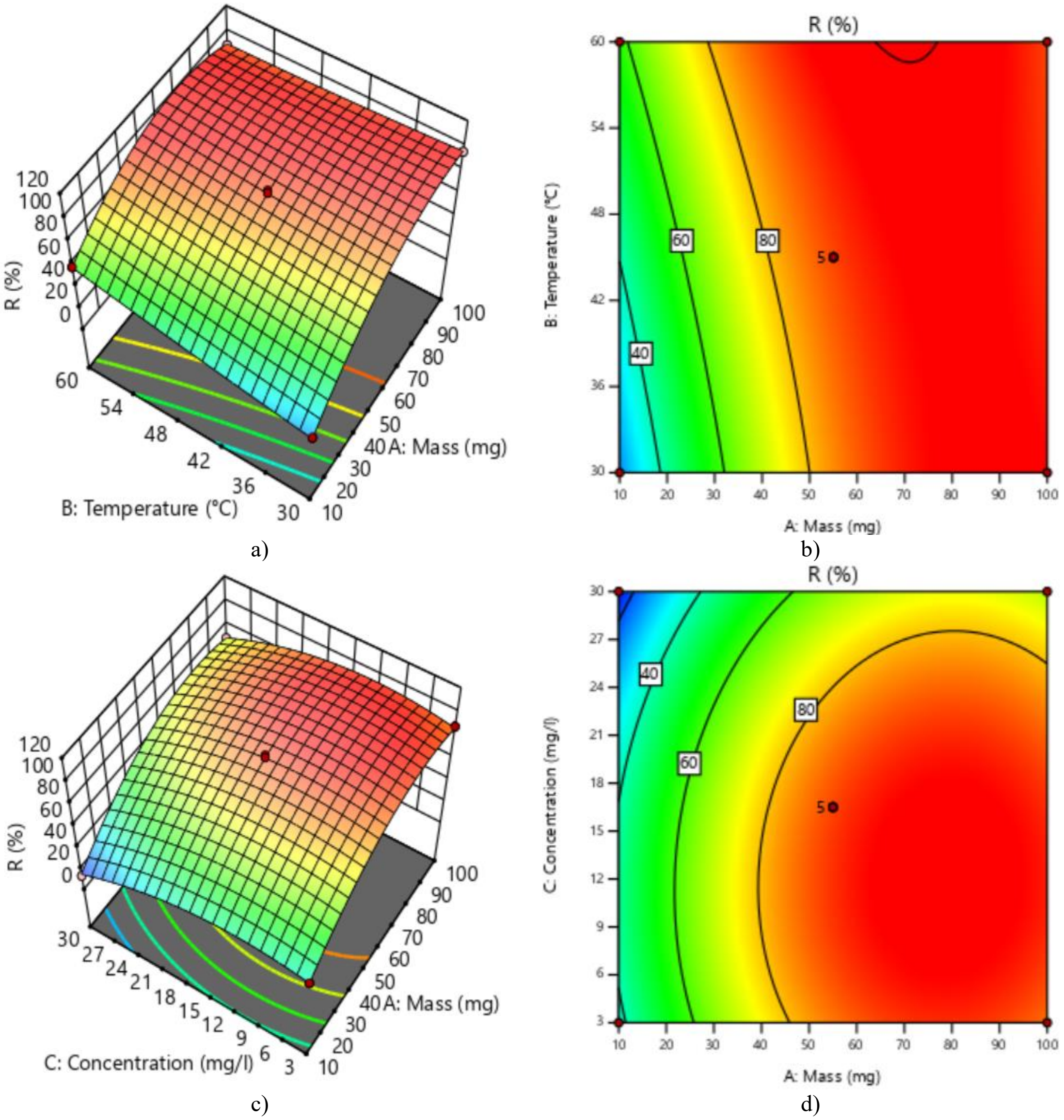


a) b)

c) d)

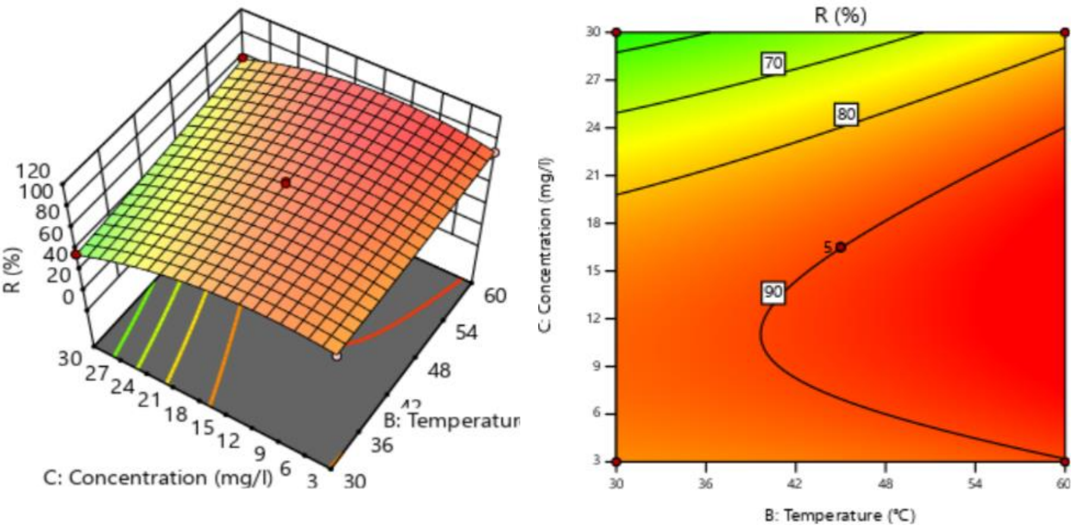
Fig. 1 : a) Normal Plot of Residuals; Figure b) Residuals vs. Predicted; Figure c) Residuals vs. Run Order; Figure d) Predicted vs. Actual

However, the overall impact of temperature is less pronounced compared to the dominant influence of concentration. The contour plot ((Fig.2(f)) reinforces this trend, with regions of high CV Removal (above 80%) confined to low initial concentrations, regardless of temperature. At high concentrations, even increased temperatures fail to substantially improve the removal efficiency. This interaction highlights the need to maintain lower concentrations to achieve optimal performance, while moderate temperature adjustments may further enhance efficiency under favorable conditions.


R (%)
120
100
80
60
40
20
0
B: Temperature (°C)
60
54
48
42
36
30
A: Mass (mg)
10
20
30
40
50
60
70
80
90
100
R (%)
B: Temperature (°C)
40
60
80
5
A: Mass (mg)
R (%)
C: Concentration (mg/l)
30
27
24
21
18
15
12
9
6
3
A: Mass (mg)
R (%)
C: Concentration (mg/l)
40
60
80
5
A: Mass (mg)

a) b)

c) d)



e) f)

Figure 2 : 3D Surface and Contour Plots Depicting the Effects of Mass, Temperature, and Concentration on the Removal Efficiency of Crystal Violet (a, b) Effect of Mass and Temperature on Removal Efficiency, (c, d) Effect of Mass and Concentration on Removal Efficiency, (e, f) Effect of Temperature and Concentration on Removal Efficiency

4 Conclusion

This research effectively applied the Box–Behnken Design (BBD) within the framework of Response Surface Methodology (RSM) to optimize the removal of Crystal Violet (CV) from aqueous solutions using a biosorbent derived from acorn waste. The study focused on three main operational variables: biosorbent dosage, solution temperature, and initial dye concentration, examining their individual and interactive effects on the adsorption process. Among these, the biosorbent mass was identified as the most influential factor, significantly enhancing dye removal, whereas higher dye concentrations were found to reduce adsorption efficiency. Temperature had a moderate but positive effect.

A quadratic regression model was successfully developed and demonstrated high predictive power, with a coefficient of determination (R^2) of 0.9975, an adjusted R^2 of 0.9942, and a predicted R^2 of 0.9828, indicating excellent agreement between experimental and predicted values. The model's validity was further supported by a low standard deviation (1.94) and a high Adeq Precision value (54.45). Analysis of variance (ANOVA) confirmed the statistical significance of the main factors and their interactions, particularly those between biosorbent mass and temperature, as well as between temperature and dye concentration. Under optimized conditions—100 mg biosorbent, 45 °C, and 3 mg/L dye concentration—a removal efficiency exceeding 92% was achieved. Diagnostic tests, including residual analysis, verified that the model met key assumptions such as normal distribution, constant variance, and independence of residuals. Additionally, response surface and contour plots provided visual insights into factor interactions, highlighting the dominant role of biosorbent dosage.

The removal of Crystal Violet (CV) dye by acorn waste is likely driven by a combination of electrostatic interactions, hydrogen bonding, and π–π interactions. Electrostatic attraction occurs between the negatively charged surface groups of the acorn waste and the positively charged CV dye. Hydrogen bonds can form between dye molecules and oxygen-containing functional groups on the acorn waste surface. Additionally, π–π interactions between the aromatic rings of the dye and the carbonaceous material in the acorn waste can contribute to adsorption. Similar mechanisms have been observed in other studies involving dye removal using various adsorbents.

In conclusion, the results demonstrate that Quercus ilex acorn waste is a promising and sustainable biosorbent for the effective removal of crystal violet dye. The use of the Box–Behnken Design (BBD) not only optimized the experimental procedure but also provided a comprehensive understanding of the adsorption mechanisms, highlighting its potential for scaling up dye removal processes in industrial applications. These findings confirm the feasibility of utilizing agricultural waste materials in wastewater treatment, presenting a sustainable, cost-effective, and locally available alternative to conventional adsorbents particularly relevant for the textile industry. However, this study is limited to batch-scale experiments with synthetic dye solutions. Future research will focus on biosorbent regeneration, fixed-bed column experiments, and the treatment of real industrial wastewater to evaluate the scalability and long-term performance of the process.

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