



Bio-removal and Detoxification of Congo Red Dye in Aqueous Solutions by Using a Sustainable Biosorbent from *Robinia pseudoacacia* L.

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Abstract

Synthetic dyes cause serious damage to the environment. If synthetic dyes are discharged without treatment, they become a serious environmental problem in aquatic ecosystems. The present study aimed to bio-remove Congo red (CR) from aqueous solutions using *Robinia pseudoacacia* L. fruit (RPF) biosorbent from aqueous solutions via a biosorption process. Optimization studies were conducted on the biosorption parameters, including stirring speed, temperature, pH, contact time, initial CR dye concentration, zero charge point (pH_{pzc}), and biosorbent dosage. As a result, it was observed that 80% CR was removed from aqueous solutions using RPF biosorbent under optimum conditions (temperature: 303 K, stirring speed: 150 rpm, pH: 6, biosorbent dose: 1 g, contact time: 30 min, and initial CR dye concentration: 10 mg/L). For kinetic studies, the R^2 value ($R^2=0.999$) of the pseudo-second-order kinetic model was a better fit than the R^2 value ($R^2=0.976$) of the pseudo-first-order kinetic model. The R^2 value of the Langmuir isotherm was 0.997, and the Langmuir isotherm model best fitted the R^2 values of the Freundlich ($R^2=0.976$), Elovich ($R^2=0.952$), and Temkin ($R^2=0.993$) isotherm models. Because ΔG° values (-3.41, -4.5, -5.59 and -6.68 kJ/mol) were calculated as negative in thermodynamic studies, the biosorption process occurred spontaneously. Moreover, detoxification studies revealed significant recovery of anatomical and physiological parameters in *A. cepa* and *B. oleracea* var. *acephala* after RPF biosorbent treatment. The germination rate of *B. oleracea* var. *acephala* seeds decreased to 40% with increasing CR concentrations. Moreover, significant decreases were observed in the fresh weight (0.46 g), dry weight (0.064 g), root length (1.16 cm), and shoot length (1.36 cm). Consequently, the present study highlights the effective treatment, low-cost, and eco-friendly potential of RPF biosorbents for the removal of CR from aqueous solutions.

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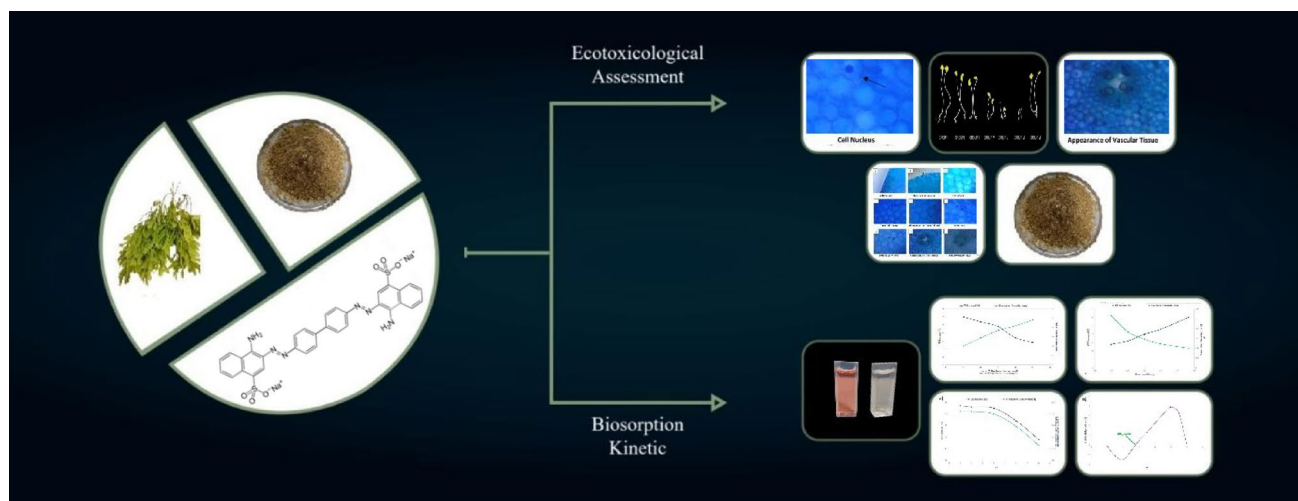
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Graphical Abstract



Highlights

CR decreased seed germination and growth of *B. oleracea* var. *acephala*.
RPF biosorbent treatment significantly reduced CR toxicity.
Biosorption process occurred spontaneous.

Keywords Biosorption · Congo red · Isotherms · Kinetics · Phytotoxicity · *Robinia pseudoacacia*

Introduction

Water resources are gradually decreasing due to the expansion of industries, rapid population growth, and global warming. The decline in water resources poses a serious hazard to organisms in the ecosystem (Akdemir et al. 2022; Rehman et al. 2024). In particular, effluents from textile industries, which use synthetic dyes in products such as paint, paper, textiles, and cosmetics, create a significant environmental problem. Textile industries consume more than 700,000 tons of synthetic dyes annually, approximately 400,000 tons of which are discharged into the environment (Abbas et al. 2023; Qaiyum et al. 2023). Synthetic dyes exhibit toxic properties including teratogenic, mutagenic, and carcinogenic effects. Therefore, the treatment of effluents containing synthetic dyes has become a global priority for mitigating their adverse environmental impacts (Fawzy et al. 2023; Gedam et al. 2019).

Congo Red (CR) is an aromatic synthetic dye that is widely used in various industries, including pharmaceuticals, printing, and dyeing. CR poses serious risks to human health owing to its harmful effects, such as skin, eye, and gastrointestinal irritation (Ahmad Aftab et al. 2023; Aounallah et al. 2024; Sathya et al. 2023). When released into aquatic environments, such as lakes, rivers, and streams, CR prevents the penetration of sunlight, thereby inhibiting

photosynthesis, which is vital for ecosystem organisms. This process ultimately leads to eutrophication, creating severe environmental threats. Hence, CR must be treated before being discharged into receiving environments (Adeleke et al. 2024; Litefti et al. 2019).

Wastewater treatment methods such as membrane filtration, ion exchange, and coagulation/flocculation have been applied for the removal of CR from aqueous solutions. However, these methods suffer from drawbacks, including chemical reagent usage, membrane fouling, large space requirements, and high energy consumption, prompting the search for alternative approaches (Munagapati et al. 2024; Suteu et al. 2024). In recent years, biosorption has emerged as a promising alternative for CR removal utilizing various biosorbents derived from natural sources. Biosorption is considered an attractive method because of its sustainability, eco-friendliness, and high efficiency for dye removal (Sarıkaya et al. 2024; Torun and Aracagök, 2024). Since the biosorbents used in biosorption offer advantages such as abundance in nature, low-cost, and effective treatment, the biosorption process is an effective treatment method for the removal of heavy metals and various organic pollutants (Ibrahim et al. 2022).

Numerous studies have investigated CR removal using biosorbents, such as *Valeriana officinalis* roots (Akdemir et al. 2022), Euroamerican poplar (Stjepanović, et al. 2021), neem bark (Sathya et al. 2023), teak leaf powder (Gedam et

al. 2019), *Vangueria infausta* fruit pericarp (Kitemangu et al. 2023), *Imperata cylindrica* (Bello et al. 2024) and *Terminalia ivorensis* (Babalola et al. 2017). However, no study has been conducted on CR removal from an aqueous solution-based *Robinia pseudoacacia* L. (Fabaceae) biosorbent. Moreover, although various types of *P. acacia*-based biosorbents have been reported in the literature for dye and heavy metal removal studies (Khorshidi and Niazi 2016; Vassileva et al. 2018), fruit-based biosorbents have recently been recognized to possess significant potential for the removal of cationic crystal violet dyes (Güllüce et al. 2024). Therefore, the effectiveness of this biosorbent against anionic dyes such as Congo Red (CR) remains unknown.

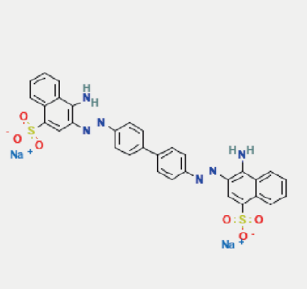
The present study aimed to investigate the bio-removal of CR dye in aqueous solutions using RPF biosorbent, as well as the phytotoxic effects of the CR solution. To determine the optimum conditions for CR removal, the parameters affecting biosorption, including stirring speed, temperature, pH, contact time, initial dye concentration, point of zero charge (pH_{pzc}), and biosorbent dosage, were evaluated. Furthermore, isotherm, kinetic, and thermodynamic models were used to elucidate the underlying biosorption mechanisms. In addition, the phytotoxicity of untreated and treated CR solutions was examined.

Materials and Methods

Preparation of Congo Red (Cr) Solutions

CR was supplied by Sigma-Aldrich (C.I. 22,120). 100 ppm stock solution was prepared by dissolving 0.01 g in 1 L of pure water. The effect of the initial CR dye concentration was investigated by diluting the working solutions from the stock solution. The general properties of the CR dyes are listed in Table 1.

Table 1 General properties of CR

Dye Common Name	Molecular weight	Class	Molecular Structure
Congo Red	696.665 g/mol	Anionic Dye	 $C_{32}H_{22}N_6O_6S_2 \cdot 2Na$

Preparation of Robinia pseudoacacia L. (Fabaceae) (RPF) Biosorbent

Robinia pseudoacacia L. (Fabaceae) fruit (RPF) biosorbent was collected from the acacia trees. To remove the pollutants from the biomass, it was washed with a galore of tap water and then washed with pure water 2–3 times. It was then dried at 80 °C for 24 h. Then, the biomass was ground into a powder, and the obtained RPF biosorbent was stored in an airtight container at room temperature until it was used for biosorption studies.

RPF Biosorbent Characterization

An FTIR spectrometer (VERTEX 70v) was used in the wavenumber range of 4000–400 cm^{-1} to determination the functional groups in RPF before and after biosorption.

Biosorption Experiments

In biosorption studies, pH (3–10), temperature (303–333 K), biosorbent dose (0.2–1 g), initial CR dye concentration (10–30 mg/L), stirring speed (75–150 rpm), and contact time (5–30 min) were investigated as parameters affecting biosorption for CR removal using RPF biosorbent from aqueous solutions. The pH was adjusted by adding 0.1 N HCl or 0.1 N NaOH. To obtain the amount of biosorption of CR dye by the RPF biosorbent, an absorbance calibration curve versus concentration was plotted (Fig. 1). After centrifugation at 6000 rpm for 5 min, the supernatant of the samples was measured using a UV–Visible Spectrophotometer (PG Instruments Limited T80+) at 500 nm (Batana et al. 2022; Ozola-Davidane et al. 2021). All experiments were performed in triplicates. CR removal (%) was calculated using Eq. (1). The biosorption capacity (q_e) was calculated using Eq. (2).

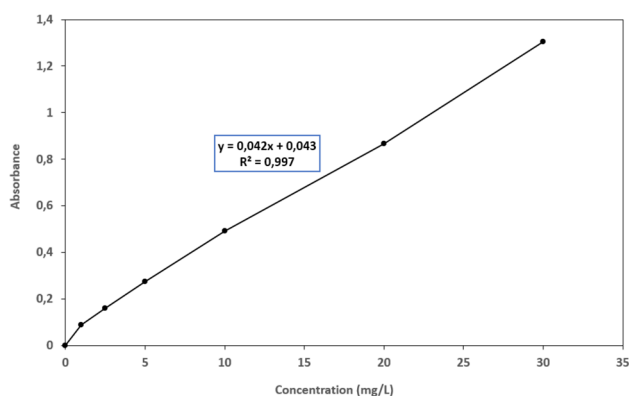


Fig. 1 Calibration curve

$$CR_{\text{removal}} (\%) = \left(1 - \frac{C_e}{C_0}\right) \times 100 \quad (1)$$

$$q_e = \frac{(C_0 - C_e)}{w} \times V \quad (2)$$

q_e is the biosorbent capacity at equilibrium (mg/g), where w is the weight of RPF biosorbent (g). C_0 and C_e (mg/L) are the initial and equilibrium CR dye concentrations, respectively. V is the solution volume (L), and all studies were carried out in a 0.05 L solution volume.

Zero Charge Point (pH_{pzc}) of RPF

pH_{pzc} is a critical parameter used to determine the net surface charge of the biosorbent. To calculate the pH_{pzc} value of RPF biosorbent, 0.15 g of RPF biosorbent was added to each of 0.05 L of 0.1 M NaCl solution. The pH of each sample was adjusted between 2 and 12 with 0.1 N HCl or 0.1 N NaOH. After the pH adjustment, the samples were shaken in an incubator at 150 rpm for 48 h. Then, the final pH values of the samples were measured, and ΔpH (initial pH–final pH) was calculated. A graph of ΔpH against pH is plotted. In this graph, the point that intersects the abscissa of pH gives the pH_{pzc} (Mubarik et al. 2025).

Biosorption Kinetics Studies

Pseudo-first-order, pseudo-second-order, Elovich and intraparticle diffusion kinetic models were calculated for the biosorption kinetic studies. The formula for the pseudo-first-order kinetic model is shown in Eq. (3).

$$\ln(q_e - q_t) = \ln(q_e) - kt \quad (3)$$

k_1 is the rate constant (min^{-1}). The k_1 and q_e values were calculated from the slope point and cut-off point of the graph drawn against $\ln(q_e - q_t)$ versus t , respectively. The formula

for the pseudo-second-order kinetic model is shown in Eq. (4).

$$\frac{t}{q_t} = \frac{1}{k_2 q_e} + \frac{t}{q_e} \quad (4)$$

q_t is the biosorption capacity at time t (mg/g), where k_2 is the rate constant ($\text{g}/(\text{mg} \cdot \text{min})$). The q_e and k_2 values were calculated from the slope point and cut-off point of the graph drawn against (t/q_t) versus t , respectively. The formula for the Elovich kinetic model is shown in Eq. (5).

$$q_t = \frac{1}{\beta} \ln(\alpha * \beta) + \frac{1}{\beta} \ln(t) \quad (5)$$

where α is the initial biosorption rate ($\text{mg}/(\text{g} \cdot \text{min})$). β is the Elovich constant (g/mg). The β and α values were calculated from the slope point and cut-off point of the graph drawn against q_t versus $\ln(t)$, respectively. The formula for the intraparticle diffusion kinetic model is shown in Eq. (6).

$$q_t = C + k_i \sqrt{t} \quad (6)$$

k_i is the intraparticle diffusion rate constant ($\text{mg}/(\text{g} \cdot \text{min}^{1/2})$). C is a constant that defines the thickness of the boundary layer. (mg/g). The C and k_i values were calculated from the slope point and cut-off point of the graph drawn against q_t versus $t^{1/2}$, respectively.

Biosorption Isotherms Studies

Langmuir, Freundlich, Elovich, and Temkin isotherm models were used in the biosorption isotherm studies. The formula for the Langmuir isotherm is given in Eq. (7).

$$\frac{1}{q_e} = \frac{1}{q_0 L} \frac{1}{C_e} + \frac{1}{q_0} \quad (7)$$

L is the constant of the Langmuir isotherm, which depends on the biosorption free energy. (L/mg), where q_0 is the biosorption capacity of the Langmuir isotherm on a monolayer (mg/g). The L and q_0 values were determined from the slope point and cut-off point of the graph drawn against $(1/q_e)$ versus $(1/C_e)$, respectively. The separation factor (R_L) values in Eq. (8) is a dimensionless value of the Langmuir isotherm model.

$$R_L = \frac{1}{1 + LC_0} \quad (8)$$

The formula of the Freundlich isotherm was shown by Eq. (9).

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \quad (9)$$

K_f is a constant in the Freundlich isotherm model. where n is the biosorption density on heterogeneous surfaces in the Freundlich isotherm. The n and K_f values were determined from the slope point and cut-off point of the graph drawn against $\ln(q_e)$ versus $\ln(C_e)$, respectively.

The formula of the Elovich isotherm was shown by Eq. (10).

$$\ln \left(\frac{q_e}{C_e} \right) = \ln (K_e q_s) - \left(\frac{q_e}{q_s} \right) \quad (10)$$

K_e is the constant in the Elovich isotherm model. where q_s is the maximum biosorption capacity in the Elovich isotherm (mg/g). The q_s and K_e values were determined from the slope point and cut-off point of the graph drawn against $\ln(q_e/C_e)$ versus (q_e) , respectively.

The formula of the Temkin isotherm was shown by Eq. (11).

$$q_e = \frac{RT}{\Delta T} \ln (KT) + \frac{RT}{\Delta T} \ln (C_e) \quad (11)$$

K_T is a constant of the Temkin isotherm model (L/g). ΔT is the energy change during the biosorption process (kJ/mol). R is the universal gas constant (8.314 J/mol*K). where T is the absolute temperature. The ΔT and K_T values were calculated from the slope point and cut-off point of the graph drawn against (q_e) versus $\ln(C_e)$, respectively.

Biosorption Thermodynamics

A thermodynamic study was conducted to determine whether the results obtained from the biosorption process were physical or chemical. ΔG° (kJ/mol), ΔH° (kJ/mol), and ΔS° (J/mol*K) values were calculated using Eqs. (12) and (13) (Van 't Hoff Equations).

$$\ln K_M = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \quad (12)$$

$$\Delta G = \Delta H - T\Delta S \quad (13)$$

K_M value was calculated using Eq. (14).

$$K_M = \frac{C_a}{C_e} \quad (14)$$

C_a is CR concentration on RPF biosorbent (mg/L).

Determination of Detoxification Potential of The Rpf Biosorbent Treatment

The phytotoxicological effects of untreated and treated CR solutions were determined using inhibition of seed germination and seedling growth parameters (Kishor et al. 2021). Isogenic kale seeds (*Brassica oleracea* var. *acephala*), were obtained from the Faculty of Agriculture. Ten healthy seeds were selected for each treatment group. Surface sterilization was performed by treating the seeds with 2% HgCl_2 solution for 2 min. The seeds were washed twice with sterile distilled water and placed in sterile petri dishes with sterile Whatman Grade 1 filter paper at the bottom. CR solutions (5 mL of CR solutions was added to the test groups and 5 mL of distilled water was added to the control groups. The Petri dishes were incubated at 28 °C in a BOD incubator for 7 days. On the second day of incubation, seed germination rates were recorded and seedling growth parameters were calculated at the end of the incubation period. The seed germination rate (%) was calculated using Eq. (15).

$$\text{Germination rate (\%)} = \left(\frac{\text{count of germinated seeds}}{\text{count of sowed seeds}} \right) \times 100 \quad (15)$$

In addition, *Allium* root tips grown in the control and test solutions were used to determine anatomical alterations in the plant roots. For this purpose, uniformly sized healthy *Allium cepa* L. bulbs were purchased from local markets. Then, the yellow–brown outer scales of selected bulbs were peeled off, and the bulbs were immersed in test tubes containing either distilled water (control group), untreated, or treated CR solutions (test groups). After a 48 h incubation period at 25 °C in the dark, the root tips were collected from the bulbs and washed with distilled water. Cross-sections were then taken with a sharp razor blade and stained with methylene blue (5%) for 2 min. Finally, cellular and structural damage was observed under a light microscope (LEICA DM 750) at 500× magnification (Çavuşoğlu et al. 2021).

All phytotoxicity experiments were conducted in triplicate with two replicates, and the results were statistically analysed by 1-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) and presented as means and standard deviations.

Results and Discussion

FTIR Analysis

FTIR analysis was performed to characterize the RPF biosorbent surface before and after biosorption of Congo Red

from aqueous solutions (Fig. 2a and b), and a large peak value was observed at 3383 cm^{-1} before the biosorption process, indicating the presence of -OH groups of phenols and alcohols in the lignin and cellulose of the RPF (Dutta et al. 2025). The peak at 2850 cm^{-1} could be assigned to the C-H stretching of the methyl group (Sorour et al. 2024). The peak values at 1731 and 1615 cm^{-1} could be attributed to the stretching of the non-conjugated C=O , C=O stretching of the carboxyl group, and -NH_2 stretching of the amino group (Shkliarenko et al. 2023; Güllüce et al. 2024). The strong peak at 1026 cm^{-1} could be assigned to C-O stretching (Gemici 2024). After biosorption, the peak at 3390 cm^{-1} can be attributed to -OH and -NH stretching (Bellaj et al. 2024). The peak at 2854 cm^{-1} could be assigned to the C-H stretching (Adeleke et al. 2024). The peak values at 1733 and 1620 cm^{-1} could be attributed to the stretching of the non-conjugated C=O , conjugated C=C stretching (Abbas et al. 2023; Shkliarenko et al. 2023). The strong peak at 1031 cm^{-1} could be assigned to C-O stretching (Galera et al. 2023). The peak values of 3383 , 2850 , 1731 , 1615 , and 1026 cm^{-1} shifted to the peak values of 3390 , 2854 , 1733 , 1620 , and 1031 cm^{-1} after biosorption. This shift indicates that the interaction between the CR dye molecules and functional groups on the RPF biosorbent surface resulted in a successful biosorption process.

Effect of pH

The pH is a critical parameter in determining the physical interaction between the dye solution and the biosorbent in the biosorption process (Mehrez et al. 2024). The effects of biosorption capacity (q_e) and CR removal (%) in the pH range of (3–11) was investigated (Fig. 3a). In addition, the pH_{pzc} value was calculated to determine the net surface

charge of the biosorbent. In the graph of ΔpH versus pH, pH_{pzc} was calculated as 5.56 (Fig. 3b). At $\text{pH}_{\text{pzc}}=5.56$, the net surface charge of the RPF biosorbent is neutral.

As the pH increased, the CR removal and biosorption capacities decreased. When $\text{pH}_{\text{pzc}}>\text{pH}$, the biosorbent surface charge was positively charged. At low pH values, H^+ ions are high, which creates electrostatic interactions between the RPF biosorbent and anionic CR dye molecules. At $\text{pH}>\text{pH}_{\text{pzc}}$, the biosorbent charge is negatively charged and OH^- ions are high, which creates electrostatic repulsion between the RPF biosorbent and dye molecules. Therefore, as the pH increases, CR removal and biosorption capacity decrease (Akdemir et al. 2022; Kitemangu et al. 2023). Many similar studies have shown that CR removal and biosorption capacity decrease as the pH increases in the literature (Alabi et al. 2024; Bellaj et al. 2024; Bello et al. 2024; Kitemangu et al. 2023).

At pH 6, CR removal was 80% and the biosorption capacity was 0.40 mg/g . All experiments were carried out at the natural pH of the CR dye ($\text{pH}=6$).

Effect of Contact Time

The effect of the contact time on CR removal (%) was determined, as shown in Fig. 4. The CR removal also increased when the contact time was increased from 5 to 25 min. This increase was observed in the first 25 min because the active sites of the RPF biosorbent were available and the dye molecules were bound to the active sites (Solmaz 2024). It was observed that equilibrium was reached equilibrium between 25–30 min. This was because the free areas of the biosorbent surface were accessible during the first 25 min. However, a repulsive force occurred because the dye molecules bound to the empty biosorbent surface between 25–30 min.

Fig. 2 FTIR spectrum **a** before **b** after biosorption of CR onto RPF

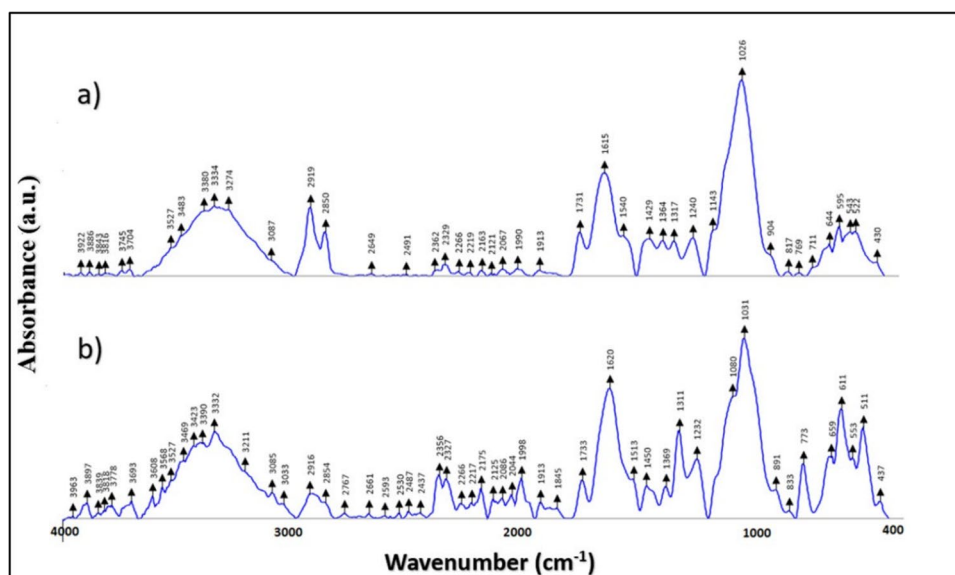


Fig. 3 Effect of pH on biosorption capacity (mg/g) and CR removal (%) based on RPF biosorbent (a); Effect of the zero charge point of the RPF biosorbent (b)

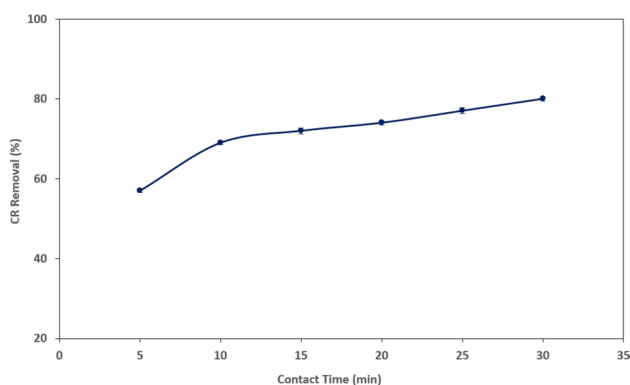
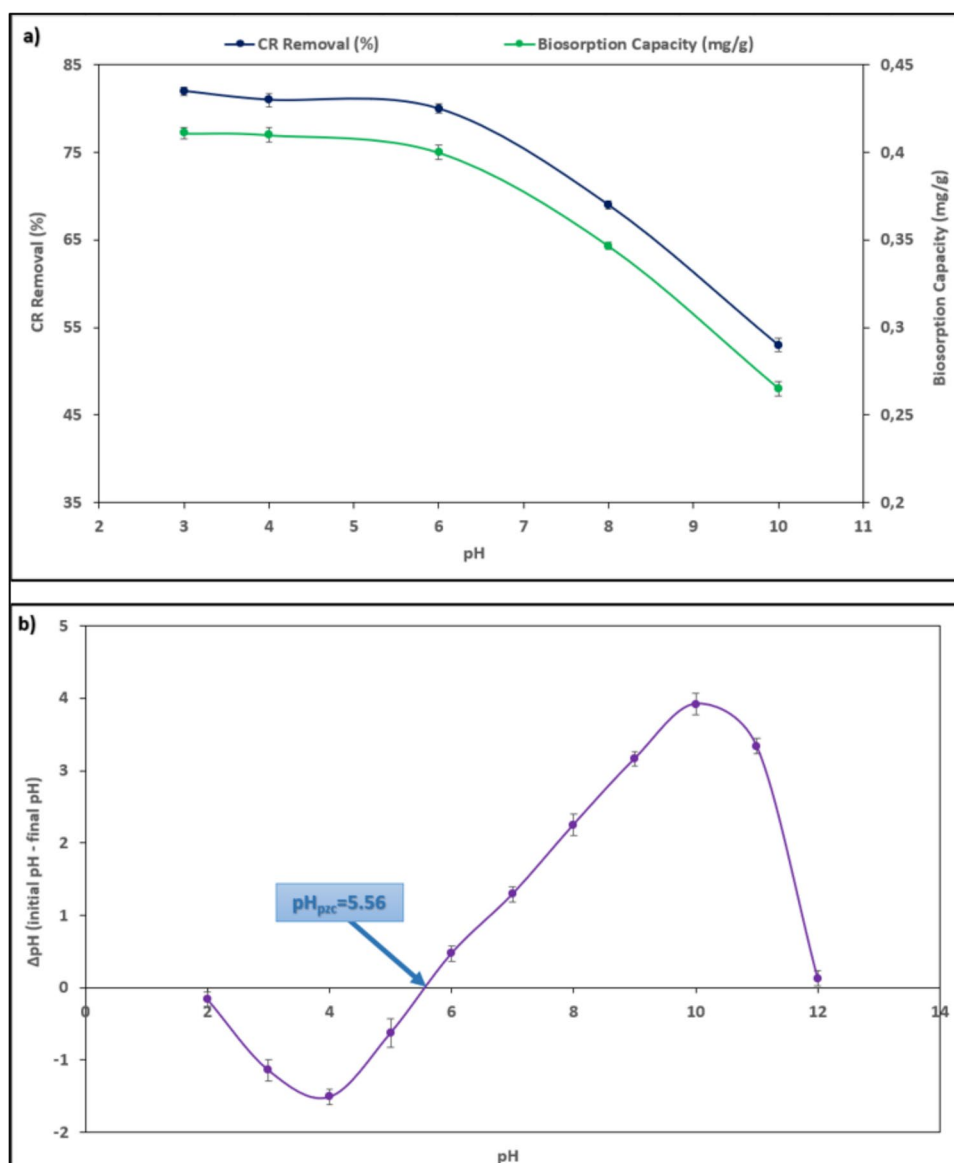


Fig. 4 Effect of contact time CR removal (%) based on RPF biosorbent (Chandarana et al. 2021). Furthermore, because the dye molecules bind sufficiently to the biosorbent surface, the biosorbent surface becomes saturated (Isik et al. 2023).

Effect of biosorbent dose

CR removal (%) and biosorption capacity are shown in Fig. 5 for their effect on biosorbent dose. According to these results, as the biosorbent dose increased, CR removal increased from 64 to 80%. The reason for this increase is explained more binding of dye molecules to the active sites of the biosorbent since the active sites of the biosorbent are more (Ahmad Aftab et al. 2023; Munagapati et al. 2024).

However, as the biosorbent dose increased, the biosorbent capacity decreased. CR dye molecules may have accumulated in the binding sites of the RPF biosorbent (Oulakhir et al. 2023). In addition, due to the extension of the diffusion path, the binding of dye molecules to the biosorbent surface is decreased (Karadayı et al. 2025).

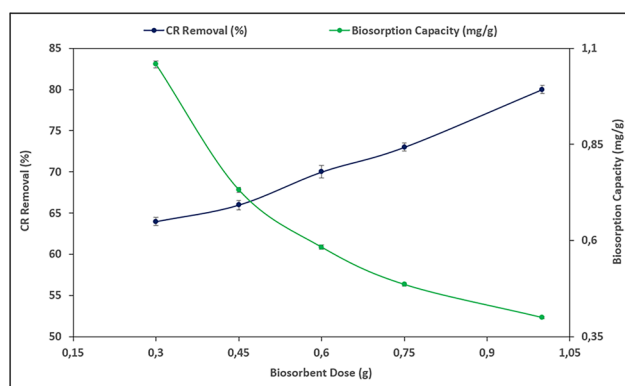


Fig. 5 Effect of biosorbent dose on biosorption capacity (mg/g) and CR removal (%) based on RPF biosorbent

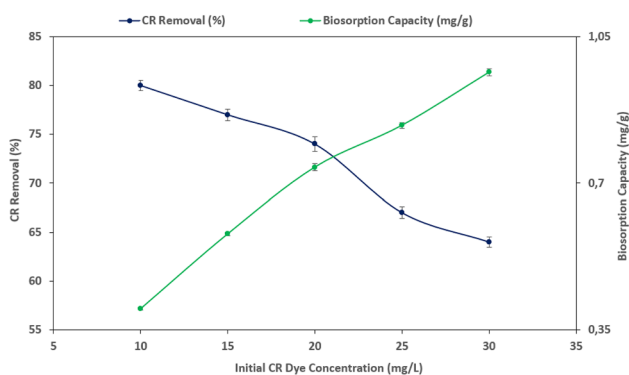


Fig. 6 Effect of initial CR dye concentration on biosorption capacity (mg/g) and CR removal (%) based on RPF biosorbent

Effect of Initial Cr Dye Concentration

The effects of biosorption capacity and CR removal (%) on the initial CR dye concentration (10–30 mg/L) were investigated (Fig. 6). When the initial CR dye concentration increased, the CR removal decreased from 80 to 64%. This is because the biosorbent surface has more porous sites compared to the number of dye molecules. These sites almost completely adsorb the dye molecules, and insufficient sites are available for the remaining dye molecules (Laghzal et al. 2024). In addition, dye molecules accumulate in the biosorbent at high dye concentrations. It was observed that CR removal decreased because diffusion in the active sites of the biosorbent decreased with the accumulation of dye molecules (Al-Saedi et al. 2023).

However, when the initial CR dye concentration was increased, the biosorption capacity increased from 0.4 mg/g to 0.97 mg/g. Increases in biosorption capacity occur with an increase in the repulsive force effect provided by the mass transfer resistance between the dye molecules and the biosorbent (Roy et al. 2024). Additionally, at high CR dye concentrations, an increase in the number of collisions between

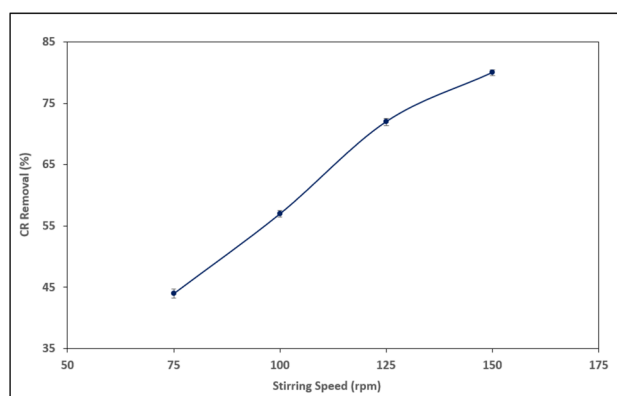


Fig. 7 Effect of stirring speed CR removal (%) based on RPF biosorbent

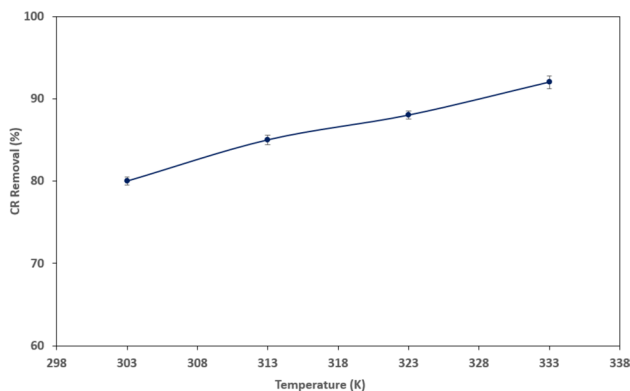


Fig. 8 Effect of temperature CR removal (%) based on RPF biosorbent

dye molecules and biosorbents increases the biosorption capacity (Güllüce et al. 2023).

Effect of Stirring Speed

The effects of biosorption capacity and CR removal (%) on the stirring speed were investigated (Fig. 7). It was found that CR removal increased from 64 to 80% as the stirring speed increased. This increase in CR removal can be explained by the excessive binding of dye molecules to the biosorbent porous sites through mass transfer (Gulluce et al. 2020).

Effect of Temperature

The effect of temperature (303–333 K) on CR removal is shown in Fig. 8. According to these results, when the temperature increased, CR removal increased from 80 to 92%. The biosorption process is naturally endothermic, as CR removal increases with an increase in temperature. The diffusion rate increases when the dye molecules pass from the outer boundary layer to the inner environment and bind to the biosorbent active sites. This increase in the diffusion rate also increases CR removal (Singh et al. 2017). This is also

thought to be due to the electrostatic interaction of van der Waals forces and H bonds between the biosorbent and dye molecules (Güllüce et al. 2024).

Kinetic Studies

Pseudo-first-order, pseudo-second-order and Elovich kinetic models are generally used for kinetic studies in order to determine the binding rate of dye molecules to the biosorbent active sites at time t in the biosorption process (Karadayı et al. 2025). The results of the kinetic models used for the biosorption process are presented in Table 2. According to these results, the R^2 values of the pseudo-first-order, pseudo-second-order and Elovich kinetic models were 0.976, 0.999, and 0.974, respectively (Fig. 9a–c). Therefore, the pseudo-second-order kinetic model provided the best fit. Moreover, the dye molecule–biosorbent interaction rate occurred via chemisorption because the pseudo-second-order kinetic model was more appropriate (Alprol et al. 2024). Similar results were obtained for the q_e value calculated using Eq. (4) in the pseudo-second-order

Table 2 Kinetic parameters values of pseudo-first-order, pseudo-second-order elovich and intraparticle diffusion kinetic models

Model	Kinetic parameters	Value
Pseudo-first-order kinetic	k_1	0.096
	$q_{e,cal}$	0.17
	$q_{e,exp}$	0.4
	R^2	0.976
Pseudo-second-order kinetic	k_2	0.867
	$q_{e,cal}$	0.428
	$q_{e,exp}$	0.4
	R^2	0.999
Elovich kinetic	α	1.483
	β	16.58
	R^2	0.974
Intraparticle diffusion kinetic	k_i	0.033
	C	0.225
	R^2	0.934

kinetic model ($q_e = 0.428$ mg/g) and experimental q_e value ($q_e = 0.4$ mg/g).

In the biosorption process, the intraparticle diffusion occurs in 3 stages: film diffusion, intraparticle diffusion, and the interaction between dye molecules and sorbent active

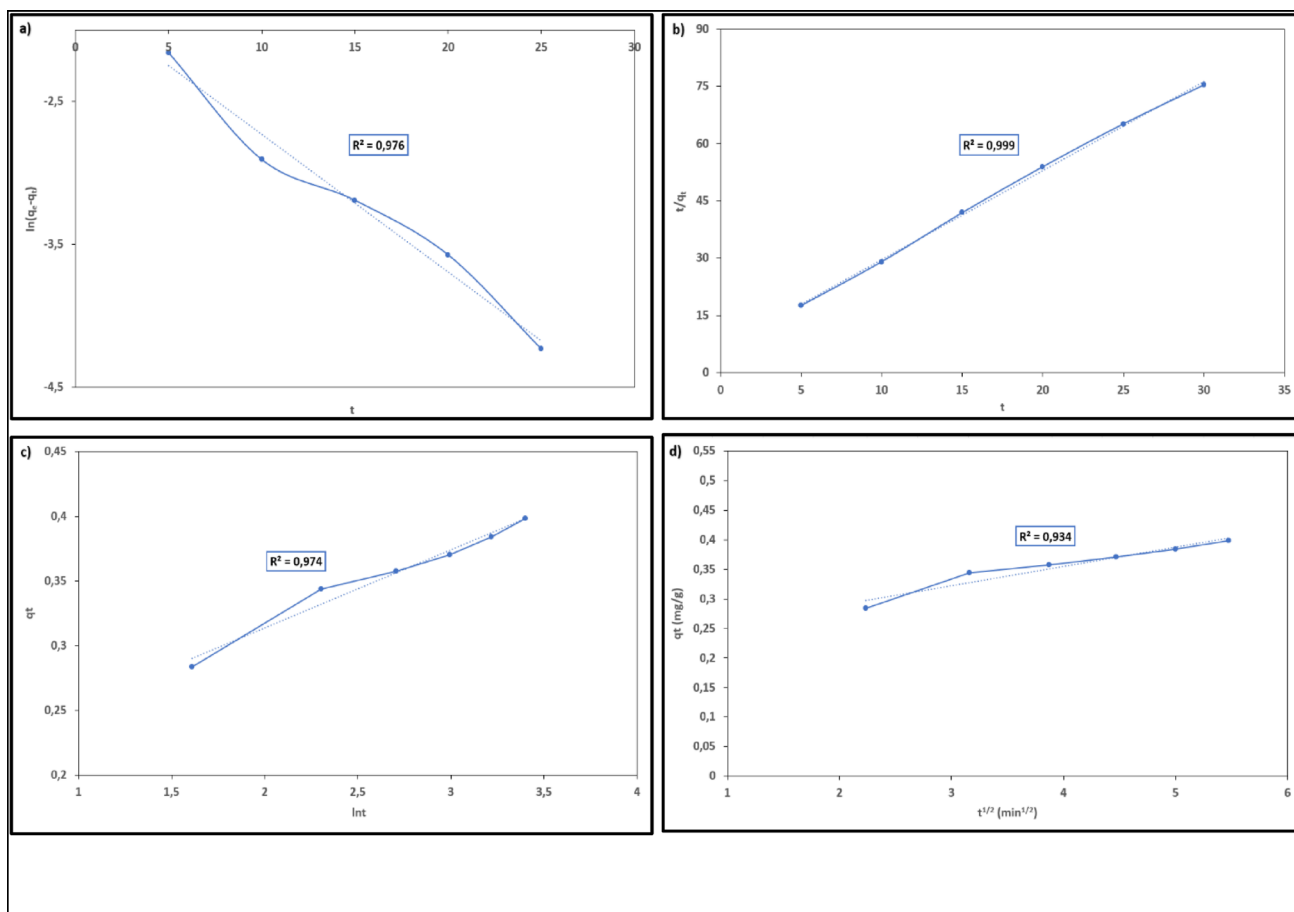


Fig. 9 Kinetic models curve of **a** pseudo-first-order **b** pseudo-second-order **c** Elovich and **d** intraparticle diffusion for the biosorption of CR dye onto RPF biosorbent

Table 3 Isotherm parameters values of Langmuir, Freundlich, Elovich and Temkin isotherm models

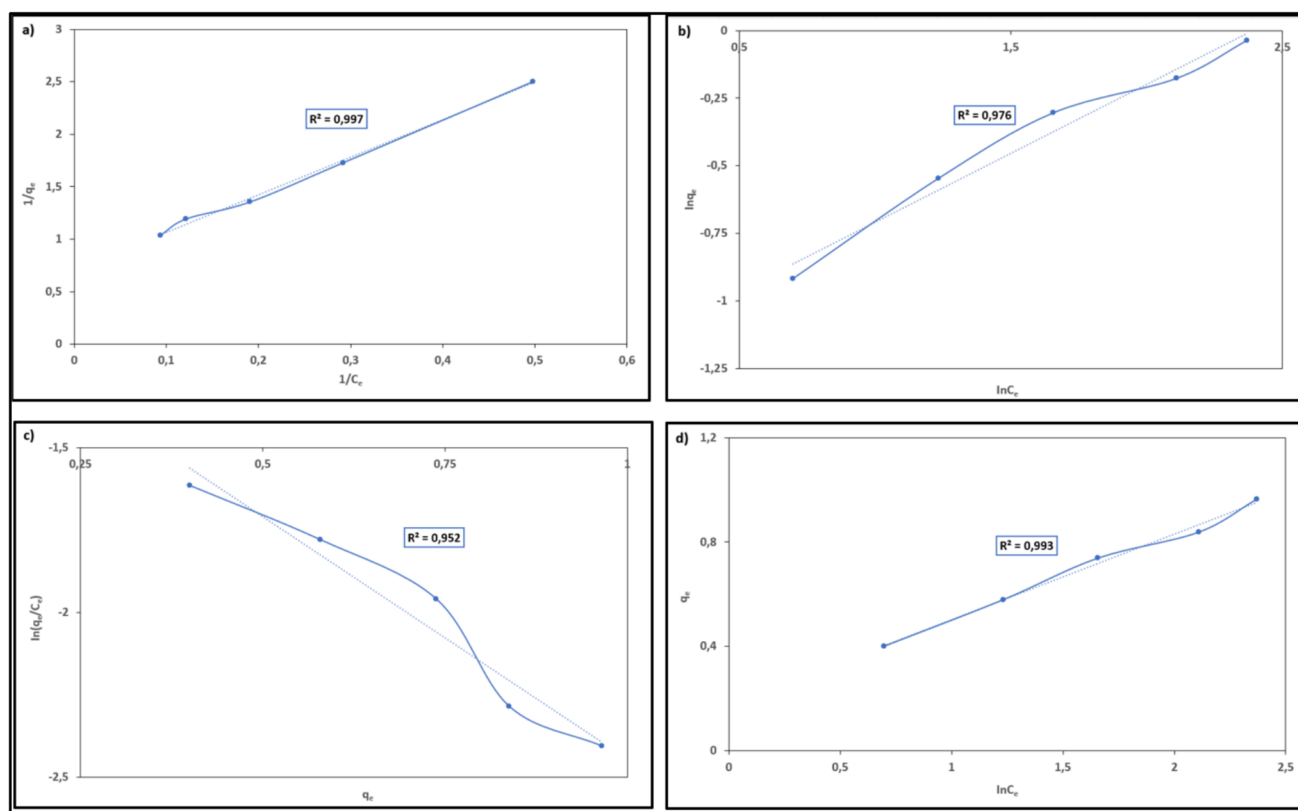
Isotherm	Kinetic parameters	Value
Langmuir	q_0	1.41
	L	0.2
	R^2	0.997
Freundlich	n	1.96
	K_f	0.295
	R^2	0.976
Elovich	K_e	0.554
	q_s	0.681
	R^2	0.952
Temkin	K_T	1.7
	ΔT	7.55
	R^2	0.993

sites (Elgarahy et al. 2020). The results of the intraparticle diffusion model used for the biosorption process are presented in Table 2. As shown in Fig. 9(d), since the curve does not pass through the origin, it indicates that the biosorption process is governed by the diffusion model, but the intraparticle diffusion model is not the only rate-determining step (Al-Saedi et al. 2023).

Isotherm Studies

Isotherm studies seek to determine how biosorption occurs and the interaction between dye molecules and biosorbents (Saadallah et al. 2024). The results of the isotherm models used for the biosorption process are presented in Table 3. According to these results, the R^2 values were calculated as 0.997, 0.976, and 0.993 for the Langmuir, Freundlich, Temkin, and 0.952 for the Elovich isotherm, respectively (Fig. 10a–d). According to the calculated R^2 values, the Langmuir isotherm provided a better fit than other isotherm models. The Langmuir isotherm model describes the monolayer homogeneous surface of the biosorbent during the biosorption process (Yildiz and Yüksel 2024). As a result, CR dye molecules were formed in the monolayer and homogeneous regions of the RPF biosorbent. The R_L values are shown in Fig. 11. According to the graph, the R_L values are in the range of (0.335–0.144). If $R_L > 1$, biosorption is unfavorable, whereas $0 < R_L < 1$ biosorption is favorable. If the R_L value is 1 or 0, biosorption is linear or irreversible, respectively (Adeleke et al. 2024). Because the calculated R_L values fall between 0 and 1, the biosorption process is considered favorable.

The n value of the Freundlich isotherm is calculated to be 1.96. The biosorption process is considered suitable when n is greater than 1 (Abdallah and Alprol 2024). The

**Fig. 10** Isotherm models curve of **a** Langmuir **b** Freundlich **c** Elovich and **d** Temkin for the biosorption of CR dye onto RPF biosorbent

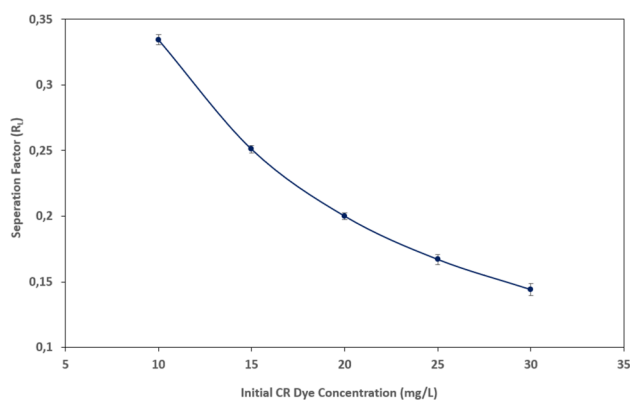


Fig. 11 Alteration of separation factor (R_L) of initial CR dye concentration

Table 4 Comparison of biosorption capacity using different biosorbents for Congo red dye removal in literature

Biosorbent	Biosorption capacity (mg/g)	Reference
<i>Vangueria infausta</i>	7.91	Kitemangu et al. 2023
Burned shoot of <i>Eichhornia crassipes</i>	5.18	Roy and Mondal 2019
Pine bark	3.92	Litefti et al. 2019
Cabbage waste powder	2.313	Wekoye et al. 2020
Banana peel	1.721	Mondal and Kar 2018
<i>Eichhornia Crassipes</i> roots	1.58	Wanyonyi et al. 2014
Eggshell	1.22	Rápó et al. 2018
Activated carbons of commercial grade	0.635	Mall et al. 2005
<i>Robinia pseudoacacia</i> L	1.41	Present study

Table 5 Thermodynamic parameters for CR biosorption on RPF biosorbent

ΔH° (kJ/mol)	ΔS° (J/mol*K)	ΔG° (kJ/mol)			
		303 K	313 K	323 K	333 K
29.592	108.93	-3.41	-4.5	-5.59	-6.68

biosorption capacity (q_e) value in the Langmuir isotherm model was calculated as 1.41 mg/g. Table 4 provides a comparison of q_e values for CR removal using various biosorbents in the literature. According to the q_0 values, RPF biosorbent has been observed to provide effective treatment performance in CR removal. RPF biosorbent is promising due to its eco-friendly and low-cost. Furthermore, because RPF biosorbent is abundant in nature and easily accessible, it can contribute to the development of sustainable and economical wastewater treatment technologies.

Thermodynamic Studies

The ΔG° (standard free energy change), ΔS° (entropy change), and ΔH° (enthalpy change) values were investigated

at different temperatures (303–333 K) in the biosorption process. The ΔH° , ΔG° , and ΔS° values are listed in Table 5. The ΔH° value was calculated to be 29.592 kJ/mol. Because $\Delta H^\circ > 0$, it is thought that the biosorption process is endothermic (Hmamouchi et al. 2024). The ΔS° value was calculated as 108.93 J/mol*K. Because $\Delta S^\circ > 0$, it increases the randomness created by the interaction between the dye molecules and the biosorbent active sites (Elgarahy et al. 2020). ΔG° values are -3.41, -4.5, -5.59 and -6.68 kJ/mol. Because ΔG° values range between -20 kJ/mol and 0 kJ/mol, the biosorption process occurs physically (Güllüce et al. 2024). When $\Delta G^\circ < 0$, biosorption occurs spontaneously (Zubair et al. 2024). As a result, CR removal from aqueous solutions based on RPF biosorbents in the biosorption process occurs spontaneously.

Detoxification of Cr by the Rpf Biosorbent Treatment

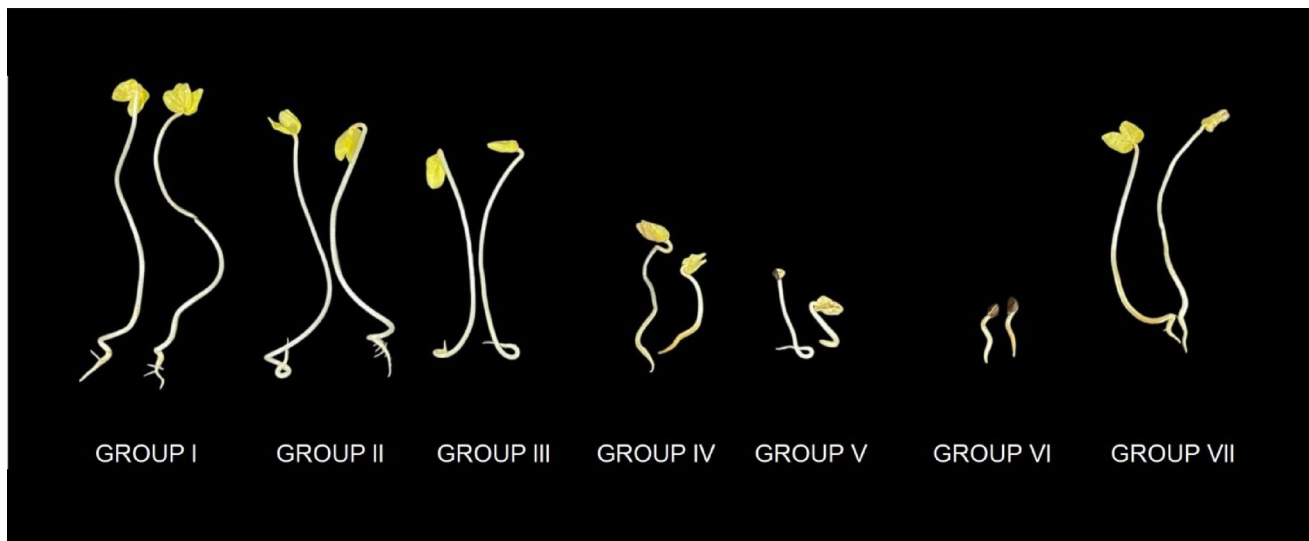
Phytotoxicity studies on *B. oleracea* var. *acephala* seed germination and seedling growth showed that significant physiological effects occurred at CR concentrations of up to 10 ppm. The germination rate of *B. oleracea* var. *acephala* seeds decreased to 40% with increasing dye concentrations. Moreover, significant decreases were observed in fresh weight, dry weight, and root and shoot lengths of seedlings. In this regard, these parameters were measured as 1.27 ± 0.021 g for fresh weight, 0.79 ± 0.0004 g for dry weight, 3.7 ± 0.26 cm for root length and 12.16 ± 1.04 cm for shoot length of the control group, while the same parameters were measured as 0.46 ± 0.004 g, 0.064 ± 0.0002 g, 1.16 ± 0.15 cm and 1.36 ± 0.15 cm respectively for the CR treated group (10 ppm). On the other hand, treatment of the CR solution with the RPF biosorbent resulted in a statistically significant recovery in all tested physiological parameters (Table 6 and Fig. 12), with germination rate increasing from 40.00 to 91.67%, root length from 1.16 to 2.60 cm, shoot length from 1.36 to 9.13 cm, fresh weight from 0.46 to 1.23 g, and dry weight from 0.064 to 0.80 g.

Anatomical alterations in plant tissues are also widely used as indicators of ecotoxicity (Çavuşoğlu et al. 2021). The results of the present study revealed that CR causes significant plant cell and tissue damage, including cellular damage to the epidermis and cortex, thickening of the cell wall, formation of flattened cell nuclei, and vascular tissue deformations in *A. cepa* roots. In addition, the RPF biosorbent treatment significantly alleviated CR-induced anatomical damage, with the epidermis cell damage rate decreasing from 1.1 to 0.1%, cortex cell damage from 0.6 to 0.0%, thickening of the cortex call wall from 1.3 to 0.0%, flattened cell nucleus rate from 0.4 to 0.0%, and unclear vascular

Table 6 The inhibitory effect of increasing CR concentrations on the physiological parameters of *B. oleracea* var. *acephala* seedlings and detoxification potential of the RPF biosorbent treatment

Test groups	Germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
GROUP I	98.33	3.70±0.26 ^d	12.16±1.04 ^f	1.27±0.021 ^e	0.79±0.0004 ^d
GROUP II	81.67	2.20±0.26 ^b	9.26±0.87 ^e	1.23±0.011 ^e	0.072±0.0005 ^c
GROUP III	73.33	2.13±0.15 ^b	7.73±0.75 ^d	1.06±0.068 ^d	0.067±0.0021 ^b
GROUP IV	58.33	1.86±0.20 ^b	4.50±0.50 ^c	0.72±0.020 ^c	0.065±0.0012 ^{ab}
GROUP V	51.67	1.36±0.15 ^a	3.13±0.41 ^b	0.55±0.005 ^b	0.064±0.0007 ^{ab}
GROUP VI	40.00	1.16±0.15 ^a	1.36±0.15 ^a	0.46±0.004 ^a	0.064±0.0002 ^a
GROUP VII	91.67	2.60±0.30 ^c	9.13±0.32 ^e	1.23±0.009 ^e	0.80±0.0022 ^d

GROUP I: control; GROUP II: 2 ppm CR; GROUP III: 4 ppm CR; GROUP IV: 6 ppm CR; GROUP V: 8 ppm CR; GROUP VI: 10 ppm CR and GROUP VII: 10 ppm CR treated with the RPF biosorbent. Data are shown as mean±SD. The mean values indicated by different letters (a–f) in the same column are significant ($p<0.05$)

**Fig. 12** Phytotoxic effect of increasing CR concentrations on the physiological parameters of *B. oleracea* var. *acephala* seedlings and detoxification with the RPF biosorbent treatment (GROUP I: control;

GROUP II: 2 ppm CR; GROUP III: 4 ppm CR; GROUP IV: 6 ppm CR; GROUP V: 8 ppm CR; GROUP VI: 10 ppm CR and GROUP VII: 10 ppm CR treated with the RPF biosorbent)

Table 7 Alleviating role of the RPF biosorbent against CR-induced cell and tissue damages in *A. cepa* roots

Test groups	Anatomical damages					
	ECD (%)	CCD (%)	TCCW (%)	FCN (%)	TD (%)	UVT
GROUP I	0.1 ^f	0.0 ^g	0.0 ^g	0.0 ^d	0.1 ^h	-
GROUP II	1.0 ^b	0.8 ^a	1.5 ^a	0.4 ^a	3.7 ^a	++
GROUP III	0.1 ^f	0.1 ^{ef}	0.1 ^f	0.0 ^d	0.3 ^f	-
GROUP IV	0.2 ^e	0.1 ^d	0.4 ^e	0.0 ^d	0.7 ^e	-
GROUP V	0.3 ^d	0.2 ^c	0.6 ^d	0.1 ^c	1.1 ^d	+
GROUP VI	0.6 ^c	0.2 ^c	0.9 ^c	0.3 ^b	2.0 ^c	+
GROUP VII	1.1 ^a	0.6 ^b	1.3 ^b	0.4 ^a	3.4 ^b	++
GROUP VIII	0.1 ^f	0.0 ^{ef}	0.0 ^g	0.0 ^d	0.1 ^{gh}	-

GROUP I: Negative control (ddH₂O); GROUP II: Positive control (EMS 25 μM); GROUP III: 2 ppm CR; GROUP IV: 4 ppm CR; GROUP V: 6 ppm CR; GROUP VI: 8 ppm CR; GROUP VII: 10 ppm CR and GROUP VIII: 10 ppm CR treated with the RPF biosorbent. Data are shown as mean±SD. The mean values indicated by different letters (a–f) in the same column are significant ($p<0.05$). ECD epidermis cell damage, CCD cortex cell damage, TCCW thickening of the cortex call wall, FCN flattened cell nucleus, TD Total damages, UVT unclear vascular tissue. (++) moderate damage, (+) little damage, (-) no damage

tissue formation from moderate to no damage (Table 7 and Fig. 13).

Although there is no information in the literature that directly matches our study, several studies have been

conducted to eliminate CR-induced hazards in aqueous solutions. In this regard, the removal and detoxification of CR and other synthetic dyes from aqueous solutions using biosorbents prepared from various biomass types is considered

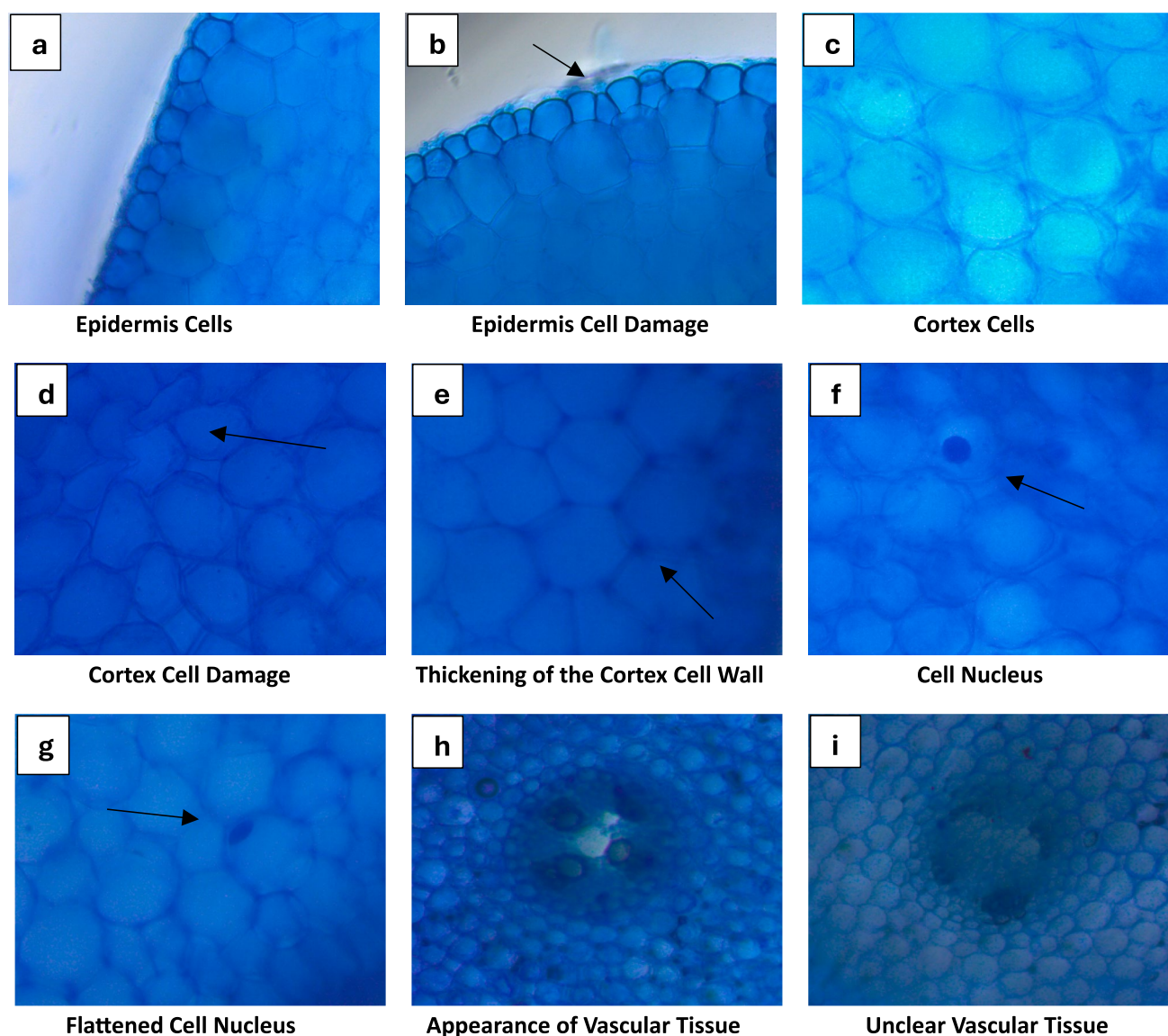


Fig. 13 CR-induced cell damages (**b,d,e,g,i**) and healthy cells (**a,c,f,h**) induced *A. cepa* roots

a promising environmental approach. In this regard, Asses et al. (2018) *Aspergillus niger* as a biosorbent for the removal of CR from aqueous solutions, and it significantly reduced CR toxicity in *Zea mais* and *Solanum lycopersicum* seeds after treatment. Similarly, Ellafi et al. (2023) developed sustainable biosorbents using *Bacillus* spp. to treat aqueous CR solutions. A significant recovery in the physiological parameters of *Raphanus sativus*, *Lycopersicon esculentum* and *Lepidium sativum* was reported after treatment of CR solutions with these biosorbents. In a recent study, Telke et al. (2010) tested the toxicity of CR wastewater before and after treatment with *Pseudomonas* sp. SU-EBT biosorbent and reported significant recovery in the physiological parameters of *Sorghum bicolor*, *Vigna radiata*, *Lens culinaris* and *Oryza sativain* the treated groups. Although there

is no information on the bioremoval and detoxification of CR using a biosorbent from *R. pseudoacacia* fruits, all these and similar studies in the literature support and explain the observed physiological and anatomical recovery after the RPF biosorbent treatment in the present study.

Conclusions

This study revealed the successful bio-removal of CR using the RPF biosorbent. CR (80%) was removed from aqueous solutions using RPF biosorbent under optimum conditions (303 K temperature, 1 g biosorbent dose, pH 6, 10 mg/L initial CR dye concentration, and 150 rpm stirring speed). In kinetic studies, the pseudo-second-order correlation

coefficient was calculated as 0.999, which was the best fit. In the isotherm studies, the R^2 value of the Langmuir isotherm was 0.997, which was a better fit than that of the other isotherms (Freundlich, Temkin, and Elovich isotherm models). Compared with the various biosorbents used in the literature for CR bio-removal, the q_0 value in the Langmuir isotherm was calculated as 1.41 mg/g. Because values of ΔG° (-3.41, -4.5, -5.59 and -6.68 kJ/mol) calculated negative for thermodynamic studies, biosorption process occurred spontaneously. The phytotoxicological effects of untreated and treated CR solutions were determined. In this regard, the germination rate of *B. oleracea* var. *acephala* seeds decreased to 40% with increasing dye concentrations. Moreover, significant decreases were observed in fresh weight, dry weight, and root and shoot lengths of seedlings. The findings of the present study demonstrate that *Robinia pseudoacacia* L. (Fabaceae) biosorbent successfully removes CR dye from aqueous solutions. In this regard, the use of *Robinia pseudoacacia* L. (Fabaceae) biosorbent for the removal of various synthetic dyes will shed light on many studies in the literature because of its advantages such as low cost, eco-friendliness, and effective treatment. However, the findings of the present study could be improved in future studies using composite dye solutions, such as those containing different pollutants, dye staff, metal salts, and textile auxiliaries. Moreover, further research is needed to evaluate the possibility of removing other wastewater contaminants, such as heavy metals and pharmaceuticals, using *Robinia pseudoacacia* L. (Fabaceae) biosorbent.

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Data Availability Data will be made available on request.

Declarations

Conflict of interest The authors declare no competing interests.

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