Adsorption of Orange-G dye by the dried powdered biomass of *Chlorella vulgaris* Beijerinck

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Decolourization potential of microalgal biomass of an unicellular green alga (*Chlorella vulgaris*) was studied using different concentrations of Orange-G dye. Several parameters were also optimized for better removal of the tested dye from its aqueous solution. Langmuir and Freundlich models were tested for equilibrium studies and experimental data of the present study are better explained by the Freundlich model. FTIR data showed that five functional groups were probably involved in the adsorption of the dye. The biosorption process using algae (including *C. vulgaris*) offers an excellent biosystem for the remediation of final discharge of textile effluents.

Keywords: Adsorption, *Chlorella vulgaris*, dyes, infra-red spectrum.

WATER resources play a central role in human development, and quality of water supports the sustainable development of various communities and ensures healthy human life[1]. Rapid growth and development of human beings in the form of rapid industrialization, urbanization and other anthropogenic activities are the main causes for change in water quality of freshwater bodies, which make it unsuitable for human consumption and aquatic life[2]. Synthetic colouring agents are among the major threats for polluting aquatic resources. The use of dyes and pigments has increased due to rapid industrialization and man's desire for colour[3]. Several industries like textiles, food, cosmetics, paper, leather, pharmaceutical, colour photography, food, etc. widely use synthetic dyes because they are cost-effective in production, have variety and firmness in colour[3,4]. Approximately, 10,000 types of synthetic colouring products have been used by different textile industries. It is estimated that more than 7×10^5 tonnes of dyes is produced worldwide annually and 10–15% of the dyes are released during the process of colouration and manufacturing[5–7]. Thus a huge volume of coloured wastewater is discharged and received by aquatic bodies every year[8]. Origin of most of the dyes is synthetic and they have a complex atomic structure that is highly durable and resistant to biological degradation[9]. The presence of dyes in aquatic resources in low concentration (1 ppm) changes the properties like transparency of water, phosynthetic activity of phytoplankton and solubility of gas in aquatic bodies. However, 300 ppm is the average concentration reported in coloured wastewater from textile manufacturing processes[10,11]. Coloured water discharged from different industries into aquatic bodies reduces the penetration of light inside and directly affects the process of photosynthesis[12]. The adverse effect on human health includes dermatitis, allergy, skin irritation[13] and mutation which may cause cancer[14,15]. The technology used for the treatment of coloured wastewater includes flocculation, ozonization, adsorption and precipitation, but these are not cost-effective, are relatively inefficient and not adaptable to different types of coloured wastewaters[16]. Biosorption is a new technology which has received great attention as a cheap, clean and efficient method for wastewater treatment[17,18]. It involves the use of biomass of photosynthetic aquatic organisms, such as algae, aquatic plants or moss (bryophytes), and represents readily available sorbents for the removal of contaminants from wastewater or polluted water systems[19]. It is well known that algae contain proteins, polysaccharides and lipids[20]. These play an important role in the process of biosorption due to the presence of different functional groups, such as hydroxyl, carboxylate, phosphate, sulphate and amino groups. Studies have been done earlier regarding microalgal role in the adsorption of dyes and heavy metals from polluted water[21–23]. In this study we explore the application of *Chlorella vulgaris*, a grass green alga, in bioremediation of dye effluents.
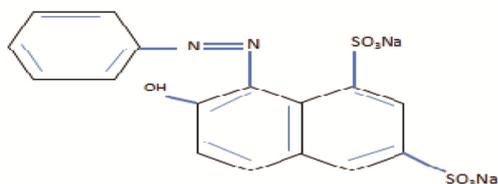
Materials and methods

Basic characteristics and molecular structure of Orange-G dye

In the present study, an orange crystalline powder synthetic azo dye (Orange-G, HiMedia) was used for

Physical appearance	CAS no.	CI no.	Molecular weight (g mol^{-1})	Formula	$\lambda_{max\ (nm)}$
Orange crystalline powder (HiMedia)	1936-15-8	16230	452.37	$C_{16}H_{10}N_2Na_2O_7S_2$	478

Figure 1. Molecular structure of Orange-G dye.

biosorption experiments. The basic characteristics and molecular formula of the test dye are given in Table 1 and Figure 1 respectively.

Determination of maximum absorption (λ_{max}) of the dye

A solution of 10 ppm dye concentration was prepared and pH adjusted to 7. The optical density was measured at 400–800 nm and maximum optical density (λ_{max}) observed at 478 nm (Figure 2).

Preparation of test dye solutions

Orange-G stock solution was prepared by dissolving weighed dose of test dye in distilled water. Working solutions of different concentrations (5–40 ppm) were prepared by dilution of stock solution.

Culture and harvesting of algal species

The alga used in the decolourization experiment was isolated from a freshwater pond and identified as *Chlorella vulgaris* (Figure 3) with the help of compound microscopy using standard monograph[24]. *C. vulgaris* was grown in Hafkin flasks using BG-11 growth medium[25]. Growth conditions comprised light intensity 1800 ± 100 lux, temperature $24° \pm 2°C$, $14:10$ h light and dark period and pH 7.4 using dilute NaOH and HCl. Algal biomass was harvested via flocculation using saturated solution of potash alum. The harvested biomass was washed thrice using tap water and dried at 50°C in an hot-air oven. After drying, the biomass was crushed with the help of mortar and pestle and then sieved (85 μm pore size) to obtain uniform-sized particles.

Decolourization experiment

Batch mode of experiments were performed to optimize different parameters effecting the decolourizing reaction.

Experimental conditions included 100 ml Erlenmeyer flasks of Borosil-make containing 50 ml solution of test dye on a rotatory shaker at 130 ± 5 rpm for 10 min at room temperature under neutral pH. Decolourization experiment was conducted using one factor at a time and rest of the factors was kept constant. Optimization of the affecting factors for maximum adsorption efficiency of algal biomass decolourization, experiments were conducted at various dye concentrations (5–40 ppm), micro-algal biomass dosage (25–200 mg), reaction time (10–160 min), temperature (10–50°C) and pH (5–9). After adsorption reaction, all the samples were filtered through 3 μm filter paper (HiMedia 237). Concentration of dye in the filtrate was calculated using a Systronics Double Beam Spectrophotometer-2202 at the absorption maxima wavelength of 478 nm. The decolourization percentage ($R\%$) was determined using the formula

$$[(C_i - C_f)/C_i] \times 100,$$

where C_i is the initial concentration of test dye (ppm) and C_f the concentration of dye filtrate (ppm) after adsorption reaction. The specific dye uptake by the algal biomass was determined as

$$Q = V(C_i - C_f)/m,$$

where Q is the dye absorption (ml of dye/mg absorbent); V the working sample volume (ml); C_i the initial dye concentration of working sample (mg/l); C_f the final dye concentration of working sample after adsorption reaction (mg/l) and m is the dose of dried adsorbent (mg).

Adsorption isotherms

Empirical models, viz. Freundlich[26] and Langmuir[27] were employed for single-solute adsorption system, while for multiple conditions modified Langmuir model was used for biosorption equilibria of the test alga.

Freundlich equation: $q_e = K_F C_e^{1/n}$,

Langmuir equation: $q_e = \dfrac{q_{max} K_L G_e}{1 + K_L C_e}$,

where q_e is amount of dye adsorbed by unit weight of adsorbent when reactions reached the stage of equilibrium (mg/g); q_{max} the maximal dose of test dye adsorbed by
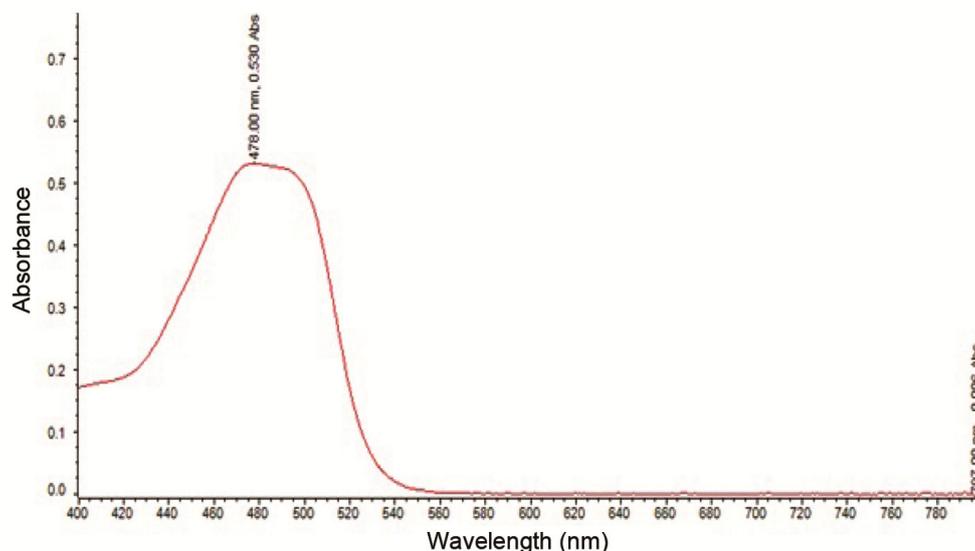
Figure 2. Determination of λ_{max} by Systronics – 2202 double beam spectrophotometer.
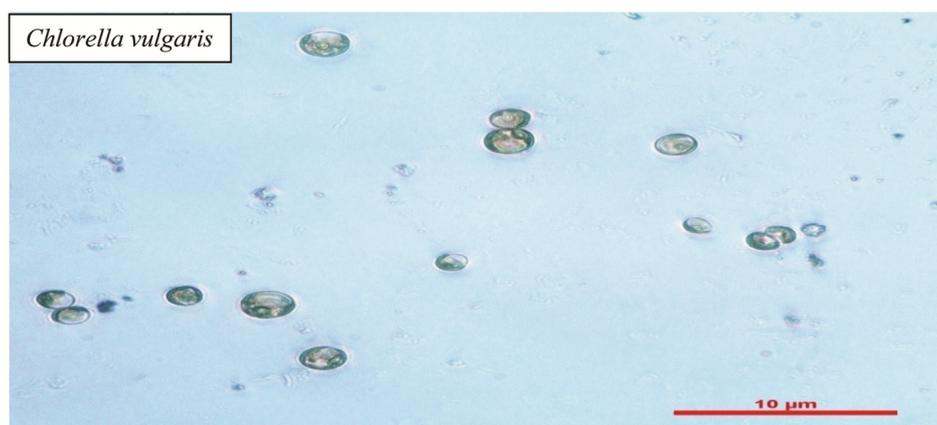

Figure 3. Microscopic image of the test alga.

unit weight of adsorbent (mg/g); K_L the Langmuir constant of adsorption reaction which characterizes affinity of binding sites for the test dye, present on the surface adsorbent (l/mg); C_e the residual concentration of test dye (mg/l) at equilibrium; K_F the constant of Freundlich adsorption reaction which represents the magnitude of adsorption reaction (mg/g) and $1/n$ is the heterogeneity factor representing adsorption intensity.

Characterization of algal biomass

Functional groups present on the algal biomass and changes in them after adsorption of dye were studied using FTIR spectroscopy. The algal biomass was mixed in potassium bromide salt (FTIR-grade) and the pellet was analysed for IR spectrum (range of IR analysis 400–4000 cm^{-1}) to identify the functional groups.

Results and discussion

Azo dyes are carcinogenic, mutagenic, toxic as well as hazardous to human health[28]. Discharge of coloured wastewater affects water transparency, aquatic photosynthesis, gas solubility and aesthetics of water bodies; it is toxic to aquatic ecosystem and ultimately results in serious environmental complications worldwide[29]. Wastewater from textile industries is characterized by high salinity, strong colour, variable pH, high temperature and high COD (chemical oxygen demand)[30]. Microalgae play a central role in the fixation of CO_2, which makes them a good candidate for bioremediation of coloured wastewater[31]. Microalgae in both viable and non-viable forms have been reported in the process of colour removal from wastewater. The procedures associated with removal of dyes include biosorption and bioconversion[32]. Microalgae such as *Chlorella* were chosen due to their ability to grow

best in wastewater and attain maximum rate of decolourization from textile wastewater[32–34].

Effect of initial dye concentration

Experiments were performed to study the effect of initial dye concentration (5–40 ppm) on the adsorption process. The results showed maximum sorption (38%) at 5 ppm. A continuous reduction in adsorption was observed with increase in dye concentration. This clearly shows that dye adsorption depends on concentration of the solution. A dye concentration of 5 ppm was selected for subsequent experiments (Figure 4).

Effect of biomass dosage

The results for dye adsorption by different biomass dosages showed that adsorption does not follow a regular pattern. Adsorption initially increased up to 38% and later decreased. Maximal decolourization was observed to be 38% with 50 mg biomass dosage. Higher dose (>50 mg) showed almost same percentage of adsorption. Therefore, a biomass dosage of 50 mg was selected for the next experiment (Figure 5).
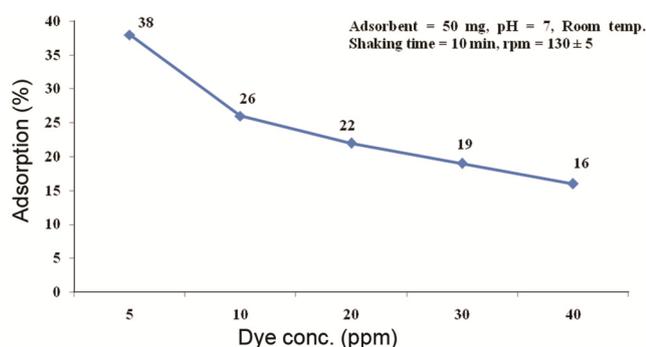
Figure 4. Per cent adsorption of Orange-G from its aqueous solutions of different concentrations.
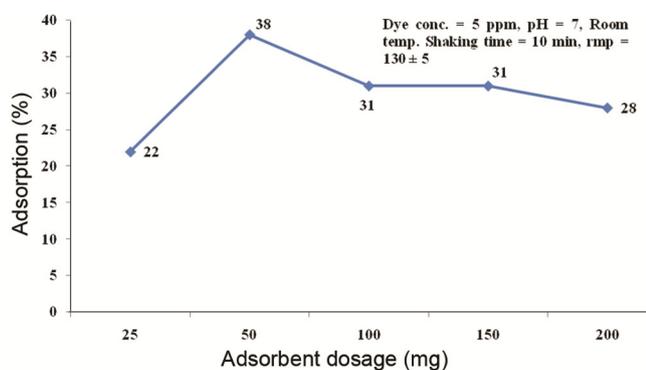
Figure 5. Per cent adsorption of Orange-G by different biomass dosages.

Effect of time

Variation in the magnitude of decolourization was observed with change in time of adsorption reaction. Maximum adsorption observed was 37% within 10 min of reaction time. After 10 min, there was no significant change in adsorption up to 160 min of reaction time. Hence optimum time period of 10 min was selected for the next experiment (Figure 6).
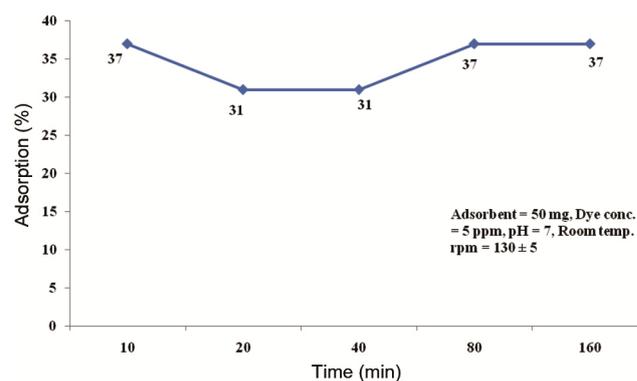
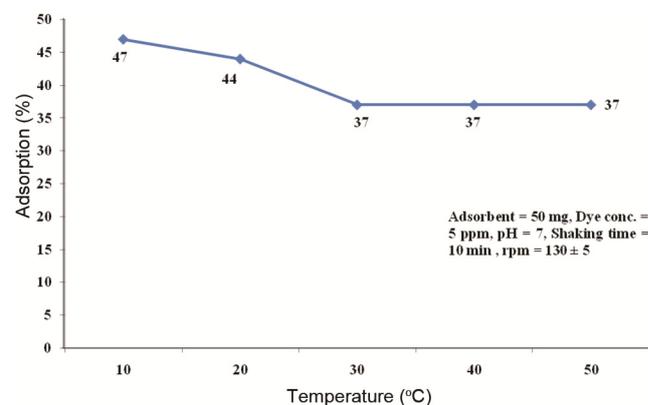**Figure 6.** Per cent adsorption of Orange-G after different time intervals.

Figure 7. Per cent adsorption of Orange-G at different temperature regimes.
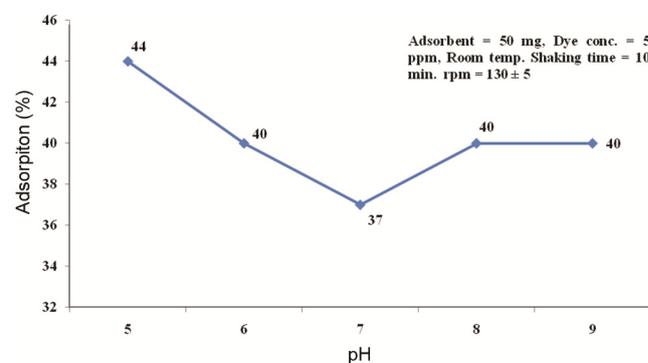
Figure 8. Per cent adsorption of Orange-G at different pH values.

Table 2. Freundlich and Langmuir isotherm constants of adsorption reaction of Orange-G by *Chlorella* species for different variables

Parameters	Freundlich isotherm constants				Langmuir isotherm constants			
	N	$1/n$	k_f	R^2	A	B	$1/ab$	R^2
Dye concentration	0.324	3.082	22.286	0.986	0.402	1.25	1.990	0.799
Adsorbent dosage	2.415	0.414	4.100	0.980	0.163	0.714	8.592	0.925
Time	1.945	0.514	4.318	1.0	0.263	0.540	7.041	1.0
Temperature	1.410	0.709	4.874	0.998	0.502	0.540	3.688	0.994
pH	1.466	0.682	4.806	0.997	0.463	0.5	4.319	0.988
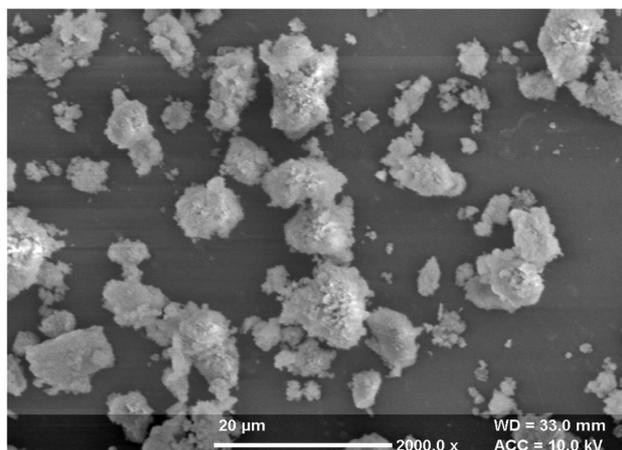
Figure 9. SEM image of unloaded biomass particles.
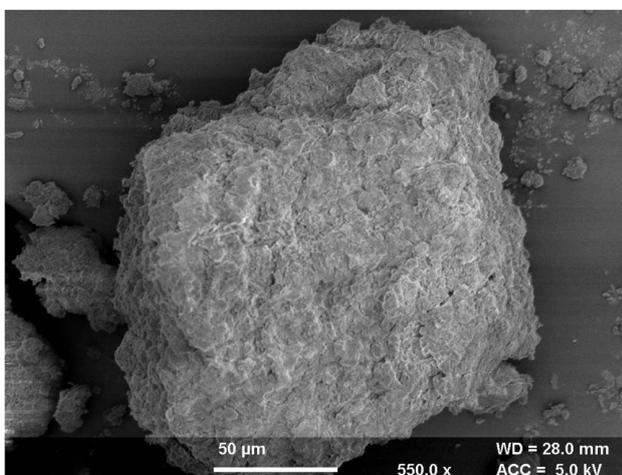
Figure 10. SEM image of algal biomass particles loaded with the dye.

Effect of temperature

It was observed that maximum adsorption was at 10°C and it decreased thereafter with increase in temperature. Thus, there is thermal deactivation of the dye-binding sites. It involves extra resources to maintain low temperatures at industrial level for the treatment of coloured wastewater and this results in increased cost of the treatment process. Therefore, the experiment was conducted at room temperature (Figure 7).

Effect of pH

The adsorption process was significantly affected by initial pH of solution, because pH changes the ionization of test dye and binding sites of the adsorbent. The results revealed that the range of acidic pH (5–7) showed more adsorption than the basic range (8–9). Maximum adsorption (44%) was observed at pH 5 and minimum at neutral pH (Figure 8).

Adsorption isotherm

The adsorption isotherm is a graphical representation of the adsorbed dose of adsorbate against the residual concentration of adsorbate in the solution[35]. It is clear from the isotherm, when all active sites of biosorbent were occupied by dye molecules then no more adsorption occur; this indicates saturation biosorbent[36]. In the present study, analysis of adsorption isotherms (Freundlich and Langmuir models) revealed that the Freundlich isotherm model was more suitable for describing the adsorption of test dye (Orange-G) effluent by powdered biomass of *C. vulgaris* at all the parameters studied. Also, much better correlation coefficients were obtained using Freundlich equation when compared with Langmuir equation (Table 2). The Fruendlich model assumes existence of multi-layered structure[37] and also heterogeneous energetic distribution in the present case. It was suggested by Freundlich that the ratio of the adsorbed dose of adsorbate and the dose of adsorbate in the solution is not constant at different concentrations of solution[35,38]. However, Giles *et al.*[39] found that the Congo Red adsorption isotherm follows the Langmuir model. Hernández-Zamora *et al.*[36] reported a higher binding affinity of *C. vulgaris* towards Congo Red dye, and also lack of strong competition between the dye molecules and solvent to occupy the binding sites of the adsorbent.

Characterization of the adsorbent material

The characterization of algal biomass (*C. vulgaris*) was performed through physico-chemical methods as well as by infrared spectroscopy (FTIR) and scanning electron
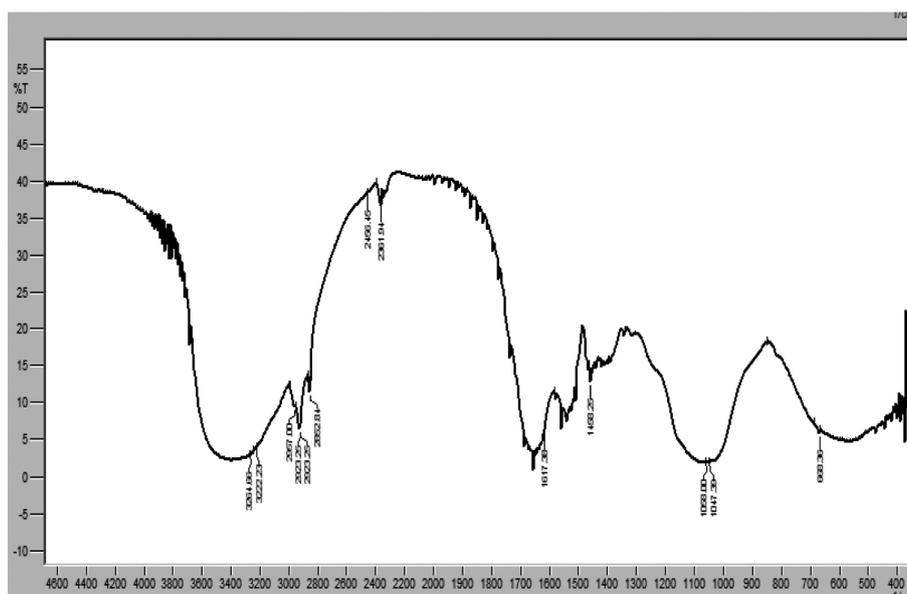
Figure 11. FTIR spectra of *C. vulgaris* biomass loaded with Orange-G dye.
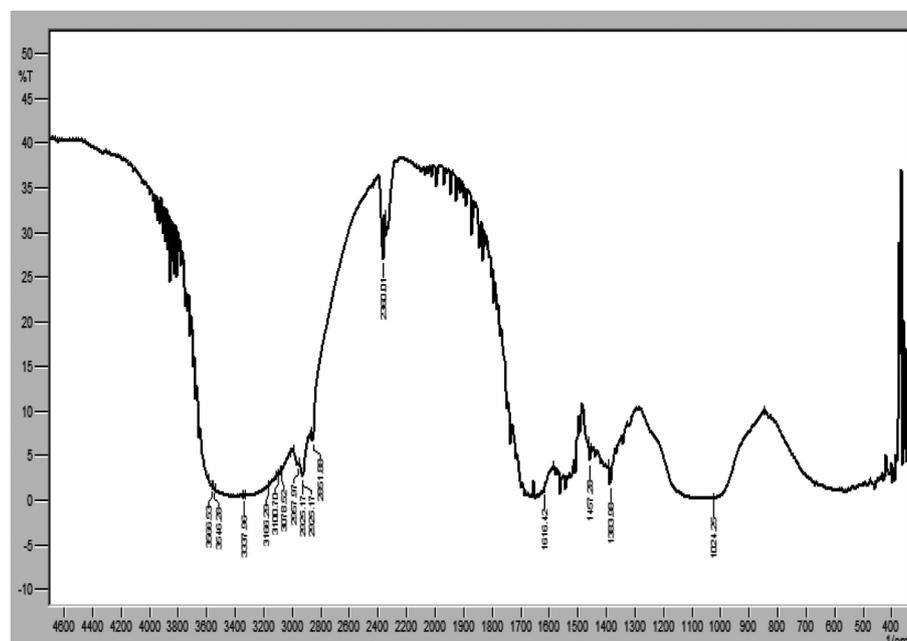

Figure 12. FTIR spectra of *Chlorella vulgaris* biomass (unloaded).

microscopy. Surface of the adsorbent was characterized before and after the decolourization reaction. The process of adsorption is associated with surface of the adsorbate. The intensity and rate of adsorption highly depend on that section of the adsorptive surface area which is available for adsorption. The dose of test dye (adsorbate) per unit weight of powdered biomass of test alga (adsorbent) is associated with size and shape of the particle, texture and composition. SEM image of unloaded particles of algal biomass (Figure 9) distinctly reveals that the surface of algal particles is mainly of irregular and porous form. As shown in Figure 10, pores in the loaded algal biomass

particles are densely packed in comparison to unloaded biomass due to the presence of dye molecules.

Infrared spectroscopic analysis

The functional groups present on the surface of the adsorbent (algal biomass) were examined to identify those groups which are involved in the adsorption of the dye. Vibrational and rotational movements of different chemical bands and molecular groups present on the surface of the adsorbent are responcible for the adsorption process

in the IR region. Spectral analysis from FTIR of the dye-loaded biomass of *C. vulgaris* showed a total of 13 peaks (Figure 11) in comparison to 15 peaks (Figure 12) shown by the unloaded biomass. The dye-treated algal biomass powder showed six new peaks while seven peaks shifted a little, but this shifting did not affect their respective functional groups. Eight peaks at wavenumbers 1024.25, 1383.98, 3078.52, 3100.70, 3166.29, 3377.96, 3546.28 and 3566.53 cm^{-1} respectively, disappeared from the spectrum after loading of dye molecules. This could be attributed to the presence of five strong functional groups, viz. C=S, aromatic rings, aromatic C–H, CH=CH, =CH$_2$, and nine moderate groups such as aliphatic chains, amide and amine. FTIR studies demonstrated that strong groups, specially aromatic C–H and =CH$_2$, are incorporated in the mechanism of decolourization of Orange-G dye. The stress developed by the binding of dye molecules with algal biomass shifted the absorption peaks and also exposed some new strong functional groups (C–Br, C–Cl, C=S, aromatic rings). These new functional groups might play a role in multilayered model of adsorption mechanism and may be responsible for dye-binding[9,40]. Different types of adsorbents have been tested for removal of colourants from wastewater, including *C. vulgaris*[41], blue-green algae and members of other algal groups. Different types of functional groups like sulphate, hydroxyl, carboxyl, phosphate and amine, including many other charged groups are present on the algal biomass, and act in the sequestration of pollutants[42]. Duygu *et al.*[43] observed typical inorganic functional group of *C. vulgaris* and defined a series of chemical groups which are vibrationally active, including cellulose (–C=O), residual water (–OH), protein (amide), lipid (–CH$_2$), starch (–C–O) and nucleic acid (>P=O).

Conclusion

Bio-removal is a sustainable approach towards the purification of coloured wastewater from different industrial processes. Result of the present study clearly indicate that the powdered biomass of *C. vulgaris* is an effective adsorbent for the removal of Orange-G dye from its aquatic solution. Adsorption profile of the algal species revealed that 50 mg/ml biomass dosage showed maximum biosorption. Optimum time, temperature and pH were found to be 10 min, 10°C and 5 respectively. Freundlich isotherm was found suitable to describe the results of adsorption experiments at equilibrium. SEM photomicrographs showed the biomass loaded with tested dye. FTIR studies confirmed the involvement of five strong binding groups and nine moderate groups. Two strong groups were found to be closely related with the bio-removal of Orange-G dye from effluents.

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