

# Phycoremediation of Phenolphthalein, Indigo Carmine, Methyl Orange and Eriochrome Black T By Using *Chlamydomonas* and *Chlorella* sp. (Chlorophyta)

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## Abstract

This work aimed to evaluate the decolorization of phenolphthalein (PP), indigo carmine (IC), methyl orange (MO) and eriochrome black T (EBT) by using *Chlorella* and *Chlamydomonas* as a biosorbent. This experiment used the batch adsorption method to study the adsorption effect of pH, biomass dosage, contact time, initial dye concentration and temperature on the dyes. The adsorption of the dyes onto the cells were fitted with the Langmuir and Freundlich isotherms. The results showed that the optimum conditions for PP removal (99%) was obtained at pH 4, contact time of 120min, 35°C, algal dosage of 0.5 mg/mL and dye concentration of 100 mg/L on to the cells. The sample prepared at 25°C, 120 min, 1 mg/mL algal dosage, 25 mg/L dye concentration and at pH 4 had the best adsorption effect on IC on to the both of cells (99-98%). In addition, the optimum conditions for MO and EBT removal (99-97%) was obtained at pH 2-4, contact time of 30 min, 25°C, algal dosage of 0.5mg/mL and dye concentration of 100mg/L. The results of this study reveal that the algae cells can be utilized as an efficient and eco-friendly biosorbent for the removal of the indicator dyes from aqueous solution.

## Introduction

Dyes used by various industries such as paper, leather, textile, pulp, food, plastic, cosmetics and rubber cause the production of dye-laden wastewater with pretreatment or without treatment directly released into the environment. Therefore, it has become inevitable to protect the environment from such pollutants and their negative effects due to the toxic, irritating even carcinogenic nature of the dyes. (Rafatullah et al. 2010; Yagub et al. 2012; Yagub et al. 2014).

Removing dyes from contaminated water is a difficult process because the aromatic structures of dyes are stable against light, heat and oxidizing agents (Chequer et. al. 2013; Brahmbhatt & Jasrai 2016). Many physical and chemical methods such as flotation,

electroflocculation, chemical flocculation, electrokinetic coagulation, electrochemical destruction, ion-exchange, irradiation, ozonation, katox, adsorption, chemical precipitation, photolysis, chemical oxidation and reduction and membrane filtration are applied for dye removal. These combined or unilateral techniques are often expensive. They also use activated carbon and air mixtures, which are ineffective methods for dye removal (Gupta & Suhas 2009). However, these are not advantageous due to low removal and more secondary sludge (Akceylan et al. 2009). Due to these limitations, the bioremediation method, which uses living things such as bacteria, yeast, fungi and micro-macroalgae, is an innovative physicochemical treatment technology to purify pollutants by breaking them down or converting them into less harmful forms. In addition, one of the main reasons why biological methods are preferred over

traditional treatment methods used in the treatment of dye-contaminated wastewater is their low cost and the absence of the possibility of producing secondary metabolites (eco-friendly). Similarly, the phycoremediation method using micro-macroalgae (especially living or non-living microalgae as photosynthetic phytoplankton like diatoms, dinoflagellates, green, yellow-brown algae and cyanobacteria) has advantages such as low cost, widely available resources, and high absorption capacity (Bhuyar et al., 1990; Abad et al. 2011; Abatenh et al. 2017; Bhuyar et al. 2020).

It has been suggested that the removal, which is generally carried out using a non-living or living algae biomass, is a more environmentally friendly study since it occurs in the cell wall and is a method independent of metabolism (such as physical and chemical adsorption, electrostatic interaction, ion exchange, complexation, chelation and microprecipitation). Studies have found that bioremediation using dead cells is more advantageous because they are not affected by toxic substances, do not need care and nutrition, and can be stored and reused (Aksu 2005).

In this study, the removal of phenolphthalein (PP), indigo carmine (IC), methylene orange (MO) and eriochrome black t (EBT) dyes, which are widely used as indicators in the textile, pharmaceutical and chemical industries, was investigated onto *Chlorella sp.* and *Chlamydomonas sp.* cells (single-celled green microalgae). This investigation provides comprehensive information on the effect of various parameters such as pH, initial dye concentration, temperature, contact time, dose of algal biomass, contact time and temperature on the absorption of dyestuff from aqueous solution. The adsorption of the dyes onto *Chlorella sp.* and *Chlamydomonas sp.* were fitted with the Langmuir and Freundlich isotherm models.

## Materials & Methods

### Materials

#### Cultivation and Preparation of Alga

*Chlorella* and *Chlamydomonas* cells were obtained from Culture Collection of Algal Biotechnology Department (Ege University, Izmir, Türkiye). Stock cultures were cultivated in BG-11 medium (Vaiciulyte et al. 2014), consists of macronutrients (1.5 g NaNO<sub>3</sub> L<sup>-1</sup>, 0.04 g K<sub>2</sub>HPO<sub>4</sub> 3H<sub>2</sub>O L<sup>-1</sup>), inorganic salts (0.036 g CaCl<sub>2</sub>·7H<sub>2</sub>O L<sup>-1</sup>, 0.075 g MgSO<sub>4</sub>·7H<sub>2</sub>O L<sup>-1</sup>, 0.02 g Na<sub>2</sub>CO<sub>3</sub> L<sup>-1</sup>), pH conditioners (0.001 g Na<sub>2</sub>EDTA L<sup>-1</sup>, 0.006 g C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> L<sup>-1</sup>, 0.006 g C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>·xFe<sup>3+</sup>·yNH<sub>3</sub> L<sup>-1</sup>), and trace elements (0.222 g ZnSO<sub>4</sub>·7H<sub>2</sub>O L<sup>-1</sup>, 1.81 g MnCl<sub>2</sub>·4H<sub>2</sub>O L<sup>-1</sup>, 0.390 g Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O L<sup>-1</sup>, 0.079 g CuSO<sub>4</sub>·5H<sub>2</sub>O L<sup>-1</sup>, 2.86 g H<sub>2</sub>BO<sub>3</sub> L<sup>-1</sup>, and 0.0494 g Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O L<sup>-1</sup>). After the BG-11 medium was sterilized in autoclave at 120°C for 15 minutes, the organism were planted in the medium. An orbital shaker with 100 rpm was used to maintain

culture in turbulent conditions at 26±1°C for 15 days under 16:8 h of light–dark cycle and white fluorescent lamps (20 E m<sup>-2</sup> s<sup>-1</sup>±20%). The growth of the cells was regularly monitored spectrophotometrically at 660 and 730 nm. After lag phase, the algal cells got into their logarithmic growth phases. The washed biomass was shade dried at room temperature for 10 days, and the dried alga was pulverised to fine powder and stored in air tight container for further studies.

### Dyes

#### Test Substances

All the chemicals used were of pure analytical grade and were purchased from Riedel, except for indigo carmin and metilen orange, which were from Merck, and from Fluca, respectively. The indicators were prepared as described by Mendham et al. (2002).

#### Phenolphthalein (PP)

PP, an indicator dye commonly used in acid-base titrations, standardization of solutions, or determination of alkalinity, is colorless at pH less than or equal to 8, red-pink color between 8 and 10, and colorless at higher than pH 10 (Kunimoto et al. 2001; Mendham et al. 2002; Rice et al. 2012).

A total of 250 mL of pre-cultivated medium at 15th day was mixed with PP. Here, the concentration of PP was varied from 25 up to 100 mg/L. After that, the dye removal efficiency was measured by using UV–vis spectrometer with wavelength of 665 nm. A calibration curve was generated to determine the correlation between the dye concentration and the absorbance value, and the mass concentration was calculated.

#### Indigo Carmin (IC)

IC, widely used in textile, cosmetics, medicine, plastic and paper mills, is a highly toxic indigoid indicator dye (Saurin 2009; Himanshu & Vashi 2010). The IC solution with a mass concentration of 1,000 mg/L was configured with ultrapure water and placed in a brown volumetric flask for use. A total of 250 mL of pre-cultivated medium at 15th day was mixed with IC. Here, the concentration of IC was varied from 25 up to 100 mg/L. The absorbance of IC was measured using an ultraviolet (UV) spectrophotometer at 600 nm, and the mass concentration was calculated.

#### Methyl Orange MO

MO, similar to phenolphthalein, is a sulfonated azo inductor dye used in acid-base titrations, standardization of solutions or determination of alkalinity in textile, pharmaceutical, printing, paper manufacturing, food industries and research laboratories. MO indicator dye is a non-biodegradable

sulfonated azo dye that has a red acid form at pH values below 2.9 and converts to an orange basic form at pH values above 4.6 (Mendham et al. 2002; Al Hamadi et al. 2017; Thiyagarajan et al. 2020).

The MO solution with a mass concentration of 1,000 mg/L was configured with ultrapure water and placed in a brown volumetric flask for use. A total of 250 mL of pre-cultivated medium at 15th day was mixed with MO. Here, the concentration of MO was varied from 25 up to 100 mg/L. The absorbance of MO was measured using an ultraviolet (UV) spectrophotometer at 464 nm, and the mass concentration was calculated.

### Eriochrome Black T (EBT)

EBT, which is blue in its protonated form and red when it forms a complex with calcium or other metals, is an azo inductor dye used in the quantity determination of some metals such as Mn, Cd, Hg, Pb (Mendham et al. 2002).

The adsorption studies was prepared by dissolving 1g of EBT dye in 1000 mL of distilled water to get a dye solution of 1000 ppm. A total of 250 mL of pre-cultivated medium at 15th day was mixed with EBT. Here, the concentration of EBT was varied from 25 up to 100 mg/L. The residual concentration of the EBT dye was estimated by determining the absorbance at maximum wavelength of 555 nm using UV spectrophotometer, and the mass concentration was calculated.

### Determination of Dry Weight

A definite volume (10 mL) of algal suspension was filtered through cellulose acetate filter membrane (47mm in diameter, 0.22  $\mu\text{m}$  in pore size) and dried overnight in an oven at 105°C. Data were given as  $\text{mg mL}^{-1}$  algal suspension.

### Growth Estimation

Growth was estimated in each sample by measuring the biomass turbidity of SP homogenized suspension at wave length 660 nm and 730 nm using spectrophotometer (Cary 50 UV-Vis spectrophotometer, Agilent Technologies, USA), it was expressed in dry mass per liter of suspension (Seely et al. 1972).

### Batch Adsorption Experiment

Experiments were conducted with 250 mL Erlenmeyer flasks containing 100 mL of aqueous solution mixed with dyes. Flasks were agitated on a rotary shaker operating at 100 rpm and 26 °C. Batch experiments were performed with different concentrations of physicochemical variables such as pH (2, 4, 6 and 8, adjusted by the addition of 1 mol/L HCl or 1 mol/L NaOH solutions, respectively), initial dye

concentration (25, 50 and 100 mg/L), contact time (30, 60, 120 and 240 min), biomass dosage (0.5, 1 and 2 mg/mL) and temperature (25, 35 and 45 °C) on adsorption were examined after the equilibrium was reached. Finally, specimens were centrifuged for 15 min at a speed of 10000 rpm in order to estimate the dye concentration. The concentrations of the two dyes in the aqueous solution were measured from UV-vis spectrophotometer in order to calculate the maximum absorption.

### Data Analysis

The adsorption effect is expressed by the adsorption capacity ( $q_e$ , mg/g; Eq.1) and removal rate (%; Eq.2). The calculation method is:

$$q_e \text{ (mg/g)} = \frac{(C_0 - C_e) * V}{m} \quad (1)$$

where,  $C_0$  and  $C_e$  ( $\text{mg L}^{-1}$ ) represent at initial and at  $t$  time concentrations of dyes in the solution, respectively.  $V$  (L) is the volume of solution,  $m$  (g) is the mass of adsorbent.

$$\text{Removal efficiency (\%)} = \frac{(C_0 - C_e) * 100}{C_0} \quad (2)$$

where  $C_0$ , the concentration of the initial dyes,  $C_e$ , the final dyes concentration (Deniz & Saygideger 2010). All treatments were repeated three times independently, and the average value was used as the measurement result.

### Adsorption Isothermal

The adsorption isotherms were fitted using the Langmuir isotherm equation and Freundlich isotherm equation. The Langmuir adsorption (Langmuir 1916) isotherm assumes that the dyestuff molecules are adsorbed in a single layer (homogeneous points) on the surface of the solid, while the Freundlich adsorption isotherm assumes that they are adsorbed in multiple layers (heterogeneous points). As a result, the system reaches equilibrium when all points on the surface are filled with dye molecules (Freundlich 1906). The linear form of Langmuir and Freundlich adsorption isotherms is given in equations 3 and 4.

$$q_e = q_m k_L C_e / (1 + k_L C_e) \quad (3)$$

$$q_e = k_f C_e^n \quad (4)$$

where  $q_e$  is the amount of biosorbed dye at equilibrium ( $\text{mg g}^{-1}$ ),  $q_{\text{max}}$  is the maximum biosorption capacity at equilibrium ( $\text{mg/g}$ ),  $C_e$  is the dye concentration at equilibrium ( $\text{mg L}^{-1}$ ) and  $K_L$  is the Langmuir constant ( $\text{L mg}^{-1}$ ). The fitness of isotherm model describing the type of adsorption was determined by regression correlation coefficient value

( $R^2$ ). The higher  $R^2$  value (near to unity) represents the fitness of the isotherm model. A dimensionless constant separation factor ( $K_L$ ) of Langmuir isotherm was used to determine the favourability of the biosorption process. The  $K_L$  values between 0 and 1 indicate the shape of isotherm to be either favourable ( $0 < K_L < 1$ ) or unfavourable ( $K_L > 1$ ) or linear ( $K_L = 1$ ) or irreversible ( $K_L = 0$ ) (Su, 2011; Igwegbe et al., 2019).

where  $K_F$  is the Freundlich constant related to adsorption capacity of adsorbent [ $(\text{mg g}^{-1}) (\text{mg L}^{-1})^{-1/n}$ ] and  $n$  is the biosorption intensity (affinity of the adsorbate for adsorbent). The value of  $n$  falling in the range 0–1 indicates favourable sorption.

$C_e/q_e$  and  $C_e$  graphs were drawn using experimental data to find the Langmuir isotherm model parameters and regression coefficient.

## Results & Discussion

### Effect of pH on Dye Removal

In this title of the study, 0.5 g adsorbent, 26°C, 30 min. and 100 mg/L initial dye concentration were taken as constant. The effect of pH on adsorption capacity was examined at variable intervals, including pH 2, 4, 6 and 8. The effect of dye removal at different pH ranges on the absorption of *Chlorella* and *Chlamydomonas* is given in Table 1.

pH is one of the most important parameters affecting algal biosorption efficiency because it affects the color and solubility of some dyes (El-Naggar et al. 2018). Since the biosorbent surface contains many functional groups such as carboxyl, hydroxyl, amino and phosphates, the pH change in the aquatic environment will affect the tendency of the biosorbent to bind to the dye. As a result, if the pH decreases, the biosorbent surface has more positively charged regions, which are favorable for the adsorption of dye anions due to electrostatic attraction (Sun et al. 2019). Conversely, at higher pH, the biosorbent surface binds with cationic dyes (Ali 2010). Since the inductor dyes (IC, MO, EBT) used in the study are in the anionic dye class, it is possible that the dye binding capacity will decrease as the pH increases.

In this study, *Chlorella* sp. observed that PP dye uptake decreased from 92.8% to 80.3% with increasing pH from 2.0 to 8.0. Similarly, it was determined that dye removal in *Chlamydomonas* cells decreased with increasing pH from 99.4% to 97.2%. However, the removal rate and adsorption capacity of PP on *Chlamydomonas* cells did not change considerably from pH 2.0 to 8.0 (Figure 1). In the IC dye removal of *Chlorella* cells, the removal rate gradually decreased from 0.35 to 0.30 mg/g as the pH of the reaction solution increased from 4.0 to 8.0, and the adsorption capacity decreased from 93.6% to 91.6%. has declined. A similar situation was observed for *Chlamydomonas* cells and the other dye (MO and EBT) uptake experiments. For MO dye uptake study on *Chlorella* cells, when the initial pH of the reaction solution was less than 4.0, with the increase of pH from 2.0 to 8.0, the removal rate decreased gradually, from 99.0% to 72.5%.

### Effect of Initial dye Concentration on Dye Removal

In this part of the experiment, 0.5 g adsorbent, 26°C, 30 min. and pH 2 were taken as constant. The effect of initial dye concentration on adsorption capacity was examined at variable intervals, including 25, 50 and 100 mg/L. The effect of dye removal at different concentration on the absorption of *Chlorella* and *Chlamydomonas* is given in Table 2.

It was determined that the adsorption capacity of both *Chlorella* and *Chlamydomonas* cells increased as a result of increasing the initial concentration in PP dye removal (Figure 2). This can be explained by the fact that the active binding points on the surfaces of both organisms are filled as the dye concentration increases and the reaction reaches equilibrium. However, as the initial concentration of IC, MO and EBT dyes increased, the dye removal efficiency on the cells slowly decreased. The adsorption rate of these dyes is high at low concentrations. This can be attributed to the greater number of vacant active sites present in the adsorbent. However, at high initial concentration, the decrease in adsorption capacity could possibly be due to the decrease in active adsorption sites on the adsorbent surface (Khan et al. 2015).

**Table 1.** The effect of dye removal at different pH ranges on the absorption of *Chlorella* and *Chlamydomonas*

pH	PP dye uptake (%)		IC dye uptake (%)	
	<i>Chlorella</i> sp.	<i>Chlamydomonas</i> sp.	<i>Chlorella</i> sp.	<i>Chlamydomonas</i> sp.
2	88,21	98,67	90,75	87,21
4	<b>92,84</b>	<b>99,40</b>	<b>93,62</b>	<b>91,47</b>
6	80,32	98,53	89,09	84,82
8	87,97	99,23	81,64	82,23

pH	MO dye uptake (%)		EBT dye uptake (%)	
	<i>Chlorella</i> sp.	<i>Chlamydomonas</i> sp.	<i>Chlorella</i> sp.	<i>Chlamydomonas</i> sp.
2	<b>99,01</b>	95,62	<b>68,47</b>	<b>61,18</b>
4	98,72	90,02	62,10	59,96
6	97,31	<b>97,74</b>	65,45	64,42
8	72,51	87,53	50,95	54,62

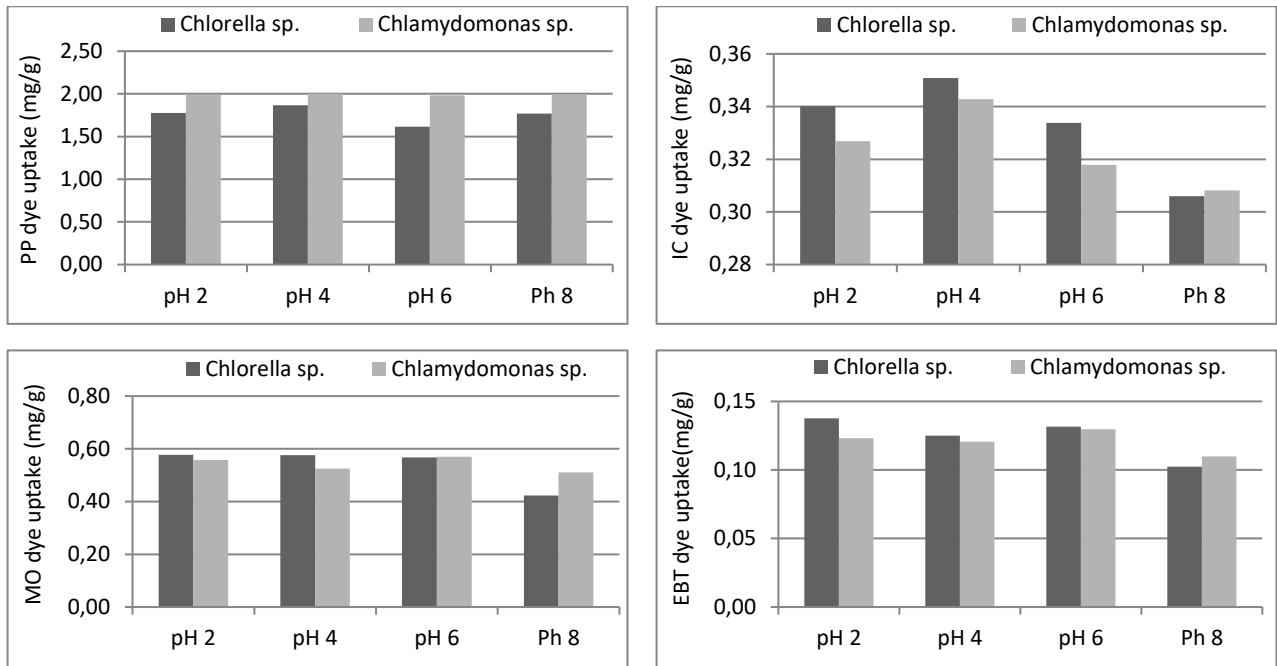


Figure 1. The effect of dye removal at different pH ranges on the absorption of *Chlorella* and *Chlamydomonas*

Table 2. The effect of dye removal at different concentration on the absorption of *Chlorella* and *Chlamydomonas*

dye conc. (mg/L)	PP dye uptake (%)		IC dye uptake (%)	
	<i>Chlorella</i> sp.	<i>Chlamydomonas</i> sp.	<i>Chlorella</i> sp.	<i>Chlamydomonas</i> sp.
25	98,48	98,19	<b>98,05</b>	<b>97,70</b>
50	98,28	98,22	85,63	77,68
100	<b>98,56</b>	<b>98,56</b>	62,29	53,44

dye conc. (mg/L)	MO dye uptake (%)		EBT dye uptake (%)	
	<i>Chlorella</i> sp.	<i>Chlamydomonas</i> sp.	<i>Chlorella</i> sp.	<i>Chlamydomonas</i> sp.
25	<b>97,79</b>	<b>97,27</b>	<b>97,42</b>	<b>97,02</b>
50	92,33	87,57	91,64	79,29
100	69,98	73,24	90,90	60,43

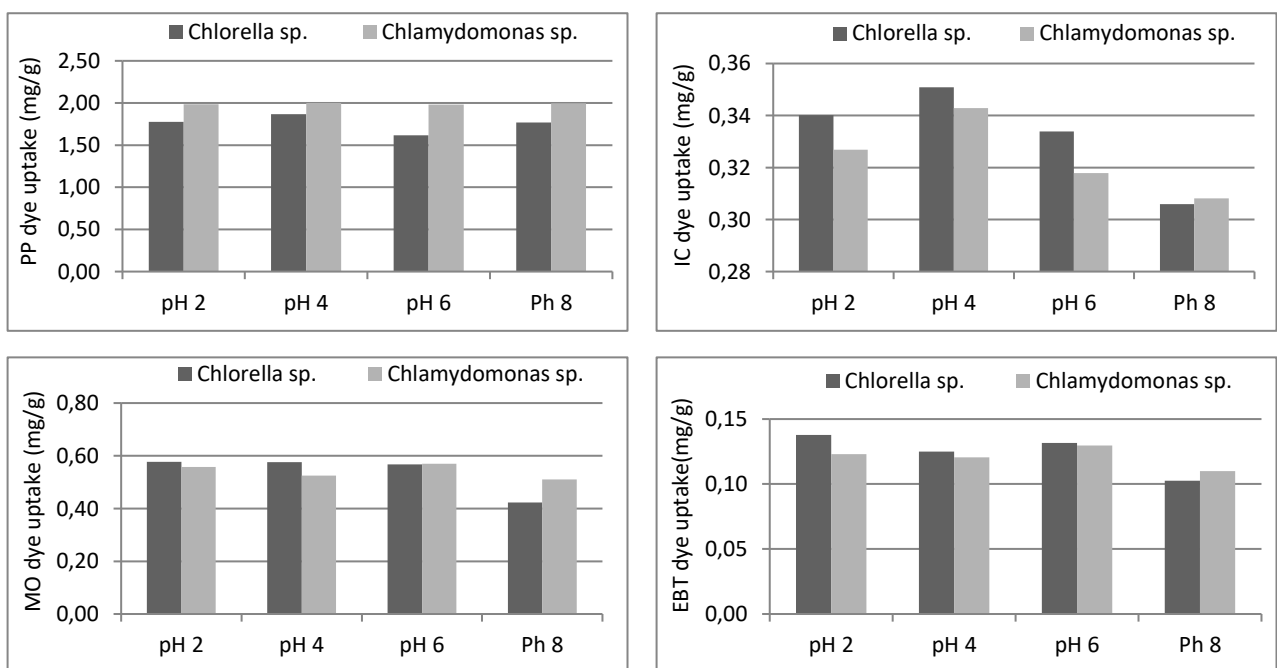


Figure 2. The effect of dye removal at different concentration on the absorption of *Chlorella* and *Chlamydomonas*

**Effect of Biomass Dosage on Dye Removal**

In this part of the study, pH 2, 26°C, 30 min. and 100 mg/L initial dye concentration were taken as constant. The effect of biomass dosage on adsorption capacity was examined at variable intervals, including 0.5, 1 and 2 mg/mL. The effect of dye removal at different adsorbent dosage on the absorption of *Chlorella* and *Chlamydomonas* is given in Table 3.

It was determined that as the amount of adsorbent increased from 0.5 mg/mL to 2 mg/mL, the adsorption capacity of all dyes used in the experiment on both *Chlorella* and *Chlamydomonas* cells decreased. When the concentration of *Chlorella* biomass dosage in the solution was 0.5 mg/mL, the adsorption amount of MO decreased from 0.44 mg/g to 0.12 mg/g, and the removal rate increased from 74.4% to 95.0%. A similar situation was observed in *Chlamydomonas* cells (Figure 3). This decrease in adsorption capacity may be due to the increase of unsaturated adsorption sites. As the amount of adsorbent increases, the surface area

also increases. As similar results have been reported in the literature, it has been determined that as the amount of dye adsorbed per unit surface area decreases, the adsorption capacity also decreases (Noorimotlagh et al. 2014; Tran et al. 2020; Munir et al. 2020; Bounaas et al. 2021).

**Effect of Contact Time on Dye Removal**

In this part of the study, 0.5 g adsorbent, pH 2, 26°C, and 100 mg/L initial dye concentration were taken as constant. The effect of contact time on adsorption capacity was examined at variable intervals, including 30, 60, 120 and 240 min. The effect of dye removal at different contact time on the absorption of *Chlorella* and *Chlamydomonas* is given in Table 4.

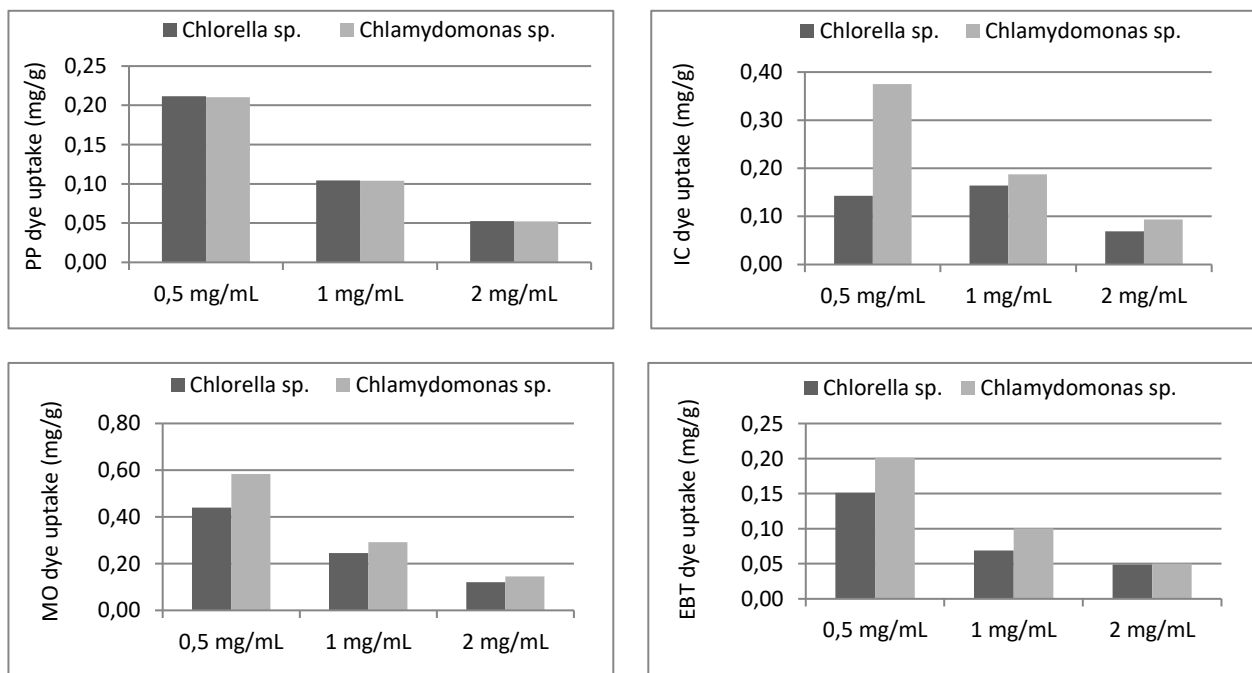
The adsorption capacity of PP and IC dyes increased slowly with the increase of contact time from 30 minutes to 120 minutes. At the 120th minute, the adsorption of PP dye reached equilibrium at 2.0091 and 2.0081 mg/g while adsorption of IC dye was 0.3060 and

**Table 3.** The effect of dye removal at different adsorbent dosage on the absorption of *Chlorella* and *Chlamydomonas*

Biomass dosage (mg/mL)	PP dye uptake (%)		IC dye uptake (%)	
	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>
0,5	99,93	99,42	46,85	38,18
1	98,67	98,35	86,83	87,75
2	99,54	98,27	76,74	73,34

Biomass dosage (mg/L)	MO dye uptake (%)		EBT dye uptake (%)	
	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>
0,5	74,46	75,42	72,98	75,17
1	46,63	84,07	98,83	68,44
2	95,03	82,46	93,53	97,26



**Figure 3.** The effect of dye removal at different adsorbent dosage on the absorption of *Chlorella* and *Chlamydomonas*

0.3282 mg/g for *Chlorella* and *Chlamydomonas*, respectively (Figure 4). This increase can be explained by the rapid diffusion of PP and IC dye ions to the cell surface whose active area is empty at the beginning of adsorption. As the contact time increases, the diffusion of the dye to the cell surface slows down in proportion to the filling of these active areas. Therefore, there is no significant change in adsorption capacity, as seen in similar results in the literature (Nwodika & Onukwuli 2017).

**Effect of Temperature on Dye Removal**

Here, the operating temperatures were fixed at 25°C, 35°C and 45°C. Meanwhile, the experiments were performed at optimum conditions determined earlier. Table 5, shows the effect of temperature on the removals of PP, IC, MO and EBT dyes.

As seen in the table, it was determined that the adsorption capacity and percentage decreased in parallel with the increase in temperature, especially for IC, MO and EBT dyes. This negative effect may be due to the fact that the optimum temperature of adsorbate molecules is 25°C degrees and the pores on the surface of the adsorbent particles decrease as the temperature increases (Figure 5).

The adsorption isotherms were fitted using the Langmuir isotherm equation and Freundlich isotherm equation. The Langmuir isotherm model for the adsorption of dyes on *Chlorella* and *Chlamydomonas* cells is given in Figure 6 and 7, respectively.

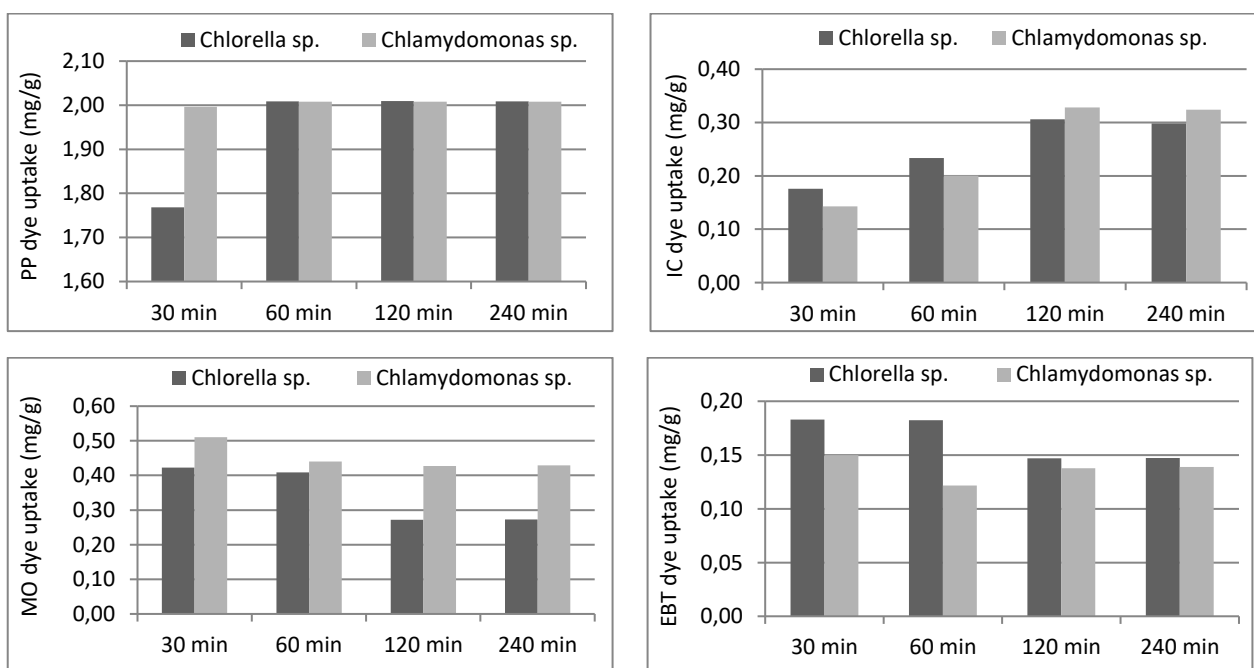
LnCe and Lnqe graphs were drawn using experimental data to find the Freundlich isotherm model parameters and regression coefficient. The Freundlich isotherm model for the adsorption of dyes on *Chlorella* and *Chlamydomonas* cells is given in Figure 8 and 9, respectively.

**Table 4.** The effect of dye removal at different contact time on the absorption of *Chlorella* and *Chlamydomonas*

PP dye uptake (%)		IC dye uptake (%)		
contact time (min)	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>
30	87,82	99,23	46,85	38,18
60	99,83	99,81	62,29	53,44
120	<b>99,87</b>	<b>99,82</b>	<b>81,64</b>	<b>87,57</b>
240	99,85	99,81	79,56	86,49

MO dye uptake (%)		EBT dye uptake (%)		
contact time (min)	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>
30	<b>72,50</b>	<b>87,53</b>	<b>90,90</b>	<b>74,50</b>
60	69,98	75,46	90,71	60,41
120	46,63	73,24	72,98	68,44
240	46,82	73,59	73,13	68,96



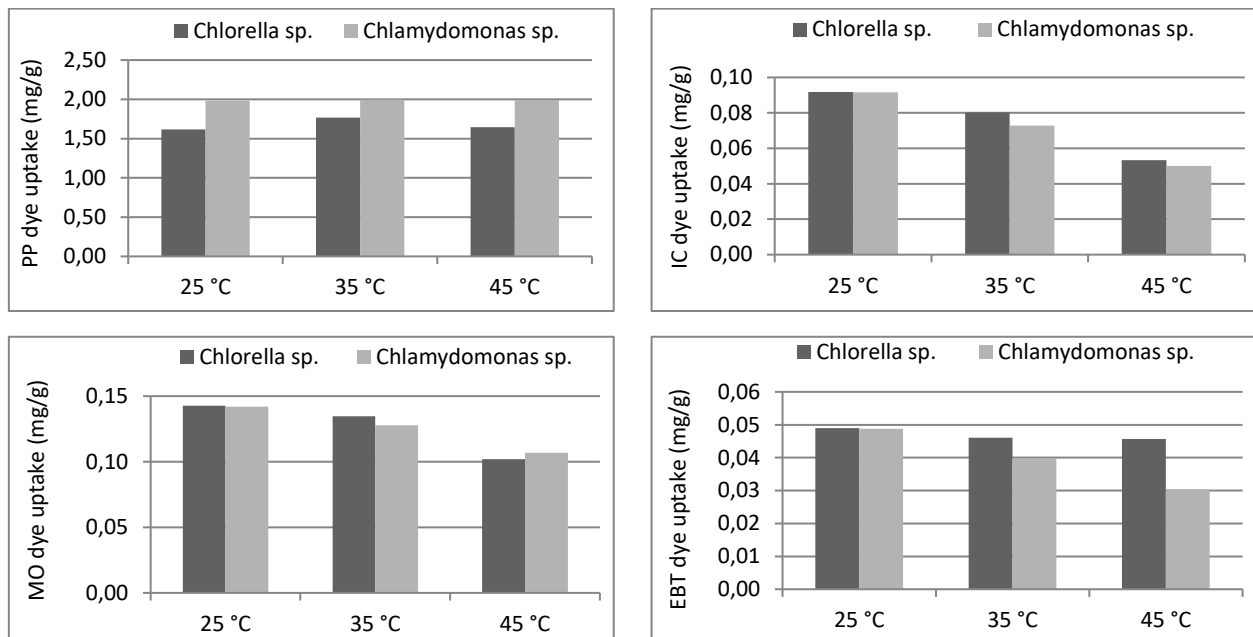
**Figure 4.** The effect of dye removal at different contact time on the absorption of *Chlorella* and *Chlamydomonas*.

**Table 5.** The effect of dye removal at different temperature on the absorption of *Chlorella* and *Chlamydomonas*.

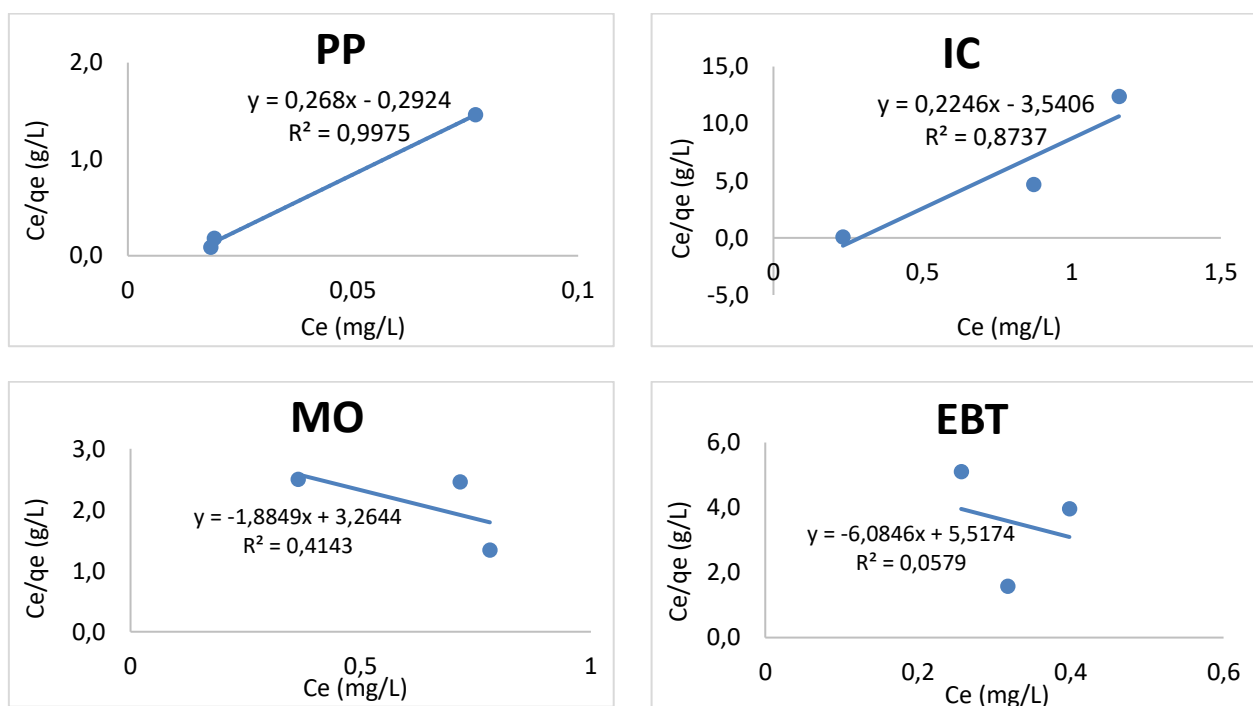
Temp. °C	PP dye uptake (%)		IC dye uptake (%)	
	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>
25	80,19	98,53	<b>98,05</b>	<b>97,70</b>
35	<b>87,82</b>	<b>99,23</b>	85,63	77,68
45	81,74	98,95	56,95	53,44

Temp. °C	MO dye uptake (%)		EBT dye uptake (%)	
	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>	<i>Chlorella sp.</i>	<i>Chlamydomonas sp.</i>
25	<b>97,79</b>	<b>97,27</b>	<b>97,42</b>	<b>97,02</b>
35	92,33	87,57	91,64	79,29
45	69,98	73,24	90,90	60,43



**Figure 5.** The effect of dye removal at different temperature on the absorption of *Chlorella* and *Chlamydomonas*.



**Figure 6.** The Langmuir isotherm model for the adsorption of dyes on *Chlorella* cells.



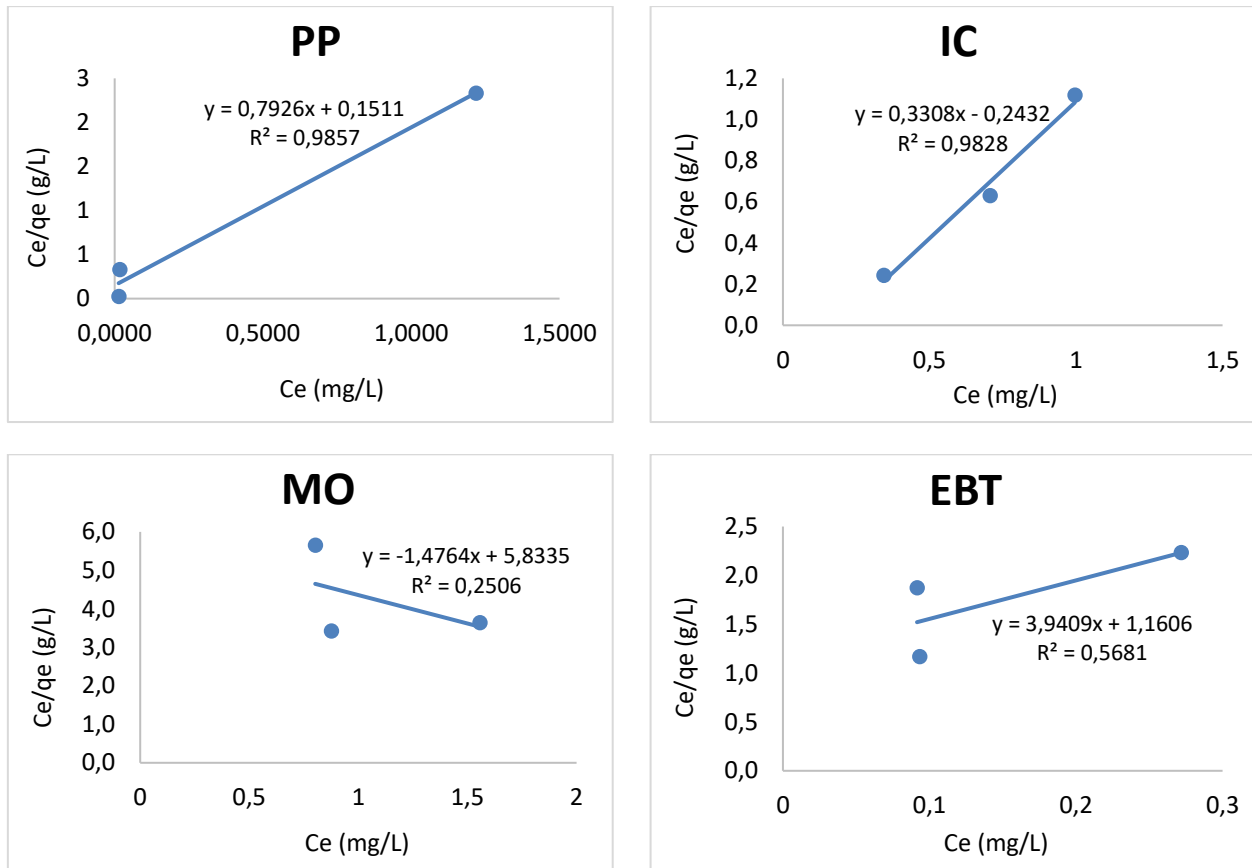


Figure 7. The Langmuir isotherm model for the adsorption of dyes on *Chlamydomonas* cells

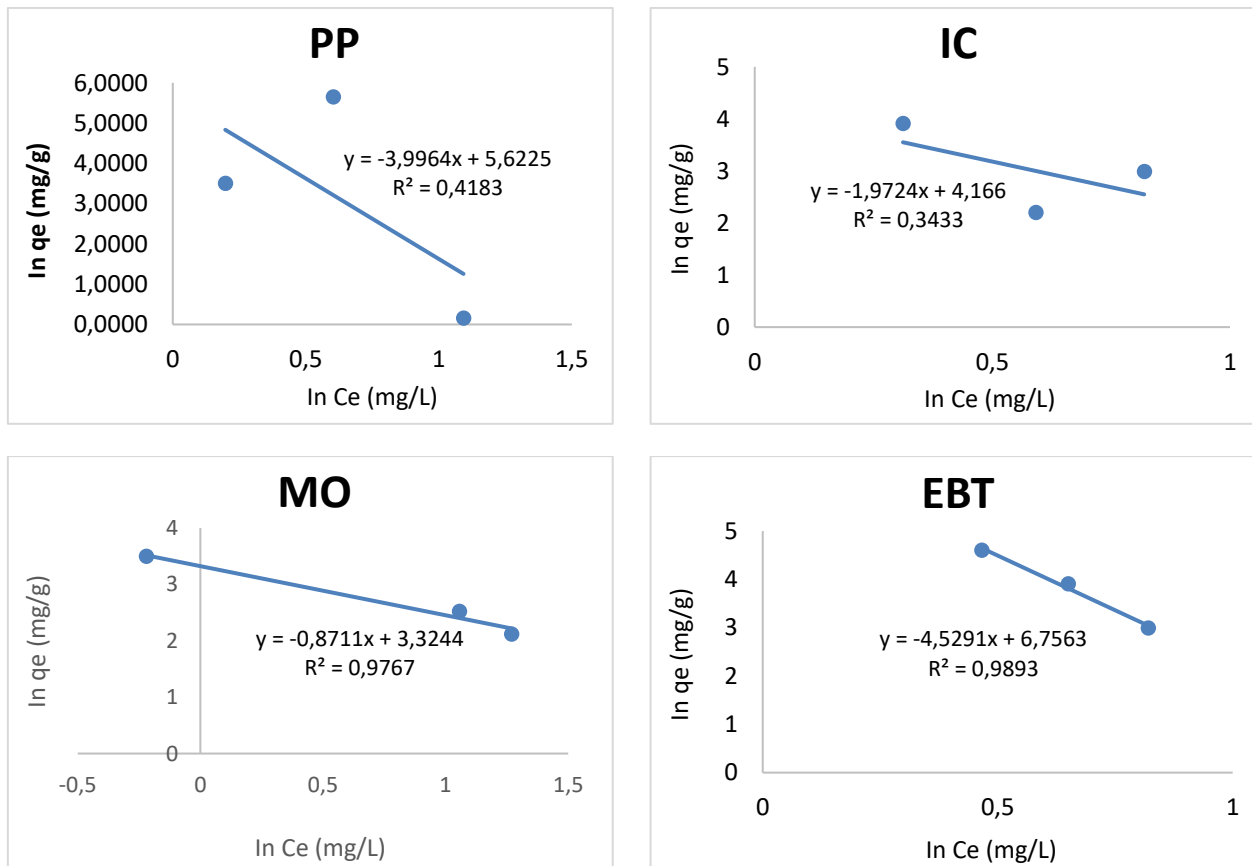


Figure 8. The Freundlich isotherm model for the adsorption of dyes on *Chlorella* cells

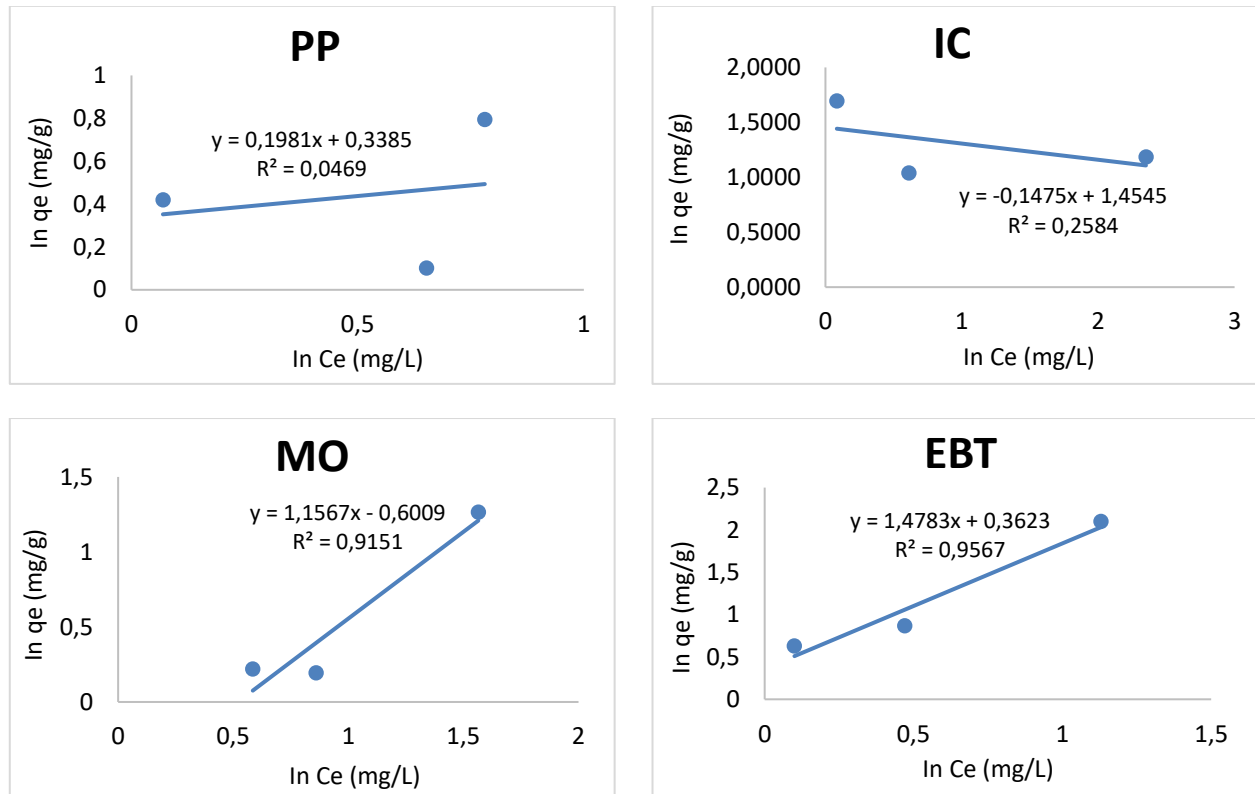


Figure 9. The Freundlich isotherm model for the adsorption of dyes on *Chlamydomonas* cells

Table 6. The Langmuir and Freundlich isotherm model for the adsorption of dyes on *Chlorella* and *Chlamydomonas* cells.

Adsorption Isotherm Constants		PP	IC	MO	EBT
<i>Chlorella</i>	<b>Freundlich</b>				
	1/n (L g <sup>-1</sup> )	1.1825	1.5069	0.8815	0.2207
	K <sub>f</sub> (L g <sup>-1</sup> )	1.251	0.81	1.285	1.162
	R <sup>2</sup>	0.6328	0.3433	0.9682	0.9893
	<b>Langmuir</b>				
	q <sub>m</sub> (mg g <sup>-1</sup> )	29.644	26.832	31.414	72.832
K <sub>L</sub> (L mg <sup>-1</sup> )	0.5649	0.2537	3.5474	1.4528	
R <sup>2</sup>	0.9975	0.8737	0.4143	0.0579	
<i>Chlamydomonas</i>	<b>Freundlich</b>				
	1/n (L g <sup>-1</sup> )	5.0479	6.7796	0.8645	0.6764
	K <sub>f</sub> (L g <sup>-1</sup> )	0.941	0.350	0.875	0.177
	R <sup>2</sup>	0.0469	0.2584	0.9099	0.9702
	<b>Langmuir</b>				
	q <sub>m</sub> (mg g <sup>-1</sup> )	30.207	26.812	28.546	66.153
K <sub>L</sub> (L mg <sup>-1</sup> )	0.6274	0.2510	5.2530	3.3955	
R <sup>2</sup>	0.9999	0.9828	0.2506	0.5681	

Table 7. The optimum adsorption conditions of dyes on different organism

The optimum adsorption conditions							
Organisms	Dyes	pH	Contact time	Algal dose	Dye conc.	Temp.	Ref.
Non live <i>Ulva fasciata</i>	Rhodamine B and methyl orange (respectively)	10-2	60 min	10 mg/L	100 mg/L		Elgarahy et al. 2023
live periphyton	Methyl orange	7	24 h		50 mg/L	30°C	Shabbir et al. 2017
Tea waste	Eriochrome Black-T	2	90 min	11g/L	200 mg/L	25°C	Bansal et al. 2020
Garcinia cola Nut Shells	Indigo carmine	2	80 min	400 mg/L	45 mg/L		Tiotsop Kuete et al. 2022
live <i>Spirulina platensis</i>	Acid Black 210 and Acid Blue 7 dyes (respectively)	2	60-75 min	0.5 mg/mL	100 mg/L	30°C	Al Hamadi et al. 2017
<i>Coelastrella</i> sp.	Eriochrome Black-T	8			10 mg/L	30°C	Deepa et al. 2019
Yam pell	Eriochrome Black-T	2	60 min	0.1g/L	100 mg/L	30°C	Ladan et al. 2021
This study							
live <i>Chlamydomonas</i> and <i>Chlorella</i> sp.	Phenolphthalein, indigo carmine (respectively)	4	120 min	0.5mg/mL 1mg/mL	100 mg/L 25 mg/L	35°C 25°C	
This study							
live <i>Chlamydomonas</i> and <i>Chlorella</i> sp.	Methyl orange and eriochrome black T (respectively)	2-4	30 min	0.5 mg/mL	100 mg/L	25°C	

The Langmuir adsorption (Langmuir 1916) isotherm assumes that the dyestuff molecules are adsorbed in a single layer (homogeneous points) on the surface of the solid. The results showed that the Langmuir isotherm model, indicating that the adsorption of dye on the surface of cells should be monolayer adsorption behavior and the properties are uniform, was the most suitable model for the adsorption of PP and IC dyes onto *Chlorella* and *Chlamydomonas* cells due to the highest regression coefficient, respectively ( $R^2=0.9975$ ; 0.9370; 0.9999; 0.9828). However, it has been shown that the Freundlich isotherm model is the most suitable model for the adsorption of MO and EBT dyes onto *Chlorella* and *Chlamydomonas* cells due to the highest regression coefficient, respectively ( $R^2 = 0.9682$ ; 0.9893; 0.9099; 0.9702) (Table 6). Freundlich adsorption isotherm assumes that they are adsorbed in multiple layers (heterogeneous points, Freundlich 1906). As a result, we can say that *Chlorella* and *Chlamydomonas* cell surface can absorb MO and EBT dyes to form more than one layer and reach equilibrium.

The results showed that the Langmuir (for PP and IC dye uptake) and Freundlich (for MO and EBT dye uptake) isotherm model best fitted the experimental data showing a straight line with a good correlation coefficient value, both of which were greater than 0.90, indicating the applicability of model for dye-algal biosorption. Based on Langmuir model, the computed maximum sorption capacities ( $q_m$ ) values were found to be 29.644; 26.832; 31.414 and 72.832 mg/g for PP, IC, MO and EBT onto the *Chlorella* cells, respectively. Alternatively, the empirical parameter ( $n$ ) calculated from Freundlich model verifies the favorability of the sorption process. The  $n$  value of Freundlich isotherm was 0.88 and 0.22 which fall between 0 and 1 and thereby indicates that the model was suitable for experimental data in the MO and EBT dye uptake study onto the both organism (Darweesh & Ahmed 2017; Elgarahy et al. 2023).

The results of the present study coincides with the findings of Pujari et al. (2023) who reported that Langmuir isotherm proves to be a better choice in interpreting the biosorption reaction between indigo carmin and *Hypnea musciformis* algae. Hence, the results indicated the monolayer coverage of the dye on the active sites of the adsorbent with homogeneous distribution. Besides, the results coincides with the findings of Chin et al. (2020) who reported that Freundlich isotherm proves to be a better choice in interpreting the biosorption reaction between metilen blue and *Chlorella vulgaris*.

Research findings are similar to previous studies. The table shows the optimum absorption values of textile dye onto the different organism groups (Table 7).

If we summarize the table in general, we can say that the optimum removal values of the four inductor dyes on different organisms are pH 2-4, 25-30°C, 0.1-10 mg/L algal dosage and 50-100 mg/L dye concentration.

## Conclusion

In this study, the removal of phenolphthalein, indigo carmine, methylene orange and eriochrome black T dyes was investigated onto *Chlorella* sp. and *Chlamydomonas* sp. cells. This experiment used the batch adsorption method to study the adsorption effect of pH, biomass dosage, contact time, initial dye concentration and temperature on the dyes. The adsorption of the dyes onto *Chlorella* sp. and *Chlamydomonas* sp. were fitted with the Langmuir and Freundlich isotherm models.

It was observed that the adsorption capacity of the cells increased as a result of increasing the initial concentration of all dyes. Using experimental data, Langmuir isotherm and Freundlich isotherm model parameters were calculated. The adsorption of PP and IC dyes onto cells was found to best fit the Langmuir isotherm model while The adsorption of MO and EBT dyes found to best fit the Freundlich isotherm model. In addition, Besides, in the Langmuir isotherm model, the maximum adsorption capacity for *Chlorella* and *Chlamydomonas* cells was calculated as 72.83 and 66.15 mg/g, respectively.

The results showed that the optimum conditions for phenolphthalein removal (99%) was obtained at pH 4, contact time of 120 min, 35°C, algal dose of 0.5 mg/mL and dye concentration of 100 mg/L on to *Chlorella* and *Chlamydomonas* cells. The sample prepared at 25°C for 120 min and at pH 4 had the best adsorption effect on indigo carmin on to the both of cells (99-98%). In addition, the optimum conditions for methyl orange and eriochrome black T removal (99-97%) was obtained at pH 2-4, contact time of 30 min, 25°C, algal dose of 0.5 mg/mL and dye concentration of 100 mg/L on to the cells. Thus, the results of this study reveal that the *Chlorella* and *Chlamydomonas* can be utilized as an efficient and eco-friendly biosorbent for the removal of the indicator dyes from aqueous solution.

## Ethical Statement

This study does not require ethics committee permission or any special permission

## Funding Information

This study was not funded

## Author Contribution

This study is performed by single author.

## Conflict of Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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