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Removal Efficiency of Synthetic Toxic Dye from Water and Waste Water Using Immobilized Green Algae – Bioremediation with Multi Environment Conditions

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ABSTRACT

The synthetic dye industry is a significant source of anthropogenic pollutants emitted into many water bodies across the world. Bioremoval is a substitute for industrial techniques for detoxifying dye-contaminated water. Green algae is an abundant microorganism processing to produce cost-effective, eco-friendly, and high-quality method to bioremediation by immobilization technique. In this present study, The effectiveness of the immobilized green alga *Chlorella vulgaris* to eliminate Congo red dye in both water and wastewater was assessed through the biodegradation Process under various conditions, including pH, concentration of dye, contact time, and NaCl. The results revealed that the removal increased with increasing contact duration, with the maximum bioremoval percentage occurring at 89.6% at a contact time of 13 days. The removal effectiveness of dye as the number of beads of immobilized *C.vulgaris* algae grew; the highest removal efficiency was achieved at 7–8 beads of immobilized *C.vulgaris* algae. There was also an inverse relationship between bioremoval and dye concentration; the maximum removal percentage was 90.1% at 0.1 M dye concentration. The highest removal efficiency was found in the range (91.3–86) at pH 6–7. The bioremoval of Congo red dye was similar in fresh and salinity water (87.2% and 85.3%, respectively). This study observed high removal efficiency for immobilized algae to Congo red under different concentrations of NaCl as an indicator of salinity, ranging between 85.3 and 87.2%.

Keywords: water and wastewater, bioremoval, immobilized algae, congo red, *Chlorella vulgaris*.

INTRODUCTION

One of the most serious problems facing our planet right now is water contamination (Al-dahri et.al., 2021). About 80 to 70 percent of water pollution problems are caused by industrial processes. Industrial wastewater products from textile mills contain large concentrations of many contaminants, such as dyes, salts, metals, acidity and alkalinity, volatile organic compounds, and others (Achour et.al., 2018).

Dyes are classified into two types according to their origin: natural dyes and synthetic dyes. Natural dyes are created using natural ingredients. while Synthetic dyes are made from different chemicals and may be hazardous. Synthetic dyes are gaining popularity due to their lower cost and higher quality when compared to natural dye sources. However, they should be used with caution due to their high toxicity (Rubangakene et.al., 2023). Synthetic dyes have many health effects on an aquatic ecosystem due to their high toxicity at low concentrations and high persistence in the environment, which may cause cancer, mutagenicity, skin diseases, and respiratory problems in humans (Huizhong, 2018; Naji and Salman, 2019; Lellis et al., 2019). They are also substances that carry multiple risks to non-target aquatic organisms in water systems (Abbas, 2020), and industries may produce several thousand tons of dyes that are discharged into lakes and rivers through wastewater (Mahalakshmi et al., 2015).

Congo red (CR) dye is a common diazo dye that contains an azo group (double bond N-N). The dye has a stable structure and is challenging to degrade. Additionally, the azo group is rapidly converted in humans to benzidine, a source of carcinogens. The colored structure, on the other hand, prevents light from entering the water, affecting aquatic life's photosynthesis and respiration processes (Arab et al., 2021; Emami et al., 2018),and It has been found to have toxic effects on aquatic vegetation and is also able to interfere with the reproductive activity of aquatic animals (Oladoye et al., 2022).

As a result of the aforementioned reasons, it has become necessary to treat wastewater before it is released into the environment (Hwang et al., 2016). There are many traditional methods through which wastewater is treated, the most important of which are chemical (Yazid et al., 2021), photochemical (Kassimi et al., 2021), and biological (Pavithra et al., 2019). And the removal techniques involve coagulation (Kassimi et al., 2021), flocculation (Ghedjemis et al., 2021), adsorption (Laamari and Haddad, 2021), and fungal decolorization (Jawad and Abdulhameed, 2020). All of these technologies are effective in one way or another, but they are complex and difficult to implement due to some environmental issues. For example, they introduce some dangerous by-products in addition to being expensive (Laamari and Haddad, 2021).Biotreatment, on the other hand, is characterized by being more economical, effective, and available. As a result, there is growing interest in its application to treatment water contaminated with azo dyes such as Congo red (Abbas, 2020; Virk, 2020). Phycoremediation is a sort of bioremediation that uses algae's ability to remove or degrade pollutants. Algae have unique metabolic capacities, and Toxins can be remedied by algae in a variety of ways, including biosorption, bioaccumulation, and degradation (Nair et al.,2023; Patel and Tiwari, 2015).

Microalgae has been widely used in bioremediation procedures to breakdown or absorb toxic dyes from aqueous solutions and to degrade other contaminants such as *Chlorella vulgaris* and *Nostoc paludosum* bioremove, crystal violet, and malachite green (Salem et al., 2021; Yun et.al., 2020). Microalgae can be a better choice for bioremediation compared to other microorganisms owing to their photosynthetic capabilities, thereby absorbing CO_2 from the atmosphere, converting solar energy into useful biomasses, and being environmentally friendly (Goh et al., 2022; Lili et al., 2019). It has been demonstrated that *C.vulgaris* able adaptable to dye stress via physiological adaptability and the ability to breakdown various contaminants, making it a potential and sustainable choice for dye removal from various aquatic systems (Salem et al., 2021).

Algae immobilized technology has received increasing attention and has been used in many applications in the environmental field, such as wastewater treatment from many pollutants such as antibiotics, heavy metals, and dyes, due to its ease of harvesting (Salman et al. 2022; Obaid et al., 2023a), higher cell density, increased cell resistance to unfavorable factors (temperature, acidity, and toxic compounds), higher productivity, better cell stability, and biomass recycling (Vasilieva et al., 2016; Eroglu et al., 2015). The current study aimed to evaluate the potential of the inactivated algae, *Chlorella vulgaris*, to bioremove Congo red dye from aqueous solutions and assess some environmental parameters to improve the bioremoval process.

MATERIAL AND METHODS

Algae cultivation

CH-10 medium was employed for the growth and development of the algae *Chlorella vulgaris*, which was described by Rippka et al. (1979) and modified by Andersen (2005). Two ml of algae culture was taken and added to 20 ml of CH-10 medium for cultivation, then the culture was incubated for two weeks under appropriate circumstances such as illumination of 200 $E/m^2/s$ (16:8 h) light and dark periods and (26 ± 1) temperature (Hussain, et al. 2022; Jain et al., 2020).

Immobilization of Algae

The microalgae (*Chlorella vulgaris)* sample was acquired by the "Environmental Research and Studies Center, University of Babylon, Iraq." For the preparation of immobilized *C. vulgaris.* 50 mL of *C. vulgaris* in stationary phase was taken on days 12 and 14 and then condensed by centrifugation for 3 min at 3000 rpm. Then, the algae concentrate was collected, blended with an equivalent amount of 2% sodium alginate solution, and then placed into a medical syringe or separation funnel, after which the mixture was dripped drop by drop into a beaker containing calcium chloride at a concentration of 3% and gently stirred for 1–2 min. It will form beads, after which they are thoroughly rinsed using distilled water by a strainer for tea to remove the bests formed from the $CaCl_2$ solution. After that, they are kept in distilled water in a cool place (Ayesha, 2022; Ayawei, 2015; Obaid et al., 2023b) as Figure 1.

Cango red dye

The dye Cango Red (CR azo) chemical formula ($C_{32}H_{24}N_6O_6S_2{\cdot}2Na_2$), commonly known as Direct Red 28, which has a molecular weight of 696.7 g and the chemical formula ($C_{32}H_{24}N_6O_6S_2{\cdot}2Na_2$) was used for the experiments. The dye was obtained from "Sigma-Aldrich" Corporation, USA (Figure 2). One gram of dye was dissolved in 100 mL of distilled water to form a stock solution containing 100 mg/L of dye. According to the dilution law, the concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 M) of the dye were prepared for the experiment and mesuring by Spectrophotometer (PD-303) at the wavelength (λ_{max}= 496 nm).

Effect interaction time bioremoval of Congo red dye

The experimental process was done in batches by changing the contact period, dye concentrations, Number of Immobilized algae beads, and pH of the medium. Immobilized algae *Chlorella vulgaris* was used to bioremoval congo red dye, where (5–6) beads of algae were placed in a plastic container, and 80% of congo red day solution was added to it at a concentration of 0.3M. after that the samples were drawn from the solution after different periods (3, 5, 7, 9, 11 and 13 days), and the absorbance was measured for each sample and then the percentage of color removal was calculated using the following method:

$$\text{Decolorization\%} = \frac{A - B}{A} \times 100$$

where: A – initial solution absorbance.
B – final solution absorbance.

Effect of Congo red dye concentration on bioremoval

Different concentrations of Congo red dye (0.1, 0.2, 0.3, 0.4, and 0.5 M) were taken, and 80% of each concentration was placed in a plastic vial. Then 5–6 beads of algae were added to each vial, samples were drawn from each solution at different periods. After that absorbance was measured (Litefti et al., 2019). The ideal bead size is between 3.6 and 4.5 mm to bioremoval of Congo red dye

Impact of pH variation on the bioremoval of Congo red dye

Five solutions of 80% of Congo red dye at a concentration of 0.3M were taken and each was placed in a plastic vial where the pH (5, 6, 7, 8, 9) of each solution was adjusted. Then (5–6) beads of algae were added to each vial.

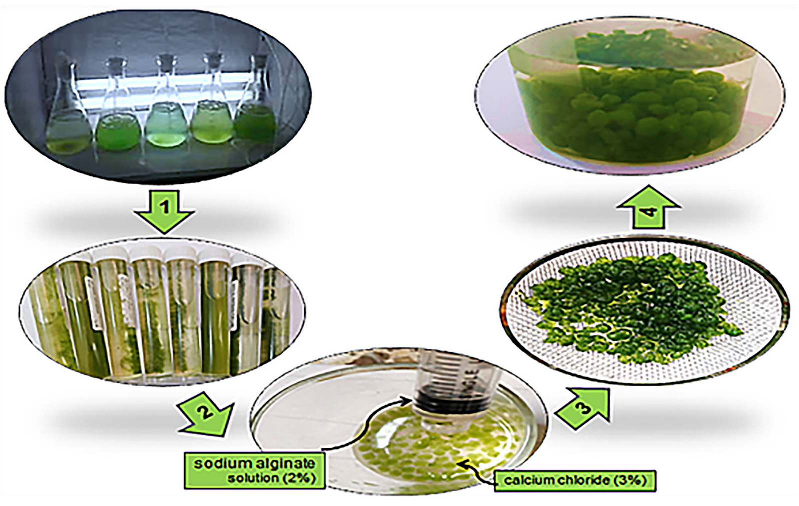

Figure 1. Steps for preparing immobilized *Chlorella vulgaris*

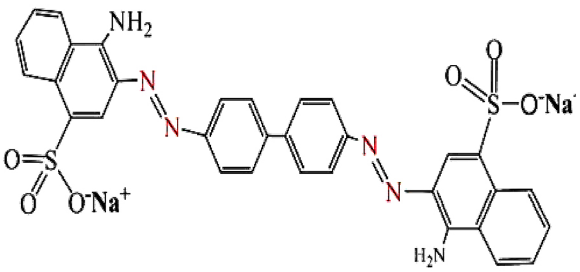

Figure 2. Congo red dye's chemical structure (Halbus et al., 2017)

Effect of immobilized algae beads on the bioremoval of Congo red dye

A different number of algae beads were taken and placed in a plastic vial, then a 50% Congo red dye solution (0.3 M) was added to each vial, and each solution was sampled at different times and its absorbance measured.

Impact of the NaCl concentration on bioremoval of Congo red dye

The solution of 80% of Congo red dye at a concentration of 0.3M and (5–6) beads of algae was prepared with NaCl concentration (0.3M) compared to other solution without salt (freshwater) at pH 7. Samples were drawn at different periods from both solutions, and the absorbance of each sample was measured for color removal.

Statistical analysis

Info are founded as mean values ± standard deviation. Data analysis was performed using SPSS (version 23). The averages of the different groups were compared using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Bioremoval of Congo red dye

Figure 3 shows that the rate of bioremoval of dye increases gradually with increasing time (3, 5, 7, 9, 11, 13 day) after exposure immobilised algae *Chlorella vulgaris*. At 13 day the bioremoval percentage was 90% after treatment.It can be attributed to the decrease in the thickness of the diffusion layer surrounding the adsorbent particles (Basharat et al., 2021).

Adsorption and biodegradation are mechanisms uses by alage for the bioremoval of azo dyes (like Congo red). Biodegradation is alteration or mineralizing the raw material of the dye to carbon dioxide and water (Samiyammal et al., 2022; Jun et al., 2020). While the adsorption is sorption techniques in the sorbent without modified (undamaged) and is performed using biomass of algae cell dead or live (Zewde et al., 2022). FTIR spectrum for dye Congo red before bioremoval and after treatment by using immobilised algae (as shown in figures 4 and 5) show that the composition of the original dye has not changed, and this indicates that the algae adsorb Congo red dye, and the only change is the initial of the dye in sample solution after five days. Generally, Congo red dye structure by FTIR spectrum shown in Figure 4. Before the bioremoval processes of dye, the stretching vibration bands of amine (N-H),

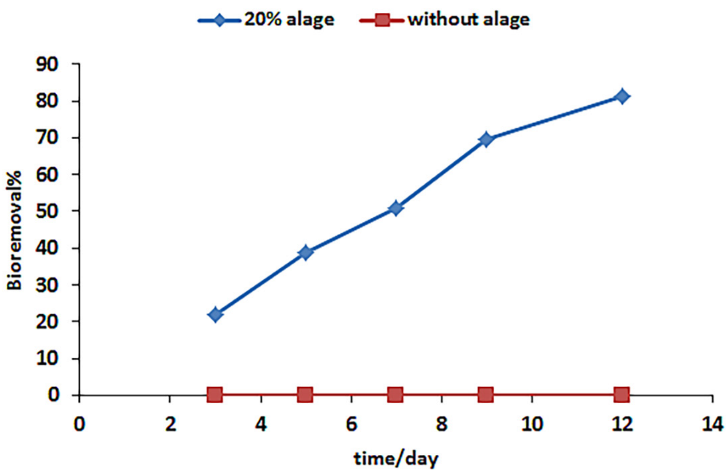

Figure 3. Effect of immobilised algae *Chlorella vulgaris* on bioremoval different conce. of congo red dye at room temperature, pH 7

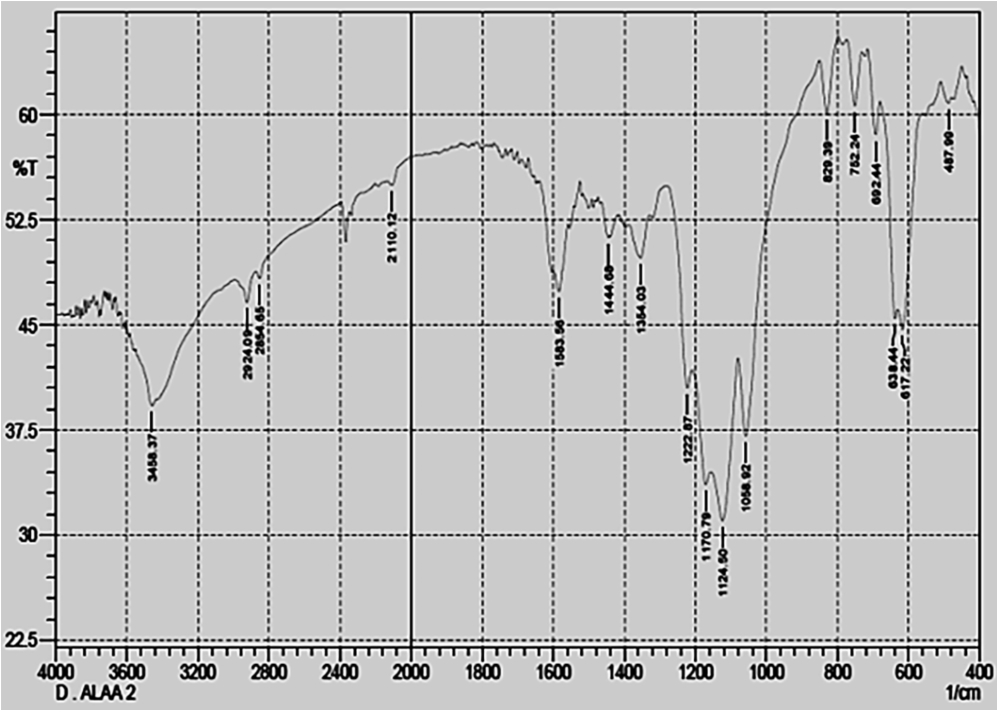

Figure 4. FTIR spectrum for analysis congo red dye before bioremoval

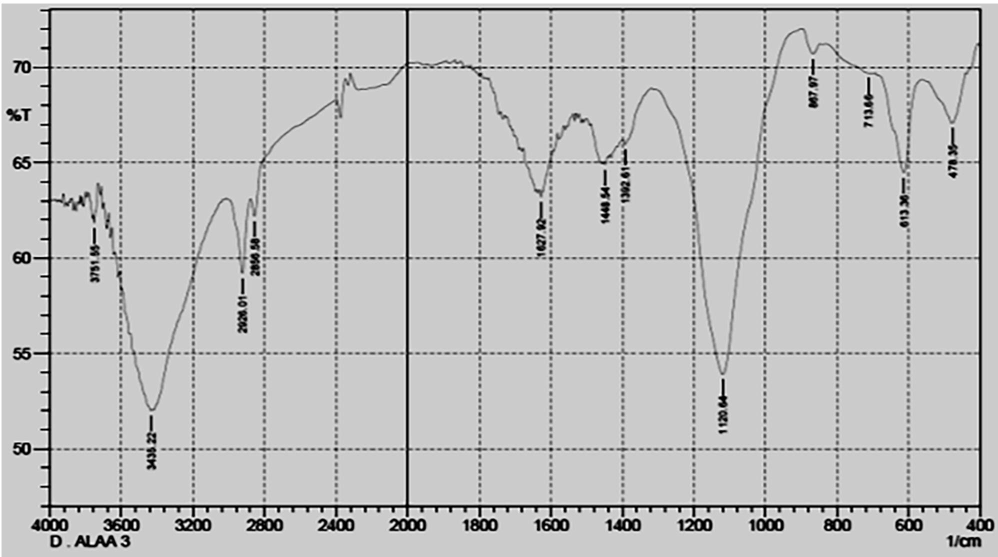

Figure 5. FTIR spectrum for analysis dye after bioremoval by using immobilized algae *Chlorella vulgaris*

aromatic (C=C), azo group (N=N), sulfonic group (S=O), and (C-N) were observed at (3458.37, 1583.56, 1449.54, 1354.03, and 1124.50) cm^{-1} respectively (Badr and Isr., 2021) as shown in Table 1. However, after the bioremoval processes, the colored dye bands were reduced, shifted, and broadened. There was a broad peak of O-H stretching vibrations at 3435.22 cm^{-1}, as a result of the existence of water, and an aromatic C=C stretching peak sheathed at 1627.29 cm^{-1}.

Impact of dye concentration on bioremoval of congo red dye

The impact of an initial dye concentration ranged (from 0.1–0.5M) at room temperature on the removal dye by using *Chlorella vulgaris* algae as adsorbents at different times (3–13 days). In Figure 6 and 7, the results were observed that the amount of bioremoval dye heighten with the deficiency in the dye

Table 1. Bans have shown in the FTIR spectrum of CR dye structure before and after bioremoval

No	Band	Description
1	3458.37 cm^{-1}	Stretching vibration band of an amine group (N-H)
2	1583.56 cm^{-1}	Stretching vibration band of aromatic (C=C)
3	1449.54 cm^{-1}	Stretching vibration band of an azo group (N=N)
4	1354.03 cm^{-1}	Stretching vibrations of C-H
5	1124.50 cm^{-1}	Stretching vibrations of (S=O) sulfonyl group

concentration, where the bioremoval percentages were 90%, but deficiency to 62.7% with the heighten in the initial dye concentration to 0.5M. this is consistent with several studies that indicated a deficiency in bioavailability with an heighten in dye concentration (Shantanu et al., 2019; Anisa et al., 2022; Yifan et al., 2022). The deficiency in bioremoval efficiency by heighting the dye concentration can be attributed to the ease of interacting of the dye molecules with the active sites of the adsorbent in the case of low concentration. On the other hand, all absorbents have a specific number of active sites (carboxyl group, hydroxyl group found on cell walls of biomass), which become saturated at a certain concentration of the adsorbent (Atef et al., 2022; Mahalakshmi et al., 2015).

Impact of pH of solution on bioremoval of congo red dye

One important factor in controlling the adsorption process is the pH of the solution. It modifies the adsorbents' superficial charge, the molecular structure of the dye, and the ionization potential of the contaminants (Astuti et al., 2020; Kube et al. 2018). And because Congo red dyes contain a group of sulfonates, they carry a negative charge (Husain et al., 2022, Juda, et al. 2019). and 20% of *Chollerella relgari s*algae solution was added to each dye solution. The obtained results are represented in Figures 7 and 8. Solutions of Congo red dye (0.3M) were taken with different PH values (5–9), and the results showed that the percentage of color removal of the dye increased with increasing time in all pH solutions (as Figure 8). But the highest decolorization percentage recorded was 89.3% and 80.2% at PH 6 and 7, respectively, as in figure.4. In other words, the best decolorization percentage for Congo red dye using (5-6) beads of *Chlorella vulgaris* algae is within the range of pH 7–8. The reason for this is attributed to the molecular structure of the azo dyes, which makes the attraction of the dye particles with the outer surface of the algae very strong at pH 7–8, followed by rapid

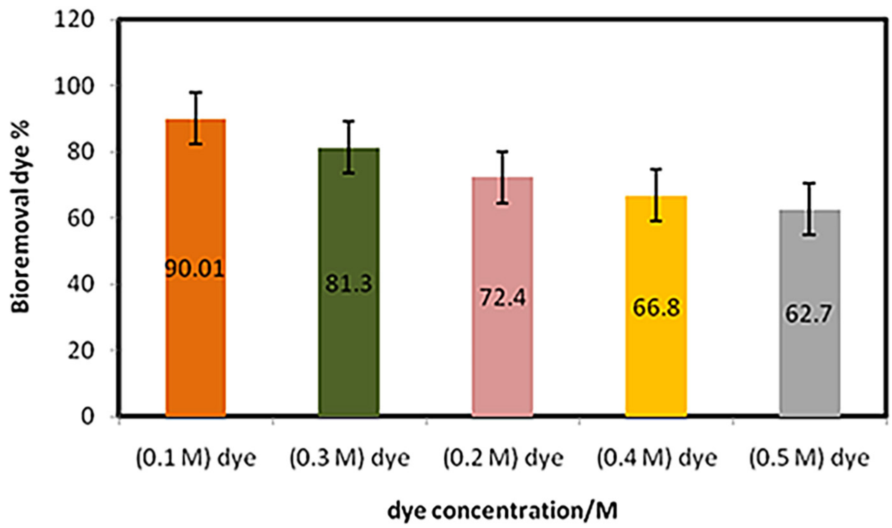

Figure 6. Effect of different concentrations of Corgo red dye (0.1, 0.2, 0.3, 0.4, 0.5M) on bioremoval of dye by using 5–6 beads *Chlorella vulgaris* algae at pH 7

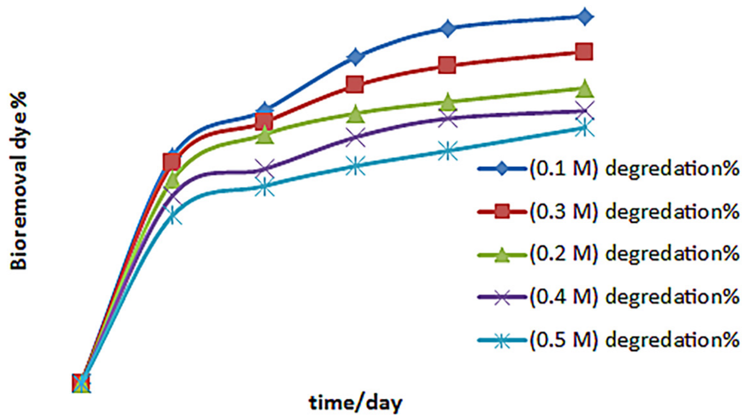

Figure 7. Effect of several concentrations of Congo red dye (0.1, 0.2, 0.3, 0.4, 0.5M) on decolonization percentage of Congo red dye dye by using 5–6 beads *Chlorella vulgaris* algae, and at pH 7

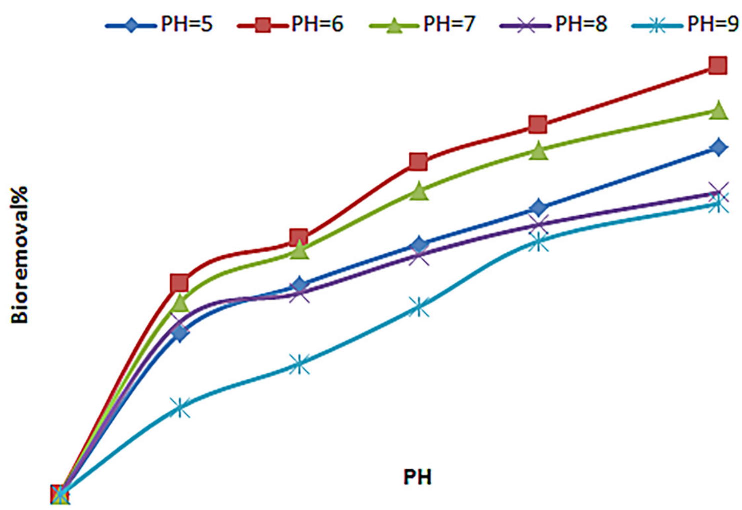

Figure 8. Impact of different pH of the solution pH = 5, 6, 7, 8 and 9 on bio removal of (0.3M) Congo red dye by using 5–6 beads *Chlorella vulgaris* algae

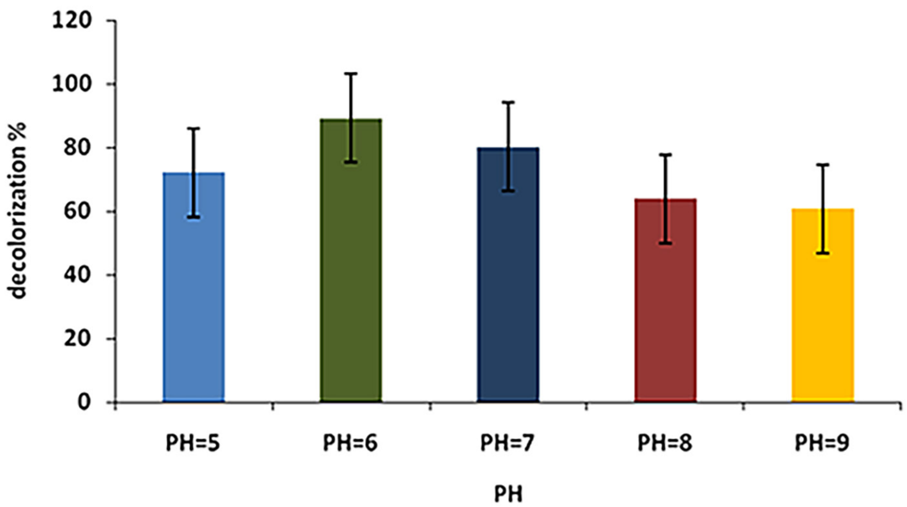

Figure 9. Impact of the pH of solution on bio removal percentage of (0.3M) Congo red dye by using 5–6 beads *Chlorella vulgaris* algae

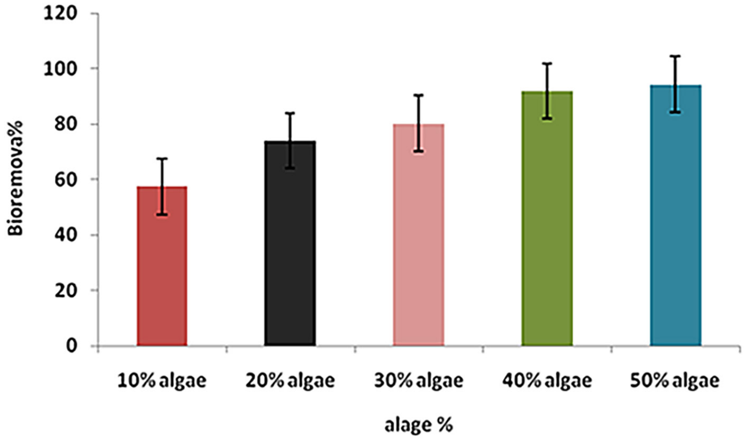

Figure 10. Effect of 5–6 beads *Chlorella vulgaris* algae content of bioremoval of (0.3M) Congo red dye

diffusion (absorption) of the dye particles into the algae cells (Hussain et al, 2022) – Figure 9.

The effects of algae concentration on bioremoval of CR dye

The effects of algae concentration on the bioremoval of CR dye were searched by preparing different percentages of algae and adding the same concentration of dye solution to each solution of prepared algae. The obtained results are plotted in Figure 10. which shows that the percentage of the bioremoval of CR dye heighten with the heighten in the percentage of algae in solution, the reason for this is due to the increase in the attraction of dye particles with the algae by increasing the concentration of algae, and this, in turn, increases the absorption of dye particles by the algae (Kowanga et al., 2016; Huizhong, 2018; Sarma et al. 2019 , Zamora et al., 2015).

Effect of the type of aquatic environment

The effect of the type of aquatic environment (saline and fresh) was studied on the bio removal Congo red dye was studied using 5–6 beads of *Chlorella vulgaris* algae, the results indicate that the percentage of bioremoval of dye is close in both aquatic environments 87.2% and 85.3% for the fresh and saline environments respectively, the obtained results are shown in Figure 11. This type of algae is characterized by a short cell doubling time, it has a rapid response in all salt and freshwater environments (Badr and Isara, 2021).

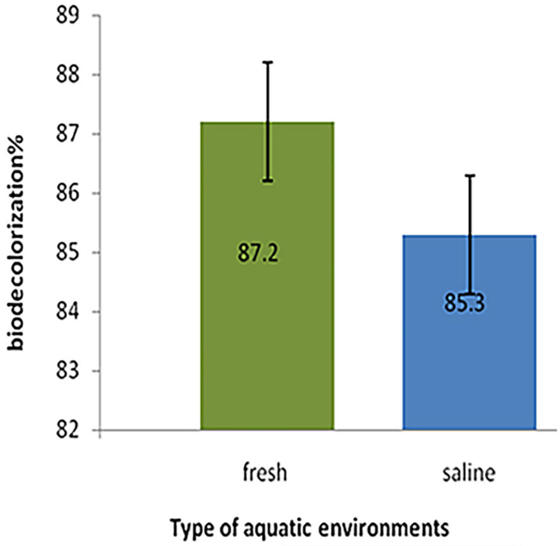

Figure 11. The effect of aquatic environments type (saline and fresh) on the bio removal of (0.3M) Congo red dye using 5–6 beads *Chlorella vulgaris* algae

CONCLUSIONS

These results demonstrate that immobilizing *Chlorella vulgaris* significantly reduced the concentration and toxicity of the Congo red dye. As a result, this may be a promising and sustainable option for treating this type of wastewater pollutant, According to the findings, the percentage of elimination of this Congo red dye dye was greatest at pH 6, and the amount of bio-removal dye increased with decreasing dye concentration, where the percentage of bio-removal was 90%, but it decreased to 62.7 percent with rising the dye concentration to 0.5M, and the percentage of bio-removal of Congo red dye rises with the number of immobilized algae in the solution. We concluded from this research that it is possible to apply this environmentally friendly and low-cost treatment method in many agricultural and industrial fields, for example in textile water treatment plants and waste water treatment plants.We recommended doing further studies to determine if immobilized *C. vulgaris* can remove additional dyes in the aquatic environment.

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