Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2019, 8 (1): 335-350

(International Peer Reviewed Journal)

A Comparative Investigation on Biosorption Performances of Non-viable *Vaucheria* sp. and *Chara* sp. for a Hazardous Basic Dye-Methylene blue

Surabhi Sagar and Arshi Rastogi*

Department of Chemistry, K.L.D.A.V. (P.G) College, Roorkee-247 667, **INDIA**

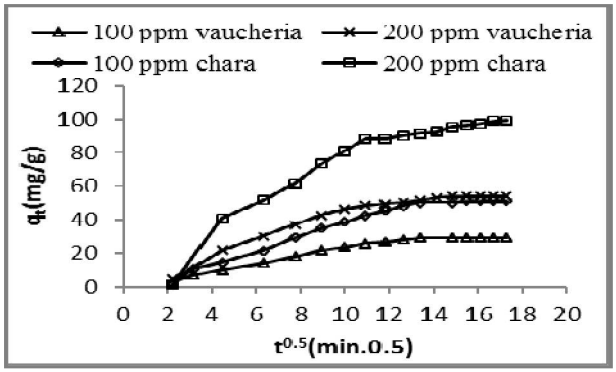
Email: arshirastogi@gmail.com

Accepted on 27th December, 2018

ABSTRACT

In this paper, a comparative account of biosorptive ability of a Xanthophyta yellow green alga Vaucheria sp. and a Charophyta green alga Chara sp. for Methylene blue, a basic dye, from synthetic wastewater is given. Various operational parameters such as contact time, biosorbent dose, pH, and temperature that affect the biosorption of Methylene blue onto algal biomass have been studied. Langmuir, Freundlich, Temkin and Dubinin-Radushkevich (D-R) isotherms were applied for equilibrium studies and the experimental data were found best fit for Langmuir isotherm model. The higher biosorption capacity was found to be 200 mg g^{-1} for Chara sp. as compared to 166.66 mg g^{-1} for Vaucheria sp. under the studied optimum conditions. The kinetic data was best described by pseudo-second-order model and the thermodynamic studies showed the endothermic nature of biosorption. Characterization part were performed to explore the physical and chemical properties of algae, the surface area calculation was done using BET method, surface morphology by scanning electron microscope images and surface functionality by FTIR. The stability and efficiency of both the algal biomass in the long term repetitive operations up to five repeated batches were also investigated. The disposal method of the used biomass is also proposed in the present study in order to retain the process eco-friendly. The present work suggests that non-viable Vaucheria sp. and Chara sp. biomass are suitable biomass for MB dye removal from synthetic wastewater.

Graphical Abstract



Intra-particle diffusion plot for the biosorption of MB on to algal biomass *Vaucheria* sp. and *Chara* sp.

Keywords: Biosorption, Methylene blue, *Vaucheria* sp., *Chara* sp.

INTRODUCTION

Water containing industrial effluents is one of the major cause of water pollution and a matter of serious concern. Dyes are used all over the world for producing many paints, printing inks, textile fabrics, cosmetics, in various food items and also in paper industries. During the dyeing process a large percentage of dye does not bind completely to the product and runs off the water streams [**1**]. Dyes adversely affect biodegradation, prevent light penetration and reduce photosynthetic activity of algae. Due to complex aromatic molecular structure of dyes, they become highly stable and difficult to biodegrade. They may retain in the environment, and due to their negative effect on the water quality they may increase the health hazards. Among a large variety of dyes Methylene blue (MB), is the most commonly used dye for colouring of cotton and wool, and if taken in human system, it may cause mental disorder, hard breathing and vomiting. Therefore, it is essential to treat the wastewater containing MB dye before being discarded [**2**].

Despite of the presence of several conventional methods such as chemical oxidation, membrane separation, reverse osmosis, solvent extraction, coagulation/flocculation for dye removal from aqueous solutions, these methods have their own limitations like accumulation of sludge concentrations, difficult and expensive operations and producing large amount of chemicals. Consequently, adsorption technique appeared to be the most promising method for dye removal because of the advantages like minimal disposal of volume of sludge, simple regeneration, less investment in terms of initial cost and high efficiency. Some of the adsorbents used earlier by researchers include activated carbon, natural materials like zeolites and clay, industrial solid wastes like fly ash and red mud and other miscellaneous adsorbents like tea waste, cow dung, egg shell etc as reported in literature [**2-5**]. Moreover, due to abundancy of the biomaterials they can also be used as low-cost and eco-friendly biosorbents. The physiochemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure is termed as biosorption. Biosorption of MB dye has been studied earlier on biomaterials such as duckweed [**6**], jackfruit peel [**7**], papaya seeds [**8**], *Couroupita Guianensis* leaves [**9**], garden grass *Paspalum notatum* [**10**] and *Posidonia oceanic(L) fibres* [**11**]. Also, there are studies indicating biosorption of MB by algae [**12-16**] with good adsorption capacity.

Biosorption studies can be carried out using viable and non-viable algal biomass. System that uses viable or living biota for biosorption studies is susceptible to many problems like ensuring conditions that are required for the maintenance of the living microorganism, and maintaining the pH that is suitable for the microorganism. Therefore, these problems can be avoided by using a non-viable algal biomass [**17**]. So, non-viable yellow green alga *Vaucheria* sp. and green alga *Chara* sp. were chosen in the present study, since these are naturally abundant, renewable and eco-friendly algae. *Vaucheria* sp. is mostly found in wetland and shows apical growth from the tip of filaments forming mats on freshwater environments and *Chara* sp. are multicellular having stem-like and leaf-like structures and resemble land plants, are also found in fresh water, mainly in limestone areas throughout the northern temperate zone, they grow submerged and are attached to the muddy bottom. Though, there have been some reports on adsorption of few dyes by *Chara* sp. and *Vaucheria* sp. [**18-22**], but there are no reports on the biosorption of commonly available MB dye present in textile wastewaters on the above test algae.

Hence, the objectives of the present study were to evaluate and compare the potential of non-viable yellow green alga *Vaucheria* sp. and green alga *Chara* sp. for the removal of MB from synthetic wastewater. For this, the experimental variables affecting optimal removal of MB dye were assessed and various adsorption isotherms and kinetic models were explored to identify the possible mechanism of dye removal and finally, to characterize the biosorbent using FTIR, SEM and BET method.

MATERIALS AND METHODS

Equipments: The pH measurements were made using a pH meter (Perfit, India) and the aqueous solution was analyzed using a UV-Vis spectrophotometer -119 (Systronics India Ltd.). Infrared spectra were recorded using KBr pellets on a Thermo Nicolet FTIR (Germany) within 4000-400 cm^{-1}. To examine the morphological characteristics of the alga before and after adsorption of MB dye, samples were viewed using a ZEISS EVO 40 EP (Cambridge, UK) with analytical software- Quantax 200. Carbon, hydrogen, and nitrogen analysis of the adsorbent was carried out on an Elementar analyse system Vario MICRO CHNS V3.1.1 (GmbH, Germany). The Brunauer-Emmett-Teller (BET) surface area of the adsorbents was measured by nitrogen adsorption isotherms on a Micrometrics ASAP 2010 surface area analyser (England, UK).

Algae and dye solution preparation: Biosorbent is the material on whose surface the adsorption takes place. The collected test algae were identified and authenticated by the botanist of botany department, KLDAV (PG) College, Roorkee. The biosorbents were washed with distilled water several times to remove all the undesired materials and were then kept on a filter paper to reduce water content. In order to obtain non-viable biomass, both the algae were Sun dried for two days followed by oven drying at 70°C for 24 h. Subsequently, they were ground on an agate stone pestle mortar and sieved, to the particles of 100 μm mesh sizes and stored in vacuum desiccators until required.

The dye used in this study is a basic dye, Methylene blue (C.I. 52015, S.D. Fine Chemicals, India, 85% dye content) having molecular formula $C_{16}H_{18}N_3SCl$, Mol. wt. 319.85 g mol^{-1} and λ_{max} 665 nm. The chemical structure of MB is shown in figure 1. The wastewater from the textile industries contains MB along with many other effluents like other dyes then MB, various salts, organic and inorganic solvents, acids, bases and organic matter. In order to avoid interference of these components in biosorption studies, artificial wastewater termed here as synthetic wastewater was prepared by keeping the concentration of MB approximately equal to the wastewater of textile industry effluent. Earlier, there have been some reports in literature also where the researchers have used synthetic wastewaters [**23-24**].

The stock dye solution was prepared by dissolving accurately weighed quantity of dye in distilled water of concentration 1000 mg L^{-1} and diluting it when needed according to experimental requirements.

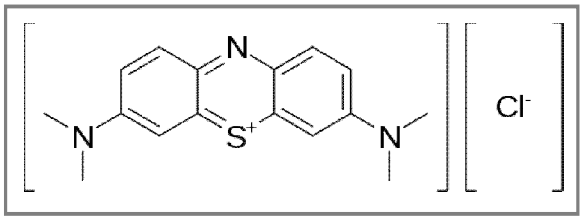


Figure 1. Chemical structure of Methylene blue dye (MB).

Chemical and physical characteristics of biomass: Elemental analysis was done to examine the percentage composition of carbon, nitrogen and sulphur. The Scanning electron micrographs were used to study the surface texture and morphology of biosorbent. BET method was used to observe the surface area of both biosorbents. The presence of various functional groups and the main effective binding sites were identified by FTIR technique.

Batch adsorption studies: Various biosorption experiments were performed to study the effects of parameters such as initial dye concentration, contact time, biosorbent dose, pH, and temperature on the uptake of dye by the biosorbent.

To observe the effect of contact time on biosorption, pre-weighed dried biomass of both the algae were suspended in 100 mL of MB solution to two different concentrations 100 and 200 mg L^{-1}. The effect of contact time was carried from 0 to 300 min. Different dose of both the algal biomass ranging from 1 to 10 g L^{-1} were suspended in the dye solution to study the effect of adsorbent dose on biosorption process. The initial dye concentration was fixed at 100 and 200 mg L^{-1} in all the studies. To study the pH effect on dye biosorption, the pH value was adjusted between 3-9.01 by the addition of 0.1M HCl or 0.1M NaOH. The effect of temperature was studied by performing experiments at three different temperatures (298, 308 and 318 K). The samples in all the above experiments were centrifuged at 200 rpm for 10 min at 298K to ensure complete separation of adsorbent particles from solution. Afterwards, MB concentration was determined by UV-Vis spectrophotometer with the aid of calibration curve. The experiments were performed in triplets and the average values were expressed. Standard deviations were found to be within ± 1.5%. Further, the error bars for the figures were so small as to be smaller than the symbols used to plot the graphs and, hence, not shown. The adsorption capacity was obtained by mass balance equation given as:

$$q_e = (C_o - C_e)\, V / M \qquad \text{--- (1)}$$

Where q_e is the adsorption capacity of algae (mg g^{-1}), C_o and C_e are the initial and the equilibrium concentration of dye (mg L^{-1}), V is the volume of reaction mixture (L) and M is the mass of adsorbent used (g).

To determine the adsorption isotherms and kinetic parameters, various theoretical equations were applied to experimental data and the validity of models is evaluated using the coefficient of determination (R^2). Thermodynamic experiments were performed by mixing MB dye solutions of different concentrations (100, 200 mg L^{-1}) with optimized dose of biomass at different temperatures (298, 308 and 318 K).

Sorption-desorption and reuse studies: Desorption studies were carried out in order to reduce secondary pollution. Repeated adsorption desorption experiments were carried out to check adsorbent reusability, using an acid, a base and a chelating agent for both the algae loaded with MB, for five consecutive cycles. After one complete cycle the resultant algal biomass loaded with MB were filtered and reintroduced into the desorption solvents and agitated for carrying out the next cycle. Desorption ratio is given as the amount of dye desorbed to the amount of dye adsorbed multiplied by 100.

RESULTS AND DISCUSSION

Characterization of biosorbents: The surface area of *Vaucheria* sp. and of *Chara* sp. was found to be 3.248 and 0.855 m^2 g^{-1}, respectively by BET method **[25]**. Elemental Analysis of both the algal biomass showed the composition of Carbon, Nitrogen, Hydrogen and Sulphur to be 35.93, 4.28, 4.590 and 0.744 respectively for *Vaucheria* sp. and 13.55, 0.32, 0.477 and 0.426 respectively for *Chara* sp. The SEM images for *Vaucheria* sp. (figure 2A and figure 2B) and *Chara* sp. (figure 2C and figure 2D) showed the surface texture and morphology of algal biomass before and after biosorption of MB dye. From the micrographs it is seen that the rough and irregular algal surface after biosorption of dye are more occupied which may be due to the possibility of dye molecules being trapped or biosorbed on the surface.

Cell walls of algal biomass have a complex chemical composition with different functional groups being responsible for dye removal. For this, the FTIR spectra of both the test algae before and after biosorption of dye were determined (spectra not shown). FTIR spectra confirmed the changes in functional group and surface properties of the biosorbent. IR spectra of *Vaucheria* sp. before biosorption showed prominent bands at 3423.09 cm^{-1}, 2925.13 cm^{-1}, 1644.72 cm^{-1}, 1535.31 cm^{-1}, 1432.55 cm^{-1}, 1068 cm^{-1} 835.00 cm^{-1}, 611.00 cm^{-1} and 478.1 cm^{-1}. Similarly, *Chara* sp. showed

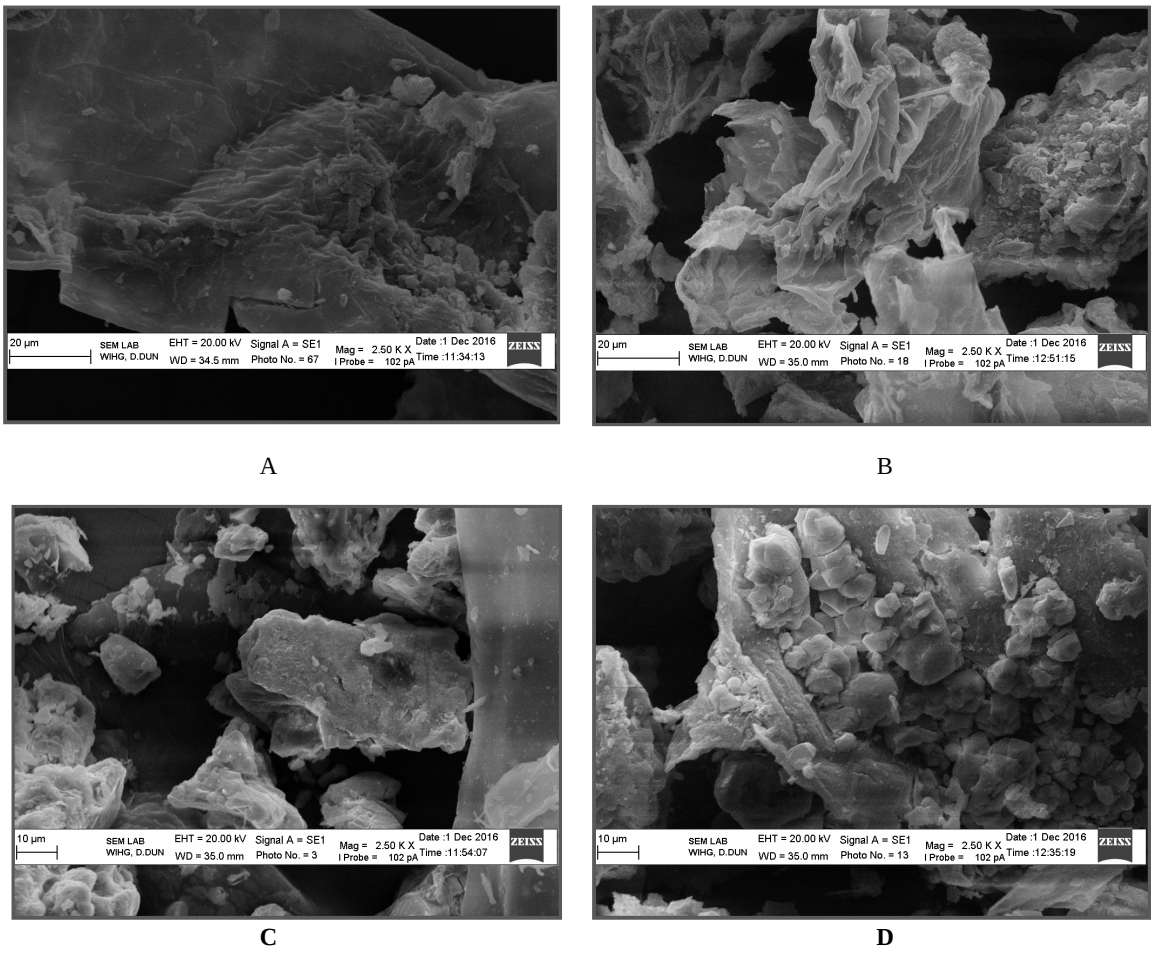


Figure 2. SEM micrographs of (A) *Vaucheria* sp. before biosorption (B) *Vaucheria* sp. after biosorption of MB (C) *Chara* sp. before biosorption (D) *Chara* sp. after biosorption of MB.

prominent bands at 3416.05 cm^{-1}, 2926.42 cm^{-1}, 2515 cm^{-1}, 1610.00 cm^{-1}, 1410.00 cm^{-1}, 1096.81 cm^{-1}, and 873.85cm^{-1} indicating biosorbent heterogeneity, i.e. the presence of functional groups like carboxyl, hydroxyl, phenolic and amino groups on the algal cell surface responsible for sequestration of contaminants (dye) from synthetic wastewater. Table 1 show the shifts in the peak after the biosorption of MB onto the algae, which suggest that there is the formation of new bonds between biosorbent and dye molecule and that the binding takes place on the surface of the biomass.

Factors affecting adsorption of dye: There are many factors affecting dye adsorption such as contact time, biosorbent dose, solution pH, temperature and initial dye concentration. Thus, the effects of these parameters are to be taken into account.

Effect of contact time: The effect of contact time on the biosorption of MB onto both the test alga at two different concentrations of dye (100 and 200 mg L^{-1}) has been shown in figure 3. The figure showed that the rate of biosorption of MB is rapid in the initial stage and then it gradually slowed down until it attained equilibrium and then there was no significant increase. The curve represented three different phases. The first phase represents high biosorption of dye which may be due to chemisorption type of interaction of dye over algal biomasses. This rapid biosorption may be regarded due to the participation of specific functional groups and active surface sites. The second phase that shows slow biosorption indicates that all the active sites were utilized and attains saturation or equilibrium phase. The third phase is the equilibrium phase in which the biosorption was almost negligible [**26**]. A large amount of MB was adsorbed within 140 and 120 min of contact time for *Vaucheria* sp. and *Chara* sp. respectively.

Table 1. IR absorption bands and corresponding possible functional groups present on algal biomass *Vaucheria* sp. and *Chara* sp.

Vaucheria sp. before MB adsorption (cm^{-1})	*Vaucheria* sp. after MB adsorption (cm^{-1})	*Chara* sp. before MB adsorption (cm^{-1})	*Chara* sp. after MB adsorption (cm^{-1})	Bonds indicative of functional groups
3423.09	3429.47	3416.05	3425.12	Carboxylic/OH stretch and N-H stretch
2925.13	2921.26	2926.42	2930.43	Phenolic/carboxylic
-	-	2515.66	2515.97	
1644.72	1643.07	1610.00	1630.00	=C=O stretch, >C=C, >C=N, Amide I band
1535.31	1546.76	-	-	Amide II band
1432.55	1427.62	1410.00	1423.29	OH bond, symmetric bending of CH3 of the acetyl moiety
1068.79	1059.51	1096.81	1116.78	≡C-N<
-	-	873.85	875.49	Plane deformation
611.00	611.98	710.3	712.71	
478.11	482.25	-	-	C-N-S scissoring

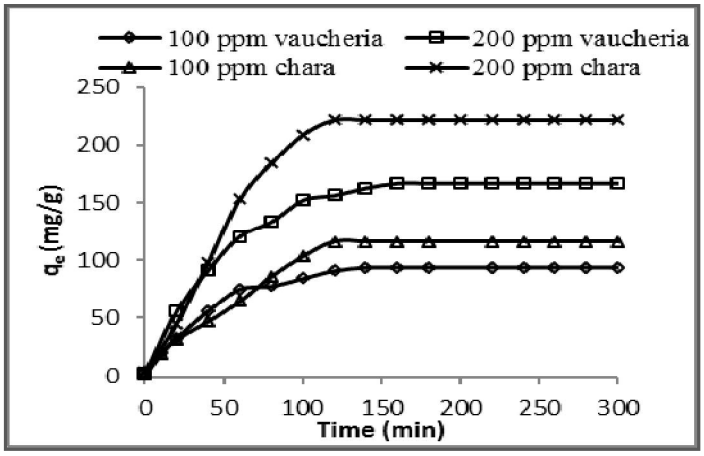


Figure 3. Effect of contact time on the extent of biosorption of MB onto two algal biomasses(*Vaucheria* sp. and *Chara* sp.*)*, MB concentration 100 and 200 mg L^{-1}

Effect of biosorbent dose: The effect of biosorbent dose on the MB biosorption was studied by taking different amounts (1-10 g L^{-1}) of algal biomass *(Vaucheria* sp. and *Chara* sp.*)* suspended in 100 mL of the working solution having 100 and 200 mg L^{-1} dye concentration. Other parameters i.e., pH and contact time were kept constant. The biosorption of MB by both the algal biomass is shown in the figure 4. The graph shows that on increasing the adsorbent dose the biosorption capacity increased up to certain dose and then decreased. This may be attributed to the fact that at low dose all the type of active sites is exposed and biosorption takes faster and attains saturation. But, when the dose is increased after a certain limit the partial overlapping or aggregation of adsorbent at higher biomass concentration result in decrease in total surface area and availability of biosorption sites [23]. The maximum biosorption was reported at 4 g L^{-1} and 2 g L^{-1} of *Vaucheria* sp. and *Chara* sp. at 200 mgL^{-1} of MB concentration.

Effect of pH regime: The pH of aqueous solution influences the biosorption of dye to a very high extent due to its influence on the binding sites of the biosorbent. The effect of pH on the biosorption of MB on test algae was studied at pH ranging from 3 to 9.01 and the results are presented in figure 5. Results indicated that as the pH varied from acidic to basic medium the biosorption of MB increased and attained maximum value at pH 7.5 for *Vaucheria* sp. and 6.33 for *Chara* sp. The pH varies with

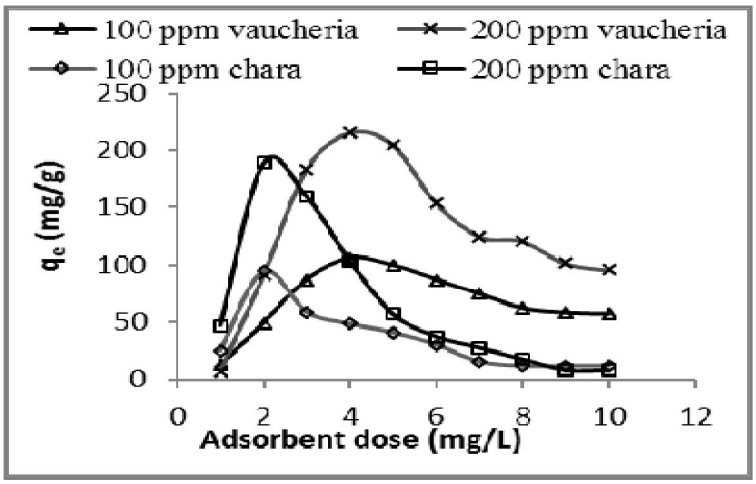


Figure 4. Effect of biosorbent dose on the biosorption of MB onto two algal biomasses (*Vaucheria* sp. and *Chara* sp.*)*, MB concentration 100 and 200 mg L^{-1}.

the degree of ionization and also the surface properties of the biosorbent. In earlier studies it is reported that adsorption process increases with increase in the electrostatic attraction [**12**]. MB when dissolved in water releases cations which get attracted towards the surface of biosorbents containing different functional groups such as carboxyl, hydroxyl and other charged groups. At lower pH the biosorbent gets positively charged and reduces the biosorption whereas at higher pH the biosorbents gets negatively charged and enhances the electrostatic attraction of MB cation. The same trend was observed on the MB uptake by *Posidonia oceanic* fibres [**11**].

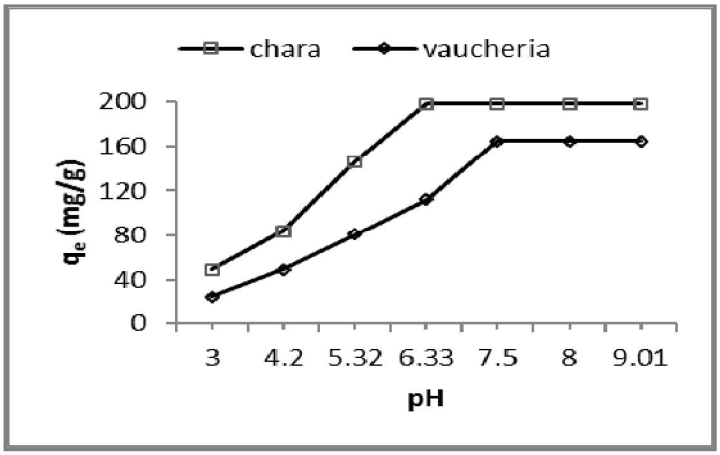


Figure 5. Effect of pH on the biosorption of MB onto two algal biomasses (*Vaucheria* sp. and *Chara* sp.*);* MB concentration 200 mg L^{-1}.

Effect of Temperature and Thermodynamic study: The effect of temperature on biosorption by both the algal biomass has been studied at three different temperatures (25, 35, 45ºC) and is shown in figure 6A and 6B. As the temperature was increased from 25 to 45ºC there was an increase in biosorption process which indicates that it is endothermic in nature. As the temperature increases the boundary layer thickness of biosorbent decreases thus leading to the increase in the availability of active sites and porosity and also increases kinetic energy of dye molecules [**27**]. The endothermic nature of dye biosorption has also been reported for the biosorption of Reactive Red 120 on *Chara contraria* [**20**]. The present results showed essentially no thermal deactivation of biosorption activity under operational temperatures.

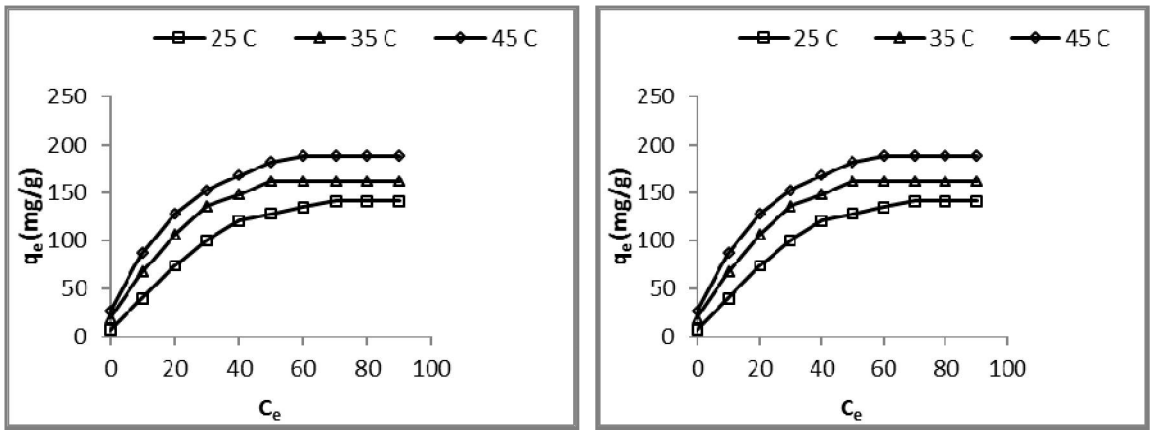


Figure 6. Adsorption isotherms at three different temperatures for algal biomass: (A) *Vaucheria* sp. and (B) *Chara* sp.

To deduce the biosorption mechanisms, thermodynamic parameters like standard free energy changes (ΔG^o, kJ mol^{-1}), enthalpy changes (ΔH^o, kJ mol^{-1}) and entropy changes (ΔS^o, kJ mol^{-1} K^{-1}) associated with the sorption of dye on algal biomass were calculated using the following equations.

$$\Delta G^o = - R\ T\ \ln (b) \qquad \text{--- (2)}$$

$$\ln (b_2 / b_1) = - \frac{\Delta H^0}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \qquad \text{--- (3)}$$

$$\Delta G^o = \Delta H^o - T\ \Delta S^o \qquad \text{--- (4)}$$

Table 2 shows the values of all the three parameters. The positive value of ΔH^o indicates that MB biosorption onto both the algal biomass is physical and endothermic reaction. The biosorption process is generally considered as physical process if ΔH^o < 25 kJ mol^{-1} and as chemical process when ΔH^o < 40 kJ mol^{-1} [**26**]. Further, the positive values of ΔS^o specifies the presence of randomness and negative values of ΔG^o shows the spontaneity of the reaction.

Adsorption Equilibrium Study: The adsorption equilibrium study indicates how the adsorbed molecules distribute between the liquid phase and the solid phase when the adsorption process reaches an equilibrium state. The analysis of the isotherm data is done by fitting them to different isotherm models equations and it is the important step to find the suitable model that can be used for design purpose. For obtaining adsorption isotherms, both algal biomass (4 g L^{-1} of *Vaucheria sp* and 2 g L^{-1} of *Chara* sp.) were suspended in MB dye solution. The experiments were carried at three different temperatures i.e. 298, 308 and 318K. Various models like Langmuir, Freundlich, Temkin and Dubinin –Radushkevich, were applied to find the model that describes the experimental data. The value of regression coefficient obtained from these models was used to find the best fit isotherm.

The Langmuir isotherm assumes that the dye biosorption occurs on a uniform surface with homogenous binding sites and equivalent sorption energies. Monolayer sorption can be expressed as:

$$\frac{1}{q_e} = \frac{1}{Q_0} + \frac{1}{bQ_0 C_e} \qquad \text{--- (5)}$$

Where q_e is the amount adsorbed (mg g^{-1}), C_e is the equilibrium dye concentration of adsorbate (mgL^{-1}), Q_0 is the Langmuir constant related to maximum monolayer adsorption capacity (mg g^{-1}) and b is

the constant related to free energy. The values of these constants were calculated by plotting a graph of 1/ q_e versus 1/ C_e. The graph gave a straight line with the correlation coefficient R^2=0.997 for both the algal biomass which shows that the biosorption of MB onto algae fits Langmuir isotherm reasonably. Furthermore, the value of q_e which is the measure of maximum biosorption capacity of MB onto *Vaucheria* sp. and *Chara* sp. was found to be 166.66 and 200 mg g^{-1} respectively. This proves both, the homogeneous distribution active sites of algal biomasses and the formation of monolayer coverage of MB dye molecule at its outer surface [**28**].

The essential characteristics of Langmuir isotherm are embodied in terms of a dimensionless separation factor R_L expressed as

$$R_L=1/ (1+ bC_0) \quad \text{--- (6)}$$

The values of R_L were computed at different temperatures and Table 2 shows the R_L value that lies between 0 and 1 thus confirming the favourable nature of the adsorption isotherm as per the criterion which is as follows:

$R_L > 1$	Unfavourable
$R_L = 1$	Linear
$0 < R_L < 1$	Favourable
$R_L = 0$	Irreversible

The Freundlich isotherm assumes a heterogeneous surface with interaction between adsorbed molecules with different surface energies. It can be expressed as:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad \text{--- (7)}$$

The plot between ln q_e versus ln C_e was drawn and the intercept and slope were used to calculate the value of K_F and n. The value of K_F and n indicated the adsorption capacity and adsorption intensity, respectively. The magnitude of K_F increased with increasing temperature value (Table 2) showed easy separation of dye molecules on the alga from aqueous solution. Besides, high values of n indicated high affinity between the solute molecules and the adsorbent.

The Temkin isotherm is based on the assumption that the heat of adsorption would decrease linearly with the increase of coverage of adsorbent. Temkin isotherm can be expressed as

$$q_e = RT/ B_T \ln (A_T C_e) \quad \text{--- (8)}$$

Where R is the gas constant, T is the absolute temperature in kelvin. The constants b_T and A_T were calculated from slope and intercept of q_e versus ln C_e. The low value of A_T and R^2 values shows that equilibrium isotherm of MB on both the algal biomasses is poorly described by Temkin isotherm. The Dubinin-Raduskevich (D-R) isotherm equation has been often used to determine the mean adsorption energy that may provide useful information with regard to whether or not biosorption is subject to a chemical or physical process. It can be evaluated using the following equations:

$$\ln q_e = \ln q_m - \beta \varepsilon^2 \quad \text{--- (9)}$$

$$\varepsilon = RT \ln (1+1/Ce) \quad \text{--- (10)}$$

Where q_m is maximum biosorption capacity(mg g^{-1}), β is constant related to biosorption energy (mol^2 kJ^{-2}), ε is a Polanyi potential, R is the gas constant (8.314 J $mol^{-1}K^{-1}$), T is temperature(K). The mean adsorption energy can be calculated from formula

$$E = 1/\sqrt{2\beta} \qquad \text{--- (11)}$$

If the value of E lies between 8 kJ mol^{-1} and 16 kJ mol^{-1}, then the biosorption process is a chemical biosorption, and if the value of E is lower than 8 kJ mol^{-1} then it is a physical biosorption [**20**]. In this study the calculated value of E for both the biosorbents is lower than 8 kJ mol^{-1}, which indicates that the biosorption of MB on to test algae is a physical biosorption.

Table 2 shows the value of the constants and the correlation coefficients for all the four isotherms. As it can be seen the values of correlation coefficient were high at different temperatures for Langmuir model followed by Freundlich, Temkin and D-R model. The value of *b* is found to be higher for *Chara* sp. indicating higher affinity of MB for *Chara* sp. than *Vaucheria* sp. Moreover, the values of *n* calculated by Freundlich isotherm are higher than unity at all temperatures which indicates favourable biosorption.

Table 2. Adsorption isotherm model and thermodynamic parameters for the biosorption of MB onto two algal biomass (*Vaucheria* sp. and *Chara* sp.*)* at different temperatures

Isotherm Parameters	*Vaucheria* sp.			*Chara* sp.		
	298K	308K	318K	298K	308K	318K
Langmuir isotherm						
B L mg^{-1})	0.02711	0.035609	0.0425	0.0274	0.311	0.446
q_e (mg g^{-1})	125	136.986	166.66	111.1	142.85	200
R^2	0.990	0.995	0.997	0.990	0.993	0.997
Thermodynamic parameters						
ΔG^o (kJ mol^{-1})	-22.466	-23.918	-25.657	-22.497	-23.572	-25.2925
ΔS^o (kJ mol^{-1} K^{-1})	0.134955	0.135386	0.1365	0.139837	0.138788	0.139836
ΔH^{o*} (kJ mol^{-1})		17.750			19.175	
Freundlich isotherm						
n	2.141	2.463	3.355	1.686	1.763	1.788
K_F(mg g^{-1})	13.157	14.85	23.359	7.869	8.775	9.088
R^2	0.963	0.957	0.960	0.969	0.954	0.951
Temkin isotherm						
A_T	0.526	0.613	0.792	0.597	0.661	0 .798
b_T	58.766	63.874	72.177	53.557	57.286	63.401
R^2	0.955	0.954	0.977	0.956	0.948	0.949
D-R isotherm						
q_m(mg g^{-1})	110	120	125	108	133	198
E(kJ mol^{-1})	0.079	0.084	0.288	0.118	0.129	0.158
R^2	0.940	0.928	0.916	0.801	0.923	0.932
Dimensionless separation factor						
R_L	0.155	0.123	0.105	0.154	0.158	0.011

Comparison with other biosorbents: The comparative data for the maximum monolayer adsorption capacity of MB dye onto both the algal biomass used in the present study and various other biosorbents have been presented in table 3. It shows that the biosorption capacity of *Chara* sp. is higher than that of *Vaucheria* sp. for MB and also were significantly high than the other biosorbents. This shows that both these algae are better biosorbents for MB removal.

Adsorption kinetic study: To study the mechanism involved in biosorption process pseudo-first-order and pseudo-second-order models were used. The best fit model was determined based on the linear regression correlation coefficient values. Kinetic studies of both the algal biomass were carried

out at two initial MB concentrations (100 and 200 mg L^{-1}) at 298K. The extent of biosorption was studied at regular time interval.

Table 3. Comparison of maximum monolayer adsorption capacity of Methylene blue onto various biosorbents

Adsorbents	qe (mg g^{-1})	References
Duckweed (Spirodela polyrrhiza)	144.93	[6]
Posidonia oceanica fibres	5.56	[11]
Caulerpa racemosa var. cylindrace (alga)	18	[12]
Cystoseira barbatula Kutzing	38.61	[13]
Ulva lactuce alga	40.2	[14]
De oiled algal biomass	107.51	[15]
Oscillatoria sp.*(alga)*	129.58	[16]
Living fungus (Aspergillus niger)	18.54	[29]
Dead fungus (Aspergillus niger)	1.17	[29]
Vaucheria sp(alga)	166.66	This study
Chara sp.*(alga)*	200	This study

The linear form of the pseudo-first order rate expression of Lagergren can be expressed as [30]

$$\log (q_e - q_t) = \log q_e - \frac{k_{1,\,ads}}{2.303} t \qquad \text{--- (12)}$$

Where q_t (mg g^{-1}) is the amount of dye adsorbed by biomass at equilibrium time t, k_1 is the pseudo-first order rate constant (min^{-1}). The graph between log (q_e–q_t) versus t (time) was plotted in order to calculate the constants.

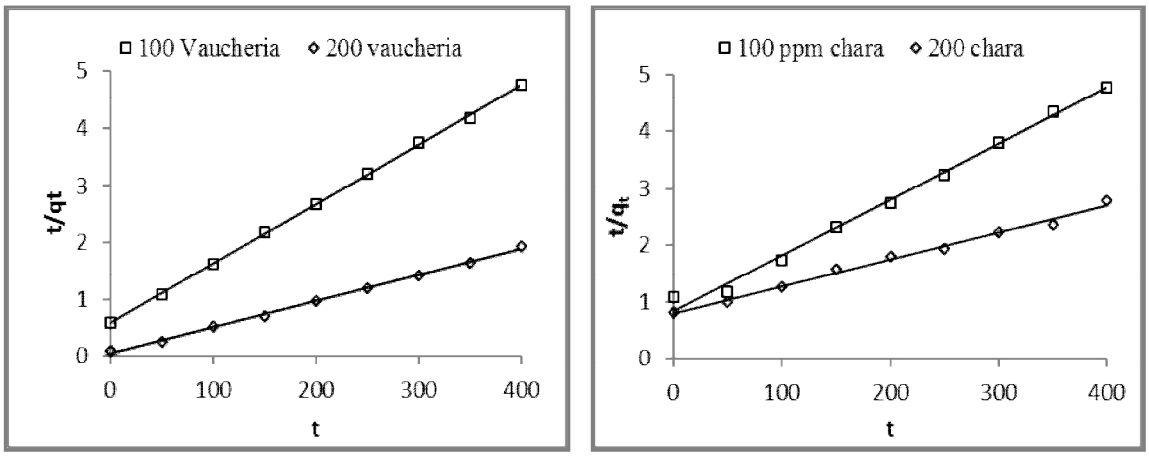
The pseudo-second order model can be expressed in its linear form by the equation [31].

$$\frac{t}{q} = \frac{1}{k_{2,ads} q_e^2} + \frac{1}{q_e} t \qquad \text{--- (13)}$$

Where k_2 (g mg^{-1} min^{-1}) is the rate constant of second order adsorption. The graph plotted between t/q versus t (figure 7A and B) was used for calculating the constants. Table 4 represents the values of constants calculated for pseudo-first order and pseudo-second order kinetics for the biosorption of MB on both the algal biomasses *Vaucheria* sp. and *Chara sp* at two different concentrations of dye. From the table the values of R^2 for pseudo first order kinetics for both the algae were lower than the R^2 value of pseudo-second order kinetics. Further, the q_e (calculated biosorption capacity) values from second order model agreed well with the q_{exp}. values (experimental). This indicates that the biosorption of MB onto both the algal biomass is not first-order kinetics but a second order kinetics. Similar results were also observed for the biosorption of MB on Mediterranean green alga *Enteromorpha* sp. and *Ulothrix* sp [32, 33].

Table 4. Kinetic parameters estimated by pseudo-first order, pseudo-second order and intra-particle diffusion models for both algal biomass *Vaucheria* sp. and *Chara* sp. at two different concentrations of dye

Algae	Initial dye Conc. (mg L^{-1})	q_e(exp) (mg g^{-1})	First-order model			Second-order model			Intra-particle model	
			K_1 (x 10^{-3} min^{-1})	$q_{e\,cal.}$ (mg g^{-1})	R^2	K_2 x 10^{-3} (g mg^{-1} min^{-1})	q_e(cal) (mg g^{-1})	R^2	K_W (mg g^{-1} $min^{0.5}$)	R^2
Vaucheria sp.	100	94.12	9.212	71.614	0.956	0.170	100	0.999	1.73	0.914
	200	216.80	11.515	76.208	0.924	1.689	232.56	0.994	2.942	0.906
Chara sp.	100	116.18	11.013	106.905	0.980	0.096	111.11	0.997	3.274	0.929
	200	221.75	13.818	89.54	0.977	0.020	250	0.991	5.558	0.916



A B

Figure 7. Second-order kinetic modelling of Biosorption of MB onto algal biomass (A) *Vaucheria* sp. (B) *Chara* sp.

Diffusion process: A numbers of steps are believed to control the rate of dye biosorption onto algal surface. These include (a) "molecular diffusion" which takes place from the bulk solution to a film layer surrounding the adsorbent particle; (b) "film diffusion" where diffusion proceeds from the film to particle surface; (c) "surface diffusion", migration inside the adsorbent particle and (d) "pore diffusion" diffusion, within liquid-filled pores, and the adsorption uptake may involve several ways of interactions such as chemisorption, physiosorption, ion exchange, or complexation [34].

In case of solid-liquid biosorption process, the solute transfer is characterized by film diffusion or intra-particle diffusion or both. In this study intra-particle diffusion based mechanism was studied in order to investigate the mechanism of MB biosorption onto the algal biomass. It was found that the biosorption of MB on both the algal biomass varied almost proportionately with the square root of the contact time ($t^{1/2}$). Weber and Morris proposed the most widely used intra-particle diffusion equation for biosorption system as:

$$q_t = k_{id}\, t^{1/2} \qquad \text{--- (14)}$$

Where q_t is the amount of MB adsorbed per unit mass of biosorbent (mg/g) at time t and k_{id} the intra-particle diffusion rate constant (mg g^{-1} $min^{-1/2}$).

If the plot between q_t versus $t^{1/2}$ is linear then intra-particle diffusion is the mechanism of adsorption process figure 8 indicates that the graph plotted between q_t and $t^{1/2}$ was not linear with time. The intra-particle diffusion took place in two phases. The first phase was attributed to macropore or boundary layer diffusion and the second linear phase was due to the micropore diffusion. Figure 8 also shows that the plot does not pass through the origin suggesting that although intra-particle diffusion is involved in the biosorption process but it is not the sole rate-controlling step, and some other mechanism are also involved [35].

Activation parameters: The activation energy (E_a) for biosorption of MB by *Vaucheria* sp. and *Chara* sp. was determined by using the Arrhenius equation [36]. The equation is expressed as:

$$\ln k = \ln A - E_a/RT \qquad \text{--- (15)}$$

Where k is pseudo-second-order rate constant, A is the Arrhenius constant, E_a is the activation energy (kJ mol^{-1}), R is the gas constant (8.314 J $mol^{-1}K^{-1}$), and T is the absolute temperature (K).

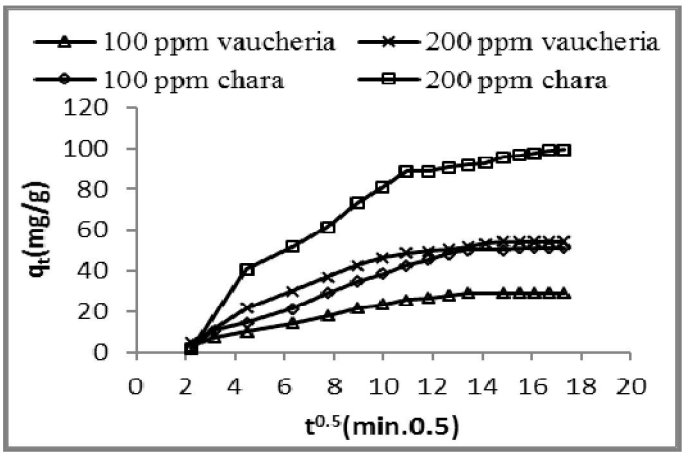
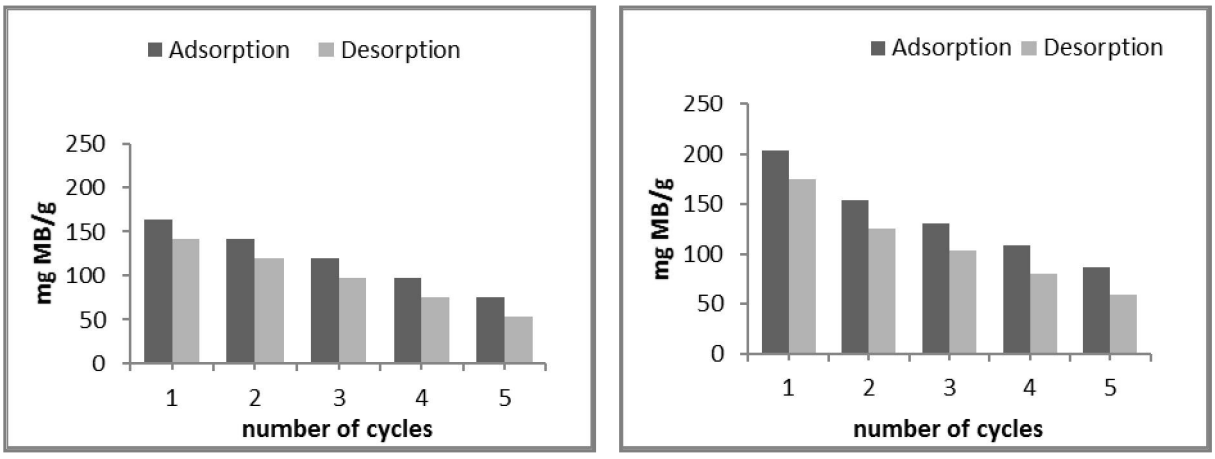


Figure 8. Intra-particle diffusion plot for the biosorption of MB onto algal biomass *Vaucheria* sp. and *Chara* sp.

The magnitude of E_a is generally used to predict the nature of biosorption process, (i.e.) whether it is a physical or chemical process. The physical biosorption ranging from 5 to 40 kJ mol^{-1} is reversible and require low energy, whereas chemical biosorption involves stronger forces and requires high activation energy (40-800 kJ mol^{-1}). The Activation energy for the biosorption of MB onto *Vaucheria* sp. and *Chara* sp. estimated from the slope of the linear plot ln k_2 vs. 1/T was 8.800 kJ mol^{-1} and 9.658 kJ mol^{-1} respectively, suggesting that the biosorption could be mainly physical biosorption. Similar result was shown on Reactive Red 120 dye biosorption on *Chara contraria* [**20**].

Sorption-desorption and Reuse study: In order to study the reusability of the both algal biomasses *Vaucheria* sp. and *Chara* sp. after the biosorption, repeated batch experiments were performed. Both the algal biomasses were eluted using 0.1M sol of an acid (HCl), a base (NaOH), and a chelating agent (EDTA (Ethylene diamine tetra acetic acid)) each, loaded with MB for five consecutive cycles using the same biosorbents. One complete cycle followed the sequence consisting of adsorption followed by desorption (temperature 298 K, agitation 200rpm, biosorbent mass (4g L^{-1} of *Vaucheria* sp. and 2g L^{-1} of *Chara* sp.), dye concentration 200 mg L^{-1}, adsorption contact time (140 min for *Vaucheria* sp. and 120 min for *Chara* sp.) and desorption contact time 120min). After one complete cycle the resultant algal biomass loaded with Methylene blue were filtered and reintroduced into the



A B

Figure 9. Adsorption/desorption cycles for (A) *Vaucheria* sp. (B) *Chara* sp. using HCl.

desorption solvents and agitated for carrying out the next cycle. More than 85% of MB was desorbed from both algae using HCl. The reversibility of biosorption depends on the presence of binding forces between biosorbent surface and the dye molecule. High desorption may occur in case of weak binding forces such as Van der waal's forces or dipole-dipole interaction, but if strong forces like ionic or covalent bonding is present then the reversibility of biosorption will be low [27]. Thus, the reusability study of the biomass has a long term benefit while studying their practical and economical applications.

Disposal of the used biomass: As we have used the same biomass repeatedly for multiple cycles, the volume of used biomass for ultimate disposal is less. For disposal, firstly, the used biomass is sterilised by microwave irradiation for 10 minutes followed by encapsulating the biomass in concrete, very deep in pits. Sterilisation of the biomass is required to prevent decay and any subsequent leakage from the disposed area. This is done to make the process eco-friendly.

APPLICATION

Abundantly available yellow green alga *Vaucheria* sp. and green alga *Chara* sp. used in the present study can be effectively used as a low cost adsorbent for the removal of Methylene blue dye from aqueous solution.

CONCLUSION

Both the algal biomasses *Vaucheria* sp. and *Chara* sp. proved to be efficient in the removal of MB from synthetic wastewater. Biosorption of MB by *Vaucheria* sp. occurred within 140 min of contact time at 7.5 pH, algal dose 4g L^{-1} at 200 mg L^{-1} of initial dye concentration, whereas for *Chara* sp. It occurred within 120 min of contact time at optimum pH of 6.33 and algal dose 2 g L^{-1} at the same initial dye concentration. Both the algae fitted well in Langmuir isotherm model that proves monolayer adsorption of dye. Analysis of data shows that the process involves second order kinetics and thermodynamic treatment of equilibrium data shows endothermic nature of the adsorption process. The calculated values of Activation Energy (E_a), Mean adsorption energy (E), and Enthalpy change (ΔH^o) confirmed the physical nature of biosorption of MB onto both *Vaucheria* sp. and *Chara* sp. IR spectra showed the presence of various functional groups. The maximum biosorption capacity for *Vaucheria* sp. and *Chara* sp. was found to be 166.66 mg g^{-1} and 200 mg g^{-1} respectively hence *Chara* sp. is a better biosorbent compared to *Vaucheria* sp. under optimum conditions. The two test biosorbents were reused in five biosorption desorption cycles with negligible decrease (up to 85% recovery) in their biosorption capacities. Both algal biomasses are abundantly available in nature, renewable, inexpensive, bio-degradable and eco-friendly with high adsorption capacity and can be efficiently used for removal of dyes from wastewater.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1]. A. Talaiekhozania, S. Rezaniab, Application of photosynthetic bacteria for removal of heavy metals, macro pollutants and dye from wastewater: A review, *J. Water Process Eng*, **2017**, 19, 312-321.

[2]. M. Rafatullah, Adsorption of methylene blue on low-cost adsorbents: a review, *J Hazard. Mater.*, **2010**, 177 (1-3), 70-80.

[3]. N. Sharma, T. Alam, A. Rastogi, H. Tarannum, Gita Saini, Adsorptive removal of Alizarin red-s from aqueous solutions by using Cobalt and Copper Ferricyanides, *J. Applicable Chem.*, **2015**, 4 (3), 871-882.
[4]. T. V. Nagalakshmi, K. A. Emmanuel, C. Sureshbabu, K. Nagaraju, K. S. Rao, Adsorption of basic textile dye from aqueous solution by prepared activated carbon, *J. Applicable Chem.*, **2016**, 5 (2), 452-465.
[5]. C. Suresh Babu, C. Chakrapani, K. A. Emmanuel, K. S. Rao, Adsorption kinetics of a cationic dye onto indigenously prepared activated Kaza's Carbon, *J. Applicable Chem.*, **2014**, 3 (6), 2462-2469.
[6]. P. Waranusantigul, P. Pokethitiyook, M. Kruatrachue, E. S. Upatham, Kinetics of basic dye (methylene blue) biosorption by giant duckweed (*Spirodela polyrrhiza*), *Environ Pollution*, **2003**, 125, 385-392.
[7]. B. H. Hameed, Removal of cationic dye from aqueous solution using jackfruit peel as non-conventional low- adsorbent, *J. Hazard. Mater.*, **2009**, 162, 344 -350.
[8]. B. H. Hameed, Evaluation of papaya seeds as a novel non-conventional low-cost adsorbent for removal of methylene blue, *J. Hazard. Mater.*, **2009,** 162, 939-944.
[9]. N. S. Rajurkar, N. S. Walvekar, Removal of Methylene Blue and Indigo Carmine from aqueous solutions using *Couroupita Guianensis* leaves as an adsorbent, *J. Applicable Chem.*, **2014**, 3 (6): 2602-2610.
[10]. K. V.Kumara, K. Porkodi, Mass transfer, kinetics and equilibrium studies for the biosorption of methylene blue using *Paspalum notatum*, *J. Hazardous Material*, **2007,** 146, 214–226.
[11]. M. C. Ncibi, B. Mahjoub, M. Seffen, Kinetics and equilibrium studies of methylene blue biosorption by *Posidonia oceanic (L) fibres*, *J of Hazard. Mater.*, 2007, 139, 280-285.
[12]. S. Cengiz, L. Cavas, Removal of methylene blue by invasive marine seaweed: *Caulerpa racemosa* var. cylindracea, *Biores. Technol.*, **2008**, 99, 2357– 2363.
[13]. D. Caparkaya, L. Cavas, Biosorption of Methylene Blue by a Brown Alga *Cystoseira barbatula* Kutzing, *Acta Chimica Slovenica.*, **2008**, 55, 547 -553.
[14]. A. ElSikaily, A. Khaled, A. ElNemr, O. Abdelwahab, Removal of Methylene blue from aqueous solution by marine green alga *Ulva lactuca*, *Chemistry and Ecology*, **2006**, 22, 149-157.
[15]. R. Maurya, T. Ghosh, C. Paliwal, A. Shrivastav, K. Choksi, I. Pancha, A. Ghosh, S. Mishra, Biosorption of Methylene Blue by De-Oiled Algal Biomass: Equilibrium, and Artificial Neural Network Modelling, *PLOS ONE*, **2014**, 9(10).
[16]. S. Sagar, A. Rastogi, Biosorption of Methylene blue from aqueous solutions by using blue green algae *Oscillatoria* sp.: Kinetic and equilibrium studies, *J. Applicable Chem.*, **2017**, 6(3), 374-384.
[17]. M. A. Khalaf, Biosorption of reactive dye from textile wastewater by non-viable biomass of *Aspergillus niger* and *Spirogyra* sp., *Biores. Technol.*, **2008**, l99 (14), 6631-6634.
[18]. A. R. Khatee, G. Dehghan, A. Ebadi, M. Zarei, M. Pourhassan, Biological Treatment of a dye solution by Macroalgae *Chara sp*: Effect of operational parameters, intermediates identification and artificial neural network modelling, *Biores. Technol.*, **2010**, 101(7), 2252-2258.
[19]. A. R. Khatee, G. Dehghan, E. Ebadi, M. Pourhassan, Central Composite design Optimization of Biological Dye Removal in the presence of Macroalgae *Chara sp*, *Clean Soil Air Water*, **2010**, 38(8), 750-752.
[20]. A. Celekli, G. Ilgun, H. Bozkurt, Sorption Equilibrium, Kinetic, Thermodynamic and desorption studies of Reactive Red 120 on *Chara contraria*, *J of Chem Eng*. **2012**, 191, 228-235.
[21]. A. Celekli, B. Tanrivedi, H. Bozkurt, Predictive Modelling of removal of Lanaset Red G on *Chara contraria*; kinetic, equilibrium, and thermodynamic studies, *J. Chem. Eng*, **2011,** 169, 166-172.
[22]. A. R. Khatee, M. Zarei, G. Dehghan, E. Ebadi, and M. Pourhassan, Bio treatment of a triphenylmethane dye solution using a Xanthophyta alga: Modelling of key factors by neural network, *J. the Taiwan Institute of Chem Eng.*, **2011**, 42(3), 380-386.

[23]. R. H. Crist, J. R. Martin, P. W. Guptill and J. M. Eslinger, D. R. Crist, Interaction of metals and protons with algae. 2. Ion exchange in adsorption and metal displacement by protons, *Environ Science and Techno*, **1990**, 24(3), 337-342.
[24]. M. Lal, K. Daga, V. Vaishnav, S. Aggarwal, Adsorption of Benzoazurin-G from synthetic wastewater by activated charcoal derived from Eichhornia leaf (water weeds), *Inter J of Scientific and Eng Res*, **2013**, 4(8), 2229-5518.
[25]. B. Stephen, P. H. Emmett, E. Teller, Adsorption of Gases in Multimolecular Layers¸ *Jj American Chem Society*. **1938**, 60 (2), 309–319.
[26]. A. Saeed, M. Iqbal, S. I. Zafar, Immobilization of *Trichoderma viride* for enhanced methylene blue biosorption: Batch and column studies, *J, Hazard. Mater.*, 2009, 168, 406 – 415.
[27]. V. K. Gupta, D. Pathania, S. Sharma, S. Agarwal, P. Singh, Remediation and recovery of methyl orange from aqueous solution onto acrylic acid grafted *Ficus caria* fiber: Isotherms, kinetics and thermodynamics, *J. Molecular Liquid*, **2013**, 177, 325-334.
[28]. T. M. Elmorsi, Equilibrium Isotherms and Kinetic Studies of Removal of Methylene Blue Dye by Adsorption onto Miswak Leaves as a Natural Adsorbent, *Journal of Environmental Protection*, **2011**, 2, 817-827.
[29]. Y. Fu, T. Viraraghavan, Removal of dye from an aqueous solution by the fungus *Aspergillus niger*, *Water quality Res J. Canada*, 2000, 35(1), 95-111.
[30]. Y. S. Ho, G. McKay, The sorption of lead(II) ions on peat response to comment, *Water Res.* **1999**, 33, 578-584.
[31]. G. McKay, Y. S. Ho, Pseudo-second-order model for sorption processes, *Process Biochemical*. **1999**, 34, 1999, 451-465.
[32]. M. C. Nibi, A. M. B. Hamissa, A. Fathallah, M. H. Kortas, T. Baklouti, B. Mahjoub, M. Seffen, Biosorptive uptake of methylene blue using Mediterranean green alga *Enteromorpha spp., J. Hazard Mater.*, 2009, 170, 1050-1055.
[33]. C. Dogar, A. Gurses, M. Acikyildiz, E. Ozkan, Thermodynamics and kinetic studies of biosorption of a basic dye from aqueous solution using green algae *Ulothrix* sp. *Colloids and surface Biointerfaces*, **2010**, 76, 279-285.
[34]. V. K. Gupta, R. Bhushan, A. Nayak, P. Singh, B. Bhushan, Biosorption and reuse potential of Blue Green alga for the removal of hazardous Reactive dyes from Aqueous solutions, *Bioremed. Journal*, **2014**, 18(3), 179-191.
[35]. S. J. Allen, G. Mckay, K. Y. H. Khader, Intraparticle Diffusion of a Basic Dye during adsorption onto Sphagum Peat, *Environ. Pollut.*, **1989**, 56(1), 39-50.
[36]. T. S. Anirudhan, P. G. Radhakrishnan, Thermodynamics and adsorption of Cu (II) from aqueous solutions onto a new cation exchanger derived from tamarind fruit shell, *J. Chem. Thermodynamics*, **2008**, 40, 702-709.