The Bioremoval of Toxic Dyes Using Chlorella Algae in Airlift Bioreactor

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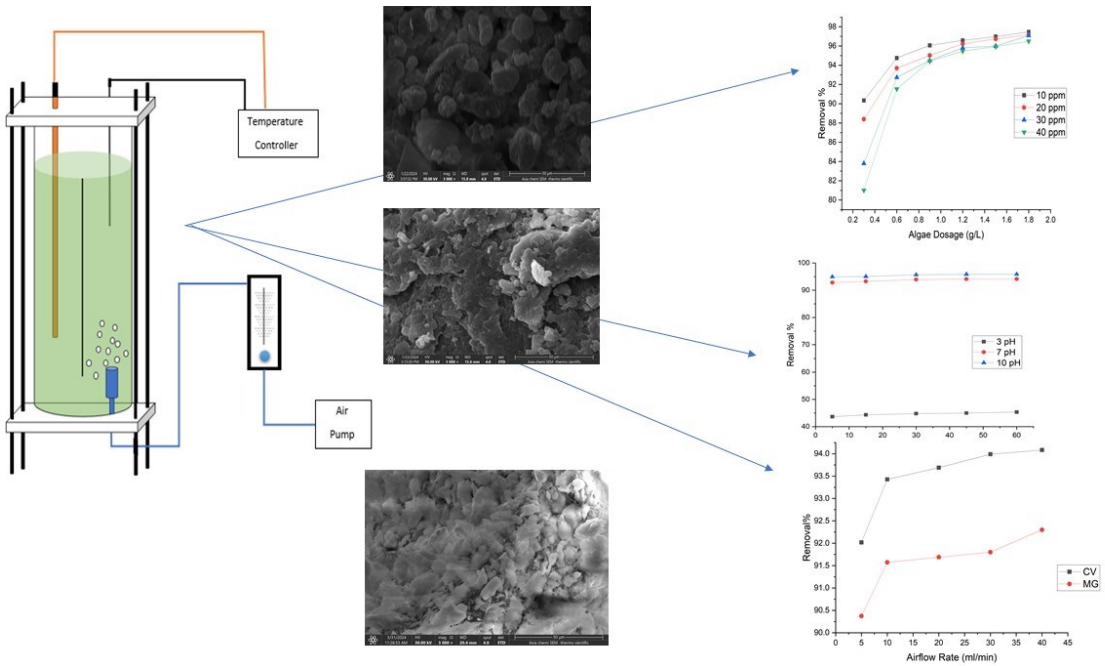
Graphical Abstract



Abstract

Malachite green (MG) and Crystal Violet (CV) are used mainly as dyestuff and antimicrobials in aquaculture. They have a severe toxic effect on the environment. Several techniques were used to remove impurities from an aqueous solution: chemical, physical, and electrical. Among all these techniques, using dry algae is a more economical and helpful process. This study aims to investigate using an airlift reactor in the removal of MG and CV by chlorella algae as a biosorbent under different variables. The experiments were carried out in an airlift bioreactor. The experiments were carried out under the effect different operating conditions of initial dye concentration (5-40 ppm), alga dosage (0.3-1.8 gm/l), pH (3–10), air flow rate (0-40 ml/min), temperature (298-318 K) and contact time (5-60 min). The results show that the introduce of air bubbles significantly enhances the removal efficiency of the dye. The best removal effectiveness was 95.2% for MG dye and 96.1% for CV dye. The thermodynamics results reveal that the

processes are exothermic for both dyes. Kinetic and adsorption isotherms results show the best fit is pseudo-second order and Langmuir model. The mass model result shows that the liquid film diffusion model was the best-fitted for both dyes.

Keywords

Algae, bioremoval, dyes, Airlift Bioreactor

1-Introduction

The world has undergone significant changes in the past few decades. The rapid increase in population led to a massive escalation in different industries, one of which is dye-based industries and the rise in the use of textiles, leather, and paper[1]. Dyes are considered one of the primary pollutants in aquatic systems; they are toxic substances that can dissolve in water but are challenging to break down and colorant. Wastewater from dye-based industries should be treated well before being disposed of in water.

The pollutants from textile, paper, and leather industries are a massive problem because of their toxic properties and their color that affect living organisms in waterbodies, affecting the photosynthesis process and chemical and biological oxygen demand that affects the oxygen quantity in water bodies[2].

Malachite green (MG) is a water-soluble cationic dye with a green color and crystalline shape. The malachite green (also called aniline green, basic green 4) was first invented in 1877 by Fischer; MG is used in food, leather, silk, and agriculture. At first, MG was used in the fish farming industry because of its good value in preventing parasite infection, an effective topical fungicide. MG is banned from being used by many countries and the US Food and Drug Administration. However, it is still widely used in the leather and silk industry in many countries. MG is very harmful to humans, and its toxicity depends on exposure time, concentration, and temperature[3,4].

Crystal Violet (CV) is a widely known dye used in the medical, pharmaceutical, and leather industries to make blue and black ink in pen and printer factories. It is toxic and has a poisonous nature, can cause irritation and skin infection, and, even worse, can cause blindness and kidney failure. The discharge of the dyes into water rivers causes violet coloration of water, which prevents light penetration into water bodies, causing a deactivation in the photosynthetic process[5,6].

wastewater from dye-based industries should be treated well before being disposed of in water bodies; various processes have been deployed to remove dyes, including chemical precipitation, electrochemical methods, adsorption, ion exchange, nanofiltration, and reverse osmosis membranes[7] but most of these processes are not compelling enough for a significantly diluted solution[8]. They are expensive to operate and construct[9]. Alternative methods have been used which are not highly cost-effective for a wide range of dye concentrations; the use of biological material as adsorbent, the biological treatment utilizes algae to remove dyes from wastewater, which has gained much attention in the last few decades for its remarkable performance on treating dyes wastewater and generate no hazard agent in the environment[10]. Low-cost adsorbents such as steel plant slag, fly ash, maize cob, and wood are examples of adsorbents being used but because of their low capacity of adsorption the researchers become more interested in microorganisms, because microorganisms are capable of bioaccumulating or biodegrading the dyes[1].

Algae are widely used in color removal in wastewater, several studies done to investigate the rule of algae in dye removal as a low-cost biosorbent, Researchers[11] test dried P. animale algae as biosorbent to remove Remazol Black B dye and achieve 99.6% removal with optimal condition of 4g/l algae dosage, 93.16 ppm (RBB) initial concentration, 2 pH, 45 ^{O}C temperature, and carried out in Batch experiment, in Moghazy [12] the study used two dry

macro-algal s (U. fasciata and S. dentifolium) as biosorbents to remove MB dye achieving 97% removal at 328 ppm initial MB concentration by U. fasciata and 85.6% removal at 26 ppm MB dye by U. fasciata and S. dentifolium, this results were achieved at 1.5 g/l and 2 g/l algae dosage for U. fasciata and S. dentifolium respectively with optimal pH 7 and 25 ^{O}C, and carried out in Batch experiment, in OMAR [13] the study used Ulva lactuca, Sargassum crassifolium, and Gracilaria corticata dry algae to remove malachite green MG, the results show that the highest removal of 98.3% at optimum condition of 2 g/l algae dosage, 35 ppm initial dye concentration, 8 pH and 25 ^{O}C temperature, the study also conducted in batch experiment.

Despite all the research above, the use of Chlorella vulgaris algae in removing MC and CV dyes using an airlift reactor has yet to be covered and needs more investigation. Hence, this work aims to show the effectiveness of air bubbles in the removal of MC and CV in airlift reactor, where it was done under flow rates (5-40 ml/min), as well as to study the effect of different variables of the algae dosage (0.3-1.8 gm/l), initial dye concentration (5-40 ppm), pH (3-10) contact time (5-60 min), and temperature (20-40 ºC) on the removal process by conducting UV examination.

2- Materials and Methods

2.1.1 Material

Malachite green oxalate ($C_{50}H_{52}N_4O_8.C_2H_2O_4$) and Crystal Violet ($C_{25}H_{30}N_3Cl$) were purchased from Himedia laboratories; the stock solution of 1000 ppm of both dyes was prepared by dissolving 1 g of dye in 1000 ml of deionized water, the stock is used to prepare different concentration solution from 5 to 40 ppm of both Malachite green and Crystal Violet.

2.1.2 Algal Biomass

C. vulgaris was supplied by the University of Baghdad/College of Science. The algae was removed from the aqueous solution using the Whatman Grade 1 Qualitative Filter Paper. The algae were dried under the sun for two days, crushed in a domestic grinder, sieved with 0.5 mm sieve paper, and stored at room temperature.

2.2 Experimental Procedure

The Experimental work was divided into two sections. The first section is a batch biosorption. The experiments were carried out using Erlenmeyer flasks (250 ml) under constant temperature, pH, and agitation speed to study the effect of the initial dosage of algae (0.3 g/l to 1.8 g/l) with different concentrations of MG and CV (5 ppm to 40 ppm) on biosorption removal process, The second section is semi-batch, biosorption using (1L) airlift bioreactor (34 cm

length and 7 cm internal diameter) as shown in figure (1). The experiments were done under different factors of initial concentration of dye (10 ppm to 40 ppm), pH (3 to 10) controlled by 0.1 M of hydrochloric acid and (0.1 M) sodium hydroxide, temperature (range from 20 ^{O}C to 40 ^{O}C), interval time (range from 5 min to 60min), and effect of airflow (range from 5 ml/min to 40 ml/min) to obtain the optimum condition for biosorption of Malachite Green and Crystal Violet by Chlorella vulgaris. The samples were taken after each interval time to detect the removal percentage of the dye. The samples were put in a centrifuge at 2500 rpm for 7 min to separate the algae from the solution and finally to measure the dye concentration. Each experiment was repeated three times, and the average value was adopted.

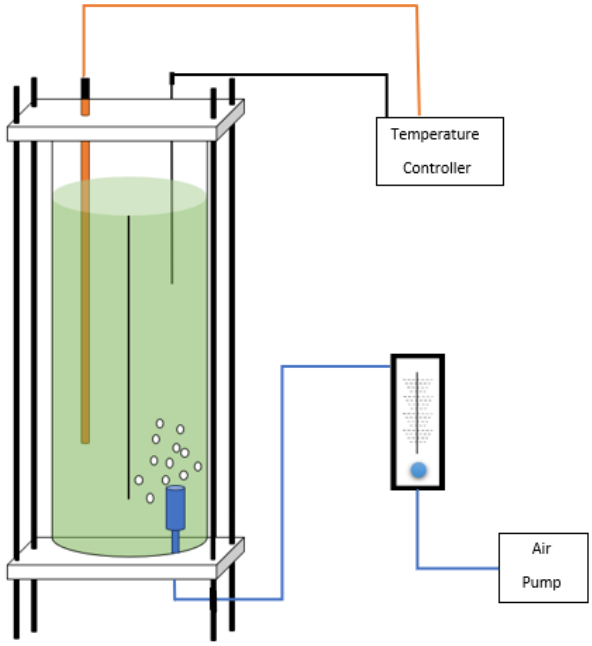


Figure (1): Airlift bioreactor

2.3 Tests and Characterization

2.3.1 UV-Test

The dye concentration is measured before and after the biosorption process by a Visible-Uv spectrophotometer (Shimadzu, UV-1800, Japan) at 618 nm and 590 nm for MG and CV, respectively. The removal percentage of dye was calculated using eq(1):

$$R\% = \frac{C_o - C}{C_o} * 100 \quad (1)$$

Where:

C_o = initial concentration of dye in solution (mg/l), C = concentration of dye in solution (mg/l), R% = removal percentage of dye in solution[14].

The amount of dye adsorbed in algae at equilibrium was also calculated using eq(2):

$$q_e = \frac{(C_o - C_e)V}{M} \qquad (2)$$

where:

qe = amount of dye adsorbed on algae (mg/g), C_e = concentration of dye in solution at equilibrium (mg/l), V = solution volume (ml), M = algae biomass (g)[15].

2.3.2 The zero charge of pH

The point of zero charge pHzpc test was made on C.Vulgaris algae; the pHzpc is pH when a positive and negative charge are equal[16]. It is an important parameter that demonstrates the biosorption ability of algae surface, and it is vital to analyze the algae's ability to remove dyes from water[17], the pHzpc was measured according to a (Mokhtar method) by preparing six solutions of 50 ml of 1M NaCl with a pH range from 2-12, adjusted using 0.1 M of NaOH and HCL. A 0.5 g of chlorella vulgaris was added for each of the six solutions, the final pH (pHf) was calculated, and the difference of pH value from initial to final value was calculated using eq (3) and plotted against pH initial where pHzpc is measured at ΔpH zero:

$$\Delta pH = pH_i - pH_f \qquad (3)$$

2.3.3 FTIR Test

The FTIR spectroscopy test was performed on algae particles before and after the biosorption of both MG and CV using (SHIMADZU, Japan) at the BPC Analysis Center in Baghdad, ranging from 4000-400 cm-1, to analyze the functional groups responsible for the biosorption process on the surface of the adsorbent particle.

2.3.4 SEM-EDS

The surface of algae was also scanned before and after the biosorption process using (Axis chemsem, Thermo Fisher, USA) Magnification: 5 to 1,000,000× (Polaroid) at Al-Khora Laboratories in Baghdad to study elements of algae surface before and after the adsorption of two dyes,

3. Result and Discussion

3.1 characterization of algae

3.1.1 pH_{zpc} point of zero charge

pHzpc has a significant effect on the biosorption process; it indicates at which pH value the surface of algae behaves as a neutral charge[18]. It can be seen from figure (2) that the surface of algae has a positive charge from 2.4 to 4.8, and when pH is more than 4.8, the algae surface becomes negatively charged. This result indicates that at higher pH values, the removal of positive molecules such as cationic dye increases.

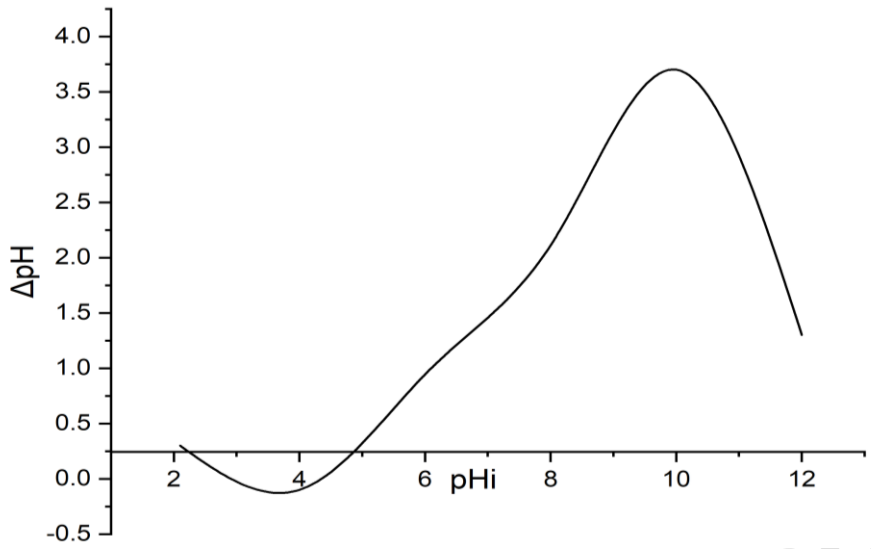


Figure (2): PZC plot for C.Vulgaris

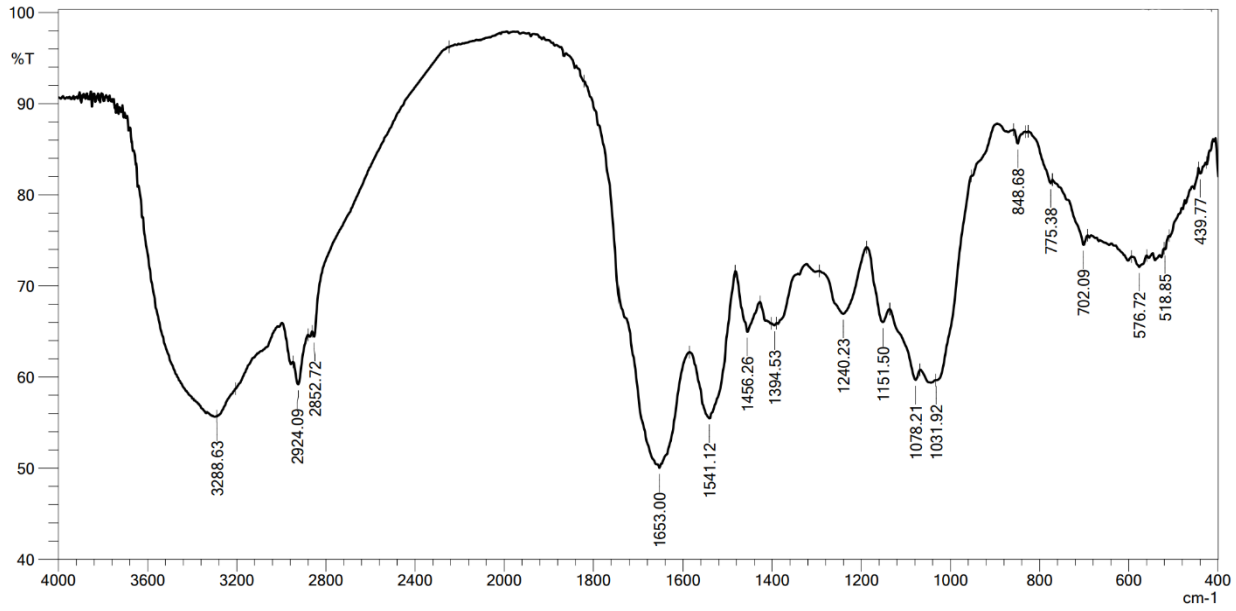
3.1.2 FT-IR

Many research shows that the functional groups present on the surface of algae are hydroxyl, amino, Carboxyl, phosphate, and sulfate; the FTIR test was done for C. Vulgaris algae before and after being loaded with MG and CV dye to understand the interaction between both dyes and functional group on the algae surface as shown in Figure (3). The peaks at 3288.63 Fig (3.a) represent the hydroxyl (-OH) group and amine (-NH) functional group. This band is shifted to 3439.08 and 3410.15, as shown in Fig (3. b and 3. c) after loading with MG and CV, indicating moisture in the sample. Another strong peak at 2924.09, 2852.72 Fig (3. a) presents alkyl group (C-H), after loading with MG and CV remains in Fig (3. b and 2. c) 2926.0, 2852.72, 2854.65.

The peak band 1653,00 peak band present C=N stretch imine /oxime group or C=O alkenes in Fig (3. a) shifted to 1651.07, 1647.21 figs (3. b and 3. c)), after loading with MG and CV respectively may be attributed to the organic form of both dyes.

The peak 1541.12 present nitro compound NO_2 stretching shifted 1583.56 and 1535.34, as shown in Fig (3. c) after loading with CV, which could be attributed to Carboxyl (C=O).

The peaks at 1394.53 Fig (3. a) represent alcohol O-H bonding. This band is shifted to 1373.32 and 1363.67, as shown in Fig (3. b and 3. c) after loading with MG and CV, respectively; these shifts in peak band due to the biosorption process, functional groups have an essential role in bioremoval both dyes.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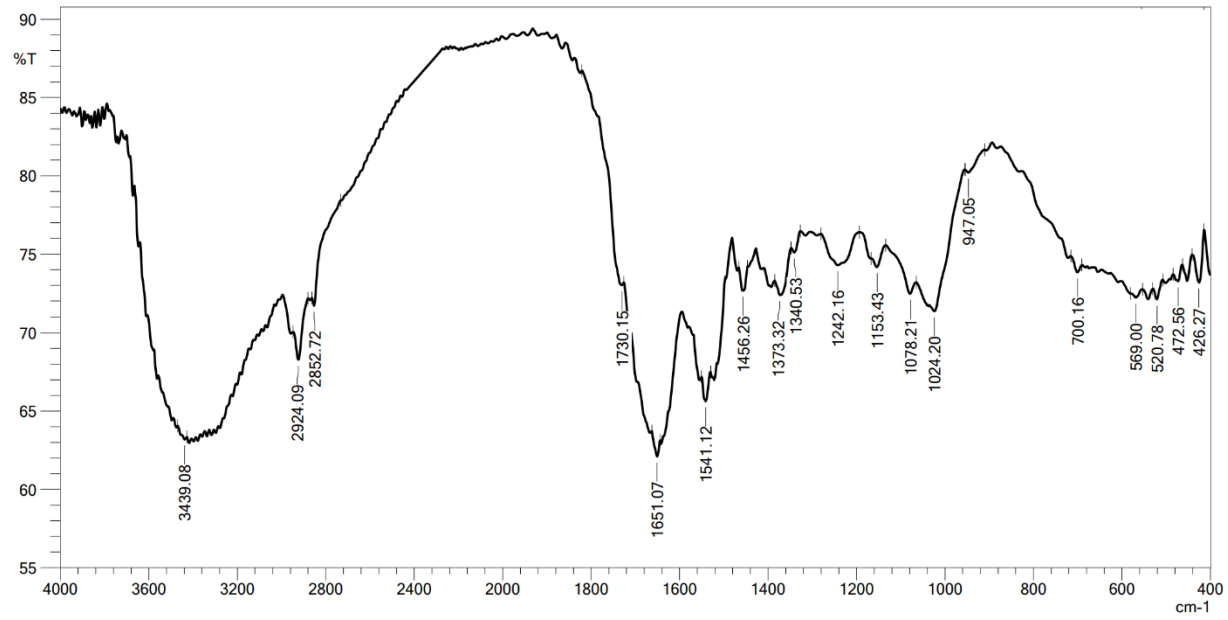
(a)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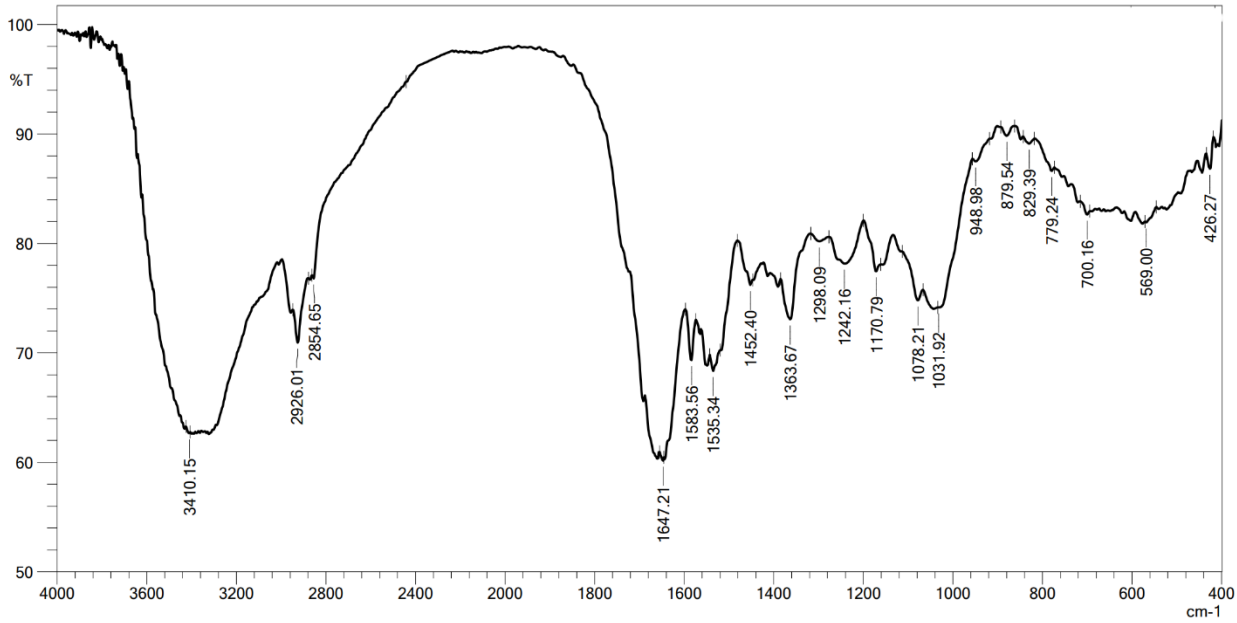
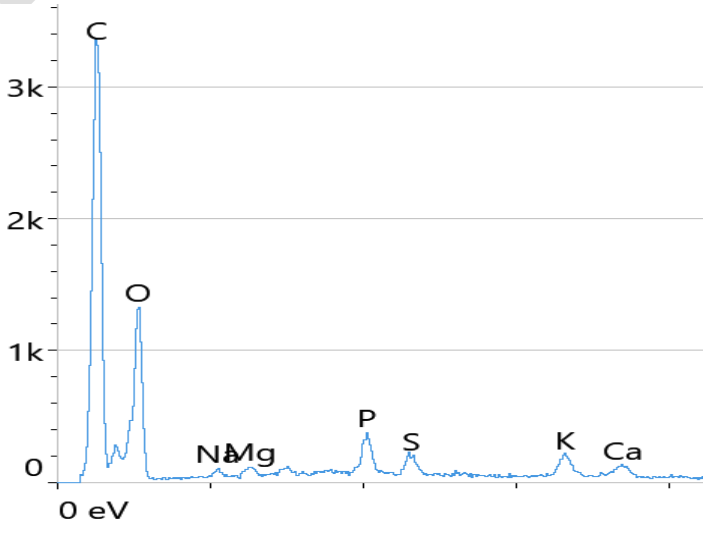


(c)

Figure (3): FTIR peaks of transmittance of C.Vulgaris (a) before biosorption (b) loaded with MG dye (c) loaded with CV dye

3.1.3 EDS analysis

The EDS of the alae before and after the removal process for both dyes MG and CV are shown in Figure (4). It can be seen from the figure that both C and O atoms are present in algae with a high composition of 59.9% and 37.8%, respectively, with other contaminants; after using the Algae to remove the two dyes, an increase in the composition of C after adsorption from 59.9% to 64.4% and 61.9% for MG and CV respectively was occurred as shown in figure (4. b) and (4. c). This result approves the biosorption occurrence since both dyes contain C in their structure.



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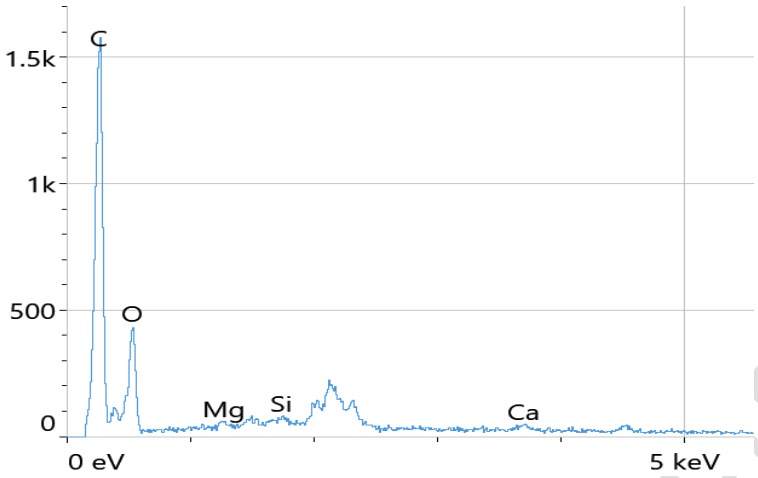

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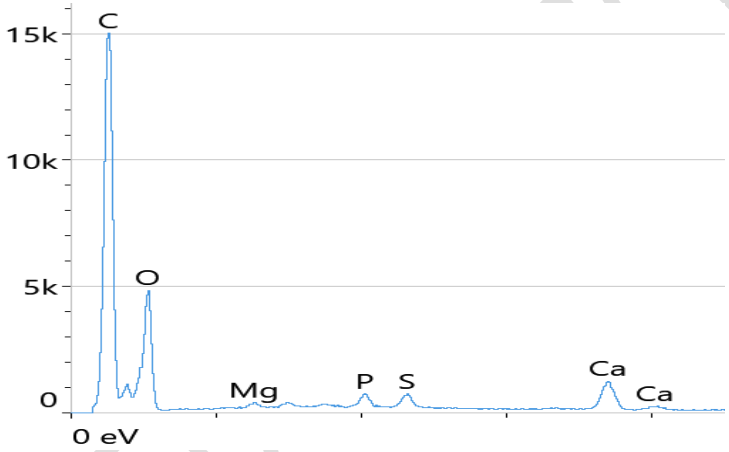


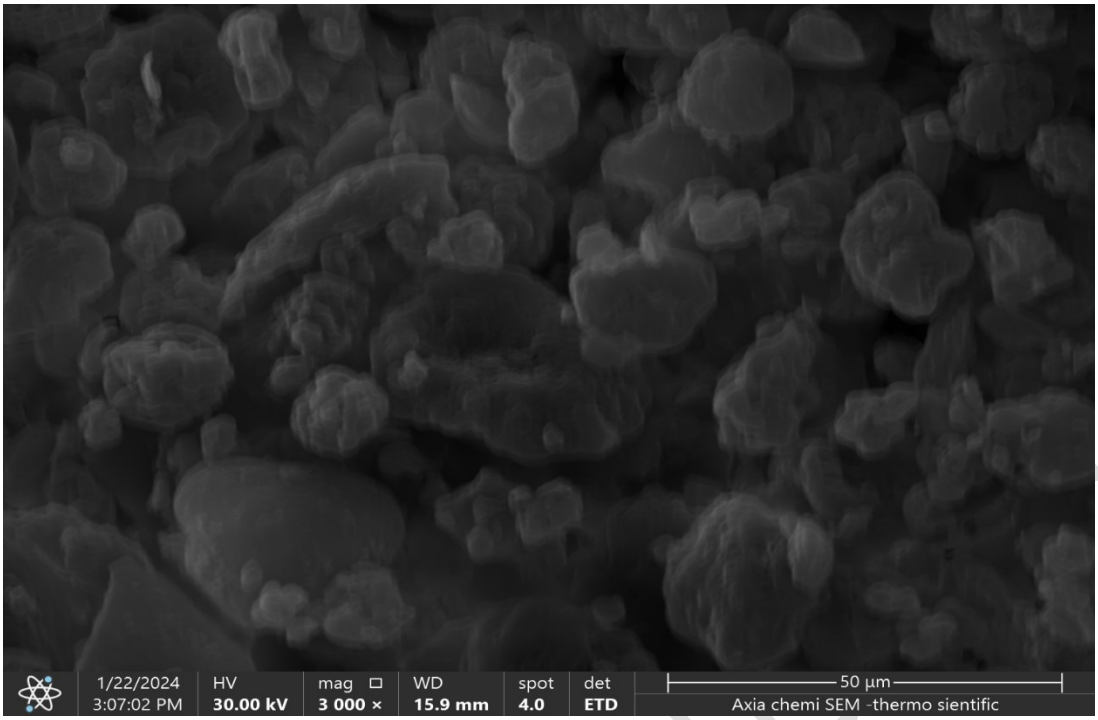
(c)

Figure (4): EDS analysis for (a) free algae (b)algae loaded with MG (c) algae loaded with CV

3.1.4 SEM

The scanning electron microscope is an important tool for studying the morphology and the change of morph of algae surface before (Fig 5. a) and after the adsorption of both dyes (Fig 5. b and c).

It can be noticed from Figure (5. a) that the small spherical particles that present algae before the adsorption, the algal surface was rough and permeable before the adsorption process began. After the adsorption process, there is a color change in particles (as shown in Figures 5. b and 5. c), which shows that algae were coated with MG and CV dye. This result suggests that the adsorption of MB dyes onto the algal surface is a physical process.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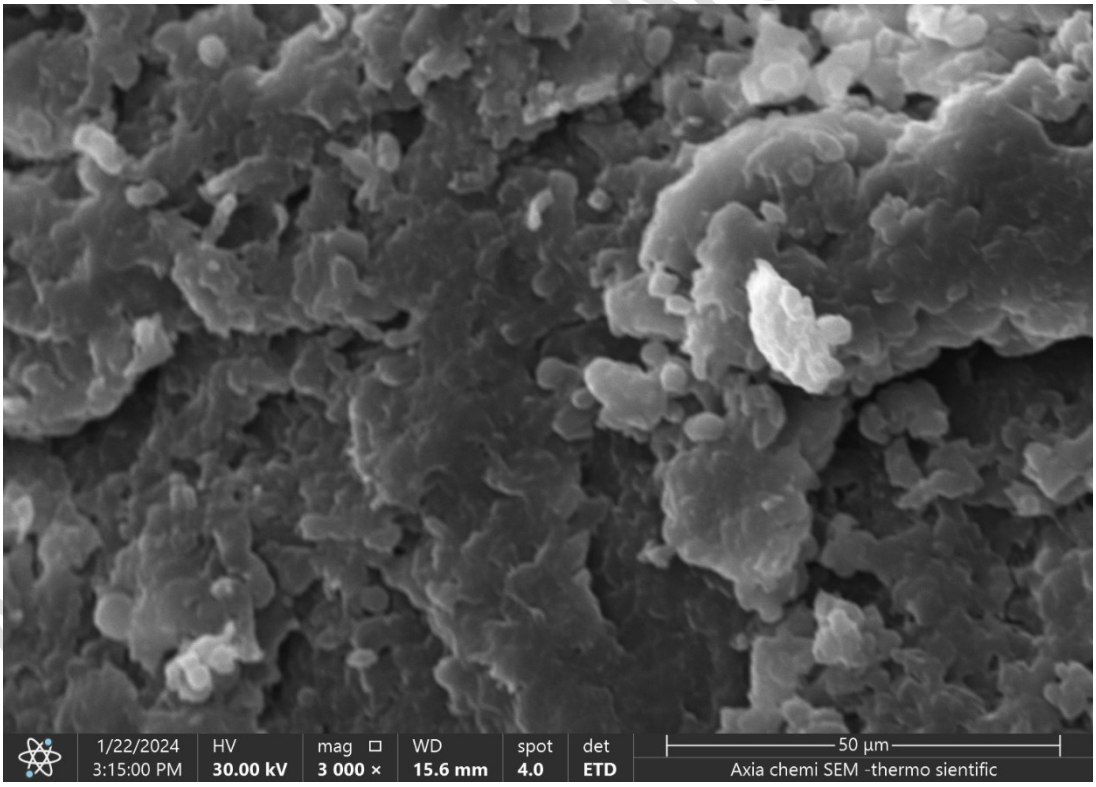
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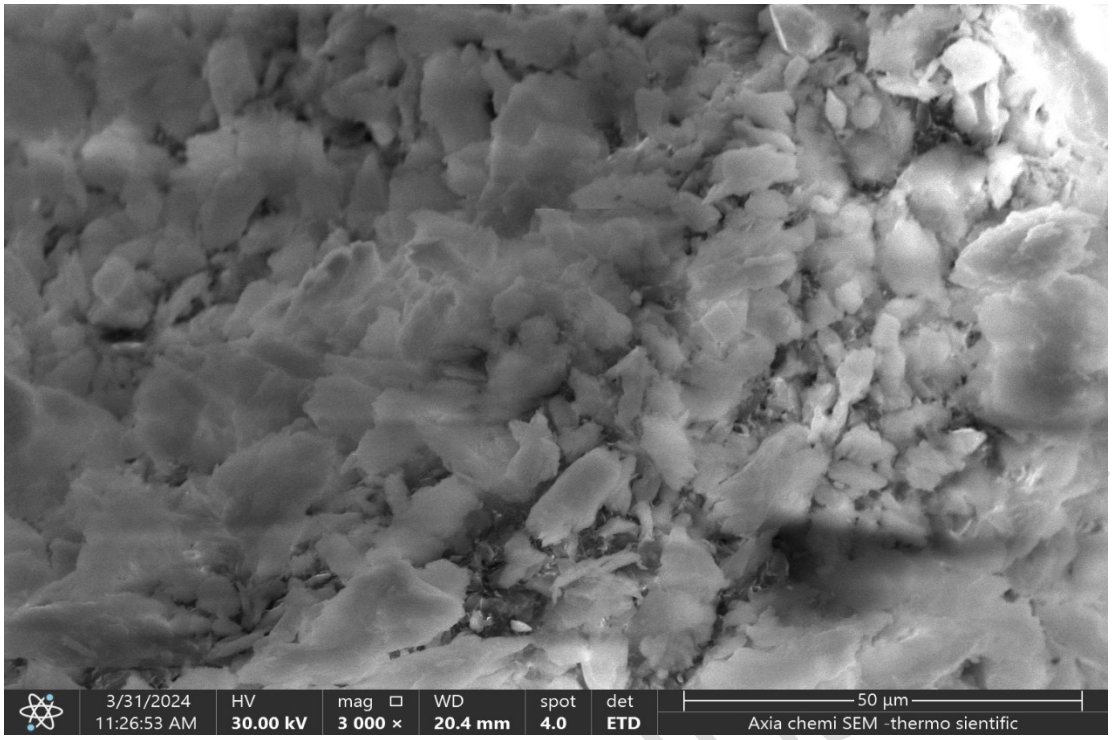
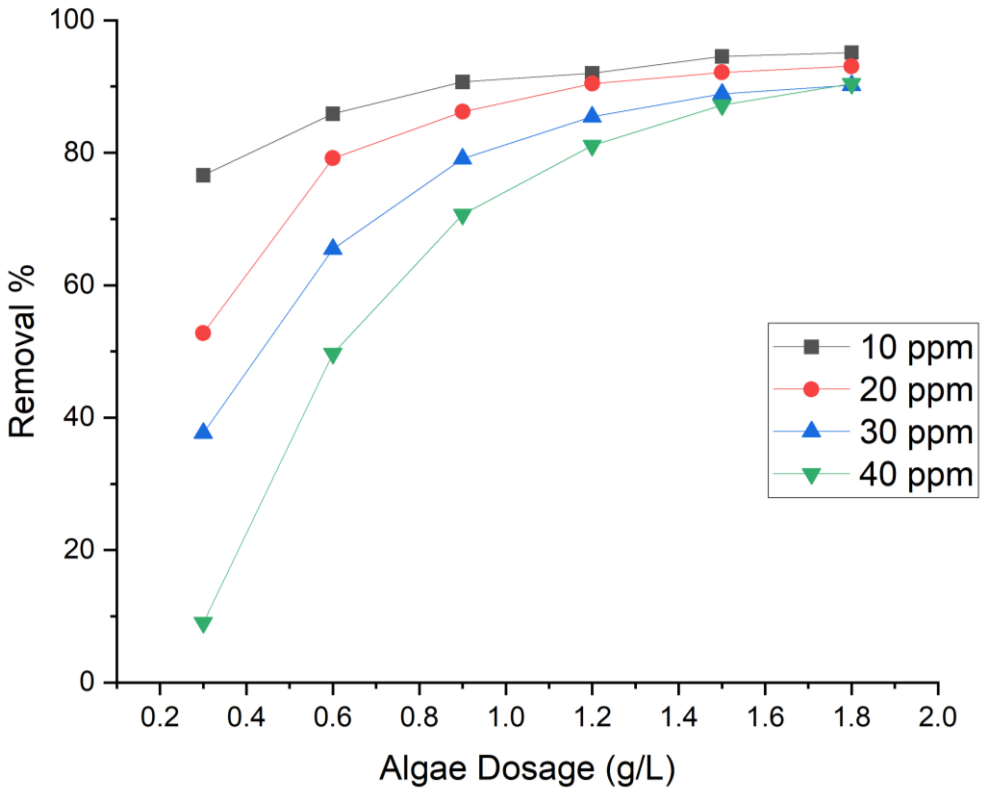


(c)

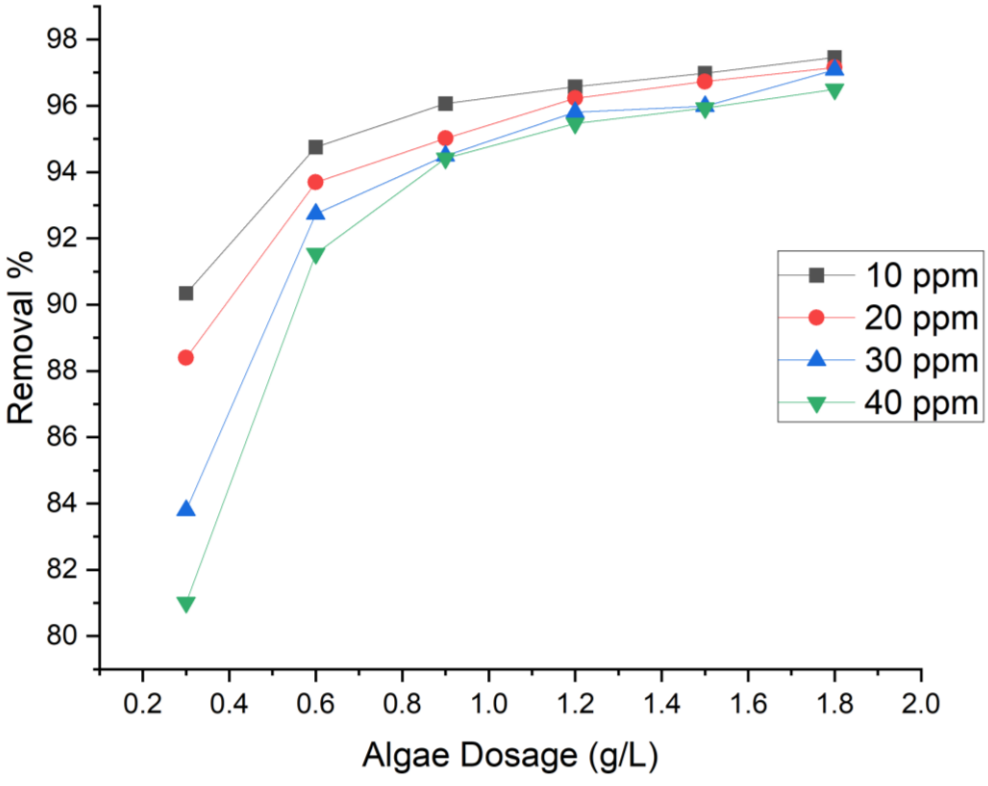
Figure (5): SEM image for (a): algae (b): algae loaded with MG (c): algae loaded with CV

3.2 The effect of algae dosage

The algae dosage is an essential factor affecting the biosorption process, which indicates the sorption capacity of algae; in this paper, C.Vulgaris algae dosage ranging from 0.3 to 1.8 g/l with different dye concentrations ranging from 5 to 40 ppm was used for this purpose. The experiment was done using 250 ml Erlenmeyer flasks (batch process) under 350 rpm shaking at 25 ºC and seven pH with 60 min contact time. The result indicates that at a 40 ppm initial dye concentration, the removal of CV dye increases from 81.2% to 96.1% using a dosage of 0.3 to 1.8 g/l, and the removal of MG dye increases from 9.05% to 90.1% using a dosage from 0.3 to 1.8 g/l, as shown in fig (6). Indeed, it appears from the figure that the dye removal percentage increases with increasing the algae dosage till it reaches equilibrium; after the equilibrium point (1.5 g/l for MG dye and 1.2 g/l for CV dye), the removal of dyes becomes nearly constant. This is because, in the beginning, there is a binding site available for the dye to be removed. After reaching the equilibrium point, a balance occurs between the dye in the binding site and the dye in the solution[19].


Removal %
Algae Dosage (g/L)
10 ppm
20 ppm
30 ppm
40 ppm

(a)


Removal %
Algae Dosage (g/L)
10 ppm
20 ppm
30 ppm
40 ppm

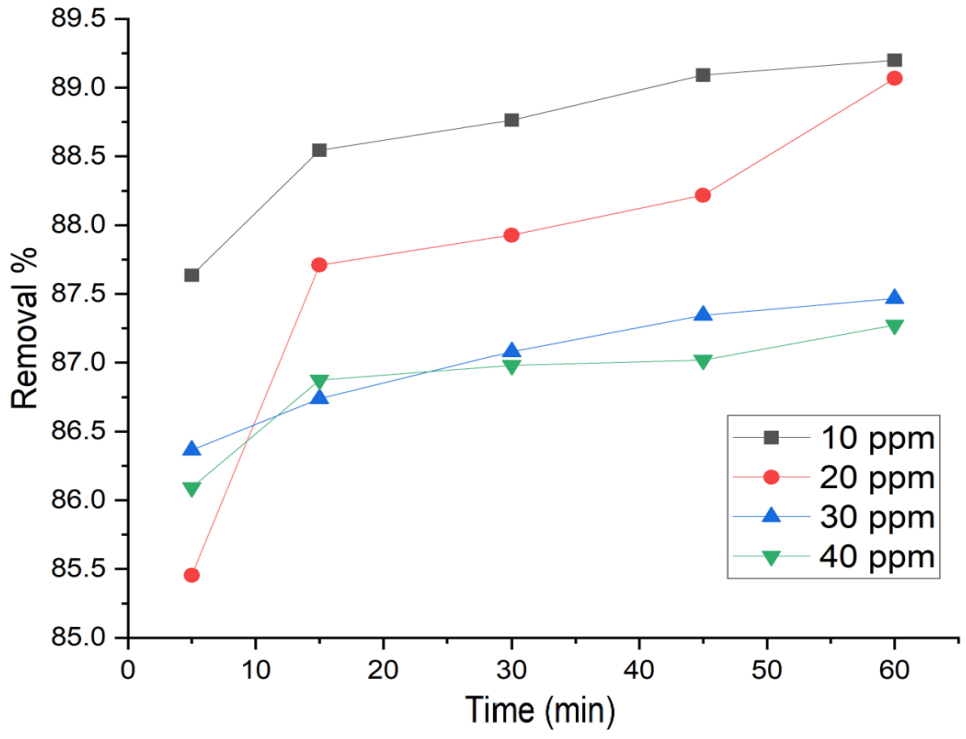
(b)

Figure (6): Effect of algae dosage on dye removal (a) loaded with MG (b) loaded with CV (at 7 pH, temperature 20 °C, shaker speed 350 rpm,)

3.3 The effect of dye concentration

The initial dye concentration is a crucial factor in the biosorption process. It provides the driving force to overcome the mass transfer between the solution liquid phase and algae particles in the solid phase[19], To study the effect of dye concentration, a range of dye concentrations from 10 to 40 ppm was used, and the results are shown in Fig (7). The figure shows that as the initial dye concentration increases, the dye removal percentage decreases.

This is due to the saturation of the active side of algae with dye particles, and there is not enough place available for more adsorption to occur. This behavior can also be explained by the increase in the repulsive force between the dye particles and algae particles and between the algae particles themselves, causing the distance between algae particles to rise, which lowers the adsorption capacity[20].



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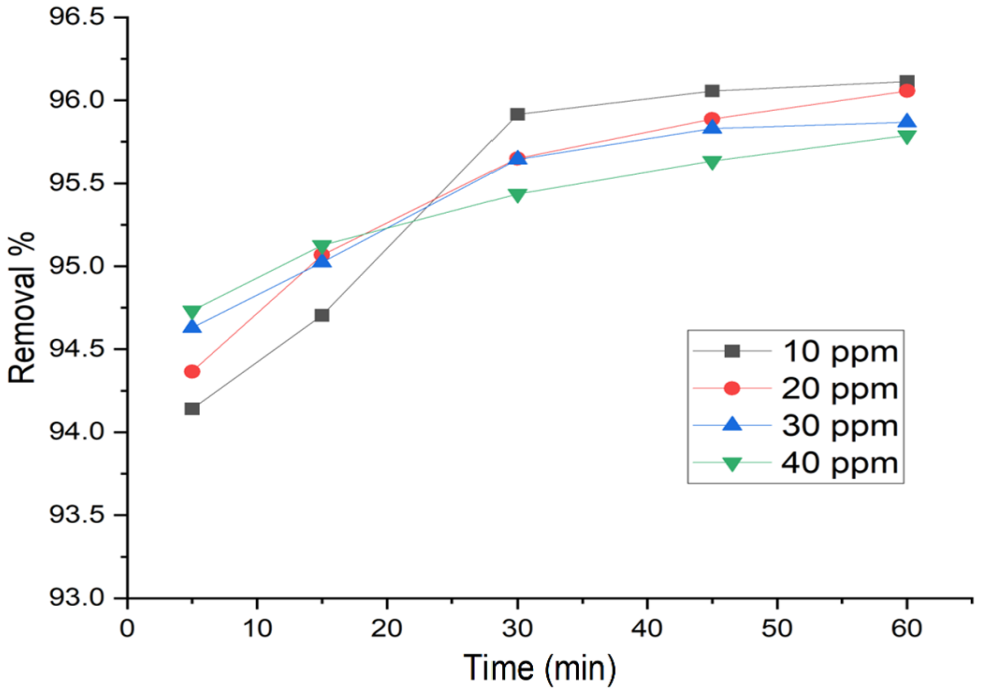


(b)

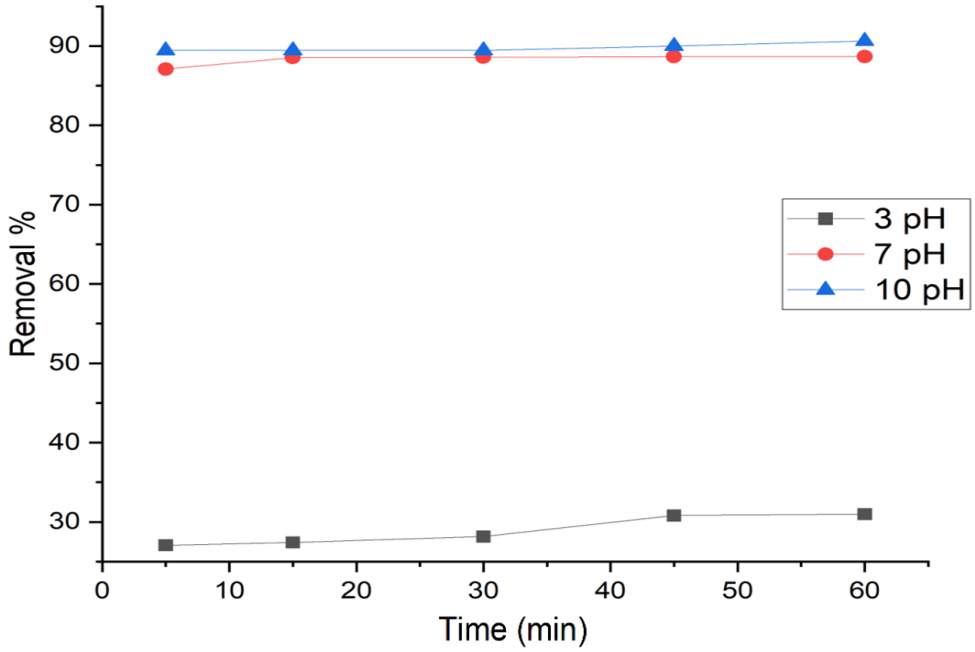
Figure (7): Effect of initial dye concentration on dye removal(a)loaded with MG (b)loaded with CV (at 7 pH, temperature 20 ºC, 20 ml/min airflow rate)

3.4 The effect of pH

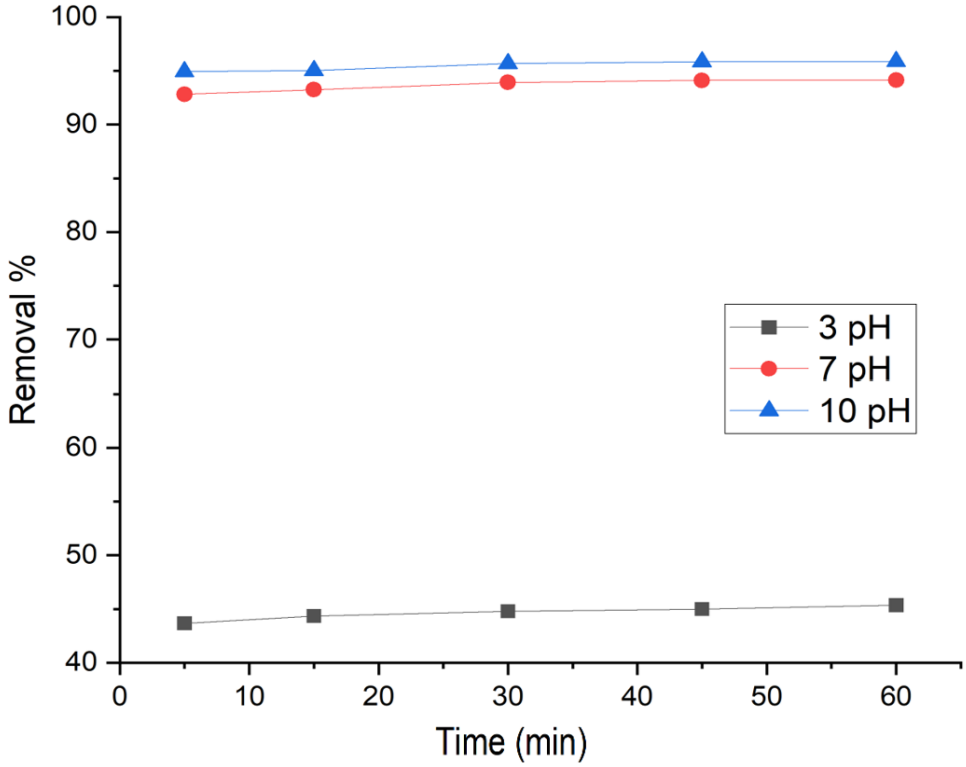
pH is a critical factor in the biosorption process; the pH effect on algae surface charge affects its biosorption ability and changes the ionic form on the algae surface. Solutions with a pH range from 3 to 10 were prepared to study its effect. The result shows that the dye removal was high when pH was higher than seven and almost constant from 7 to 10 pH, the removal percentage increased slightly from 86.36% to 89.29% when pH increased from 7 to 10 for MG removal, and 94.58% to 95.76% when pH increase from 7 to 10 for CV removal, both at initial dye concentration 20 ppm, temperature 20 ºC, 20 ml/min airflow rate,

The removal percentage of both dyes significantly decreased at lower pH levels, reaching 27.63% and 46.27% for MG and CV respectively under the same conditions. This finding aligns with the results of the zero-point charge test. For C.Vulgaris, the pHzpc is 4.8. At pH > 4.8, the algae surface becomes negatively charged, increasing removal at higher pH levels for cationic dye removal.

The decrease in removal efficiency at a lower value pH is due to the presence of the H+ ion concentration higher at low pH, which results in the algae surface becoming positively charged and reducing the attraction force that occurs between the algae surface and dyes (positive charge dye) causing less removal compare with higher pH[19], this phenomenon called protonation causes the surface of algae to become nonideal for biosorption removal[20].



(a)



(b)

Figure (8): Effect of pH on dye removal (a)loaded with MG (b)loaded with CV (at initial dye concentration 20 ppm, temperature 20 ^{o}C, 20 ml/min airflow rate)

3.4 The effect of airflow rate

To demonstrate the effect of airflow rate in the removal process, the range of airflow rates 5,10,20,30,40 ml/min were tested, and the results are shown in Figure (9). A significant rise in the percentage of removal is observed with the increase in the velocity of the air under the experimental conditions used in the present investigation. This is because as the airflow rate increases, the turbulence increases, causing a decrease in the mass transfer resistance of algae biomass. This result indicates that the airflow serves a vital role as it delivers the dye molecule to the active binding site on the algae surface.

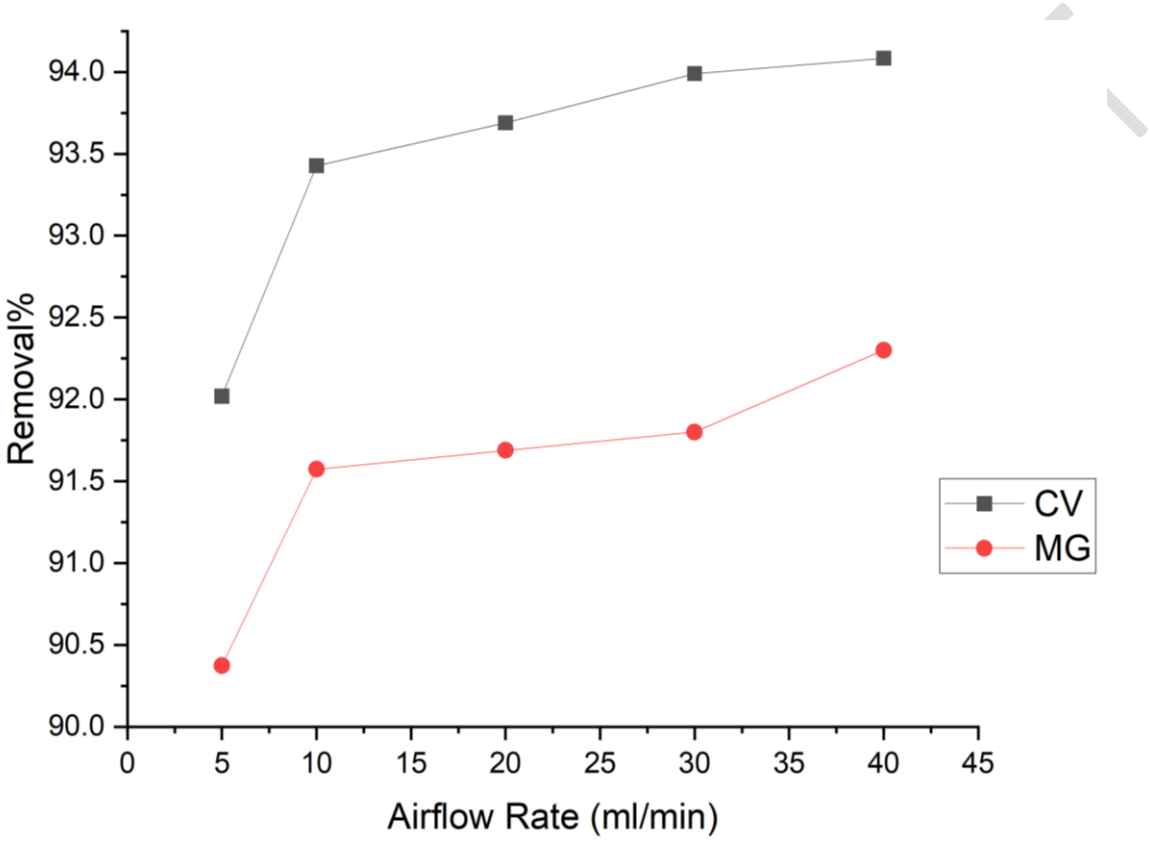


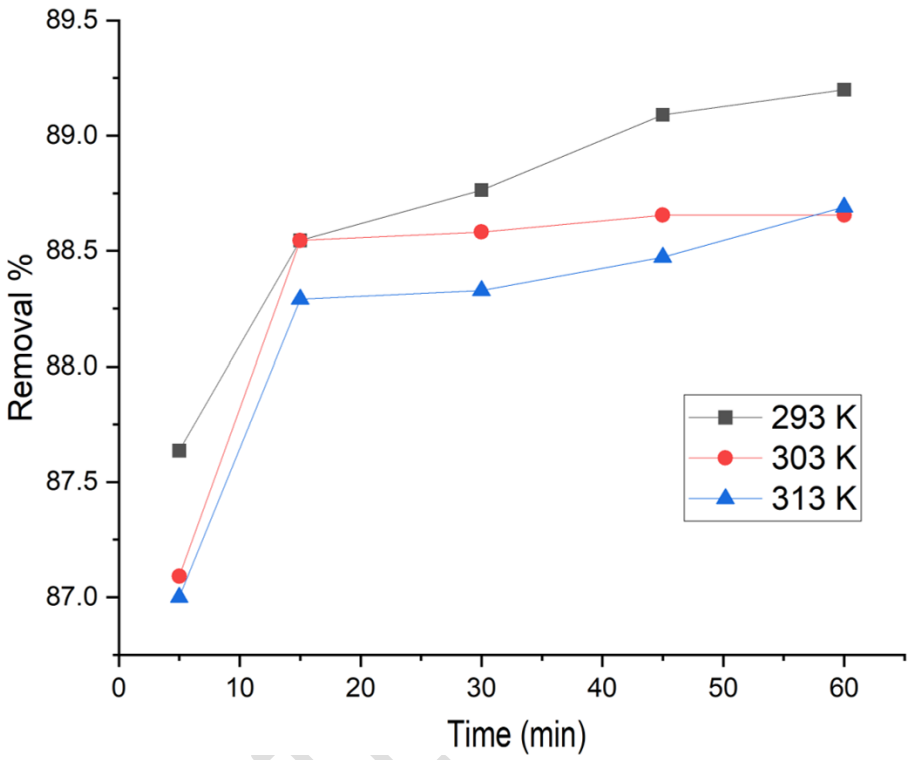
Figure (9): Effect of Airflow rate on dye removal (at initial dye concentration 30 ppm, algae dosage 1.5 g/l, temperature 20 ºC, pH 7, contact time 30 min)

3.4 the effect of contact time

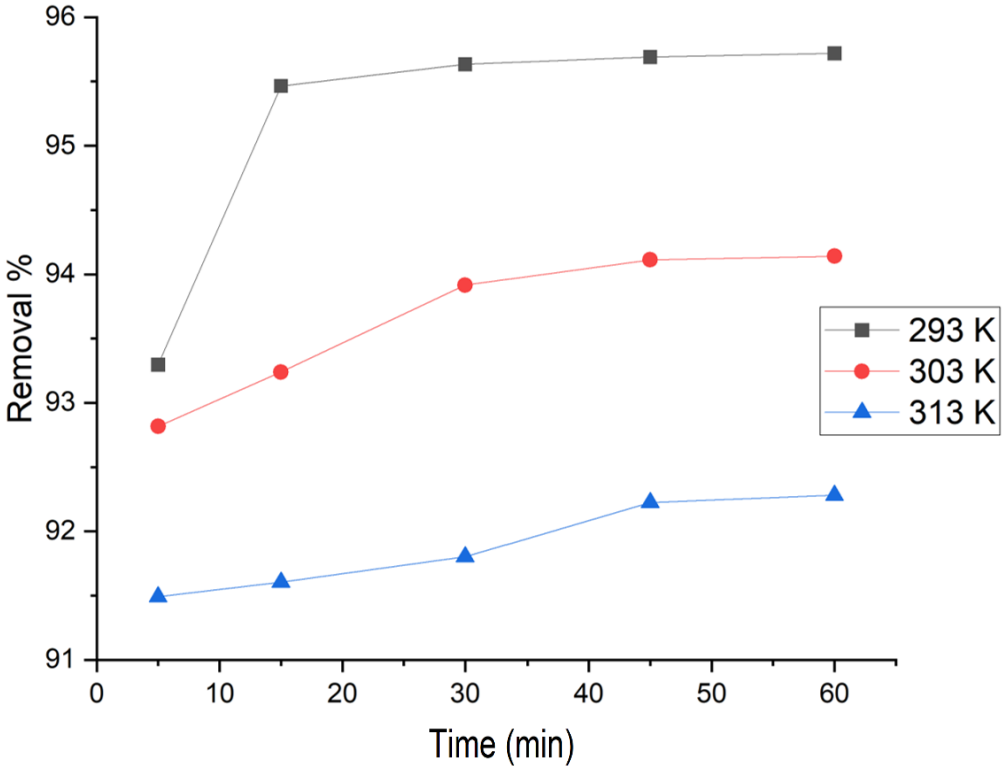
The contact time is an essential factor in the biosorption removal process; the experiments were conducted at different times 5,10,15,30,45,60 minutes to analyze the time effect on the removal process, as shown in Figure (6,7,8). The biosorption process occurred rapidly within 5 minutes, and the removal percentage was nearly 88% and 85 % for both CV and MG, respectively. The removal percentage increased slightly till it reached an equilibrium state with 94% and 93% removal at 45 min for both CV and MG, respectively; the available binding site saturated early at 10 min or less, especially at higher dye concentrations, causing the system reaching equilibrium at an early stage, as seen in the Figure the removal percentage does not change a lot after 15 min contact time.

3.5 The Effect of Temperature

One of the factors affecting the removal process is temperature. To study its effect, a set of experiments at 20,30, and 40 OC with four different dye concentrations of 10,20,30,40 ppm was conducted, and the results are shown in Figure (10). The results show that as the temperature rises, the dye removal decreases and adsorbent capacity decreases; that is because, at the higher temperature, the connection between the active binding site at the algae surface and dye molecule is controlled by endothermic interaction, and at lower temperature, it controlled by exothermic interaction[20], this result indicates that the removal process of both dyes is exothermic.



(a)



(b)

Figure (10): Effect of Temperature on dye removal (a)loaded with MG (b)loaded with CV (at initial dye concentration 20 ppm, Airflow rate 20 ml/min, pH 7)

2.6 The Thermodynamic study

Thermodynamics have also been studied, including enthalpy changeΔH^O, Gibbs free energy change ΔG^O and Entropy change ΔS^O for removal of the CV and MG dyes using the C. Vulgaris algae. The entropy and enthalpy values both were estimated according to equation 4 and from the value of slope and intercept of Log KC versus 1/T as shown in Figure(11)

Based on this equation:

$$\text{Log } (K_C) = \frac{\Delta S^0}{2.303R} + \frac{-\Delta H^0}{2.303R} \qquad (4)$$

$K_C = q_e / C_e$

Where q_e is the amount of molecule adsorbed at equilibrium per unit mass of algae (mg/g), C_e is dye concentration (mg/L) and T is the temperature in K

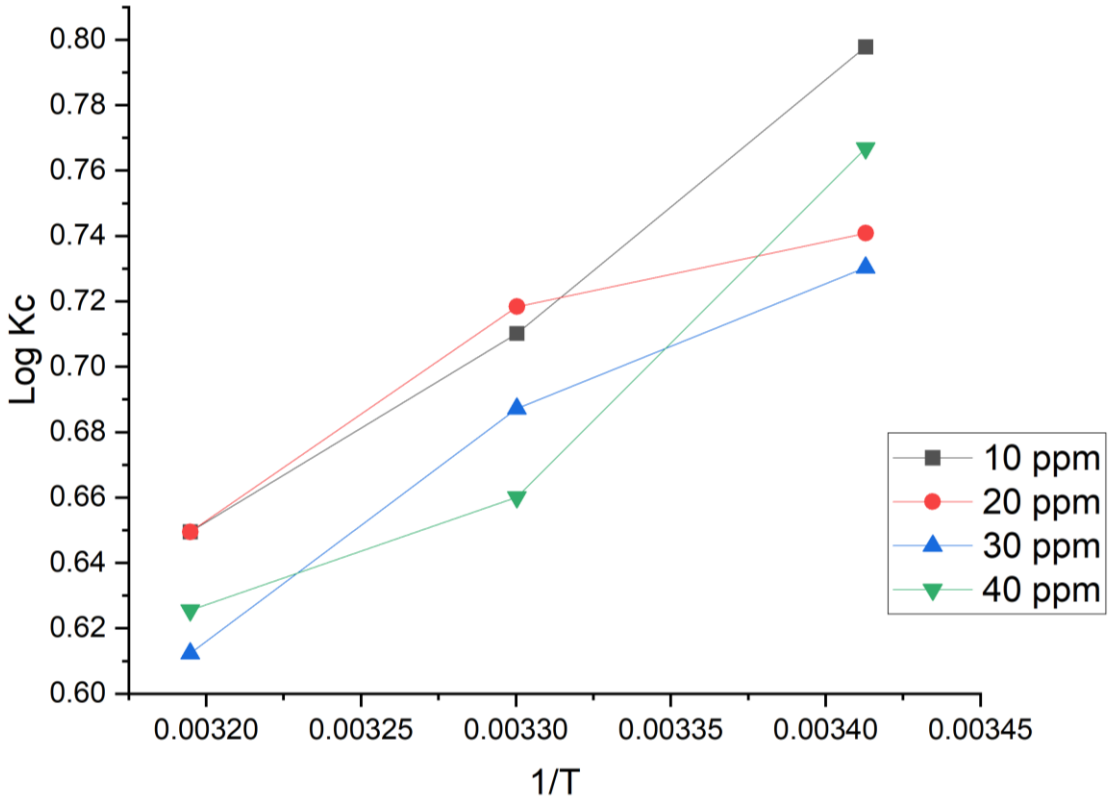
The results are tabulated in Tables 1 and 2. The negative values of ΔH^O at all concentrations show that the process is exothermic; this result can also be indicated as the removal efficiency decreases when the temperature of the solution increases. The negative values of ΔS^O at all concentrations show that the adsorption of dye at the algae surface is more organized than in the aqueous phase, and the negative value of ΔG^O at all concentrations indicates that adsorption on the algae surface is spontaneous and occurs rapidly.

Table (1): Thermodynamic parameter values for CV removal

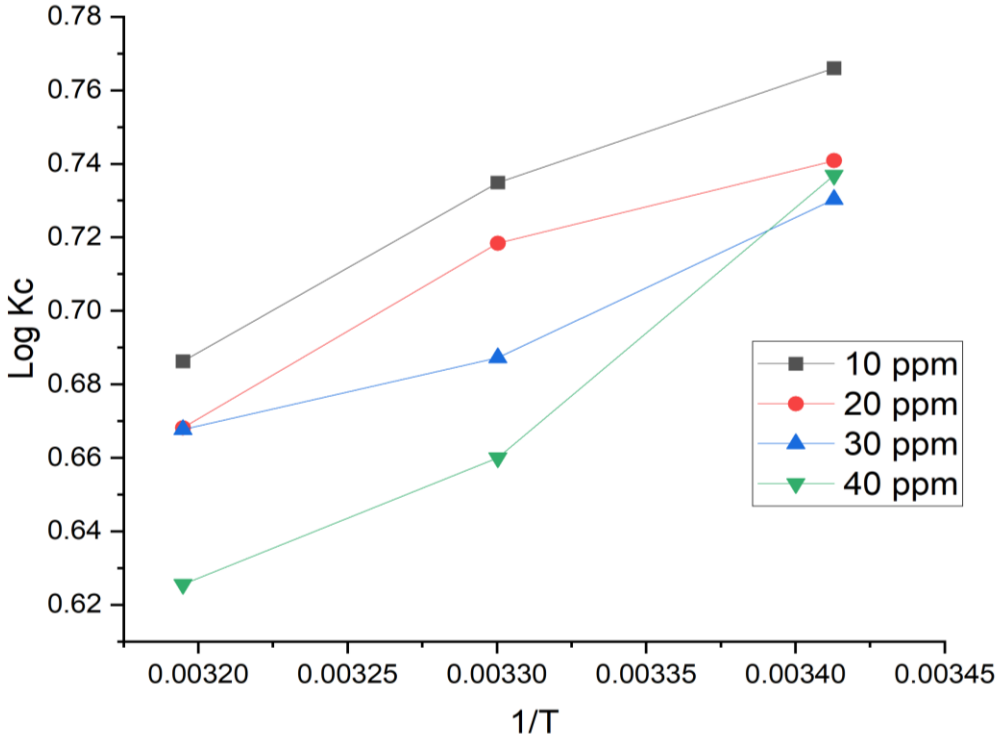
Dye concentration (mg/L)	$-\Delta H^O$ (KJ/mol)	$-\Delta S^O$ (j/mol.k)	$-\Delta G^O$ (KJ/mol)		
			293 K	303K	313 K
10	18.76	44.20	5.808	5.366	4.92
20	18.23	39.98	6.516	6.321	5.92
30	18.20	39.22	6.71	6.32	5.92
40	13.94	25.62	6.434	10.445	10.18

Table (2): Thermodynamic parameter value for MG removal

Dye concentration (mg/L)	$-\Delta H^O$ (KJ/mol)	$-\Delta S^O$ (j/mol.k)	$-\Delta G^O$ (KJ/mol)		
			293 K	303K	313 K
10	13.04	29.31	4.455	4.162	3.86
20	7.974	12.8	4.20	4.07	3.94
30	10.32	21.1	4.12	3.91	3.70
40	12.47	28.09	4.24	3.960	3.68



(a)



(b)

Figure(11): Thermodynamic parameter for algae (a)loaded with MG (b)loaded with CV

3.6 The kinetic model

The study of biosorption kinetic is essential to have a clear idea about the dye biosorption behavior of algae. In the Adsorption mechanism of both dyes by algae and the adsorption process favorability, two kinetics have been studied: pseudo-first-order (PFO) and pseudo-second-order (PSO)pseudo-first-order (PFO) eq:

$$log(q_e - q_t) = log\, q_e - K_1 \frac{t}{2.303} \quad (5)$$

q_e=amount of particle been adsorbed at equilibrium (mg/g), q_t= amount of particle been adsorbed at the time (t) (mg/g), K_1= pseudo-first order sorption equilibrium rate constant (1/min)[21].

pseudo-second-order (PSO) eq:

$$\frac{t}{q_t} = \frac{1}{K_2\, q_e{}^2} + \frac{t}{q_e} \quad (6)$$

K_2= rate constant of pseudo-second-order (g/mg. min)[22].

The pseudo-first-order model suggests that adsorbate's adsorption rate is restricted by physisorption; on the other hand, the pseudo-second-order model suggests that adsorbate's adsorption rate is controlled by chemisorption [23].

The removal of both dyes by algae is best fitted in pseudo-second-order, a finding that is underscored by the remarkably high correlation coefficient (R^2=0.998), as shown in Tables 3 and 4. This result, which aligns with previous studies [10,15,17,18].

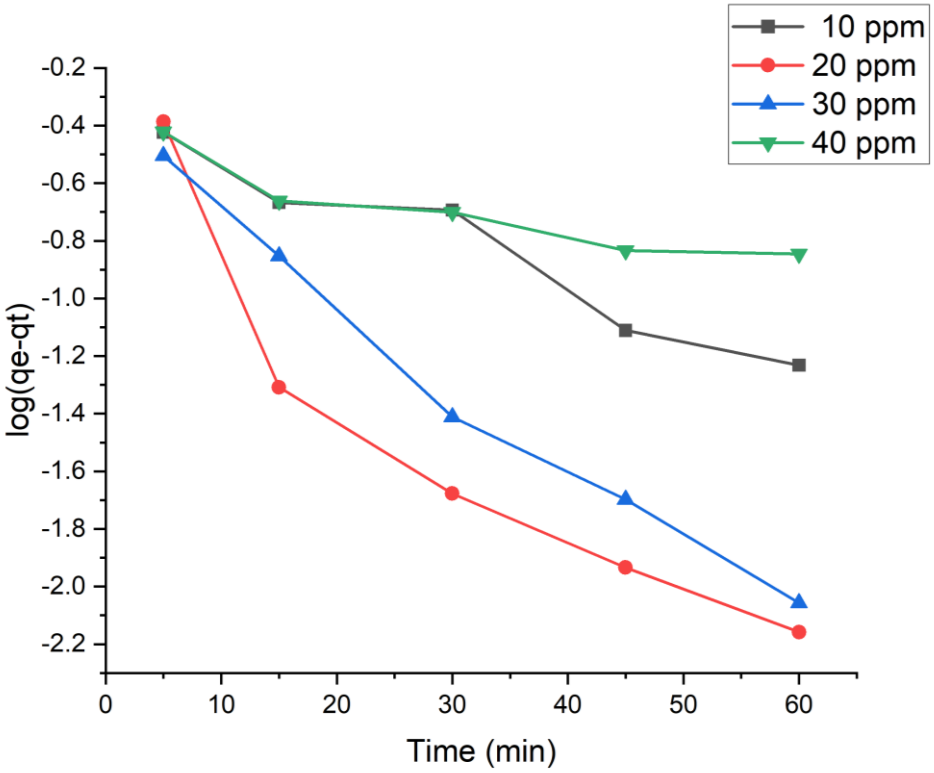
Table (3): Kinetic model parameter value for CV removal

Kinetic model	Parameter	10 ppm	20 ppm	30 ppm	40 ppm
Experimental	q_e	6.67	13.3	15.034	20.1
Pseudo first order	q_e	0.932	2.09	2.763	3.975
	K_1	0.00423	0.0056	0.0018	0.0016
	R^2	0.801	0.6909	0.897	0.915
Pseudo second order	q_e	5.948	11.818	17.52	23.07
	K_2	0.5434	0.24	0.42	0.286
	R^2	0.998	0.999	1	1

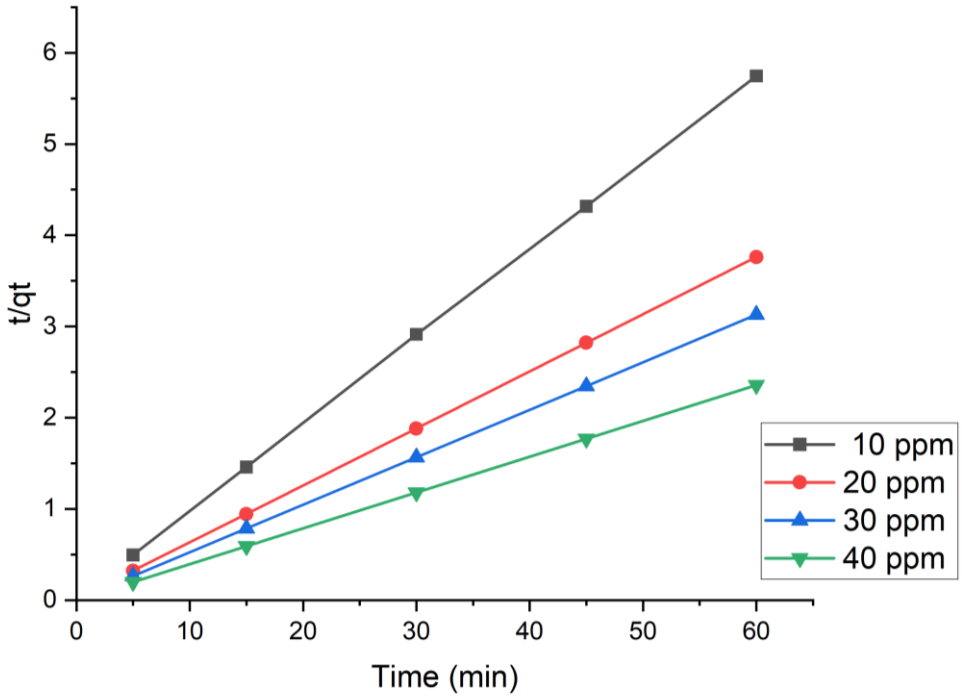
Table (4): Kinetic model parameter value for MG removal

Kinetic model	Parameter	10 ppm	20 ppm	30 ppm	40 ppm
Experimental	q_e	10.67	16.04	19.1962	25.581
Pseudo first order	q_e	0.55	3.487	0.3389	0.319
	K_1	0.0153	0.00132	0.055	0.0179

	R^2	0.9225	0.5193	0.9648	0.8451
Pseudo second order	q_e	10.478	12.787	19.215	25.482
	K_2	0.33	0.76	0.49	0.624
	R^2	1	0.998	1	1

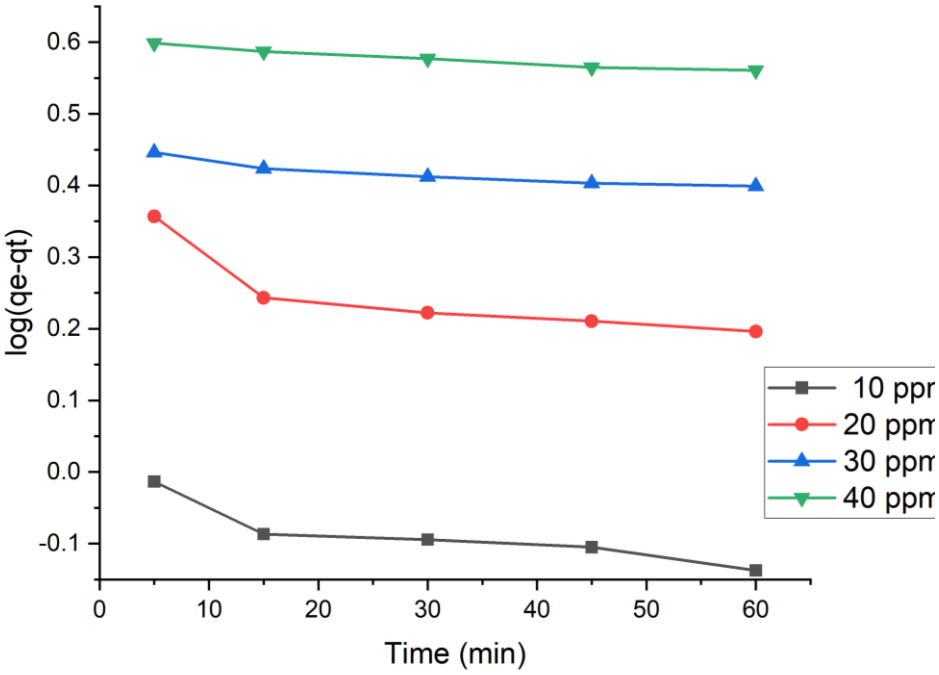


(a)

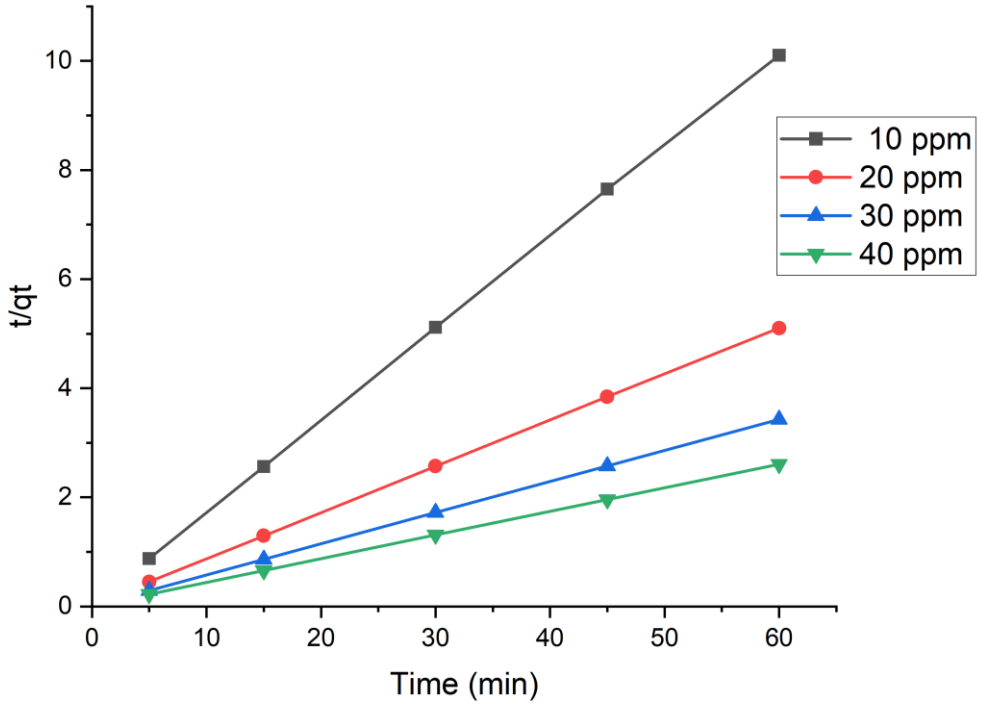


(b)

Figure (12): Kinetic data for CV adsorption a: PFOR b: PSOR



(a)



(b)

Figure (13): Kinetic data for MG adsorption a: PFOR b: PSOR

3.7 Isotherm model

The isotherm model calculation is vital to understanding the type of interaction that occurs between the algae (adsorbent)and dye (adsorbate); three adsorption isotherms have been studied: Langmuir, Freundlich, and Temkin isotherms. The Langmuir isotherm depends on whether the adsorption process is physical or chemical and assumes that each site of adsorbent adsorbs only one adsorbate and that there is no interaction between the surrounding and adsorbent[24], The Langmuir equation:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{q_m K_L C_e} \quad (7)$$

Where q_m maximum monolayer coverage capacity (mg/g), K_L Langmuir isotherm constant (L/mg).

Freundlich isotherm, which assumes the formation of a multilayer of adsorbate on the adsorbent surface, there is interaction between molecules[24] Freundlich equation:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (8)$$

Where K_f indicator of adsorption capacity

Temkin isotherm assumes that the adsorption heat decreases linearly rather than logarithm with increased adsorbent coverage surface, and adsorption is a homogenous distribution of binding energy over an adsorbent surface, Temkin equation[25]:

$q_e = B \ln A_T + B \ln C_e$ (9)

Temkin isotherm equilibrium binding constant (L/g)

B = Constant related to heat of sorption(J/mol).

Tables 5 and 6 show the parameter values of the above equations for both dyes. Those tables show that the best-fitted equation was the Langmuir equation, and the last one was the Freundlich equation for both dyes, which means only one layer of dye was formed on the surface of the algae.

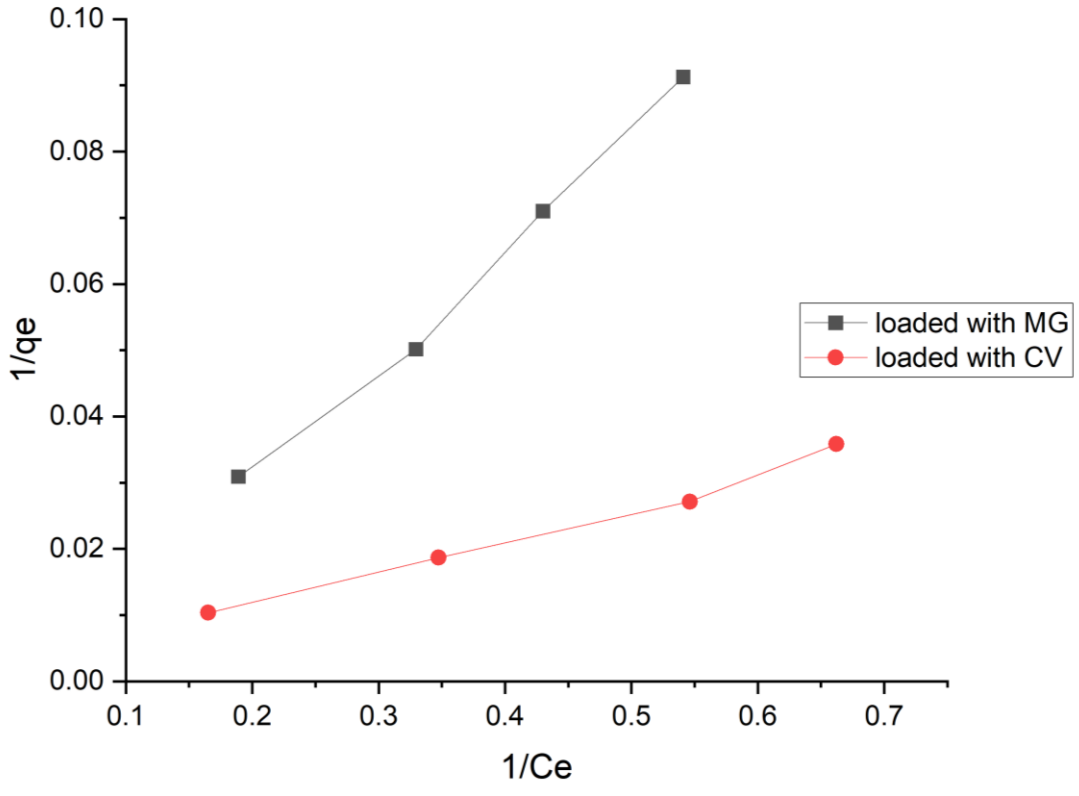
Table (5): Isotherm model parameter value for CV removal

Type of isotherm	Parameters	Value
Langmuir	q_m (µg/g)	605.2028626
	b (L/mg)	0.03330089
	R^2	0.9974
	R_L	0.461780475
Freundlich	K_F	20.75534
	1/n	0.865813791
	R^2	0.8588
Temkin	A	1.132990153
	B	49.10868426
	R^2	0.956

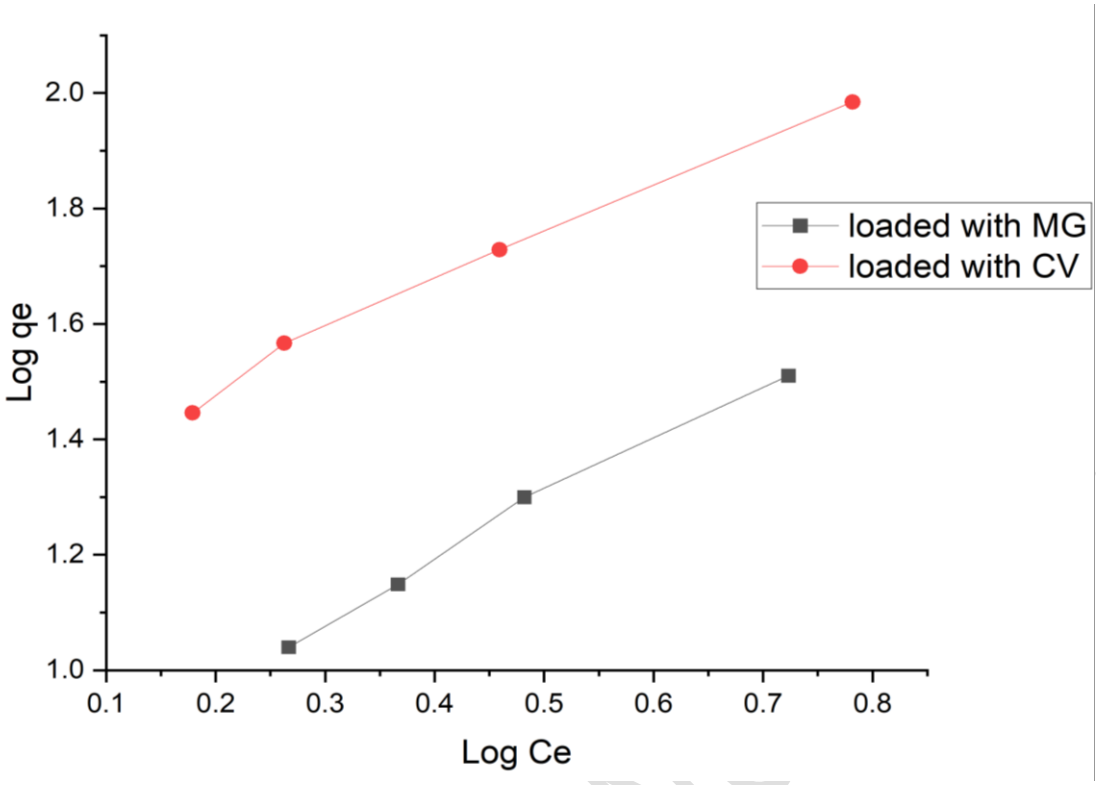
Table (6): Isotherm model parameter value for MG removal

Type of isotherm	Parameters	Value
Langmuir	q_m (µg/g)	261.3373016
	b (L/mg)	0.022044242
	R^2	0.9987

	R_L	0.75150472
Freundlich	K_F	5.95307829
	1/n	1.033141002
	R^2	0.954
Temkin	A	0.87829089
	B	20.82734805
	R^2	0.997



(a)



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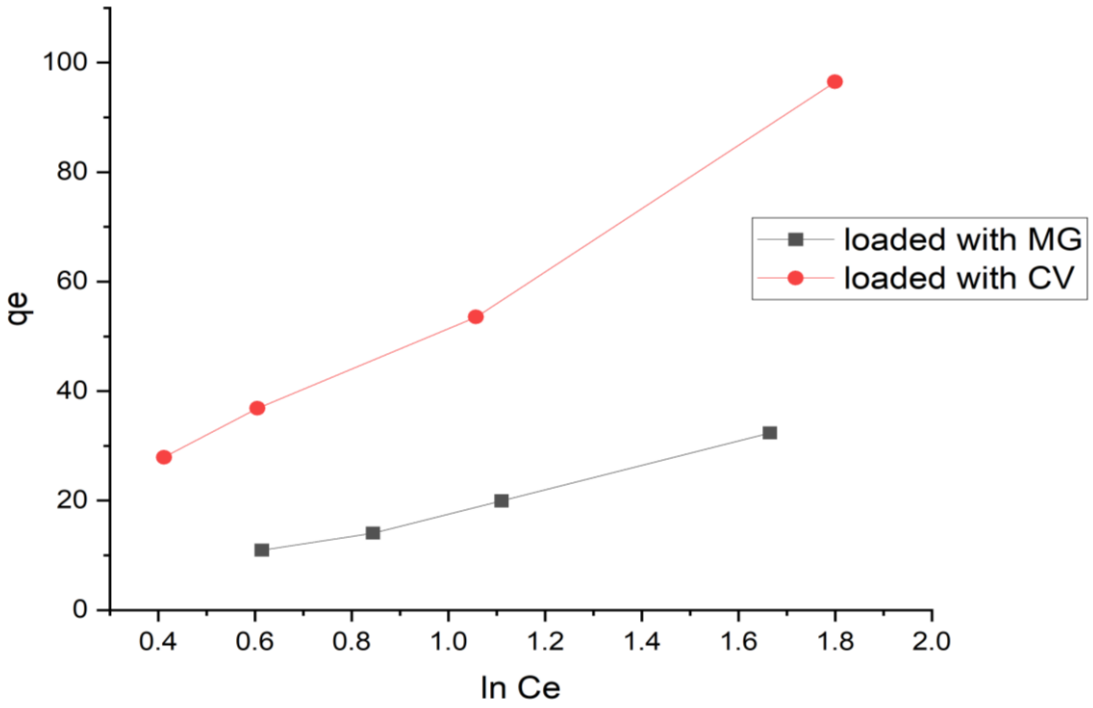


(c)

Figure (14): isotherm data a: Langmuir equation, b: Freundlich equation c: Temkin equation

3.8 Mass transfer adsorption model

The mass transfer adsorption models are based on a gradual adsorption process: (1) adsorption through liquid film attached to the algae surface (adsorbent surface) called film diffusion, (2) diffusion inside the pores or the pore walls called intraparticle diffusion, and (3) the adsorption and desorption between the active site on the surface of adsorbent and adsorbate[26].

The adsorbent surrounding is an important factor in film diffusion, which controls the transfer of the adsorbate from the liquid phase to the surface of the adsorbent, The liquid-film-diffusion model equation is as follows:

$Ln\ (1-F) = -K_{fd}\ t$

Where $F = q_t/q_e$, K_{fd} is The liquid-film-diffusion constant and t is the time[27].

The best model used to describe intraparticle diffusion is the Weber and Moris model, where a change in concentration of adsorbate as function $t^{1/2}$, the Weber and Morris equation is:

$q_t = K_{int}\ t^{1/2}$

where

K_{int} is intraparticle-diffusion-model constant [28].

The rate of the adsorption process is governed by the slowest, the rate-limiting process which is characterized by properties of adsorbent and adsorbate[29].

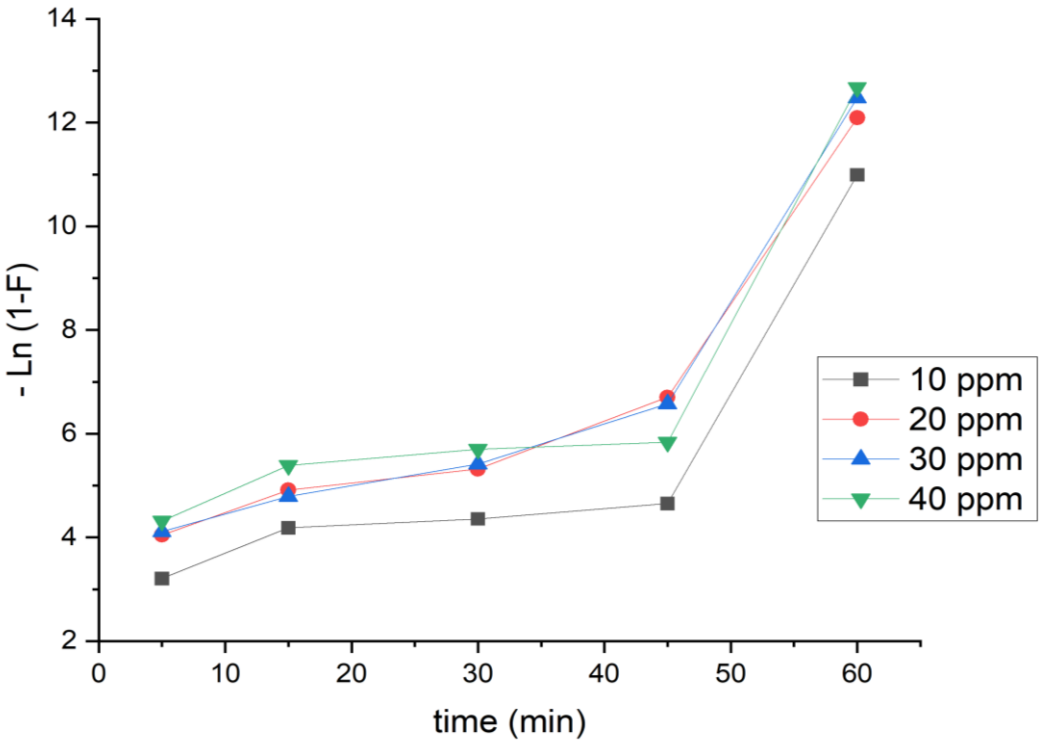
Table (7): mass transfer model parameter for algae (a) loaded with MG (b) loaded with CV

(a)

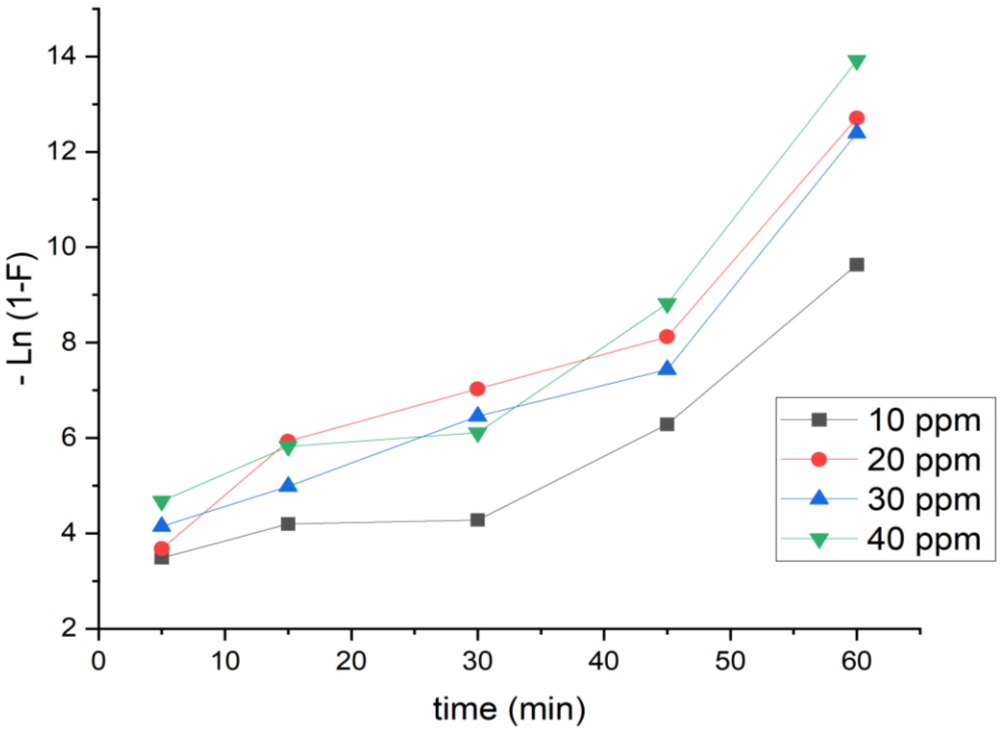
Mass transfer models	Mass transfer model parameter		
Weber and Morris model	Concentration ppm	K_{int}	R^2
	10	0.0071	0.7201
	20	0.00677	0.7144
	30	0.0097	0.7142
	40	0.009	0.7127
Liquid film diffusion model	Concentration ppm	K_{fd}	R^2
	10	0.116	0.9071
	20	0.13	0.9313
	30	0.135	0.9296
	40	0.124	0.9028

Mass transfer models	Mass transfer model parameter		
Weber and Morris model	Concentration ppm	K_{int}	R^2
	10	0.011	0.7186
	20	0.0112	0.7156
	30	0.01	0.7139
	40	0.0075	0.7119
Liquid film diffusion model	Concentration ppm	K_{fd}	R^2
	10	0.104	0.9361
	20	0.1445	0.9482
	30	0.137	0.9432
	40	0.155	0.9426

(b)

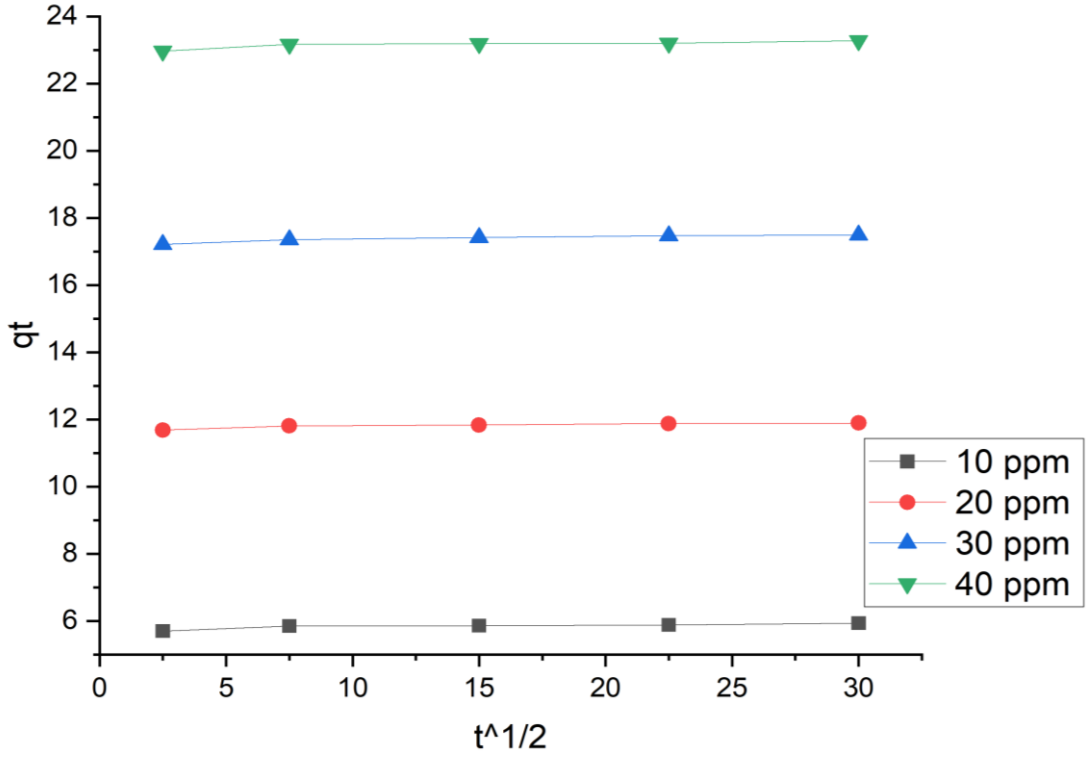


(a)

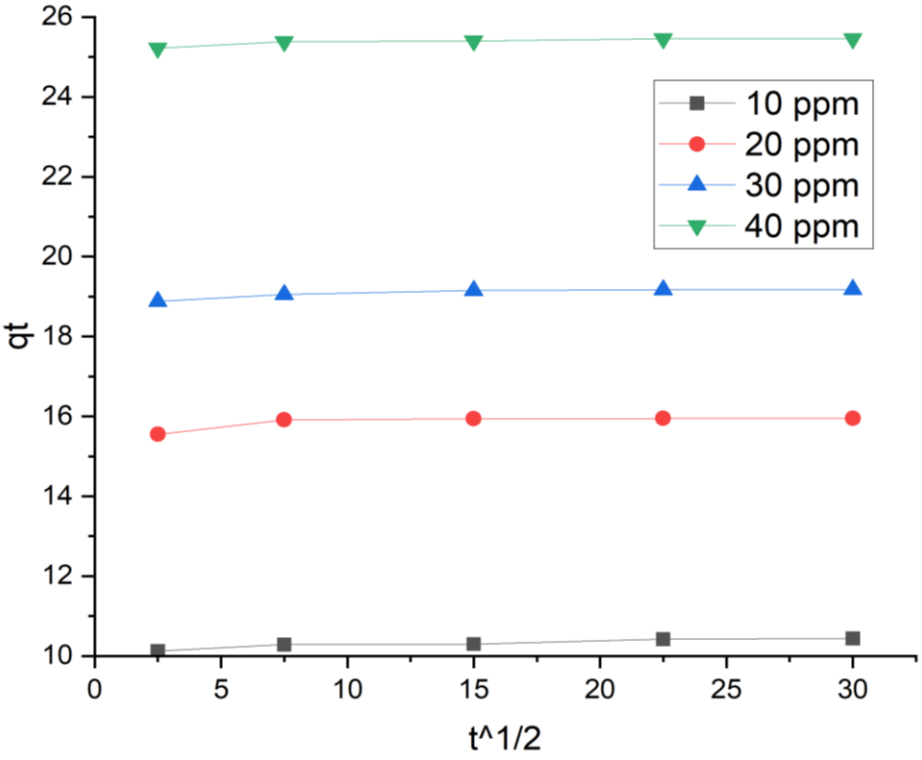


(b)

Figure (15): Liquid film diffusion model for algae (a) loaded with MG (b) loaded with CV



(a)



(b)

Figure (16): Weber and Morris model for algae (a) loaded with MG (b) loaded with CV

4. Model Fitting

Analysis of variance (ANOVA) was used to determine the model's significance and factors that impact the process; four factors were used in Design-Expert, 3- a level factorial design was used, and the model was chosen based on the value of the predicated and the adjusted R^2, the best model to describe the process is a quadratic model with the highest value of the predicated, and the adjusted R^2 for both dyes as shown in table (8), and the response of dye removal % to input factors follow these equations:

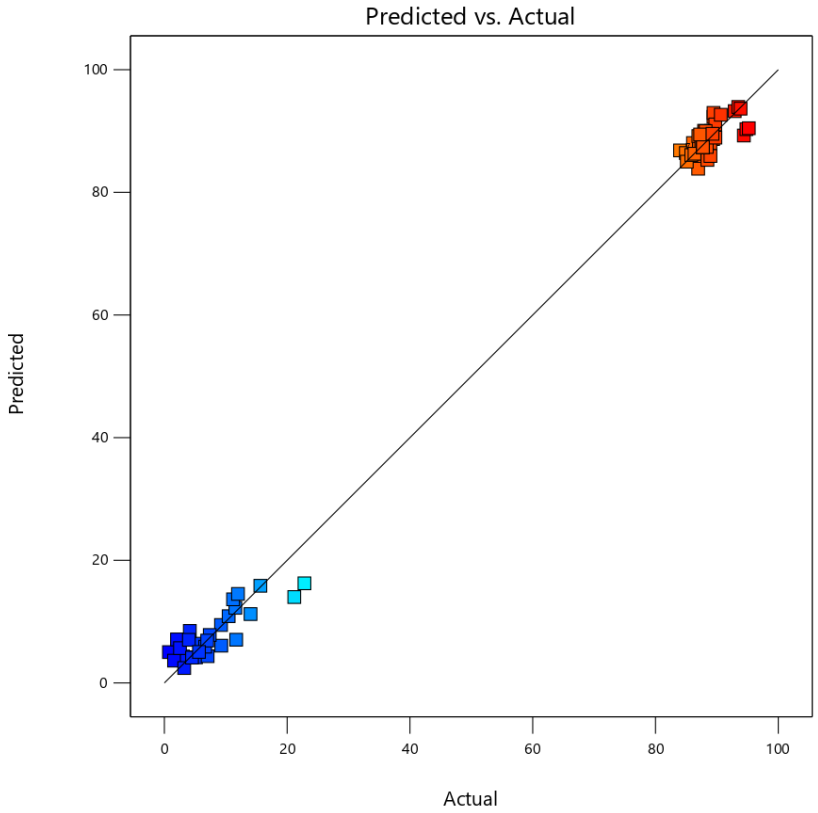
MG dye removal percentage % = -111.827 + 46.4411 * A + 0.808218 * B + -0.645154 * C + 0.16948 * D + -0.0161689 * AB + 0.0587311 * AC + -0.00604525 * AD + -0.00527688 * BC + -9.68414e-05 * BD + -0.0014167 * CD + -2.74704 * A^2 + -0.0105826 * B^2 + 0.00810928 * C^2 + -0.000662551 * D^2

CV dye removal percentage % = -15.2244 + 26.9079 * A + -1.38578 * B + 1.47086 * C + 0.077638 * D + 0.0877879 * AB + -0.122795 * AC + -0.00346363 * AD + 0.013375 * BC + 1.88046e-05 * BD + -0.000492168 * CD + -1.5098 * A^2 + 0.00205635 * B^2 + -0.0148869 * C^2 + -0.000168078 * D^2

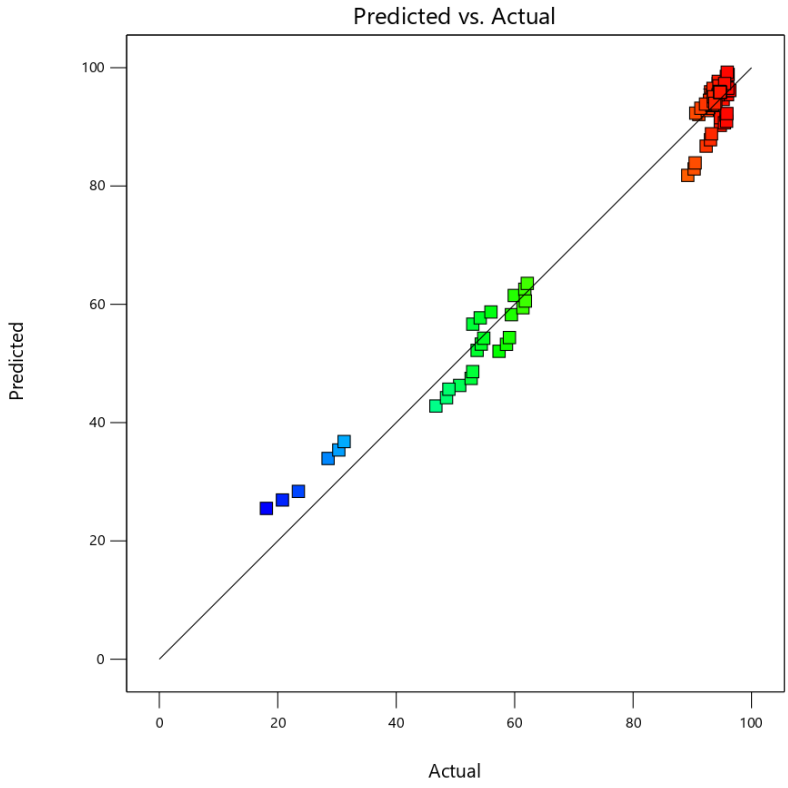
The graph depicting the experimental Vs. The predicted value for both dye removal %, obtained from the 3-level factorial design model, is a key validation tool for our model. As shown in Fig (17), this graph is crucial in obtaining removal values that can't be directly obtained from the model[30]. The 45-degree line in the middle of the graph evenly divides the data points. As shown in Figure (17), the data points are very close to the 45-degree line, indicating a strong connection between the experimental and predicted values. This close alignment confirms that the quadratic model accurately represents the actual value.

The p-value in the model is used to indicate the significance of the model parameter; the p-value for a parameter less than 0.005 means the parameter is significant; there are three general categories: first order (A, B, C, D) and 2-way interaction (AB, AC, AD, BC, BD, CD), and pure-quadratic (A2, B2, C2, D2), from the table () show that linear parameter A, C, and D are significant with p-value <0.0001, 0.0065, and 0.0002 respectively for MG removal, and for CV value A, B, and C are significant with p-value <0.0001 for three-parameter, based on F-value the order of parameter that effects the removal of MG dye: pH(A), Time (D), initial

concentration(C), and Temperature (B), and for CV dye removal: pH(A), initial concentration(C), Temperature (B), and Time (D).



(a)



(b)

Figure (17): the predicated value vs the actual value for (a) MG dye removal and (b) CV dye removal

Table (8): model summary static for (a) MG dye removal (b) CV dye removal

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	16.75	0.8101	0.8008	0.7888	25580.50	
2FI	17.23	0.8137	0.7891	0.7640	28579.68	
Quadratic	**2.44**	**0.9965**	**0.9958**	**0.9949**	**621.01**	**Suggested**
Cubic	1.81	0.9985	0.9977	0.9962	455.51	Aliased

(a)

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	11.06	0.7668	0.7554	0.7361	11346.50	
2FI	10.25	0.8143	0.7898	0.7549	10539.60	
Quadratic	**3.53**	**0.9791**	**0.9751**	**0.9679**	**1378.96**	**Suggested**
Cubic	1.67	0.9963	0.9944	0.9903	416.47	Aliased

(b)

Table (9): ANOVA analytical for (a) MG dye removal (b) CV dye removal

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.207E+05	14	8620.86	1446.29	< 0.0001	significant
A-pH	86086.82	1	86086.82	14442.44	< 0.0001	
B-Temperature	23.64	1	23.64	3.97	0.0502	
C-Initial Concentration	46.92	1	46.92	7.87	0.0065	
D-Time	95.22	1	95.22	15.98	0.0002	
AB	11.61	1	11.61	1.95	0.1672	
AC	357.37	1	357.37	59.95	< 0.0001	
AD	12.30	1	12.30	2.06	0.1551	
BC	23.39	1	23.39	3.92	0.0514	
BD	0.0256	1	0.0256	0.0043	0.9479	
CD	12.79	1	12.79	2.14	0.1474	
A^2	21308.90	1	21308.90	3574.91	< 0.0001	

B^2	22.12	1	22.12	3.71	0.0580	
C^2	49.94	1	49.94	8.38	0.0050	
D^2	4.86	1	4.86	0.8159	0.3694	
Residual	429.17	72	5.96			
Lack of Fit	429.17	66	6.50			
Pure Error	0.0000	6	0.0000			
Cor Total	1.211E+05	86				

(a)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	42102.84	14	3007.35	241.32	< 0.0001	significant
A-pH	29414.72	1	29414.72	2360.37	< 0.0001	
B-Temperature	672.22	1	672.22	53.94	< 0.0001	
C-Initial Concentration	1189.47	1	1189.47	95.45	< 0.0001	
D-Time	42.13	1	42.13	3.38	0.0701	
AB	342.18	1	342.18	27.46	< 0.0001	
AC	1562.22	1	1562.22	125.36	< 0.0001	
AD	4.04	1	4.04	0.3241	0.5709	
BC	150.27	1	150.27	12.06	0.0009	
BD	0.0010	1	0.0010	0.0001	0.9930	
CD	1.54	1	1.54	0.1238	0.7260	
A^2	6436.75	1	6436.75	516.51	< 0.0001	
B^2	0.8354	1	0.8354	0.0670	0.7964	
C^2	168.29	1	168.29	13.50	0.0005	
D^2	0.3130	1	0.3130	0.0251	0.8745	
Residual	897.26	72	12.46			
Lack of Fit	897.26	66	13.59			

Pure Error	0.0000	6	0.0000			
Cor Total	43000.09	86				

(b)

Conclusion

The potential use of C.Vulagris as biosorbent for removing two toxic dye has been studied, and result show that C.Vulagris has remarkable performance for removing CV and MG dye, the article study different factor (Initial concentration, algae dosage, pH, Airflow rate, Temperature, and Contact time), and optimum is occur at pH 7, airflow rate 20 ml/min, temperature 20 ^{o}C, contact time 30 min for both dyes, the C.Vulgaris was characterized by FT-IR, SEM, EDS, and pHzpc, the FT-IR test show that the surface of C.Vulgaris contain several functional group that are responsible on dye adsorption, SEM show that there is change in morphology of algae surface before and after adsorption process the algae was coated with both dyes, the EDS show that there is increase in C element on surface of algae that approved the adsorption was occurred since both dyes contain C in their structure, pHzpc show that when pH >4.8 the algae surface become negatively charge, both Kinetic and isotherm model assessment have been carried out, and show the pseudo-second order and Langmuir equation was best fitted for adsorption process.

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