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Utilization of Immobilized Green Algae *Scenedesmus qudricauda* as A Sustainable Method to Remove Some Industrial Textile Dyes

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Abstract

The textile industry is a growing global sector that creates many jobs and boosts the country's economy. However, it is a major source of water pollution that threatens aquatic life and human health. Therefore, it has become necessary to find new methods that are effective, sustainable, and low-cost to remove these dyes from wastewater. The research aims to investigate the capacity of *Scenedesmus quadricauda algae* to adsorb textile dyes Reactive Yellow 145, and Disperse Yellow 114 from aquatic media by studying the effects of 5 variables on the dye removal efficiency, including initial dye concentration (10-50 ppm), contact time (3-12 days), pH (5-9), temperature (4-40 C°), and adsorbent dosage (0.1-2.5 g L^{-1}) The current study's results revealed that the immobilized algae achieved the highest color removal percentages of 98% and 97% for the dyes Reactive Yellow 145 and Disperse Yellow 114, respectively. Most decolorization activity was found at an initial concentration of 40 mg L^{-1}, 9 days of contact, a pH value of 5, a temperature of 25°c, an algae dosage of 2.5 g L^{-1}, and a stirring speed of 120 rpm. The spectrophotometer method was used to verify this. In addition, SEM and FTIR analysis of the immobilized biomass of *S. quadricauda* were performed before and after dye treatment. It was found that the immobilized algae biomass had high biosorption properties associated with its surface structure and active groups, proving the immobilization effectiveness.

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Introduction

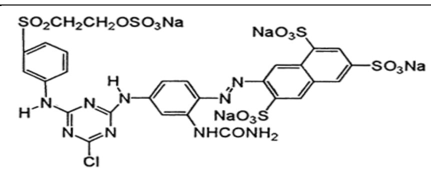
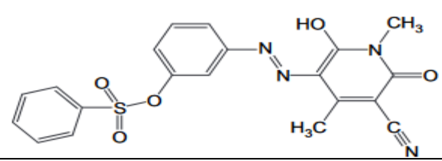
Water scarcity and pollution are among the most serious issues facing our world today, with industrial activities accounting for 70 to 80 percent of total water pollution. Textile wastewater contains a variety of pollutants, including dyes, metals, and salts, acidity, alkalinity, and volatile organic compounds (Jan *et al*., 2023). The textile industry is considered a major global polluter, it needs large quantities of water to operate its many units, as one ton of product in the textile sector typically requires 230–270 tons of water to process, which leads to the release of massive volumes of a variety of synthetic dyes and materials toxic to the environment (Partal *et al*., 2022).

Synthetic dyes leak into aquatic environments. It has a variety of consequences, including a detrimental impact on the aquatic food web by preventing light from entering water bodies and impeding primary producers' ability to photosynthesize (Kumar *et al*., 2020). It reduces the biodiversity and aesthetic appeal of water bodies, in addition to its carcinogenic and genotoxic properties, and causes many diseases such as allergies, skin diseases, and respiratory problems in humans (Wargala *et al*., 2021; Obaid, 2022; Benjelloun *et al*., 2024). Sewage treatment is crucial to prevent hazardous materials from contaminating aquatic environments (Mokif *et al*., 2024). Biological removal provides a promising alternative to conventional methods for purifying contaminated water, as algae are among the most effective bio sorbents for eliminating contaminants, including artificial colors, pharmaceuticals and heavy metals (Obaid *et al.,* 2024). This is due to their inexpensive and environmentally friendly nature, in addition to distinctive qualities like vast surface area such as large surface area, high capacity, High abundance, diversity of functional groups, rapid growth, high affinity, and resistance to harsh environmental conditions (Abdelfattah *et al.,* 2023; Mahlangu *et al.,* 2024). *S. quadricauda* belongs to the green microalgae, widely utilized in wastewater treatment, and the production of biodiesel, the most widespread algae in fresh water, and is characterized by high growth rates, this strain can be cultivated in a variety of environments, and high adsorption capacity compared to other strains (Nag Dasgupta *et al.,* 2018; Qader *et al.,* 2023). Immobilized algal cells have many important advantages such as high enzyme activity, maintaining high biomass concentrations; easy harvesting, low cost, and tolerance to many environmental changes, such as pH and temperature changes, and high concentrations of pollutants; they have been developed as an effective technology for eliminating contaminants, including toxic metals and dyes including toxic metals and dyes (Liu *et al.,* 2022; Obaid *et al.,* 2023) Several studies show that immobilized algae can effectively remove a range of industrial colors. El-Sheekh *et al.* (2023), study demonstrated that, following seven days of incubation, immobilized algae eliminated a higher percentage of the azo dyes reactive orange 122 and reactive red 194 than did suspended algae. In a related study, Juda et al. (2023), employed the immobilized microalga *Chlorella vulgaris* to remove Congo red pigment from water solutions, with a maximum removal rate of 89.6% after 13 days of contact. a maximum removal rate of 89.6% after 13 days of contact. Several studies have shown that algal masses have a high adsorption capability for a wide range of textile colors. For instance, Chin *et al*. (2020) observed that the algae *C. vulgaris* might eliminate methylene blue dye at high rates of up to 83.04% after three days of adsorption through surface adsorption via electrostatic contact. An investigation by Hamouda *et al*., (2022), reported that *S. obliquus* algae removed dispersed 2RL Azo orange dye, and the achieved removal rate was 98.14%. In another study, Khalaf *et al.* (2023), employed the macroalga *Lychaete pellucida* to eliminate four azo dyes and achieved a clearance rate of 95% for all azo dyes tested. Al Shra'ah *et al*. (2024) reported that after 75 minutes of contact, the microalgae Bracteacoccus sp. could eliminate 97% of the methyl orange dye from water solutions. The goal of this study was to evaluate the possibility of employing immobilized *S. quadricauda* algae as a sustainable bio-sorbent for the elimination of reactive 145, and dispersed 114 dyes through the study of the impacts of operational parameters such as temperature, weight of immobilized algae, contact time, starting dye concentration, and the pH value in the biosorption test.

Materials and Methods

Microalgae *S. quadricauda* was provided by the" Environmental Research and Studies Center (ER.R.A.S.C.), University of Babylon, Iraq". The experiment was carried out at the same center's labs and continued for about 6 months in 2024**.** As indicated in Table 1, reactive yellow 145 and diffused yellow 114 dyes were selected as azo and non-ionic dyes acquired from Sigma-Aldrich Company. To prepare a stock of the above-mentioned dyes, 0.250 grams of the dye was dissolved in 250 ml of distilled water and placed in an orbital shaker (Edmund Buhler SM25, Germany) to ensure optimal dissolution. UV-vis spectroscopy was utilized to assess the devitalization of reactive and dispersed dyes in the test samples at wavelengths of 422 and 445 nm, respectively.

Table 1. Characteristics Reactive Yellow 145 and Disperse Yellow 114

Type dyes	Molecular Weight	Molecular Formula	Chemical structure	Reference
Reactive yellow 145	1026.25	$C_{28}H_{20}ClN_9Na_4O_{16}S_5$		(Kifetew *et al.*, 2023)
Disperse Yellow 114	408.43	$C_{20}H_{16}N_4O_4S$		(Huang and Qian, 2008)

Cultivation and immobilized of algae

S. quadricauda was grown with medium blue (BG-11) for the algal growth (Pandey *et al.*, 2023). To immobilize the algae, 50 milliliters of the algal culture was placed in the stationary phase and centrifuged at a speed of 3000 rpm for two to three minutes, after which a 2% sodium alginate was added and well agitated to homogenize the combination (algae and alginate). Finally, the mixture was then progressively distilled. In a 3% calcium chloride ($CaCl_2$), beads with 4±0.2 mm diameter are formed, then the formed beads are isolated in a calcium chloride solution and rinsed well with distilled water using a tea strainer (Obaid *et al.*, 2023) Figure 1.

Impact of initial dye concentration

The effect of starting concentration dye concentration on adsorption was studied at 10, 20, 30, 40, and 50 mg L^{-1}; Other parameters (pH = 6, at 25°C, dose 2 g L^{-1} of the solution, and stirring speed 120 rpm) were kept constant throughout the test (El-Sheekh *et al.*, 2023)

Impact of contact time

The effect of contact duration on the removal efficiency of active dyes by immobilized algae was investigated. In the present work, several contact periods are employed for the experimental adsorption experiments, ranging from 1 to 12 days (El-Sheekh *et al*., 2023). Other parameters (pH = 6, at 25°C, dose 2 g L^{-1} of the solution, and stirring speed 120 rpm).

Impact of pH value

The impact of altering the pH on the effectiveness of eliminating dye from the solution was investigated since the elimination of dyes from an aqueous medium relies on the pH of the dye solution being used. The impact of pH was examined in the present study at pH values of 5, 7, and 9. While keeping the remaining parameters constant, solutions of 0.01 M HCl or NaOH were added to the dye solution as needed to modify its pH value (Juda *et al.,* 2023).

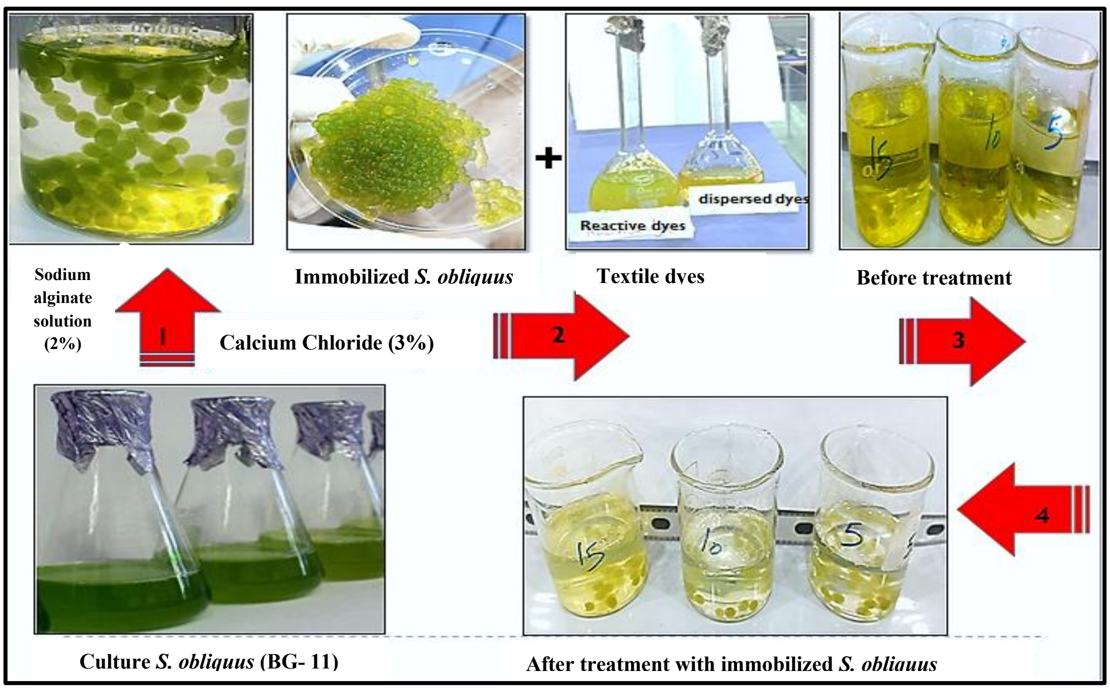


Figure 1. Experiment design scheme

Impact of Temperature

One of the most significant variables influencing the dye absorption procedure is temperature. The effect of temperature on the effectiveness of eliminating dyes by microalgae from an aquatic solution was investigated within a temperature range of 4°C to 40°C while maintaining other parameters constant (Goda *et al*., 2023; Mustafa *et al*., 2023).

Impact biosorbent mass (immobilized *S. quadricauda*)

We investigated the influence of the mass of the absorbent material (immobilized algae) on the biosorption phenomenon of the dye; the mass range specified was between 0.1 and 2.5 mg L^{-1}, while keeping the rest of the parameters constant. Dye solutions were centrifuged and absorbance values were measured using a UV-Vis spectrometer (Cary-100 conc., USA) at wavelengths of 421 and 445 nm for reactive and dispersed dyes, respectively. The percentage of decoloration was calculated utilizing Equation 1, (Omokpariola, 2021):

$$R\% = \frac{\mathrm{Ci} - \mathrm{Ct}}{\mathrm{Ci}} \times 100$$

Where: R% is the removal efficiency of dye, Ci is the initial concentration of dyes, Ct is the dye concentration at time t, respectively.

Algal characterization by FTIR and SEM

In order to determine the active functional groups on the surface of *S. quadricauda* that are in charge of dye adsorption, "Fourier transforms infrared (FTIR)" spectroscopy analysis was carried out in the Department of Polymer Engineering, College of Materials Engineering, University of Babylon" using IRAffinity-1S from Shimadzu products in the spectral range of 400-3000 cm^{-1}. The exterior morphology of the surface of *S. quadricauda* algae was examined employing "a scanning electron microscope (SEM)" ("JEOL-JCM-5200 LV, USA") both before and after the dye was removed (Youssef *et al.*, 2023).

Analysis of statistics

The SPSS (version 26) software was employed to do the statistical computations, and all results were displayed as mean ± standard error, with a significant grade ($P < 0.05$), (Alkatrani *et al.*, 2024).

Result and Discussion

Impact of contact time for decolorization

Finding out how successfully the immobilized algae *S. quadricauda* eliminated reactive yellow 145 and dispersed yellow 114, two textile colors, from aqueous solutions was the aim of this investigation. In this study, five lab tests were conducted. It was crucial to look into the optimal contact time between *S. quadricauda* and the dye adsorption indicated above. Figure 2 shows how contact time affects the removal of reactive yellow 145 and diffused yellow 114 dye from the aqueous media. According to the current findings, the reactive dye and dispersed dye clearance rates were lowest on day 3 (40.03% and 34.6%, respectively) and highest on day 9 (86.5% and 73.67%, respectively). The absorption rate was initially high because there were so many potential absorption sites, but as time went on and these absorption sites became saturated, the absorption rate dropped and an equilibrium plateau developed (Imessaoudene *et al.*, 2023). The length of time it takes to eliminate the dye depends on the types of algae, the dyes employed, and the dye concentration (El-Sheekh *et al.*, 2018). These results are consistent with those of other research that demonstrated the significance of the contact time element in dye elimination (El-Naggar *et al.*, 2022; Hamouda *et al.*, 2022; Al-Fawwaz *et al.*, 2023).

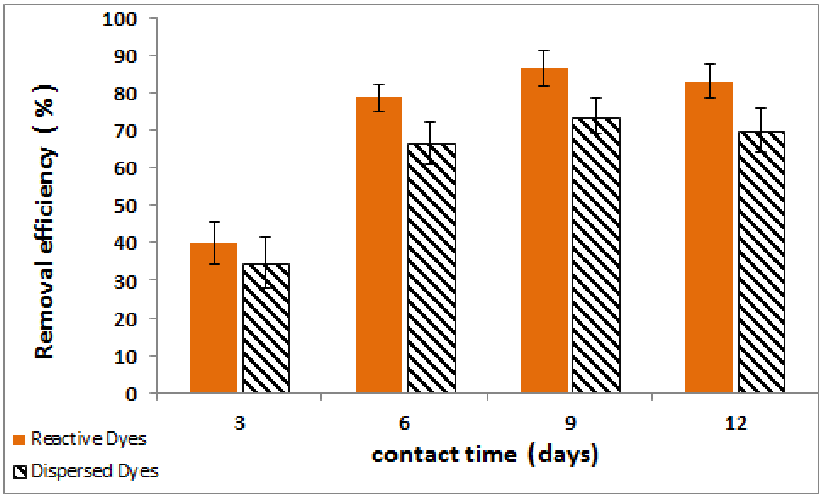


Figure 2. Impact of contact time for decolorization (mean ± S.E.)

Impact of initial dye concentration on the decolorization

Another factor influencing dye removal success is the initial dye concentration, which may have an indirect effect by changing the number of binding sites on the surface of the absorbent material (Rápó and Tonk, 2021). Figure 3 shows how the initial dye concentrations (10, 20, 30, 40, and 50 mg L^{-1}) impacted the dye removal efficiency. The highest removal percentage was recorded for reactive dyes and dispersed dyes with rates of 92.8 and 87.5% at 10 mg L^{-1} concentration and the lowest removal efficiency of 67.3, 64.85% for reactive dyes and 64.85% for dispersed dyes at a concentration of 50 mg L^{-1}. The rest of the concentrations ranged between these two limits. The current study's findings showed that the removal efficiency progressively declined as the concentration of the dyes mentioned above increased. Due to the high proportion of available active sites to pigment molecules, all dye molecules ma I think one reference is good because all these in the same year y interacts with the sorbent at low initial concentrations and be immediately extracted from the solution. The overall number of accessible adsorption sites will be limited or occupied at high starting concentrations, though, which may cause the dye removal rate to drop (de Farias *et al.,* 2020). This is supported by the results of several studies (El-Sheekh *et al.,* 2023; Raghad and Abeer, 2023).

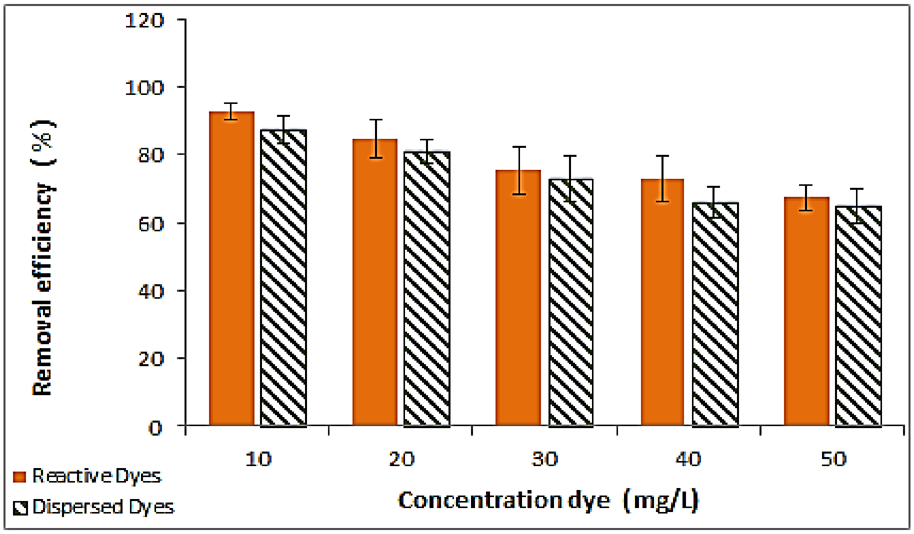


Figure 3. Impact of initial dye concentration on the decolorization (mean ± S.E.)

Impact the temperature on the decolorization

The biodegradation of dyes in aqueous media is significantly impacted by temperature (Shetty and Krishnakumar, 2020). As shown in Figure 4, the current investigation obtained the highest dye removal rates (70% and 80%) for reactive and dispersed dyes at 25°C. The temperature stability of the algal cells during their connection with alginate may have been the source of this, since it created a different monolayer for adsorption (Wang *et al.*, 2019). However, when the temperature was raised to 40 °C, the color removal efficiency decreased to 16% for dispersed dyes and 33% for reactive dyes,, the color elimination effectiveness dropped to 16% for dispersed dyes and 33% for reactive dyes, which can be explained by the adsorbent's propensity to migrate away from the active sites into the aqueous solution and the changes in the adsorbent's active sites brought on by the temperature increase (Sun *et al.*, 2019; Mustafa *et al.*, 2023). A related study (Wang *et al.*, 2019) found that the immobilized algae *Chlorella pyrenoidosa* reduced color removal from commercial dyes to 97.5% at 60 °C, whereas the highest color removal (98%) was reached at 50°C. El-Sheekh *et al.* (2023) reported that the highest removal rates of azo dyes (reactive orange 122 and reactive red 194) were (89.52% and 91.28%), respectively, achieved at 25 °C and well-aerated conditions by the immobilized algae *Chlorococcum sp.* and *Scenedesmus*. These findings are consistent with the current study.

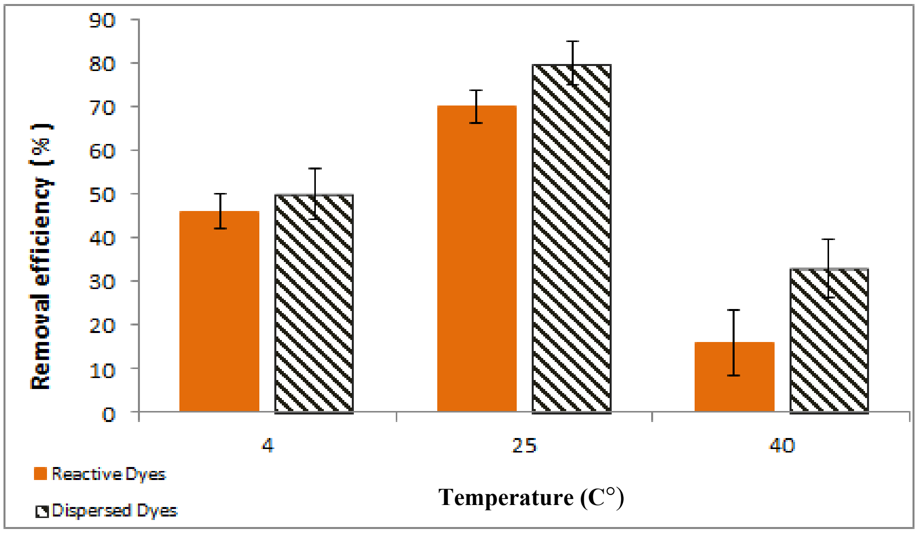


Figure 4. Impact the temperature on the decolorization (mean ± S.E.)

Impact the pH value on the decolorization

The pH of the dye solution is the most significant variable in almost all adsorption processes because it influences the properties of the adsorbent, the adsorption mechanism, the activity of the adsorbent's functional groups, the dissociation of the dye molecules, the chemistry of the contaminant solution, the degree of ionization of the adsorbed ion, and the surface charge. (Sun *et al*., 2019; Juda *et al*., 2023). Different pH values 5, 7, and 9 were examined in this experiment, and we found that pH significantly affected how well the immobilized algae removed color from the aqueous media. At pH 5, the reactive dye's maximum removal effectiveness was 84%. By contrast, the dispersed dye's elimination effectiveness at pH 9 was 70%. Because it encourages a change in the adsorbent's surface characteristics, the initial pH of the solution has a major effect on the dye's biosorption, as seen in Figure 5 (Ayele *et al*., 2021). It has an impact on the adsorbent's surface functional groups, which control how well pollutants dissolve in aqueous media. The charge of the algae adsorbent is dependent on pH since its surface has numerous functional groups, including "amino, carboxyl, hydroxyl, and phosphate." The surface of the biosorbent becomes negatively charged at high pH and positively charged at low pH (Sun *et al*., 2019). The maximum adsorption of reactive yellow 84 from aqueous solution happened on the hydroxyapatite complex at pH 5, which is comparable to the result by Barka *et al*. (2011).

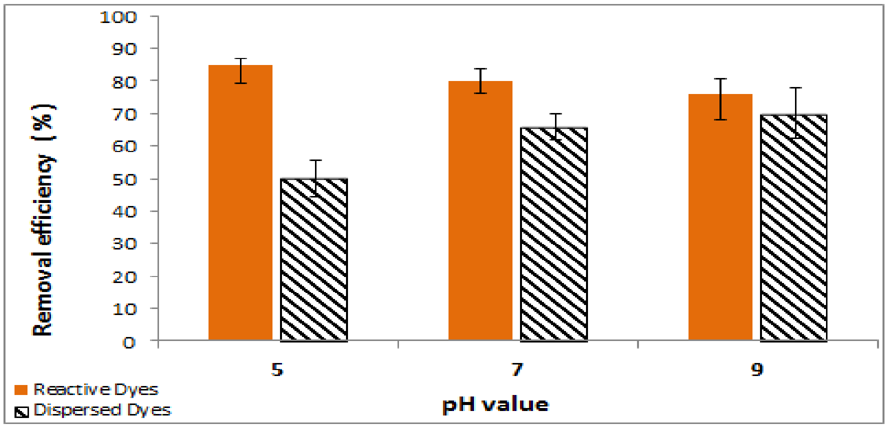


Figure 5. Impact the pH value on the decolorization (mean ± S.E.)

Impact adsorbent dosage (immobilize algae) on the decolorization

The quantity of adsorbent is an important factor in the adsorption process since it determines the adsorbent's capacity for a certain starting concentration (Alhujaily *et al.,* 2020). As seen in Figure 6, the maximum removal efficiencies for reactive dye and dispersed dye in this investigation were 90% and 95% at the algal dose of 2.5 g L^{-1}. It was discovered that increasing the dosage of the adsorbent is positively correlated with the efficiency of eliminating dyes because it produces more accessible adsorption sites at a certain pollutant concentration (Rehman *et al.,* 2023). Algae are among the best types of bio-adsorbents for dyes due to their high bio-adsorption capacity, which is caused by the structure and composition of their cell wall, which contains a lot of polysaccharides, alginic acid, chitin, and mannan as well as proteins with amino acid, hydroxyl, phosphate, amino, and sulfate groups to provide good sites for dye adsorption (Singh *et al*., 2018). This study is in line with others that have demonstrated the important roles that temperature, agitation, nutrient availability, pH, salt, and dye concentration play in algae's capacity to remove color (Silva *et al*., 2020; Motik *et al*., 2023).

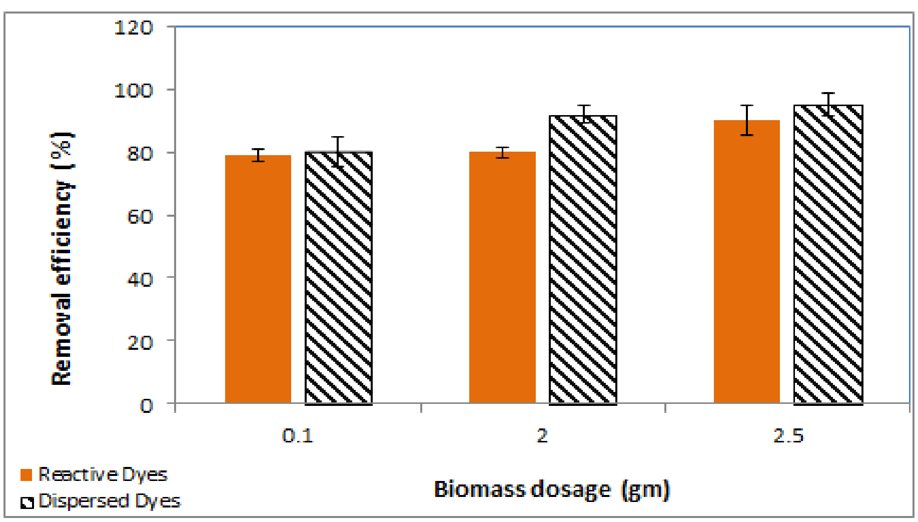


Figure 6. Impact adsorbent dosage (immobilize algae) on the decolorization (mean ± S.E.)

FTIR spectra analysis of Algae immobilize

The functional groups on the outer surface of microalgae biomass have a major impact on the biosorption of contaminants (Silva *et al*., 2020). FTIR analysis is utilized to identify the various functional groups on the surface of algal biomass responsible for the adsorption process. (Raghad and Abeer, 2023). Figure 7 displays the peaks of the immobilized algae that were adsorbed with dyes and those that were not (control) as follows: the presence of -OH groups is indicated by the bands around 3417.86, 3981.08 cm^{-1}, 3373.06, 34117.86 cm^{-1}, and 3417.86, 387.06 cm^{-1}. On the other hand, the bands at 2198.85 cm^{-1}, 2924.09 cm^{-1}, and 2268.29 confirm an aldehyde C-H extension. However, the bands are verified between 1620.21 and 1427.34, indicating that -C=O exists. The present investigation verified that the restriction was successful in the adsorption process by determining that the -OH, C-H, and -C=O groups were most crucial for the biosorption of reactive and dispersed yellow pigments on immobilized algae. According to other research, restriction played a significant part in the adsorption of pigments from media, as reported by (Abbas *et al*., 2023; Juda *et al*., 2023).

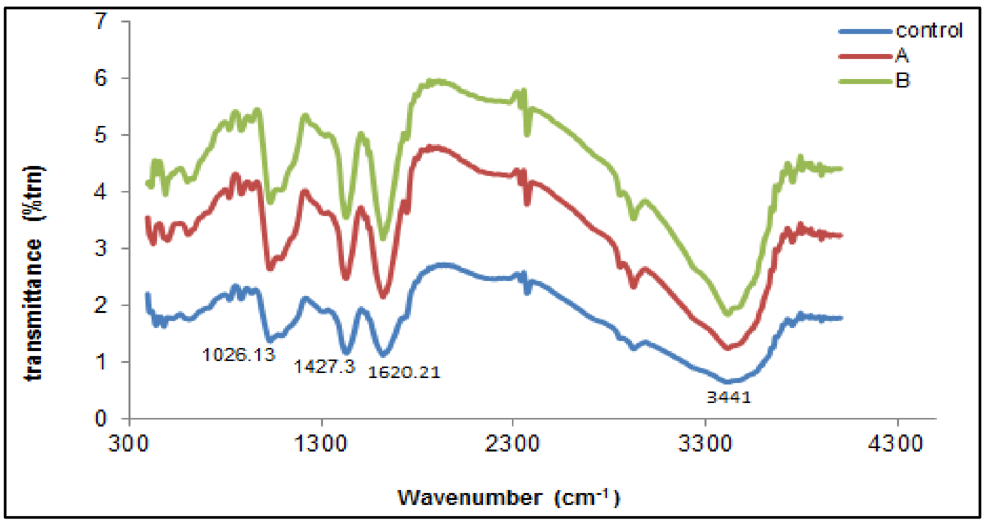


Figure 7. FTIR spectra analysis of Algae immobilize before (control) and after (a) reactive dye, and (b) direction dye biosorptions

SEM analysis of immobilized Algae

The surface morphology of the immobilized *S. quadricauda* was characterized through analysis. Figure 8a shows the SEM images prior to dye adsorption. The attachment of dye molecules and functional groups to the surface, which improved the sorption process, was caused by cavities of different sizes (Aslam *et al*., 2024). The adsorbent's curved surface was rough, uneven, and irregular; it had many cavities and no impurities. Figure 8b, c illustrates how the rough surfaces of the immobilized S changed following the adsorption of the two colors. *S. quadricauda* was found, exhibiting a high concentration of contaminants and cell surface shrinkage, confirming the hypothesized mechanisms of tiny dye interactions leading to dye adsorption on *S. quadricauda*. Given that the surface structure and functional groups of the algal cell wall are crucial for the biosorption capacity of dyes, the current study's findings show that the immobilized *S. quadricauda* algae is efficient at absorbing the dye because of its rough and porous structure (Şentürk and Alzein, 2020). This is in line with the findings of other investigations that showed that dyes alter the algal cell surface's porosity when they are removed (El Naggar *et al*., 2022; Yasir *et al*., 2022; Youssef *et al*., 2023).

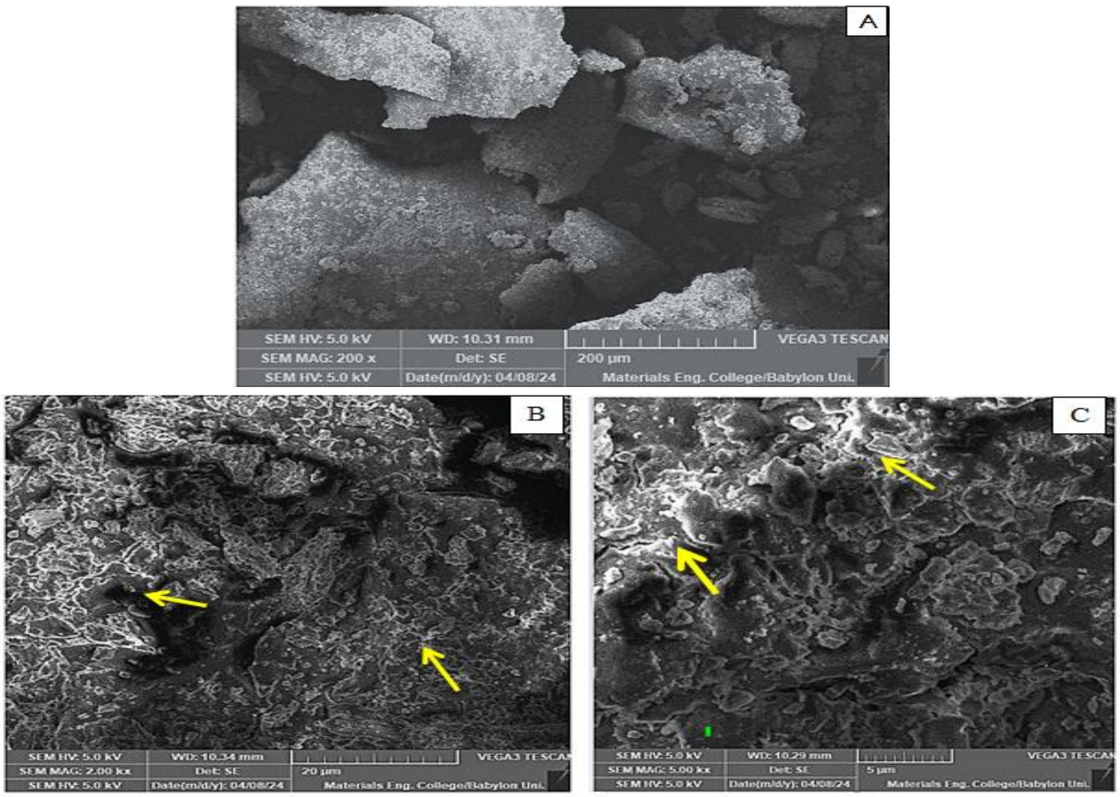


Figure 8. SEM analysis of immobilized Algae before (a) and after (b) reactive dye, and (c) direction dye decolorization, (c) Yellow arrows indicate changes on the surfaces of algal cells due to dye adsorption

Conclusions

Because of the possible environmental risks associated with large-scale discharges of synthetic dyes into aquatic habitats, it was vital to seek a sustainable and economically viable alternative. This study demonstrated the possibility of applying the immobilized green algae *S. quadricauda* as a promising approach to remove industrial dyes such as (reactive yellow dye 145 and dispersed yellow dye 114) from the medium, with removal rates of up to 98% and 97% for reactive dyes and disperse dyes, respectively. This was achieved by modifying some important factors, including pH, initial dye concentration, immobilized algae dosage, and contact time, to improve the efficiency of dye removal. The study's findings indicate that the immobilized green algae *S. quadricauda* have good dye adsorption potential based on multiple relevant properties. Therefore, we recommend using immobilized *S. quadricauda* as an economical, environmentally friendly, and efficient bio-absorbent for eliminating dyes from industrial wastewater.

Conflict of Interest

The authors declare no conflicts of interest with the publication of this work.

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