



Enhanced cationic dye adsorption using *Stenotrophomonas indicatrix*-modified MnFe_2O_4 : performance evaluation and adsorption mechanism

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Abstract

In this study, MnFe_2O_4 magnetic nanoparticles were modified with the *Stenotrophomonas indicatrix* bacteria for the first time in the literature to develop an effective and novel bio-adsorbent for the remediation of toxic cationic dyes, specifically Rhodamine 6G (R6G) and Methylene Blue (MB), from contaminated aqueous media. MnFe_2O_4 and bacteria-loaded MnFe_2O_4 were thoroughly characterized using FTIR, XRD, and SEM-EDX analyses. XRD results confirmed that the cubic spinel structure of MnFe_2O_4 was preserved after bacterial modification, while FTIR spectra evidenced the successful incorporation of biological functional groups, including $-\text{NH}$, $-\text{OH}$, amide, and phosphate moieties, onto the nanoparticle surface. Adsorption performance of the dyes was evaluated in terms of parameters including aqueous solution pH, equilibrium time, bacteria-loaded MnFe_2O_4 dosage, and initial R6G and MB concentration. After investigating the effect of salt on adsorption efficiency, the applicability of the proposed adsorbent was assessed using real water samples. According to the experimental results, the optimum pH and equilibrium period for the uptake of both dyes were determined to be 8.0 and 60 min, respectively. Kinetic analyses showed that the pseudo-second order kinetic model exhibited a superior fit in representing the adsorption rates of both dyes, while isotherm studies indicated that both Langmuir and Freundlich isotherm models fit the experimental data well. While the adsorption capacities of pure MnFe_2O_4 for MB and R6G were 19.5 mg g^{-1} and 15.3 mg g^{-1} , respectively, these values reached 67.6 mg g^{-1} and 59.5 mg g^{-1} for the bacteria-loaded MnFe_2O_4 . The obtained data revealed that bacterial modification significantly increased the adsorption capacity of MnFe_2O_4 , suggesting that the developed bio-adsorbent stands out as an effective and promising candidate for the remediation of dye-contaminated effluents.

Keywords Adsorption · Bacteria-modified MnFe_2O_4 · Rhodamine 6G · Methylene Blue · Wastewater treatment

1 Introduction

Synthetic dyes, commonly utilized in a variety of industries including textiles, paper production, plastics, and leather processing, cause serious environmental problems in aquatic ecosystems [1]. Cationic dyes, especially those with high solubility such as methylene blue (MB) and rhodamine 6G (R6G), persist for long periods in the receiving environment due to their stable, complex aromatic structures and low biodegradability [2]. Release of dyes into aquatic environments can lead to reduced light penetration and disruption of photosynthetic balance [3]. In addition, some dyes exhibit toxic, mutagenic, or potentially carcinogenic properties, exerting detrimental effects on both aquatic biota and human health [4, 5]. Their persistence and potential for

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bioaccumulation can also pose a risk to humans through drinking water and the food chain. Therefore, effective treatment of wastewater containing such pollutants before discharge into the receiving environment is critical for sustainable environmental management [6].

Various physical and chemical treatment technologies, including coagulation-flocculation, membrane filtration, adsorption, ion exchange, ozonation, Fenton, and photo-Fenton processes, are applied for dye removal, depending on the structural characteristics of the target dyes, the properties of the wastewater, and the desired treatment level [7]. Of the available treatment approaches, adsorption is widely selected due to its high removal efficiency, ease of application, and low cost [8]. The performance of the adsorption process is primarily governed by the characteristics of the adsorbent employed. In previous studies, various adsorbents such as poly(N-vinylpyrrolidone)-graphene oxide composite [9], cassava rhizome activated carbon [10], nanocrystalline and heterostructural Zr MOF@bentonite composite [11], Cu@Zr-MOFs-PAN nanofiber composites [12], Fe₃O₄ modified biochar obtained from rice husk [13], acrylic acid-based super-absorbents [14], Cu/Al-LDHs@Gelatin nanobiosorbent [15], CuFe₂O₄@g-C₃N₄ [16], activated biochar yeast@magnetic-TiO₂@Brewer's yeast [17], crosslinked nanosilica with nanobiochar and chitosan [18], and nanobiochar impregnated layered double hydroxides [19] have been used for dye removal, but their synthesis is often complex and requires extensive preparation steps. Besides these, magnetic nanoparticles offer significant advantages, especially owing to their extensive surface area and the ability to be recovered from aqueous solutions using a magnet without the need for lengthy and labor-intensive centrifugation or filtration [20]. MnFe₂O₄ with spinel ferrite structure, characterized by high magnetic susceptibility and chemical stability, is stable over a wide pH range and has the potential to adsorb pollutant species thanks to the metal-oxygen groups present in its structure [21]. Several studies in the literature have highlighted the adsorption potential of MnFe₂O₄-based materials for dye removal. Abbas et al. [22] reported the efficient adsorption of methyl orange using a co-precipitated MWCNT/MnFe₂O₄ nanocomposite, while Shihab et al. [23] demonstrated that sol-gel-synthesized MnFe₂O₄ nanoparticles rapidly and effectively removed crystal violet dye, as confirmed by kinetic and isotherm analyses.

In recent years, approaches aimed at biological surface modification of magnetic nanoparticles to increase the adsorption efficiency of pollutant species have received significant attention. El-Kalliny et al. [24] developed a low-cost, environmentally friendly, and reusable MnFe₂O₄-encapsulated *Scenedesmus obliquus*

composite that demonstrated high adsorption efficiency toward methylene blue and crystal violet dyes. Pi et al. [25] prepared a bacteria-loaded Fe₃O₄ bio-nanocluster through mixing bacterial suspension and Fe₃O₄ nanoclusters and used it to achieve high-efficiency elimination of Congo Red from contaminated water sources. Nadi et al. [26] developed an environmentally friendly water treatment approach that enabled efficient degradation of azo dyes by immobilizing *Bacillus subtilis* cells on superparamagnetic Fe₃O₄ nanoparticles. Cheng et al. [27] reported effective adsorption of crystal violet dye using bacteria decorated with Fe₃O₄ magnetic nanoparticles and demonstrated rapid and efficient dye removal from aqueous solutions. In the present study, for the first time in the literature, MnFe₂O₄ magnetic nanoparticles were modified with *Stenotrophomonas indicatrix* and the adsorption-based removal of cationic dyes, MB and R6G, from aqueous solutions was investigated. The selection of *Stenotrophomonas indicatrix*, a Gram-negative bacterium, is based on its cell wall composition and surface chemistry, which are highly suitable for adsorption applications. Bacterial biomass, particularly from Gram-negative species, contains a variety of functional groups such as hydroxyl, amino, carboxyl, amide, and phosphate groups that can effectively interact with cationic dye molecules. Moreover, the use of dead biomass offers several advantages, including enhanced operational stability, resistance to experimental conditions, and no requirement for nutrients. Coating of the MnFe₂O₄ surface with bacteria allows the introduction of active functional sites into the magnetic nanoparticles, which allows the dye molecules to bind to the adsorbent through electrostatic interactions, ion exchange, π - π interactions, and hydrogen bonding [27]. Adhesion of bacterial cells to the surface contributes to a rougher and heterogeneous surface structure of the magnetic nanoparticles, increasing porosity and facilitating the adsorption of dyes. Bacterial modification also significantly prevents the aggregation of magnetic nanoparticles, increasing the surface area and improving the adsorption efficiency [28, 29].

To the best of our knowledge, no previous study has reported the modification of MnFe₂O₄ with *Stenotrophomonas indicatrix* for dye removal applications. Therefore, this work presents a novel bio-based strategy for designing high-performance magnetic adsorbents. The adsorption process was analyzed using kinetic and isotherm models to gain insights into the nature of the dye-adsorbent interaction mechanism. The results are intended to contribute to the literature by demonstrating that bacteria modified magnetic nanoparticles offer environmentally friendly and high-performance alternatives for the treatment of dye-containing wastewater.

2 Materials and methods

2.1 Apparatus and chemicals used in batch experiments

Methylene blue (MB; $C_{16}H_{18}ClN_3S$) and Rhodamine 6G (R6G; $C_{28}H_{31}N_2O_3Cl$) were chosen as the cationic dyes to be removed by adsorption from aqueous solutions. All chemicals utilized in the experiments were of analytical-grade quality and were used as received without further purification. MB, R6G, NaOH, HNO_3 , NaCl, $BaCl_2$, $FeCl_3 \cdot 6H_2O$ and $MnCl_2 \cdot 4H_2O$ tryptic soy agar, and tryptic soy broth were acquired from Sigma-Aldrich (Darmstadt, Germany). All experiments were conducted using distilled/deionized water. For the experimental work, 1.250 g of MB and 1.250 g of R6G were dissolved separately in 250 mL of distilled water to prepare the stock solutions of both dyes at a concentration of 5000 mg L^{-1} . Calibration and working solutions were prepared by appropriately diluting aforementioned stock solutions.

The surface characteristics and elemental distribution of $MnFe_2O_4$ and bacteria-loaded $MnFe_2O_4$ were evaluated through a scanning electron microscope (SEM–EDX, FEI Quanta FEG 450). Fourier transform infrared (FTIR) spectroscopy was conducted using a PerkinElmer Spectrum Two instrument to characterize the functional groups present on the surfaces of both adsorbents. X-ray diffraction (XRD) analysis was executed by employing $Cu K\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) with a 2θ range of $5\text{--}80^\circ$ using a PANALYTICAL EMPYREAN instrument to examine the crystal structure of the adsorbents.

A Perkin Elmer Lambda 25 model UV-Vis spectrophotometer was applied for the quantification of dye concentrations. To prepare solutions at desired pH values and to measure the pH values of the solutions, a WTW inoLab pH 7110 model desktop pH meter, a BOECO PSU-15i model mechanical shaker for adsorption experiments, a NUVE NF 200 model centrifuge to separate the adsorbent from the solution, a KERN ABJ 220-4NM model analytical balance for weighing the adsorbent and other chemicals, and an IKA RCT Basic model magnetic stirrer were used. A Weightlab WF-UD6 model ultrasonic bath was employed for the synthesis of $MnFe_2O_4$, while a Protech model muffle furnace was used during the calcination step. A Santen SE 125 model drying oven was utilized to dry the adsorbents.

2.2 Culture of bacteria

Stenotrophomonas indicatrix strain Z.10.3.9.1, isolated from olive tree roots at a depth of 10 cm in Havran, Balıkesir, Türkiye (NCBI accession number: PX588044) [30], was used in this study. Strain preparation was performed with

some modifications from the study of Kılınç et al. [31]. The strain was first inoculated on tryptic soy agar to confirm the absence of contamination and incubated at 28°C for 24 h. Pre-cultures were then prepared by inoculating the strain into 500 mL tryptic soy broth in glass bottles and shaking at 28°C and 150 rpm for 48 h using a rotary shaker. For harvesting, the cultures were centrifuged at 8000 rpm for 10 min (Hettich Universal 320R, Germany), and the bacterial biomass pellets were collected. The pellets were washed three times with sterile 0.9% NaCl solution to remove residual medium and subsequently autoclaved at 121°C for 15 min to ensure cell death. The sterilized pellets were then lyophilized (Christ Loc-1 m, Germany) to obtain dry biomass.

2.3 Preparation of $MnFe_2O_4$ and bacteria-loaded $MnFe_2O_4$

$MnFe_2O_4$ nanoparticles were synthesized using the co-precipitation method. $FeCl_3 \cdot 6H_2O$ and $MnCl_2 \cdot 4H_2O$ salts were weighed according to the stoichiometric 2:1 molar ratio and dissolved in 250 mL of deionized water. The solution was stirred at $35\text{--}40^\circ\text{C}$ for 30 min using a magnetic stirrer and subsequently treated in an ultrasonic bath at 80°C for 2 h. During this process, 100 mL of 3 M NaOH solution was heated to 80°C and added dropwise to the reaction mixture to maintain the pH within the range of 10–11. The mixture was kept at the same temperature for an additional 30 min. After the reaction was completed, the suspension was allowed to cool to room temperature. The formed precipitate was then separated by filtration and washed with deionized water until the pH of the washing solution reached neutrality. The solid product was dried at 105°C for 6 h and subsequently calcined at 400°C for 4 h. Finally, the calcined material was ground into a fine powder and stored in sealed containers to prevent moisture exposure [32].

The preparation of the bacteria-loaded $MnFe_2O_4$ was carried out using a wetting–drying method. In this procedure, 0.200 g of dried *Stenotrophomonas indicatrix* was physically mixed with 1.0 g of $MnFe_2O_4$ nanoparticle. 2.0 mL of distilled water was introduced into the bacteria- $MnFe_2O_4$ nanoparticle mixture. The slurry-like mixture was dried in an oven at 65°C for 12 h. To ensure the successful loading of the bacteria onto the $MnFe_2O_4$ surface, the wetting–drying procedure was repeated at least three times.

2.4 Batch adsorption procedure

The adsorptive removal of MB and R6G was assessed by batch process in 15 mL of polypropylene centrifuge tubes with 10 mL of dye solutions and agitated at 250 rpm. The influences of different experimental parameters including

pH (2.0–8.0), contact time (1–240 min), initial dye concentration (20–300 mg L⁻¹), and amount of adsorbent (5–50 mg) were investigated on the adsorption efficiency. While the effect of any parameter was being examined, all other parameters were kept constant. After each experiment, the dye loaded adsorbent was separated via centrifugation and the concentration of MB and R6G in the aqueous solution was analyzed at $\lambda_{\max}$ of 663 nm and 526 nm, respectively by UV–Vis spectrophotometer. The uptake of MB and R6G by bacteria-loaded MnFe₂O₄ (q_e (mg g⁻¹)) was calculated according to Eq. 1.

$$q_e = \frac{V(C_o - C_e)}{m_s} \quad (1)$$

Here, C_o and C_e denote the dye concentrations at the beginning of the experiments and at equilibrium (mg L⁻¹), respectively, V represents the volume of the solution (L), while m_s corresponds to mass of bacteria-loaded MnFe₂O₄ (g).

Data obtained from experiments evaluating the initial dye concentration were applied to isotherm models, whereas the data from experiments assessing the effect of contact time were subjected to kinetic modeling.

2.5 Adsorption kinetic models

Kinetic models were applied to investigate the time-dependent changes in the concentrations of MB and R6G adsorbed on the bacteria-loaded MnFe₂O₄ surface to elucidate the kinetic characteristics and mechanistic pathway of adsorption. These models help determine whether the rate-controlling step is governed primarily by chemical interactions, physical forces, or diffusion phenomena. Among the conventional kinetic models, the pseudo-first order (PFO), pseudo-second order (PSO), and intraparticle diffusion (IPD) models were employed in this study. While PFO model typically characterizes physical adsorption processes [33], the PSO model provides a better description when chemisorption is the dominant mechanism [34]. Additionally, the IPD model examines how intraparticle diffusion contributes to the overall rate of adsorption and clarifies whether the adsorption proceeds through a single step or multiple sequential stages [35].

The non-linear forms of PFO, PSO and IPD models are provided in Eqs. 2, 3, and 4, respectively.

$$q_t = q_e(1 - e^{-k_1 t}) \quad (2)$$

$$q_t = \frac{k_2 q_e^2 t}{1 + k_2 q_e t} \quad (3)$$

$$q_t = k_{id} t^{1/2} + c \quad (4)$$

Here, q_e denotes the adsorption capacity of bacteria-loaded MnFe₂O₄ at equilibrium whereas q_t refers to the dye uptake at a given contact time. The constants k_1 (min⁻¹), k_2 (g mg⁻¹ min⁻¹), and k_{id} (mg g⁻¹ min^{-1/2}) correspond to the rate coefficients associated with the PFO, PSO, and IPD kinetic models, respectively. The C value provides insight into the boundary-layer thickness.

The linearized forms of PFO, PSO and IPD models are shown in Eqs. 5, 6, and 7, respectively.

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (5)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (6)$$

$$q_t = k_{id} t^{1/2} + c \quad (7)$$

For the PFO model, plotting $\ln(q_e - q_t)$ against t yields a straight line, from which k_1 (slope) and q_e (intercept) can be obtained. In PSO model, plotting t/q_t against t yields a linear relationship, from which the slope and intercept are used to calculate q_e and k_2 , respectively. In the case of IPD model, the slope and intercept of the $q_t - t^{1/2}$ plot yield k_{id} and C , respectively.

2.6 Adsorption isotherm modeling

To describe the variation in the amounts of MB and R6G adsorbed onto bacteria-loaded MnFe₂O₄ depending on concentration at a fixed temperature, Langmuir, Freundlich, and Dubinin–Radushkevich (D–R) isotherm models were adopted.

Langmuir isotherm model assumes that the active sites on the adsorbent surface are homogeneous and that the adsorbate is adsorbed in a monolayer onto these sites. The model also assumes that all adsorption sites possess the same energy. The Langmuir isotherm is widely employed for the assessment of the adsorption capacity of an adsorbent [36]. The non-linear expression of the model is presented in Eq. 8. An important parameter of the model is the separation factor, R_L , which is used to assess whether the adsorption is favorable or unfavorable, and is mathematically defined as shown in Eq. 9. According to Eq. 9, a R_L value ranging from 0 to 1 signifies that the adsorption is favorable.

$$q_e = \frac{b q_{max} C_e}{1 + b C_e} \quad (8)$$

$$R_L = \frac{1}{1 + b \cdot C_o} \quad (9)$$

C_e denotes the equilibrium level of dyes (mg L^{-1}), q_e represents the quantity of dye adsorbed per unit bacteria-loaded MnFe_2O_4 mass (mg g^{-1}), $q_{\max}$ indicates the maximum adsorption capacity (mg g^{-1}), and b corresponds to free adsorption energy (L mg^{-1}).

The linear form of the Langmuir model is given in Eq. 10.

$$\frac{C_e}{q_e} = \frac{C_e}{q_{\max}} + \frac{1}{bq_{\max}} \quad (10)$$

The adsorption constants b and $q_{\max}$ were derived from the linear plot of C_e/q_e versus C_e , with the intercept and slope corresponding to b and $q_{\max}$, respectively.

Unlike Langmuir model, the Freundlich isotherm considers that the adsorption energy is not uniform across the surface and effectively describes multilayer adsorption processes [37]. The Freundlich isotherm model can be expressed in its non-linear form as shown in Eq. 11.

$$q_e = K_f C_e^{1/n} \quad (11)$$

K_f represents the adsorption capacity (mg g^{-1}), while n is a non-dimensional constant related to the adsorption intensity. An n value within the range of 1–10 reveals that the designed process is suitable.

The linearized form of the Freundlich isotherm model is expressed in Eq. 12.

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \quad (12)$$

For the determination of K_f and n , a plot of $\ln q_e$ versus $\ln C_e$ is obtained, with the slope and intercept of this plot used to calculate n and K_f , respectively.

The Dubinin–Radushkevich (D–R) isotherm model was developed to distinguish between physical and chemical adsorption processes [38]. One of the model's parameters, the adsorption energy, E , is used to make this distinction. An E value within 8–16 kJ mol^{-1} suggests that the adsorption primarily proceeds via an ion-exchange mechanism. Values below 8 kJ mol^{-1} suggest that the adsorption is physical in nature, whereas values above 16 kJ mol^{-1} signify that chemical adsorption has taken place [39]. The equations of the D–R model are presented in Eqs. 13–15.

$$q_e = q_m \exp(-\beta \varepsilon)^2 \quad (13)$$

$$\varepsilon = RT \ln(1 + 1/C_e) \quad (14)$$

$$E = 1/(-2\beta)^{1/2} \quad (15)$$

Here, q_m denotes the monolayer adsorption capacity (mol g^{-1}), β is a coefficient related to the average adsorption energy ($\text{kJ}^2 \text{mol}^{-2}$), ε is the Polanyi potential.

Equation 16 represents the linear form of the D–R isotherm model.

$$\ln q_e = \ln q_m - \beta \varepsilon^2 \quad (16)$$

The D–R model constants, q_m and β , were determined from the intercept and the slope of the linear plot of $\ln q_e$ versus ε^2 , respectively.

3 Results and discussion

3.1 Characterization of adsorbent

XRD patterns illustrated in Fig. 1 demonstrate the structural comparison between MnFe_2O_4 and bacteria-loaded MnFe_2O_4 . The diffraction peaks observed at 2θ values of 28.2° , 35.8° , 42.5° , 55.9° , and 62.2° can be indexed to the (220), (311), (400), (333), and (440) crystallographic planes, respectively. These diffraction reflections correspond well with the standard cubic spinel structure of MnFe_2O_4 (JCPDS card No. 74-2403), confirming the successful formation of MnFe_2O_4 [40–42]. The XRD patterns of MnFe_2O_4 and bacteria-loaded MnFe_2O_4 exhibit similar peak positions. This indicates that the spinel crystal structure of MnFe_2O_4 is preserved after bacterial loading due to the amorphous nature of the biomass.

The FTIR spectra of MnFe_2O_4 and bacteria-loaded MnFe_2O_4 are depicted in Fig. 2. Examination of the FTIR spectra of both MnFe_2O_4 and bacteria-loaded MnFe_2O_4 reveals the presence of absorption bands around 3000 cm^{-1} , 2913 cm^{-1} , and 1081 cm^{-1} , which are attributed to surface –OH groups, aliphatic C–H stretching, and C–O stretching

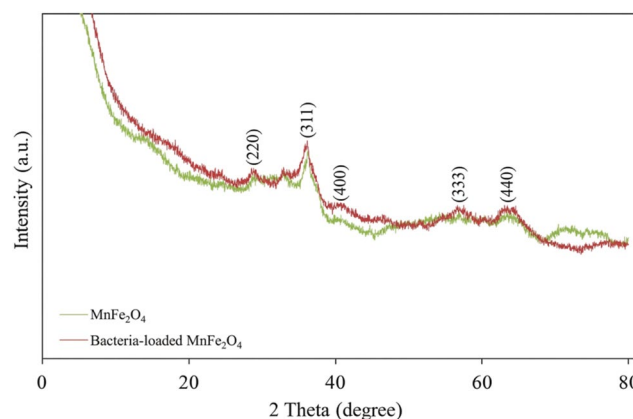
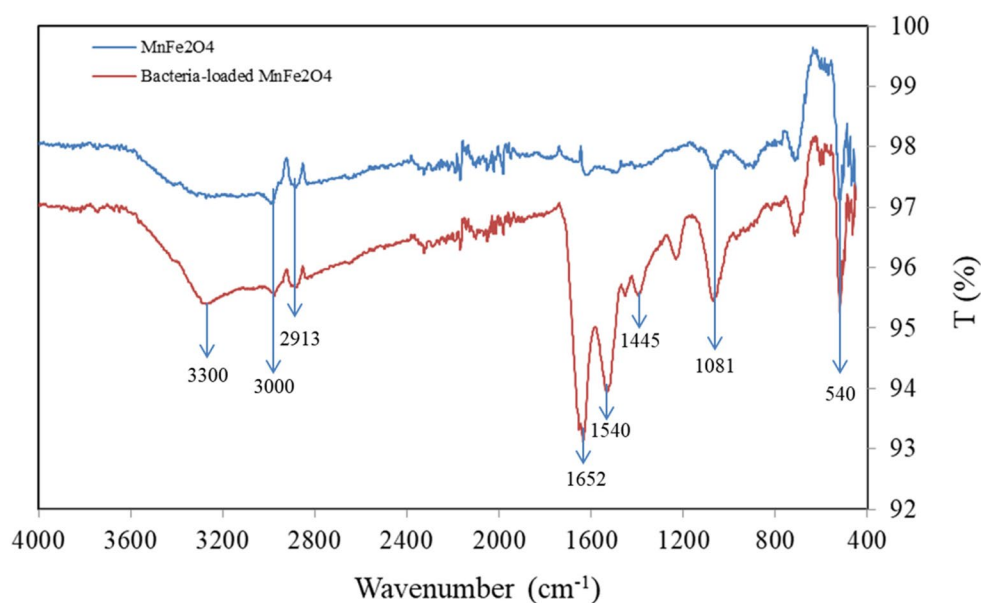


Fig. 1 XRD spectra of MnFe_2O_4 and bacteria-loaded MnFe_2O_4

Fig. 2 FTIR spectra of MnFe_2O_4 and bacteria-loaded MnFe_2O_4



vibrations, respectively. In both spectra, sharp absorption bands observed in the $540\text{--}580\text{ cm}^{-1}$ and $450\text{--}480\text{ cm}^{-1}$ regions confirm the successful formation of the MnFe_2O_4 structure. These bands correspond to the stretching vibrations of Fe–O bonds at tetrahedral sites and Mn–O bonds at octahedral sites in the MnFe_2O_4 lattice [41]. In the FTIR spectrum of bacteria-loaded MnFe_2O_4 , the appearance of several new absorption bands is clearly observed. The broad absorption band appearing at 3300 cm^{-1} originates from the stretching vibrations of –OH and –NH groups present in the bacterial cell wall, indicating the presence of biological functional groups. The most prominent evidence of bacterial immobilization onto the MnFe_2O_4 surface is provided by the bands observed at 1652 cm^{-1} and 1540 cm^{-1} , which are assigned to amide I (C=O stretching vibrations originating from peptide bonds) and amide II (N–H bending coupled with C–N stretching vibrations), respectively [43, 44]. These bands confirm the binding of protein and peptide structures from the bacterial biomass onto the MnFe_2O_4 surface. Furthermore, the absorption band observed at 1080 cm^{-1} can be attributed to P=O stretching of phosphate groups in the bacterial structure [45]. The small band detected at 1445 cm^{-1} is associated with the vibrations of carboxylate (–COO[–]) groups and –CH₂ bending modes [46], further supporting the successful bacterial loading.

SEM images of pristine MnFe_2O_4 and bacteria-loaded MnFe_2O_4 are presented in Fig. 3 to evaluate the surface morphology before and after bacterial immobilization. The pristine MnFe_2O_4 exhibited irregularly shaped particles with pronounced aggregation, forming dense agglomerated clusters, which is likely attributed to magnetic interactions between the ferrite nanoparticles [41]. Significant changes in the surface morphology of MnFe_2O_4 were observed as

a result of modification with bacteria. Following bacterial loading, the particle surfaces exhibited a more irregular and heterogeneous structure, with increased agglomeration in some areas and the surface appearing to be covered with a biological layer. According to the EDX analysis, the pristine MnFe_2O_4 consisted of C, N, O, Fe, Mn, and S with weight percentages of 9.51%, 4.10%, 25.86%, 25.83%, 34.50%, and 0.20%, respectively (Fig. 4(a)). After bacterial immobilization, the elemental composition changed noticeably; Fe, Mn, C, N, O, P, and S were detected at 36.22%, 18.33%, 17.23%, 12.55%, 14.56%, 0.65%, and 0.13%, respectively, together with 0.33% of other minor elements (Fig. 4(b)). The significant increase in C and N contents, together with the appearance of P, clearly indicated the successful loading of bacterial biomass onto the MnFe_2O_4 surface.

3.2 Influences of the initial pH

The initial solution pH simultaneously modulates the ionization degree of dye molecules and the surface charge density of the adsorbent, thereby governing the ionization state of its functional groups [47]. The adsorption of MB and R6G onto bacteria-loaded MnFe_2O_4 nanoparticles was investigated in the pH range of 2.0–8.0, with the initial dye concentration maintained at 60 mg L^{-1} . The results revealed that pH had a pronounced effect on the adsorption capacity of the adsorbent for both dyes. As the solution pH was elevated from 2.0 to 8.0, the adsorption capacities of MB and R6G increased markedly from 5.7 mg g^{-1} to 37.1 mg g^{-1} and from 6.8 mg g^{-1} to 32.0 mg g^{-1} , respectively (Fig. 5 (a)). The significant influence of solution pH on the adsorption of MB and R6G is attributed to the ionization behavior of both the MnFe_2O_4 nanoparticles and the functional groups originating from

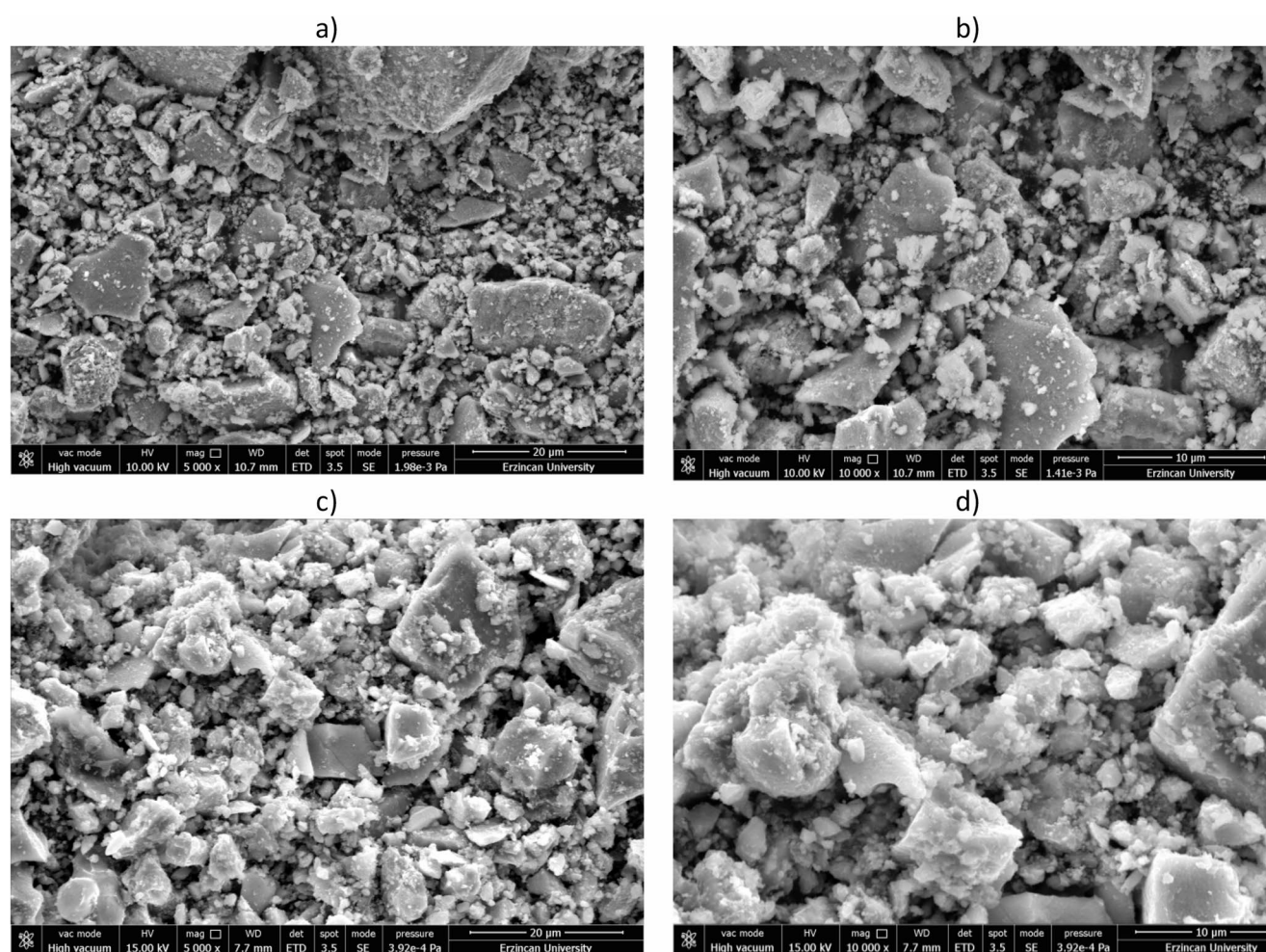


Fig. 3 SEM images of (a–b) MnFe_2O_4 and (c–d) Bacteria-loaded MnFe_2O_4

the bacterial structure. The bacterial cell surface contains a diverse range of pH-responsive functional groups, including carboxyl, phosphate, hydroxyl, amino, and carbonyl groups, derived from biomolecules including lipids, proteins, carbohydrates, phospholipids, and nucleic acids present in the cell wall [48]. The pH_{pzc} value of bacteria-loaded MnFe_2O_4 was obtained as 6.7 (Fig. 5 (b)). At low pH values ($\text{pH} < \text{pH}_{\text{pzc}}$), these functional groups become protonated, resulting in a positively charged adsorbent surface. Accordingly, electrostatic repulsion is established between the protonated surface and the cationic dye molecules MB and R6G. In addition, the competitive interaction of the abundant H_3O^+ ions with the dye molecules for the active binding sites further suppresses adsorption at low pH values. Upon rising pH levels ($\text{pH} > \text{pH}_{\text{pzc}}$), the bacterial functional groups undergo deprotonation, leading to a negatively charged surface that electrostatically attracts the cationic dye molecules [49, 50]. The deprotonation of these groups exposes carboxylate, phosphate, and hydroxyl functional groups, enabling additional interactions such as ionic bonding, hydrogen bonding, and

ion–dipole interactions. Therefore, the observed increase in adsorption efficiency for both MB and R6G with increasing pH is a direct consequence of the pH-dependent ionization of bacterial functional groups. Based on the experimental results, the optimum pH for the adsorption of both dyes was identified as 8.0. Supporting these findings, Ghoniem et al. [51] reported that the maximum removal of MB using *Pseudomonas alcaliphila* NEWG-2 as a biosorbent occurred at pH 8.0. Similarly, in the study conducted by Modi et al. [52], which employed a bacterial-consortium–zinc-oxide nanoparticle-based approach for MB removal from wastewater, the optimal MB removal was also achieved at pH 8.0.

3.3 Influences of the contact time and the kinetic studies

The investigation of equilibrium time provides essential insight into the adsorption rate and the underlying mechanism governing the adsorptive affinity of dye molecules toward the active sites of the adsorbent surface. A critical

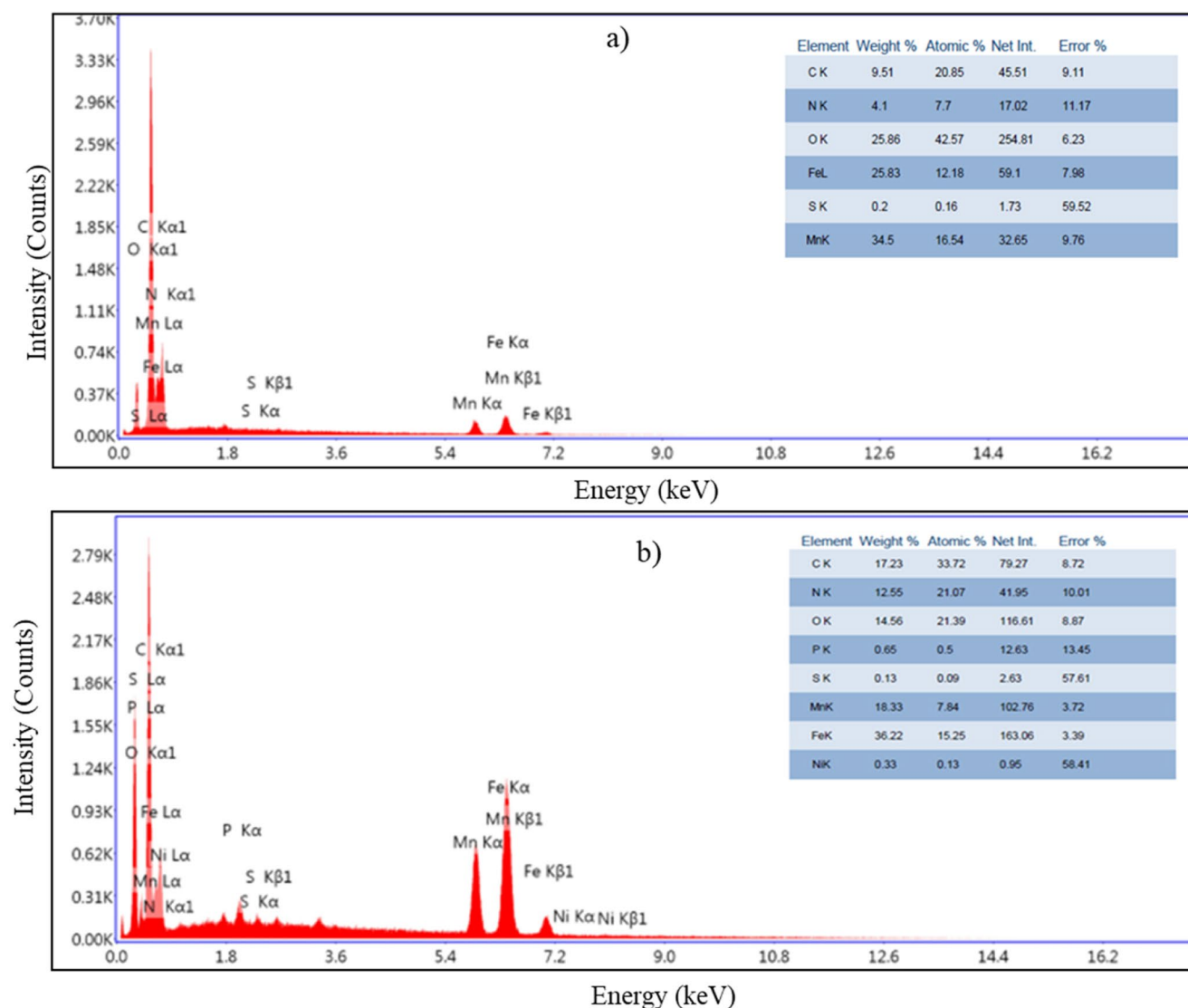


Fig. 4 EDX analysis of (a) MnFe₂O₄ and (b) Bacteria-loaded MnFe₂O₄

parameter for determining the adsorbent performance is its performance to achieve high removal efficiency within a short period. Therefore, to demonstrate the practical applicability of the developed adsorbent, the time-dependent adsorption behavior of MB and R6G on bacteria-loaded MnFe₂O₄ was investigated over a contact time range of 1–240 min at an initial dye concentration of 60 mg L⁻¹. The removal efficiencies at the first minute were 15.3% for MB and 19.8% for R6G. During the initial 30 min, adsorption proceeded rapidly owing to the high density of accessible binding sites on the surface of the bacteria-loaded MnFe₂O₄ nanoparticles. As a result, the adsorption efficiencies reached 52.3% for MB and 43.0% for R6G at 30 min (Fig. 6). With increasing occupation of active sites by dye molecules, the adsorption rate progressively slowed after the first 30 min [53]. After approximately 60 min, the adsorption capacity

approached a plateau, and this time was identified as the optimum equilibrium time for both dyes. Similarly, in the study conducted by Abbas and Trari [54] using activated coconut shells as the adsorbent, the equilibrium time for MB adsorption was also reported as 60 min. Al-Asadi et al. [2] reported an equilibrium time of 60 min for MB removal using fig leaf-derived activated carbon.

To evaluate the adsorption kinetics and propose a mechanism for the adsorption of R6G and MB onto bacteria-loaded MnFe₂O₄, the pseudo-first order (PFO), pseudo-second order (PSO), and intraparticle diffusion models (IPD) were applied to the experimental data (Table 1). The experimental adsorption capacities ($q_{e, \text{exp}}$) of R6G and MB were calculated as 28.3 mg g⁻¹ and 36.8 mg g⁻¹, respectively. Based on the kinetic evaluation, the adsorption capacities ($q_{e, \text{cal}}$) obtained from the PFO model were

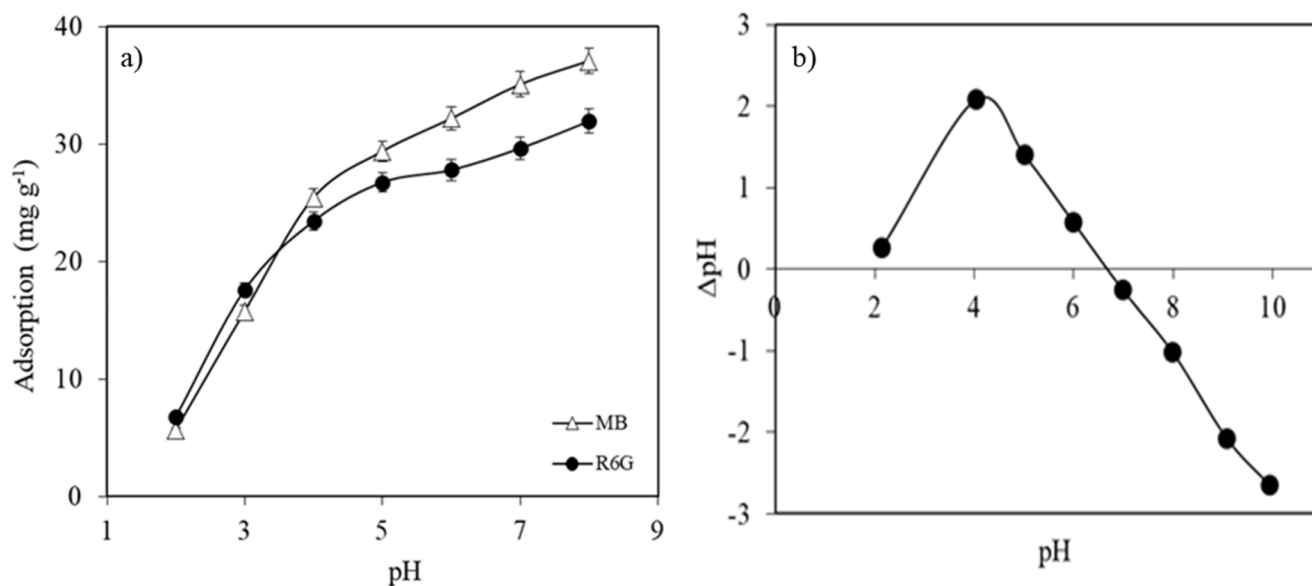


Fig. 5 **a** Impact of pH on the removal of target dyes by the bacteria-loaded MnFe_2O_4 **(b)** pH_{pzc} curve of bacteria-loaded MnFe_2O_4

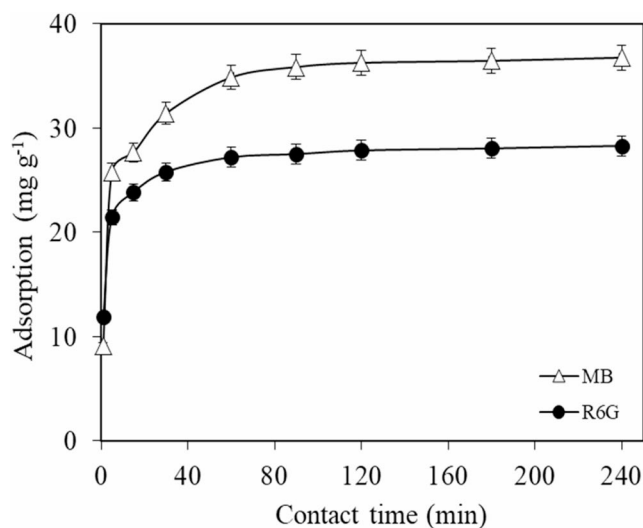


Fig. 6 Influences of equilibrium time for the removal of target dyes

determined to be 8.19 mg g^{-1} for R6G and 12.8 mg g^{-1} for MB, whereas those obtained from the PSO model were 28.5 mg g^{-1} and 37.3 mg g^{-1} , respectively. For both dye adsorption systems, the R^2 values obtained from the PSO model were higher than those derived from the PFO model. When these results are evaluated as a whole, the close agreement between the experimental q_e values and the q_e values calculated from the PSO model, together with the higher correlation coefficients, clearly demonstrates that the adsorption kinetics of both dyes are better described by the PSO model (Fig. 7). This result indicates that the adsorption process of R6G and MB onto bacteria-loaded MnFe_2O_4 is mainly governed by chemical bonding and strong electrostatic interactions between the dye molecules

Table 1 Comparison of the kinetic constants for the adsorption of MB and R6G onto bacteria-loaded MnFe_2O_4

	Adsorbate	
	MB	R6G
$q_e \text{ exp}$	36.8	28.3
PFO		
k_1	-0.0243	-0.0265
$q_e \text{ cal}$	12.8	8.19
R^2	0.9146	0.9004
PSO		
k_2	0.0068	0.014
$q_e \text{ cal}$	37.3	28.5
R^2	0.9998	0.9999
IDM		
$k_{\text{id},1}$	1.71	1.04
R^2	0.9868	0.9611
$k_{\text{id},2}$	0.14	0.12
R^2	0.9515	0.9569
C	19.4	18.2

and functional groups such as $-\text{OH}$, $-\text{COOH}$, and $-\text{NH}$ present on the adsorbent structure. The obtained kinetic results are consistent with literature reports, such as Azlan Zahari et al. [6], where MB adsorption also followed the PSO model, suggesting a chemisorption-dominated mechanism. In addition to the PFO and PSO models, the adsorption process is considered to occur in three stages according to the intraparticle diffusion (IPD) model. In the first stage, film diffusion, dye molecules are adsorbed onto the external surface of the bacteria-loaded MnFe_2O_4 . In the second stage, intraparticle diffusion, adsorption proceeds from the external surface into the pores of the adsorbent particles. The final stage corresponds to the equilibrium state, which occurs very rapidly and therefore does not contribute

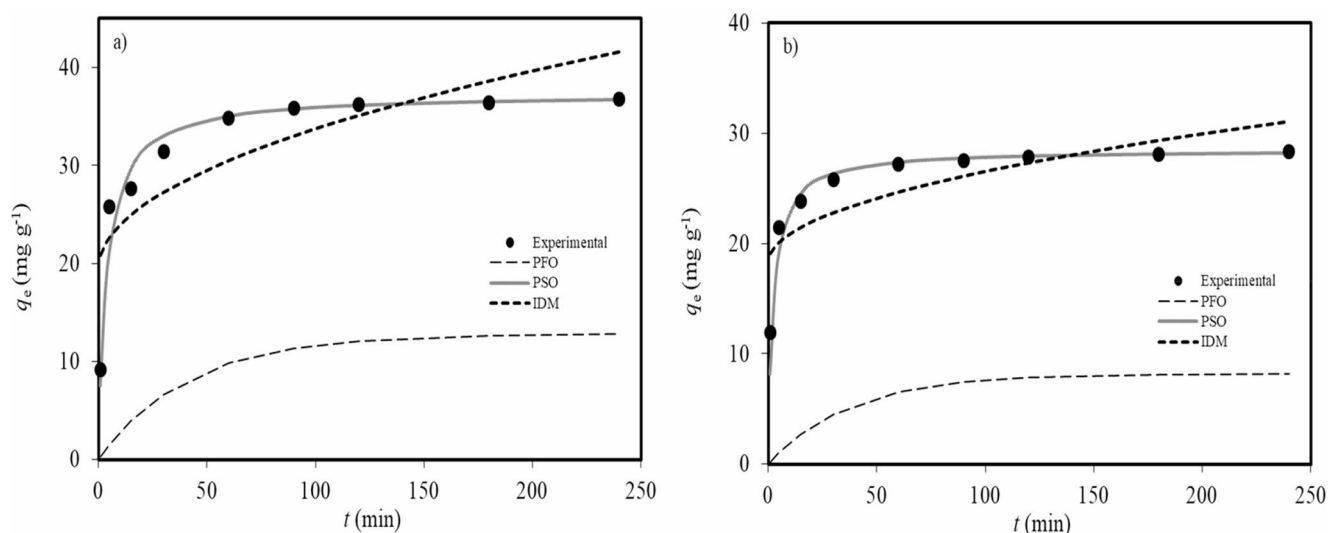


Fig. 7 Adsorption kinetics of (a) MB (b) R6G onto bacteria-loaded MnFe_2O_4

significantly to determine the overall adsorption rate. For both dyes, the rate constant obtained for the intraparticle diffusion stage is lower than that obtained for the film diffusion stage. Therefore, although intraparticle diffusion is considered to play a role in the adsorption mechanism, the fact that the C constant is different from zero indicates that both film diffusion and intraparticle diffusion are involved in controlling the adsorption process.

3.4 Influences of the bacteria-loaded MnFe_2O_4 dosage

Optimizing the dosage of adsorbent in removal processes is critically important. Insufficient adsorbent results in limited active sites, leading to low adsorption efficiency, whereas excessively high adsorbent dosages may increase removal efficiency but cause unnecessary material consumption. Using more adsorbent than required not only elevates operational costs but also reduces the overall sustainability of the process [55]. Therefore, determining the optimum adsorbent dosage is essential to achieve maximum adsorption performance while minimizing cost and resource waste. Hence the effects of bacteria-loaded MnFe_2O_4 amount on MB and R6G uptake from aqueous media were evaluated in the range of 0.5–5.0 g L^{-1} of bacteria-loaded MnFe_2O_4 dosage, under fixed experimental conditions (contact time: 60 min, initial solution pH: 8.0, initial dye concentration: 120 mg L^{-1}). The equilibrium adsorption capacity and dye removal percentages were plotted as a function of the bacteria-loaded MnFe_2O_4 dosage. When the bacteria-loaded MnFe_2O_4 amount was elevated from 0.5 g L^{-1} to 5.0 g L^{-1} , the maximum adsorption capacity (q_e) for MB and R6G decreased from 48.0 mg g^{-1} to 22.2 mg g^{-1} and from 46.4 mg g^{-1} to 18.7 mg g^{-1} , respectively, whereas the adsorption

percentage increased from 20% to 92.5% and from 19.3% to 77.8%, respectively (Fig. 8 (a) and (b)). While the increase in active adsorption sites and the resulting increase in surface area at high adsorbent concentrations led to a notable rise in the removal percentage, the presence of excessive amounts of adsorbent in the medium caused agglomeration, resulting in a decline in adsorption capacity [47, 56].

3.5 Influences of dye concentration and adsorption isotherm studies

The influence of initial MB and R6G concentrations on adsorption efficiency was assessed within a range of 20–300 mg L^{-1} initial dye concentration, keeping the amount of bacteria-loaded MnFe_2O_4 constant (1.0 g L^{-1}). Increasing the initial dye concentration from 20 to 300 mg L^{-1} enhances the concentration gradient, providing a potent driving force to overcome the mass transfer resistance between the aqueous and solid phases, and thus the adsorption amount (q_e , mg g^{-1}) increased from 17.0 mg g^{-1} to 68.8 mg g^{-1} for MB and from 16.0 mg g^{-1} to 55.8 mg g^{-1} for R6G, respectively. However, increasing the initial dye concentration induces oversaturation in the active adsorption regions on the adsorbent, thereby leading to a decline in the removal efficiency of MB and R6G from 85.0% to 22.9% and from 80.1% to 18.6%, respectively (Fig. 9 (a) and (b)). A similar finding was also obtained in the MB removal study conducted by Fang et al. [57] using humic acid–sucrose modified red mud as the adsorbent.

The comparison of equilibrium isotherms (Langmuir, Freundlich, and Dubinin–Radushkevich) between the experimental and theoretical data is presented in Figure (Fig. 10 (a) and (b)). For both dyes, the correlation coefficients obtained from the Langmuir and Freundlich isotherm

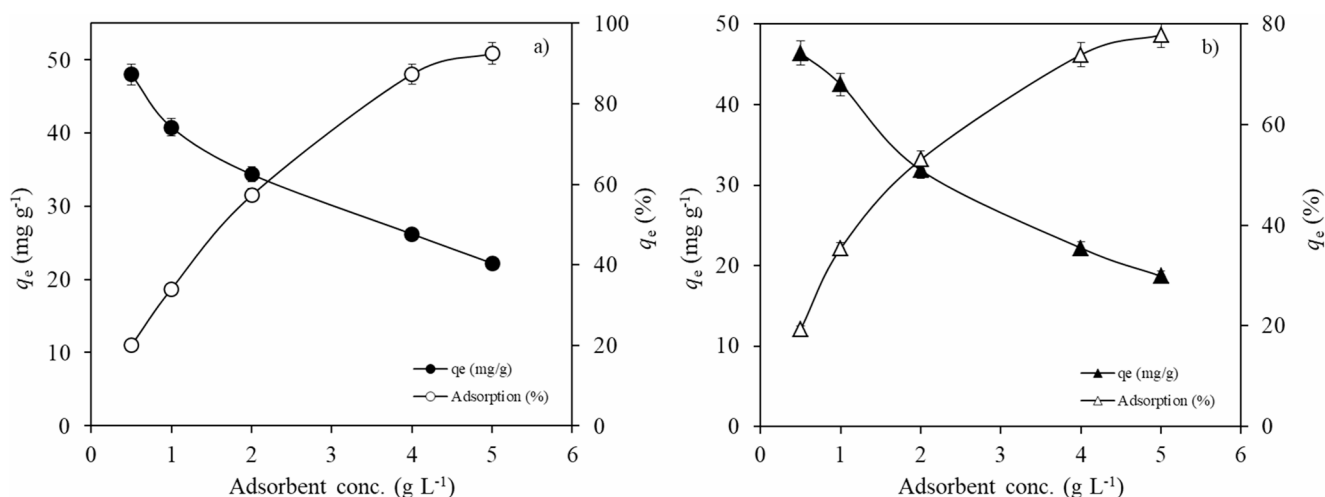


Fig. 8 Effect of bacteria-loaded MnFe_2O_4 amount on (a) MB adsorption (b) R6G adsorption

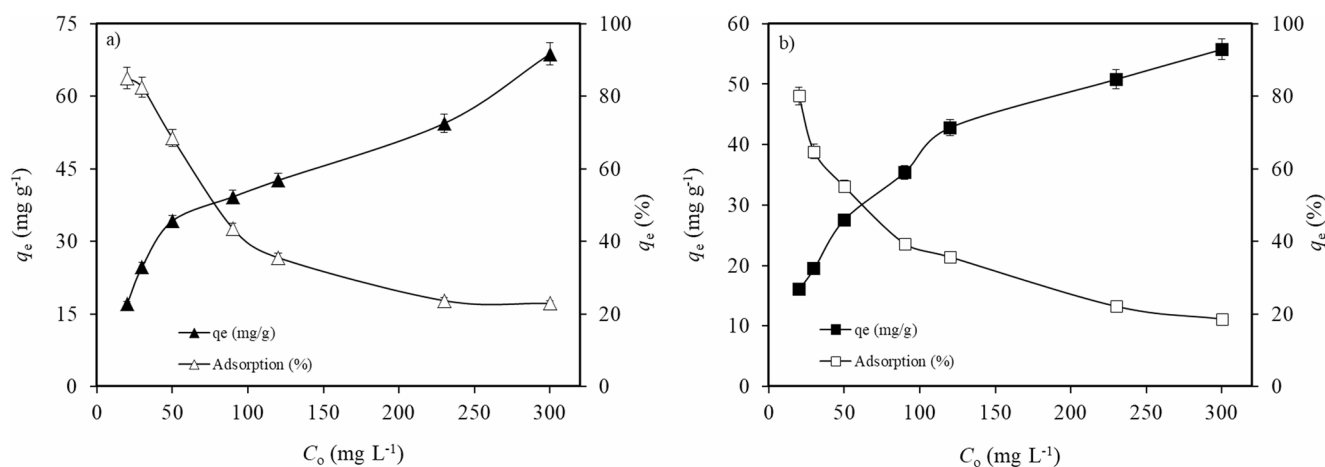


Fig. 9 Influences of initial (a) MB concentration (b) R6G concentration on their uptake

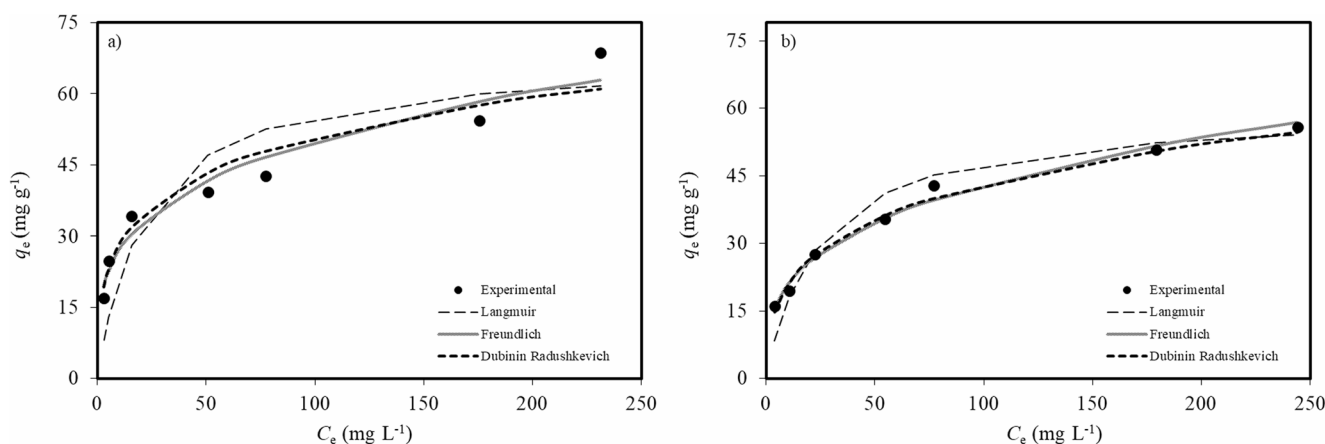


Fig. 10 Comparison of equilibrium isotherms between the experimental data and theoretical data for (a) MB (b) R6G

models were found to be higher than 0.95, indicating a good fit (Table 2). The good agreement of R6G and MB adsorption onto bacteria-loaded MnFe_2O_4 with both the Langmuir and Freundlich isotherm models suggests the presence of

energetically uniform and homogeneous sites on the surface of bacteria-loaded MnFe_2O_4 , where dyes are adsorbed as a monolayer, as well as heterogeneous regions formed as a result of bacterial modification.

Table 2 Isotherm constants and correlation coefficients for the adsorption of MB and R6G onto the developed bio-adsorbent

	MB	R6G
Langmuir isotherm model		
$q_{\max}$	67.6	59.5
b	0.045	0.041
R^2	0.9557	0.9905
Freundlich isotherm model		
K_f	14.3	10.0
n	3.68	3.16
R^2	0.9511	0.9887
D-R isotherm model		
q_m	11.3	11.9
β	-0.0023	-0.0027
E	14.7	13.6
R^2	0.9515	0.9864

Using the Langmuir isotherm model, the maximum adsorption capacities of bacteria-loaded MnFe_2O_4 for R6G and MB were calculated as 59.5 mg g^{-1} and 67.6 mg g^{-1} , respectively. To evaluate whether bacterial modification affects the adsorption capacity of MnFe_2O_4 , the Langmuir isotherm model was also applied to calculate the adsorption capacities of pristine MnFe_2O_4 for R6G and MB. According to the results, the adsorption capacities of pristine MnFe_2O_4 for R6G and MB were determined to be 15.3 mg g^{-1} and 19.5 mg g^{-1} , respectively. These findings demonstrate that bacterial modification increases the adsorption capacity of MnFe_2O_4 by more than 3.5-fold. Moreover, the adsorption capacity of bacteria-loaded MnFe_2O_4 is superior to that of most other difficult-to-prepare and costly sorbents reported in the literature [1, 6, 13, 58–64] and summarized in Table 3.

For initial R6G and MB concentrations in the range of 20–300 mg L^{-1} , the R_L values were found to vary between 0.55 and 0.08 and 0.53–0.07, respectively. In addition, the n values obtained from the Freundlich isotherm model were

calculated as 3.16 for R6G and 3.68 for MB. The R_L and n values in these ranges indicate that the adsorption of both dyes onto bacteria-loaded MnFe_2O_4 is favorable. Furthermore, according to the D-R isotherm model, the E values for both dyes fall within the range of 8–16 kJ mol^{-1} , suggesting that ion-exchange mechanisms may also be involved in the adsorption process.

3.6 Desorption of dye molecules

To determine whether R6G and MB adsorbed onto the bacteria-loaded MnFe_2O_4 surface can be recovered under appropriate conditions and to evaluate the reusability of the adsorbent, the desorption experiments were carried out using ethanol-water and acetone-water mixtures at different ratios. For this purpose, the adsorbent at a concentration of 1.0 g L^{-1} was first separately treated with 50 mg L^{-1} solutions of R6G and MB at pH 8.0 for 60 min. After this step, the adsorbent was recovered by centrifugation, dried, and subsequently contacted with 10 mL of desorption solution for 60 min. The adsorbent was then separated, and the concentrations of desorbed R6G and MB were quantified using UV–Vis spectrophotometry. Based on the obtained results, the ethanol–water (1:1) mixture was found to be the most suitable solution for the desorption of both MB and R6G, achieving desorption efficiencies higher than 70% (Fig. 11).

3.7 Influences of ionic strength and application of real samples

To evaluate the applicability of the developed adsorption process in complex water matrices containing dyes, the effect of ionic strength was investigated using NaCl and BaCl_2 at different concentrations (0.01–0.1 M). When the NaCl concentration was increased from 0.01 M to 0.10 M,

Table 3 Comparison of adsorption performance of reported adsorbents for R6G and MB

Adsorbent	Dye	pH	Contact time (min)	Adsorbent amount (g/L)	$Q_{\max}$ (mg g^{-1})	Reference
Fe_3O_4 -composited biochar derived from rice husk	R6G	7.0	-	10.0	9.42	[13]
Commercial activated carbon	R6G	7.0	45	1.0	44.7	[58]
Cellulose-lactone hybrid material	R6G	7.0	40	80 mg	8.14	[59]
Phosphorus-containing polybutadiene rubber-bentonite composite	R6G	-	1440	1.0	25.76	[60]
<i>Gossypium hirsutum</i> stalk fiber extract coated pozzolan powder	R6G	-	30	2.0	30.63	[61]
	MB	-	30	2.0	53.24	
Silica gel	MB	11.0	180	2.0	9.488	[1]
Activated carbon from bamboo waste	MB	7.62	8.0	0.8	85.6	[6]
Sawdust	MB	7.0	30	5.0	24.97	[62]
Activated carbon of <i>Parthenium hysterophorus</i>	MB	11.0	100	20.0	11.37	[63]
Magnetite nanoparticles loaded Azolla	MB	6.0–10.0	25	1.5	25.0	[64]
<i>Stenotrophomonas indicatrix</i> loaded MnFe_2O_4	R6G	8.0	60	1.0	59.5	This work
	MB	8.0	60	1.0	67.6	

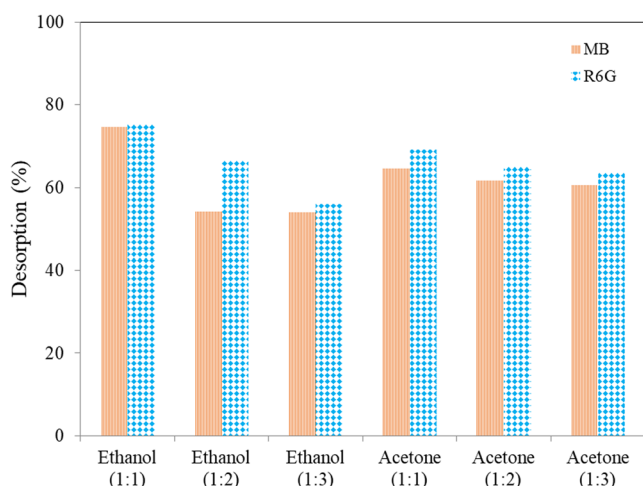


Fig. 11 Desorption of dye molecules

the adsorption capacity for MB and R6G decreased from 31.5 to 22.2 mg g^{-1} and from 26.0 to 21.8 mg g^{-1} , respectively. Similarly, when the BaCl_2 concentration was increased from 0.01 M to 0.10 M, the adsorption capacity for MB and R6G decreased from 37.7 to 11.1 mg g^{-1} and from 24.8 to 15.9 mg g^{-1} , respectively (Fig. 12 (a) and (b)). The presence of ionic strength in the medium causes a decrease in the adsorption efficiency of both MB and R6G. This decrease can be attributed to the competitive effect of Na^+ and Ba^{2+} ions with cationic dye molecules to bind to the active adsorption sites of bacteria-loaded MnFe_2O_4 . Furthermore, since Ba^{2+} has a higher charge density than Na^+ , BaCl_2 caused a greater decrease in adsorption efficiency [65].

To further validate the applicability of bacteria-loaded MnFe_2O_4 beyond synthetic systems, the adsorption process was applied to environmental samples with complex matrices, including stream water (Harshit Stream, Gümüşhane)

and wastewater collected from an organized industrial zone. In each case, dye solutions at an initial concentration of 10 mg L^{-1} were spiked into 50 mL of the water samples, followed by the addition of the adsorbent at a dosage of 1.0 g L^{-1} to initiate the adsorption process. The removal efficiencies of MB and R6G from river water were found to be 74.2% and 70.2%, respectively, while the corresponding removal efficiencies from wastewater collected from the organized industrial zone were 79.4% and 73.3%, respectively. These results highlight the effectiveness of bacteria-loaded MnFe_2O_4 under real environmental conditions.

4 Conclusion

In the present research, MnFe_2O_4 magnetic nanoparticles were successfully modified with the *Stenotrophomonas indicatrix* bacteria, and the performance of the resulting bio-adsorbent in removing Rhodamine 6G (R6G) and methylene blue (MB) from aqueous solutions was investigated in detail. Characterization studies demonstrated that bacterial loading significantly enriched the surface functional groups and active adsorption sites while substantially preserving the MnFe_2O_4 structure. The optimum pH and equilibrium time for the removal of both dyes were established as 8.0 and 60 min, respectively, indicating that the adsorbent achieves high removal efficiency within a short period. Kinetic data exhibited a high correlation with the pseudo-second order kinetic model, which implies that the chemical interactions are predominantly dominant in the mechanism. Isotherm studies, successfully explained by both Langmuir and Freundlich models, indicated that the adsorbent possesses both homogeneous and heterogeneous surface characteristics. The most striking result of this study is the remarkable enhancement in the adsorption capacity of the magnetic nanoparticles

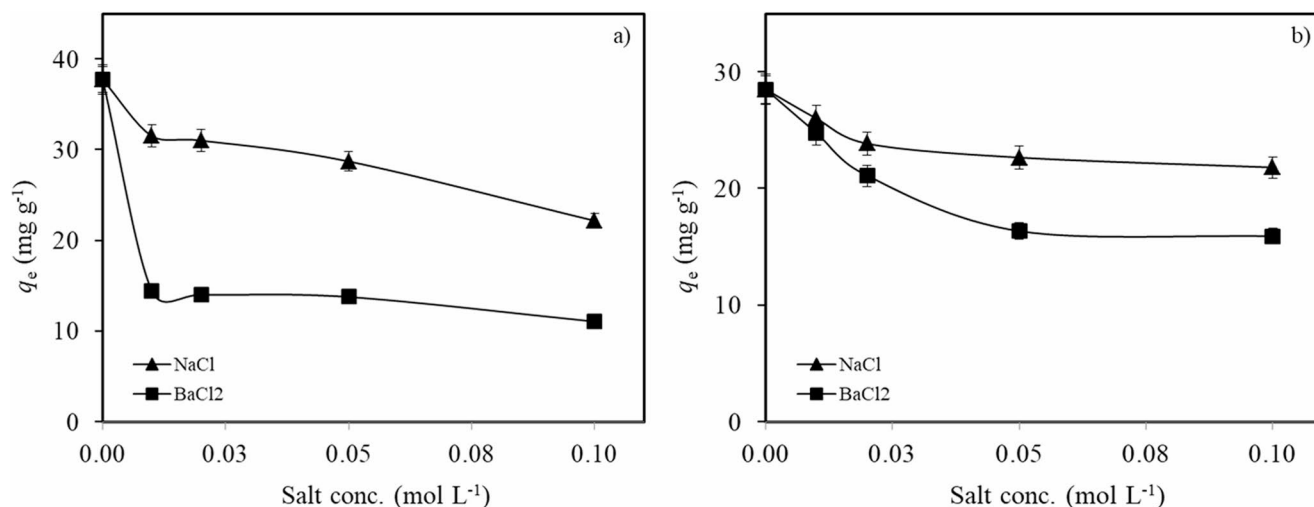


Fig. 12 Effect of salt concentration on the adsorption of (a) MB (b) R6G

provided by bacterial loading; specifically, adsorption capacities for MB and R6G exhibited 3.5-fold and 3.9-fold increases, respectively, compared to the unmodified adsorbent. Furthermore, the cost effectiveness of the developed bio-adsorbent was also considered. MnFe_2O_4 nanoparticles can be synthesized from inexpensive metal salts through a simple co-precipitation route, while the bacterial biomass is low-cost and easily produced, eliminating the need for expensive chemical modification steps. In addition, the magnetic nature of the adsorbent enables rapid separation from the treated solution without filtration or centrifugation, thereby reducing operational and energy costs.

In conclusion, *Stenotrophomonas indicatrix*-modified MnFe_2O_4 stands out as an environmentally benign, effective, and sustainable adsorbent for the treatment of dye-containing wastewater, offering high adsorption capacity and ease of recovery due to its magnetic properties. Future studies will focus on reusability performance and long-term stability of the prepared adsorbent, and continuous-flow or column-scale applications to further evaluate its practical feasibility. Such investigations will support the real industrial implementation of this bio-adsorbent for cost-effective wastewater treatment.

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Author contributions DO: designed methodologies, data curation and analysis, writing—review & editing, conceptualization. AD: culture of bacteria, review & editing. FAT and SS: investigation, data curation and analysis.

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Data availability All data obtained have been included in the manuscript and are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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