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Research Paper

Selective Adsorption of Mixed Dyes Using Freshwater Macroalgae (*Cladophora* sp.) Biomass: Spectroscopic Analysis

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Abstract

This study investigates the selective adsorption behavior of freshwater macroalgae *Cladophora* sp. for the removal of mixed dye systems containing cationic (rhodamine B (RB), malachite green (MG), and methylene blue (MB)) and anionic dyes (congo red (CR) and remazol red (RR)). The biomass was characterized using Fourier-transform infrared spectroscopy (FTIR), Brunauer–Emmett–Teller (BET) analysis, and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDX), confirming the presence of abundant functional groups and a porous surface structure suitable for adsorption. The point of zero charge (pHpzc) was determined to be 6.5, indicating that the adsorbent surface becomes negatively charged under near-neutral conditions. Selective adsorption was evaluated using UV–Visible spectrophotometry combined with Gaussian peak deconvolution to distinguish individual dye contributions in mixed systems. The results showed a significantly higher adsorption efficiency for cationic dyes, with methylene blue exhibiting the highest removal efficiency (93.56%), followed by malachite green (91.48%) and rhodamine B (51.09%). In contrast, anionic dyes showed considerably lower adsorption, with congo red (33.99%) and remazol red (32.68%). The enhanced selectivity toward cationic dyes is primarily attributed to electrostatic attraction between the negatively charged adsorbent surface and positively charged dye molecules, supported by additional interactions such as π–π stacking and hydrogen bonding. These findings demonstrate that *Cladophora* sp. is a promising low-cost and sustainable bioadsorbent with selective adsorption capability, making it suitable for the treatment of complex dye-contaminated wastewater.

Keywords

Cladophora sp., Bioadsorbent, Selective Adsorption, Mixed Dye System, UV–Vis Spectroscopy

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1. INTRODUCTION

Natural biomass-based materials have attracted considerable attention as sustainable and low-cost adsorbents for environmental remediation (Pablo et al., 2023). Among these, algae-derived materials have emerged as promising candidates due to their abundance, renewability, and environmentally benign nature (Inkoua et al., 2024). Algal biomass is rich in functional groups such as hydroxyl (–OH), carboxyl (–COOH), and sulfate groups, which can interact with various pollutants through multiple mechanisms (Zhang et al., 2021), including electrostatic attraction, hydrogen bonding, and π–π interactions (Ramadhan et al., 2025a) In addition, the inherent porous and filamentous structure of certain macroalgae provides a large surface area and accessible active sites, further enhancing their adsorption performance (Lesbani et al., 2024).

Among various algal species, freshwater macroalgae such as *Cladophora* sp. have gained increasing interest as bioadsorbents (Badaruddin et al., 2026). *Cladophora* sp. is widely distributed in aquatic environments and is often considered an underutilized biomass resource (Prazukin et al., 2025). Its filamentous morphology and high polysaccharide content contribute to its favorable adsorption characteristics (Wang et al., 2025). Previous studies have demonstrated the potential of algal biomass for the removal of dyes, heavy metals, and emerging contaminants from aqueous solutions (Bai et al., 2026). However, most of these studies have primarily focused on single-component systems, which do not adequately represent real wastewater conditions (Wang et al., 2026).

In practical applications, wastewater from textile and dyeing industries typically contains a complex mixture of dyes with different chemical structures and charges (Lesbani et al., 2025). These dyes may include both cationic and anionic species (Arora et al., 2024), leading to competitive interactions during the adsorption process (Wijaya et al., 2025a). Such interactions significantly influence adsorption efficiency, as different dye molecules compete for the same active sites on the adsorbent surface (Wibiyan et al., 2024). Therefore, understanding adsorption behavior in multi-component systems is essential for evaluating the real applicability of bioadsorbents (Palapa et al., 2024; Ramadhan et al., 2025b).

Selective adsorption has recently become an important focus in adsorption studies, particularly for mixed dye systems (Ramadhan et al., 2025b). The ability of an adsorbent to preferentially remove specific dyes from a mixture is governed by several factors, including surface charge, functional groups, and solution pH (Putri et al., 2022). Electrostatic interactions are often the dominant mechanism (Bai et al., 2026), especially in systems containing both positively and negatively charged dye molecules (Goswami et al., 2024). For instance, adsorbents with negatively charged surfaces tend to favor the adsorption of cationic dyes, while repelling anionic dyes . However, other interactions such as hydrogen bonding and $\pi-\pi$ interactions may also contribute to the overall adsorption behaviour (Cano et al., 2025).

Despite growing interest in selective adsorption, studies investigating the performance of *Cladophora* sp. in multi-dye systems remain limited. Most existing works emphasize adsorption capacity without providing a detailed understanding of competitive adsorption mechanisms and selectivity behaviour (Arora et al., 2024). This highlights a significant research gap in the development of low-cost, natural adsorbents capable of selectively removing dyes from complex wastewater systems.

Therefore, this study aims to investigate the selective adsorption behavior of *Cladophora* sp. toward mixed dye systems consisting of cationic dyes (RB, MG, MB) and anionic dyes (CR, RR). Prior to adsorption experiments, the physicochemical properties of the prepared biomass were characterized using Fourier-transform infrared spectroscopy (FTIR) to identify functional groups, Brunauer–Emmett–Teller (BET) analysis to determine surface area and porosity, and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) to examine surface morphology and elemental composition. The adsorption performance was evaluated using UV–Visible spectroscopic analysis combined with peak deconvolution techniques to distinguish the contribution of individual dyes in the mixture. In addition, the role of surface characteristics and charge properties in governing adsorption selectivity was analyzed. This work provides new insights into the application of freshwater macroalgae as a sustainable bioadsorbent for selective dye removal in complex wastewater systems.

2. EXPERIMENTAL SECTION

2.1 Materials and Instruments

The freshwater macroalgae *Cladophora* sp. was collected from South Sumatra, Indonesia. Sodium chloride (NaCl) and sodium hydroxide (NaOH) of analytical grade were obtained from Merck. Hydrochloric acid (HCl, analytical grade) was supplied by MallinckrodtAR®, USA. The dyes used in this study were collected from a local textile industry in Indonesia and used without further purification. All solutions were prepared using distilled water.

The characterization of *Cladophora* sp. was carried out using Fourier-transform infrared spectroscopy (FTIR, Shimadzu Prestige-21), Brunauer–Emmett–Teller (BET, BELSORP-mini X) surface area analysis, and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDX, JEOL JSM-6510LA). The absorbance of dye solutions was measured using a UV–Visible spectrophotometer (EMC Lab-18 PC).

2.2 Preparation of Macroalgae *Cladophora* sp.

The collected *Cladophora* sp. was thoroughly washed with distilled water to remove adhering impurities such as dust, sand, and other contaminants. The cleaned biomass was then oven-dried at 105 °C for 24 h to remove moisture content. After drying, the material was ground using a mortar and pestle to obtain a fine powder. The resulting powder was subsequently sieved through a 200-mesh sieve to ensure uniform particle size. The prepared adsorbent was stored in an airtight container for further use in adsorption experiments.

2.3 Determination of pHpzc

The point of zero charge (pHpzc) of *Cladophora* sp. was determined using the pH drift method (Ahmad et al., 2023). A series of 50 mL sodium chloride (NaCl) solutions (0.01 M) were prepared, and the initial pH values were adjusted in the range of 2–11 using 0.1 M HCl and 0.1 M NaOH solutions. A fixed amount of the prepared *Cladophora* sp. adsorbent (e.g., 0.1 g) was added into each solution. The suspensions were then agitated using a shaker at room temperature for 12 h to reach equilibrium. After equilibration, the final pH of each solution was measured. The difference between the final and initial pH values was calculated and plotted against the initial pH.

2.4 Selectivity Adsorption

Binary dye systems were prepared to evaluate the selective adsorption behavior of *Cladophora* sp. toward cationic and anionic dyes (Badaruddin et al., 2026). For the cationic system, RB, MG, and MB were mixed, while CR and RR were used for the anionic system. Each dye was prepared at an initial concentration of 10 mg/L. The dye mixtures were prepared in a 20 mL solution and transferred into glass beakers. Subsequently, 0.02 g of *Cladophora* sp. adsorbent was added to each solution. The suspensions were then agitated using a shaker for 120 min at room temperature to reach adsorption equilibrium. After the adsorption process, the solutions were analyzed using a UV–Visible spectrophotometer. Wavelength scans were recorded over an appropriate spectral range to identify the characteristic absorption peaks of each dye. The obtained spectra were further analyzed using Gaussian peak deconvolution techniques to evaluate the contribution of each dye in the mixed system and to determine the selective adsorption behavior of the adsorbent. As illustrated in Figure 1, the experimental workflow of the selective adsorption process involves binary dye preparation, adsorbent addition, and spectroscopic analysis.

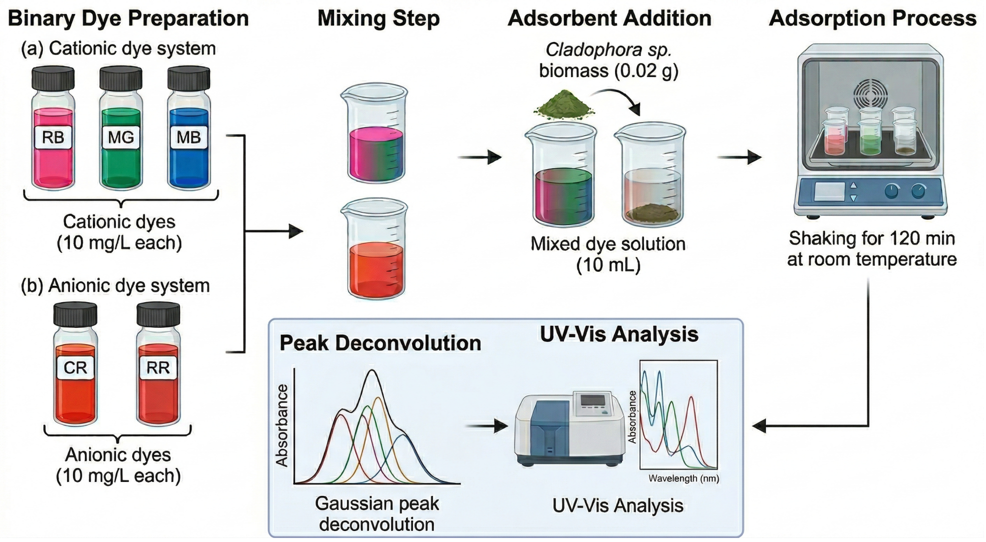

Figure 1. Schematic Illustration of the Selective Adsorption Experiment Using Cladophora sp. Biomass

3. RESULTS AND DISCUSSION

The microscopic observation of the collected biomass (Figure 2) reveals a characteristic filamentous structure consisting of elongated, thread-like cells forming interconnected networks. The filaments appear green in color and exhibit a cylindrical morphology, which is typical of freshwater macroalgae belonging to the genus *Cladophora*. The observed structure also indicates the presence of branching filaments, which further supports the identification of the sample as *Cladophora* sp. (Zhao et al., 2025).

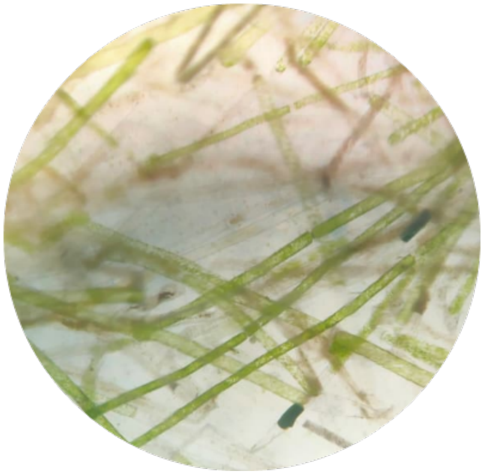

Figure 2. Microscopic Image of *Cladophora* sp.

These morphological features are consistent with previous reports describing *Cladophora* as a filamentous green algae composed of uniseriate cylindrical cells arranged in long filaments, often forming branched networks or dense mats in aquatic environments. The filamentous thallus structure is a distinctive characteristic that differentiates *Cladophora* from other freshwater algae such as *Spirogyra*, which typically exhibit unbranched filaments. In addition, the presence of visible branching and irregular filament orientation observed in this study aligns with the known morphological plasticity of *Cladophora*, where both branched and unbranched forms may occur depending on environmental conditions (Pablo et al., 2023). Therefore, the observed filamentous, cylindrical, and branched morphology is in strong agreement with the reported characteristics of *Cladophora* sp., confirming the correct identification of the collected biomass.

The FTIR spectrum presented in Figure 3a reveals the presence of various functional groups on the surface of *Cladophora* sp. biomass that are essential for adsorption processes. A broad absorption band observed in the range of 3200–3500 cm^{-1} is attributed to the stretching vibration of hydroxyl (–OH) groups, which are commonly associated with polysaccharides such as cellulose present in algal cell walls. These hydroxyl groups play a significant role in adsorption through hydrogen bonding and electrostatic interactions.

The peak observed around 2900 cm^{-1} corresponds to C–H stretching vibrations, indicating the presence of aliphatic structures within the biomass. Meanwhile, the absorption band in the region of 1600–1700 cm^{-1} can be assigned to C=O stretching and/or C=C vibrations, suggesting the presence of carbonyl and aromatic groups. These functional groups may contribute to π–π interactions between the adsorbent surface and dye molecules, particularly for aromatic dyes (Deivayanai et al., 2025). Furthermore, the peaks in the region of 1000–1200 cm^{-1} are attributed to C–O and C–O–C stretching vibrations, which are characteristic of polysaccharide structures. The abundance of these oxygen-containing functional groups indicates that *Cladophora* sp. possesses multiple active sites capable of interacting with dye molecules through various mechanisms (Cano et al., 2025). The FTIR results confirm that the biomass surface is chemically active and suitable for adsorption applications, particularly in multi-component dye systems.

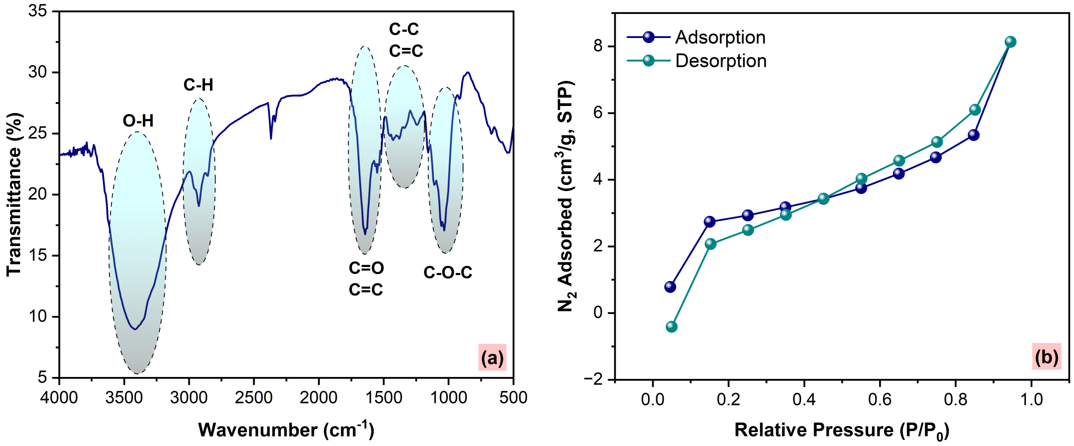

Figure 3. (a) FTIR spectrum and (b) N_2 Adsorption–desorption Isotherm of *Cladophora* sp.

Table 1. BET Surface Area and BJH Pore Characteristics of *Cladophora* sp.

Surface Area	3.142 m^2/g
Pore Size Average	2.051 nm
Total Pore Volume	0.013 cm^3/g

The nitrogen adsorption–desorption isotherm shown in Figure 3b provides insight into the textural properties of the adsorbent. The isotherm exhibits a gradual increase in nitrogen uptake with increasing relative pressure (P/P_0), indicating the presence of porous structures within the biomass (Wijaya and Yuliasari, 2023). The absence of a sharp plateau at low relative pressure suggests that the material does not exhibit typical microporous behavior but is more consistent with mesoporous characteristics.

Additionally, the slight hysteresis between the adsorption and desorption curves at higher relative pressures indicates the presence of capillary condensation within mesopores. This type of isotherm behavior is generally associated with Type IV isotherms according to IUPAC classification, which is characteristic of mesoporous materials (Laxmi Deepak Bhatlu et al., 2023) The presence of mesopores is advantageous for adsorption processes involving relatively large molecules such as dyes, as it facilitates diffusion and accessibility to internal adsorption sites.

The quantitative textural properties obtained from BJH analysis (Table 1) indicate a surface area of 3.142 m^2/g, an average pore diameter of 2.051 nm, and a total pore volume of 0.013 cm^3/g. The pore size value, which lies at the boundary between microporous and mesoporous regions, suggests that *Cladophora* sp. possesses narrow mesopores that are suitable for the adsorption of dye molecules. Although the surface area is relatively low compared to engineered adsorbents, the presence of accessible pore channels combined with abundant surface functional groups compensates for this limitation.

The combination of surface functional groups identified by FTIR and the porous structure indicated by BET analysis suggests that *Cladophora* sp. possesses both chemical and physical properties favorable for adsorption. The functional groups provide active binding sites, while the mesoporous structure enhances mass transfer and accessibility. These complementary characteristics are particularly important in mixed dye systems, where competition between molecules requires both sufficient surface reactivity and accessible pore structures. Therefore, the FTIR and BET results collectively confirm that *Cladophora* sp. biomass is a promising bioadsorbent with suitable surface chemistry and porosity for selective adsorption of dyes in complex aqueous systems.

The surface morphology of *Cladophora* sp. was further examined using SEM analysis with different detectors, as presented in Figure 4a and 4b. The image obtained using the CBS detector (Figure 4a) shows a relatively compact and layered surface with irregular structures. This morphology indicates the presence of natural folds and aggregated structures within the biomass, which can contribute to the formation of adsorption sites. The uneven surface and the presence of cavities suggest that the material has accessible regions for dye interaction.

In contrast, the SEM image obtained using the LVD detector (Figure 4b) provides a clearer visualization of the surface texture, revealing a more pronounced fibrous and wrinkled structure. The presence of intertwined fibers and rough surfaces enhances the surface heterogeneity of the adsorbent. Such morphological characteristics are advantageous for adsorption processes, as they increase the probability of contact between dye molecules and active sites. The rough and folded structure also facilitates diffusion pathways, particularly important in mixed dye systems where molecular competition occurs.

The elemental composition of *Cladophora* sp. was analyzed using EDX, as shown in Figure 4c. The spectrum indicates that the biomass is predominantly composed of carbon (C) and oxygen (O), which are the main constituents of organic materials such as polysaccharides and cellulose in algal cell walls. The dominance of these elements is consistent with the FTIR results,

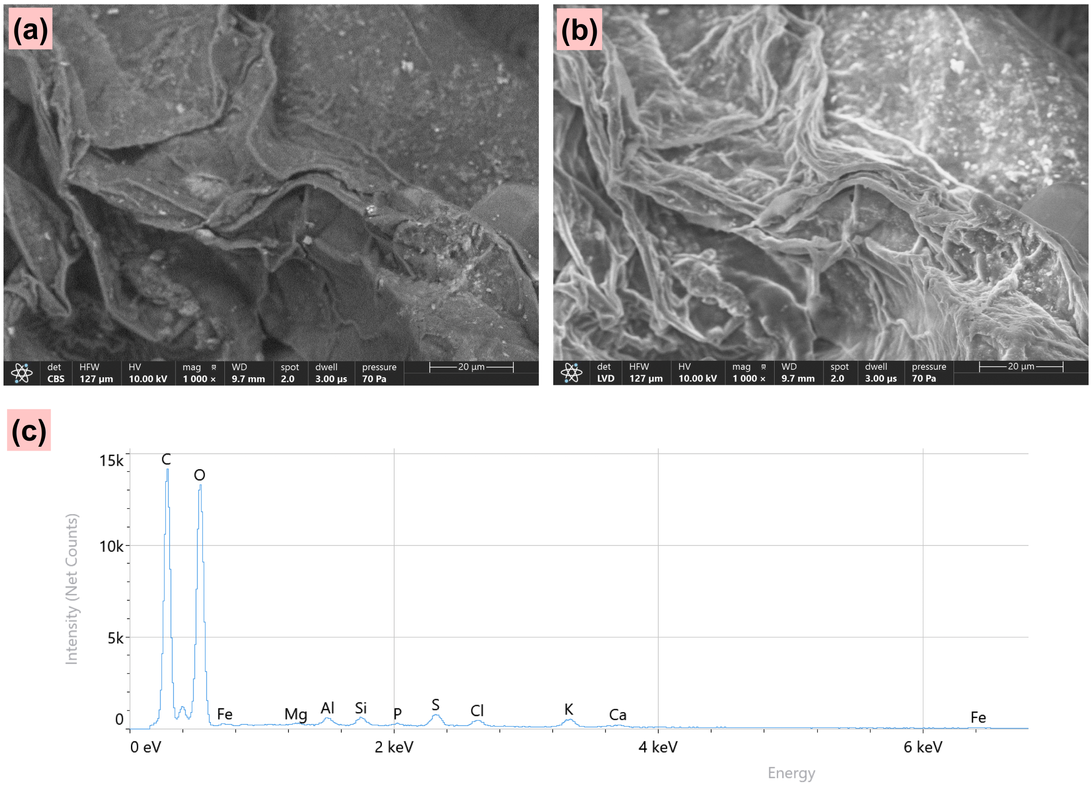

Figure 4. (a) SEM Image Observed Using CBS Detector; (b) SEM Image Using LVD Detector; (c) EDX Spectrum of *Cladophora* sp.

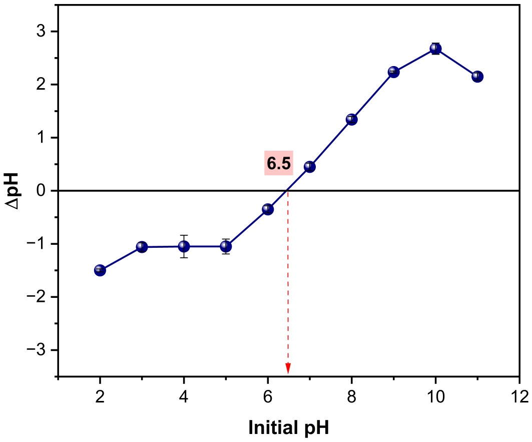

Figure 5. pH pzc of *Cladophora* sp.

which confirmed the presence of oxygen-containing functional groups such as hydroxyl and carboxyl groups. In addition to C and O, several minor elements were detected, including magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), and iron (Fe). These elements are commonly found in natural biomass and may originate from the aquatic environment where the algae were collected.

SEM–EDX analysis demonstrates that *Cladophora* sp. possesses a rough, fibrous, and heterogeneous surface morphology combined with a chemically active elemental composition. These characteristics complement the findings from FTIR and BET analyses, confirming that the biomass has favorable structural and chemical properties for adsorption. The combination of surface roughness, porosity, and functional elements plays a crucial role in supporting the selective adsorption of dyes in multi-component systems.

The point of zero charge (pHpzc) of *Cladophora* sp. was determined using the pH drift method, and the results are presented in Figure 5. The pHpzc value was found to be 6.5, corresponding to the intersection point where ΔpH equals zero. This indicates that the surface of the adsorbent is electrically neutral at pH 6.5. At solution pH values below pHpzc, the surface of *Cladophora* sp. becomes positively charged due to the protonation of functional groups such as hydroxyl (−OH) and carboxyl (−COOH). In contrast, at pH values above pHpzc, deprotonation occurs, resulting in a negatively charged surface. This behavior is consistent with the FTIR analysis, which confirmed the presence of abundant oxygen-containing functional groups that are sensitive to pH variations.

The variation of ΔpH with initial pH further reflects the buffering capacity of the biomass surface, indicating active interaction between the adsorbent and the surrounding solution. The gradual transition from negative to positive ΔpH values sug-

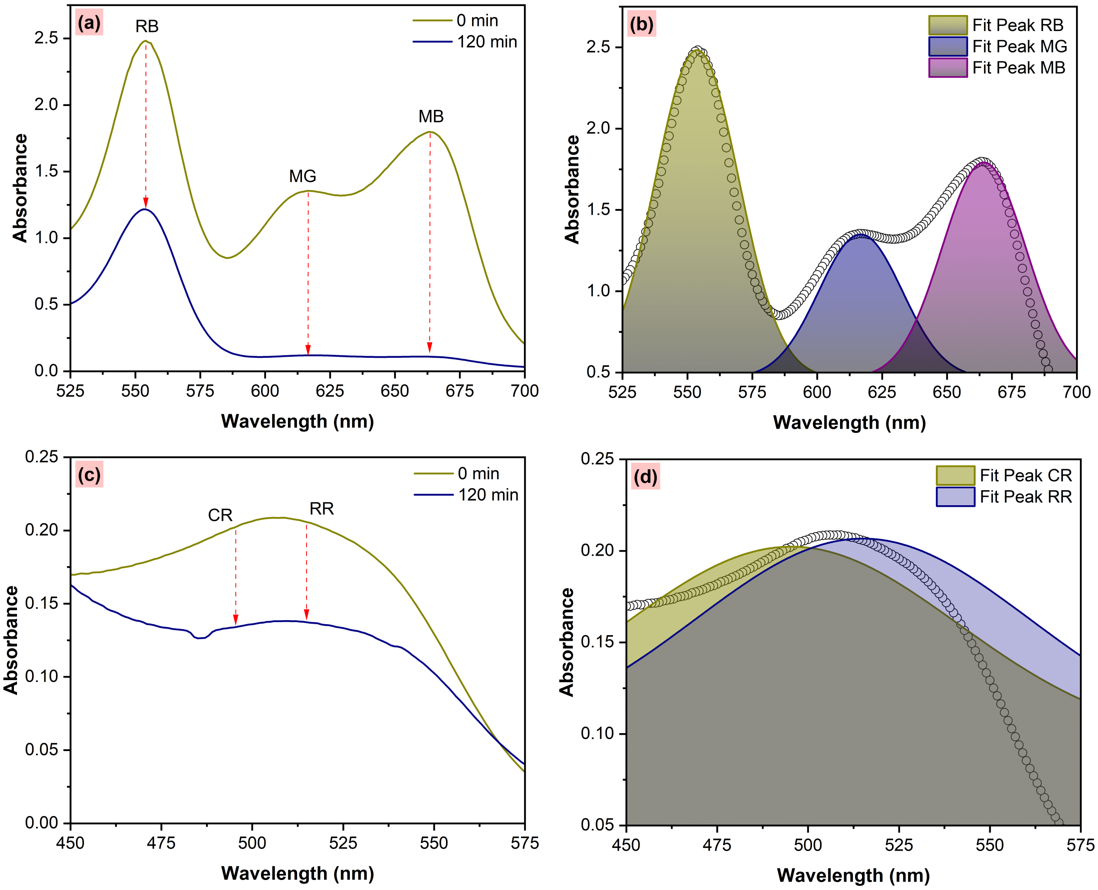

Figure 6. (a) UV–Vis Absorption Spectra of Mixed Cationic Dyes (RB, MG, MB) Before and After Adsorption; (b) Gaussian Peak Deconvolution of Cationic Dye Mixture; (c) UV–Vis Absorption Spectra of Mixed Anionic Dyes (CR, RR) Before and After Adsorption; (d) Gaussian Peak Deconvolution of Anionic Dye Mixture

gests the progressive change in surface charge characteristics across the studied pH range. Understanding the surface charge behavior of *Cladophora* sp. is essential, as it influences the interaction between the adsorbent and charged species in aqueous systems. This characteristic is expected to play an important role in governing adsorption behavior under different pH conditions, particularly in systems containing multiple types of dye molecules.

Table 2. Selective Adsorption Efficiency of *Cladophora* sp. Toward Mixed Cationic and Anionic Dye Systems

Dyes		Adsorption Efficiency (%)
Cationic Dyes	RB	51.09
	MG	91.48
	MB	93.56
Anionic Dyes	CR	33.99
	RR	32.68

The selective adsorption behavior of *Cladophora* sp. toward mixed dye systems was evaluated using UV–Visible spectroscopic analysis combined with Gaussian peak deconvolution, as shown in Figure 6. In the cationic dye system (Figure 6a), the absorption peaks corresponding to RB, MG, and MB were clearly observed in the initial spectrum. After 120 min of adsorption, a significant decrease in absorbance was observed for all dyes, indicating effective adsorption by the biomass. However, the extent of reduction varied among the dyes, suggesting selective adsorption behavior.

To further distinguish the contribution of each dye, Gaussian peak deconvolution was applied (Figure 6b). The deconvoluted peaks revealed that MB exhibited the most significant decrease in peak intensity compared to RB and MG. This observation is consistent with the adsorption efficiency data presented in Table 2, where MB showed the highest removal efficiency (93.56%), followed by MG (91.48%) and RB (51.09%). The higher selectivity toward MB can be attributed to its molecular structure and charge distribution. MB possesses a planar aromatic structure that facilitates stronger π–π interactions with the adsorbent surface, as well as favorable electrostatic attraction with negatively charged functional groups on *Cladophora* sp. under near-neutral conditions. In contrast, RB has a more complex structure with steric hindrance, which may limit its accessibility to active sites, resulting in lower adsorption efficiency.

In the anionic dye system (Figure 6c), the absorption spectra of CR and RR showed a relatively smaller decrease in absorbance after adsorption, indicating weaker interaction with the adsorbent compared to the cationic system. The Gaussian deconvolution results (Figure 6d) demonstrate that CR experienced a slightly greater reduction in peak intensity compared to RR, which is consistent with the adsorption efficiency values in Table 2 (33.99% for CR and 32.68% for RR). The relatively higher selectivity of CR may be related to its molecular size and structure, which allow better interaction with the available surface sites compared to RR, although both dyes are limited by electrostatic repulsion.

The overall lower adsorption efficiency observed for anionic dyes compared to cationic dyes can be explained by the surface charge characteristics of *Cladophora* sp., as indicated by the pHpzc value of 6.5. Under the experimental conditions (near-neutral pH), the surface of the adsorbent is negatively charged, which promotes electrostatic attraction toward cationic dyes while causing repulsion toward anionic dyes (Jin et al., 2025). This electrostatic effect is the dominant factor governing adsorption behavior in mixed dye systems. In addition to electrostatic interactions, other mechanisms such as hydrogen bonding and π–π interactions may also contribute to the adsorption process. However, their influence is secondary compared to electrostatic forces, particularly in determining selectivity between oppositely charged dye molecules. The combination of spectroscopic analysis and deconvolution techniques provides a more detailed understanding of the competitive adsorption behavior in multi-component systems (Wijaya et al., 2025b).

The results clearly demonstrate that *Cladophora* sp. exhibits selective adsorption behavior, with a strong preference toward cationic dyes, particularly MB, while showing limited affinity toward anionic dyes. This finding highlights the potential of this biomass as an effective and selective bioadsorbent for treating complex dye-contaminated wastewater.

4. CONCLUSIONS

The present study demonstrates that *Cladophora* sp. biomass can serve as an effective bioadsorbent for the selective removal of dyes in multi-component systems. Characterization results confirmed the presence of functional groups and a porous structure that facilitate adsorption processes. The determined pHpzc value of 6.5 indicates that the adsorbent surface is negatively charged under near-neutral conditions, which significantly influences its adsorption behavior. Selective adsorption analysis revealed a strong preference toward cationic dyes, particularly MB, followed by MG and RB, while anionic dyes exhibited lower adsorption efficiency. This behavior is mainly governed by electrostatic interactions, where negatively charged surfaces favor the adsorption of positively charged species and repel negatively charged ones. The results highlight the potential of *Cladophora* sp. as a low-cost, environmentally friendly, and selective adsorbent for practical wastewater treatment applications involving complex dye mixtures.

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