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BIOSORPTION OF ANIONIC REACTIVE DYE FROM AQUEOUS SOLUTION USING RED SEAWEED: *KAPPAPHYCUS ALVAREZII*

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Abstract – Potential of *Kappaphycus alvarezii,* low cost marine seaweed has been studied for adsorption of azo dye from aqueous solution. Batch mode adsorption experiments were performed to remove anionic dye Congo red under various experimental conditions. It was found that the extent of Congo red adsorption by red weed biomass increased with initial dye concentration, contact time, initial solution pH and quantity of adsorbent. The adsorption process was pH dependent and the maximum adsorption of 45 mg/g occurred at pH of 8 for an initial dye concentration of 50 ppm by *K. alvarezii.* Adsorption isotherms and kinetic models were applied in the adsorption process. Equilibrium data were best represented by Langmuir isotherm model, which implies the monolayer adsorption of dye molecules on the adsorbent. Langmuir constant 'K_L' also indicated the favourable adsorption. Overall, kinetic studies showed that the dye adsorption process followed the pseudo-first-order kinetics. The present investigation reveals that the dry biomass of red seaweed can be an economical alternative adsorbent for the treatment Congo Red from aqueous solution.

INTRODUCTION

Synthetic dye stuffs are extensively used as a colouring agent in the textile, paper, leather, gasoline, pharmaceutical and food industries. A large quantities of colourants are utilised by these industries which are lethal and even pose carcinogenic effects, as well as being harmful to aquatic organisms and mammalian animals (Kumar *et al.,* 2013; Arumugam *et al.,* 2018). Discharge of dyeing wastewater becomes a major source of pollution due to its recalcitrance nature, producing undesirable colour, reduction in sunlight incursion, resistivity to photochemical and biological attack and their toxic by-products during degradation (Omar *et al.,* 2018). Due to toxicity, textile wastewater needs to be treated to eliminate dyes before its discharge (Kooh *et al.,* 2016). The synthetic origin, aromatic structure and complexity of the dyes consumed in the fabric industries are making them stable to the heat, oxidisers, photo degradation and resistant to aerobic breakdown (Garg *et al.,* 2004; Kumar *et al.,* 2013; Arumugam *et al.,* 2018). Number of physical, chemical and biological methods including adsorption, biosorption, coagulation/flocculation, advanced oxidation, ozonation, membrane filtration and liquid–liquid extraction have been formulated and applied globally for the treatment of wastewater containing dyes (Jegan *et al.,* 2016). Despite the development of various technologies, economic, effective and rapid water treatment at a commercial level is still a challenging problem. Among the methods studied, adsorption recognized as the most versatile process as it does not generate sludge or harmful by-products (Anjaneyulu *et al.,* 2005; Jegan *et al.,* 2016; Khamparia and Kaur, 2017). Activated carbon is the standard adsorbent widely adopted for effluent treatment containing toxic contaminants (Salleh *et al.,* 2011; Pathania *et al.,* 2017). However, use of activated carbon for the treatment of dyes fails in developing countries because of the high cost, massive demand and low regeneration of activated carbon (Khamparia and Kaur, 2017). Therefore, the development of adsorbents with high adsorption capacity and better regeneration capability is an imperative.

More recently, there has been a growing interest in using seaweeds as potential agents to treat wastewater via. Adsorption. Seaweeds have

extremely high bio-adsorption capacities and are abundantly available in most of the world's oceans (Gisi *et al.,* 2016). Algal cell wall possesses different types of functional groups such as carboxyl, amide, hydroxyl, sulphur-hydryl, etc., which are responsible for adsorption of dye molecules (Omar *et al.,* 2018). Several types of algae or seaweed, such as *Ulva lactuca, Sargassum crassifolium* and *Gracilaria corticate* (Omar *et al.,* 2018), *Nizamuddinia zanardinii* (Daneshvar *et al.,* 2012; Kousha *et al.,* 2012), *Gracilaria parvispora* (Daneshvar *et al.,* 2017), *Sargassum glaucescens* (Kousha *et al.,* 2012), *Sargassum muticum* (Rubin *et al.,* 2005), *Sargassum horneri* (Angelova *et al.,* 2016), *Gracilaria edulis* and *Kappaphycus alvarezii* (Jegan *et al.,* 2016) have already been used for wastewater dye removal. However, there is still a need for low cost, highly efficient adsorbent for the treatment of textile effluents. Hence, the present study is focused to investigate the biosorption potential of seaweed towards azo dye from wastewater.

Congo Red (CR) [1-naphthalenesulfonic acid, 3,3'-(4,4'-biphe-nylenebis (azo)) bis(4-amino-) disodium salt] is a benzidine-based anionic diazo dye (Chakravarty *et al.,* 2015). Effluent containing CR is produced from textiles, printing, dyeing, paper and plastic industries. CR is toxic to many organisms and is a suspected carcinogen and mutagen (Chisutia *et al.,* 2014). Hence, keeping the unsafe existence and noxious effects in view, it is much important that these dyes must be removed before gets-off into the natural water bodies.

In this context, the present study founded of the removal of Congo red from aqueous solution was studied using *Kappaphycus alvarezii* biomass as a natural, renewable and low-cost bio-sorbent. The effect of several factors such as pH, adsorbent dose, contact time and initial concentration was investigated. Kinetics and adsorption isotherm models were investigated to evaluate experimental data. Additionally, error analysis was carried out to test the adequacy and the accuracy of the model equations.

MATERIALS AND METHODS

Collection and preparation of adsorbent

The red seaweed *Kappaphycus alvarezii* was obtained from sea6 Energy Pvt Ltd, Tuticorin, Tamil Nadu, India. The collected fresh seaweed samples were thoroughly washed with tap water to remove wastes, salt debris and other contaminants. They were subsequently washed with deionized water and then dried in an oven at 70 °C overnight. Biosorbent particles were prepared by grinding the dried red seaweeds in a blender and subsequently sieved to obtain desired particle size used for dye removal.

Adsorbate preparation

The dye used in this study, Congo Red (MW- 696.67 g/mol, λ_{max} = 497 nm) having respective formulae $C_{32}H_{22}N_6Na_2O_6S_2$ as shown in Fig. 1. All the chemicals used in this study were of analytical reagent grade obtained from S.D. Fine Chemicals, Mumbai, India. The stock dye solution at a concentration of 1000 ppm was prepared by dissolving 1g of CR dye in 1000 mL of distilled water. The stock solution was further diluted with distilled water to obtain the required concentrations.

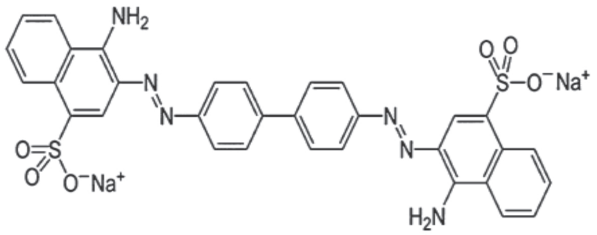


Fig. 1. Chemical structure of Congo Red

FTIR and SEM analysis

Infrared spectra of the adsorbate (CR) and adsorbents (before and after adsorption) were detected using a Fourier transform infrared spectrometer (Model: Shimadzu IR affinity 1) in the range of 600-4000 cm^{-1}. For the FT-IR study, grained adsorbent has been intimately mixed with KBr in a ratio of 1:100 to prepare a translucent pellet. From these FT-IR spectra the occurrence of functional groups on the surface of adsorbent was confirmed. The morphology and surface characteristics of biomass before and after treatment were obtained from scanning electron microscopy (SEM) using ZEISS EVO 18 scanning electron microscope under 500X magnification at a voltage of 10 kV.

Batch adsorption experiments

The adsorption of dye on red seaweed was investigated in batch mode sorption equilibrium experiments. All batch experiments were carried out in 250 mL Erlenmeyer flasks containing a fixed amount of adsorbent with 100 mL dye solution at a

known initial concentration. Samples were collected from the flasks at predetermined time intervals for analysing the residual dye concentration in the solution. The residual amount of dye in each flask was investigated using UV/VIS spectrophotometer (Model: Systronics Visiscan-167). The pH of the dye solution was initially adjusted to the desired level using 0.1 M HCl or 0.1 M NaOH. The influence of pH (5-9), contact time (6, 12, 24, 48, 72 and 96 hrs), initial dye concentration (10-100 ppm) and adsorbent dosage (5-20 g/L) were evaluated during the present study. The amount of dye adsorbed per unit of adsorbent was calculated according to a mass balance on the dye concentration using equation (1):

$$q_e = (C_e - C_i) \times V/M \quad .. (1)$$

Where C_i and C_e are the initial and equilibrium dye concentrations in solution (mg/L), V is the volume of the solution (l), M is the weight of the seaweed in gram. Percent dye removal (%) was calculated using the following equation:

$$\text{Dye removal (\%)} = ((C_i - C_e)/C_i) \times 100 \quad .. (2)$$

Adsorption isotherms

Adsorption isotherms were projected at the optimal conditions (such as pH, contact time and adsorbent dose) for the maximum removal of dye from an aqueous solution with different concentrations. The adsorption isotherm experiments were conducted by contacting 10 g/L of selected biomass with 100 mL of CR solutions of various introductory concentrations (10, 25, 35, 50, 60, 75, 90 and 100 mg/L). The Langmuir (1916) and Freundlich (1906) isotherm models were used. The Langmuir isotherm model is suitable to monolayer adsorption onto a surface with a predetermined quantity of identical sites. The Langmuir equation is written as:

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

The shape of this isotherm can also be expressed in terms of separation factor (R_L), which is given as follows:

$$R_L = 1/(1+K_L C_i) \quad .. (4)$$

Where, K_L is Langmuir constant (l/mg) related to the affinity of binding sites and the free energy of sorption. q_e is the dye concentration at equilibrium onto sorbent (mg/g). C_e is the dye concentration at equilibrium in solution (mg/L). q_m is the dye concentration when monolayer forms on adsorbent (mg/g).

The Freundlich model is used to define non-ideal sorption on heterogeneous exteriors, as well as multilayer adsorption. The linearized form of the Freundlich model is represented in equation (5):

$$\log q_e = \log K_F + (1/n) \log C_e \quad .. (5)$$

Here, q_e is the value of dye adsorbed onto biomass at equilibrium (mg/g), K_F is a sign of the adsorption capacity and denotes the relative adsorption ability (mg/g), n is a constant that belongs to sorption strength, and C_e is the dye concentration in the equilibrium state (mg/L).

Adsorption kinetics

In an effort to estimate the kinetics of dye uptake and the changes in the biosorption kinetic rates, the experimental data were modelled using the pseudo-first-order model (Mittal *et al.*, 2009), Pseudo second-order model (Ho and Mckay, 1999) and Elovich model (Wu *et al.*, 2009). The mathematical expression of the kinetic models is represented as follows:

The pseudo first-order rate expression is expressed as:

$$\log (q_e - q_t) = \log q_e - (k_1 t/2.303) \quad .. (6)$$

Where, q_e and q_t are the amount of dye adsorbed on sorbent at equilibrium (mg/g) and time (t) and k_1 is the first order rate constant (min^{-1}). A plot of log (q_e-q_t) vs t gives a linear relationship, from which the value of k_1 and q_e can be determined from the slope and intercept.

The pseudo second-order rate expression is stated in the following equation:

$$t/q_t = 1/k_2 q_e^2 + t/q_e \quad .. (7)$$

$$h = k_2 q_e^2 \quad .. (8)$$

Where the equilibrium adsorption capacity (q_e) and the second order constant k_2 (g/mg/min) can be determined experimentally from the slope and intercept of plot t/q_t vs t. Elovich parameters, α and β were obtained from the slope ($1/\beta$) and intercepts (($1/\beta$) ln ($\alpha\beta$) from ln (t) vs q_t plots using the equation (9):

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t) \quad .. (9)$$

RESULTS AND DISCUSSION

Surface Characteristics

FTIR spectral analysis

The adsorption capacity of adsorbent depends upon

the porosity as well as the functional groups present in the surface of the adsorbent. Thus, knowledge of surface functional groups would give an understanding of the adsorption capability of the biomass (Kumar *et al.*, 2010). FT-IR spectra of the Congo red and the algae biomass (before and after absorption) were presented in Fig. 2. The IR spectral analysis of Congo red showed the presence different functional groups at 3100 to 3360 cm^{-1} (N-H functional groups), 1211 cm^{-1} (Sulphur compounds $R\text{-}SO_3$), 1519 and 1643 cm^{-1} (amine group). As illustrated in Fig. 2, the spectrum of *K. alvarezii* cell wall displayed several absorption peaks, indicating the complex nature of the biomass. However, some characteristic peaks can be assigned for red algae. IR spectral analysis of biomass before dye treatment clarified the presence of adsorption bands on the cell wall at 2900-3100 cm^{-1} (-OH band),1211-1320 cm^{-1}(-CO) and 1743 cm^{-1} (-C=O).

The change in adsorption position and appearance of new peaks was observed after biosorption of Congo red onto the tested biomass (Fig. 2). Appearance of new peak at the wavelength 1226 cm^{-1}, which is a character of Congo red dye (belongs to a Sulphur functional group) in the sample after absorption indicates that the dye was absorbed onto the algal biomass. This is basically due to the participation of binding groups during interaction with Congo Red and thus causing the changes in the observed wave numbers. The position of adsorption peaks of OH, N-H and C = O functional groups of the algae changed after dye treatments, which confirms the involvement of these groups in dye removal.

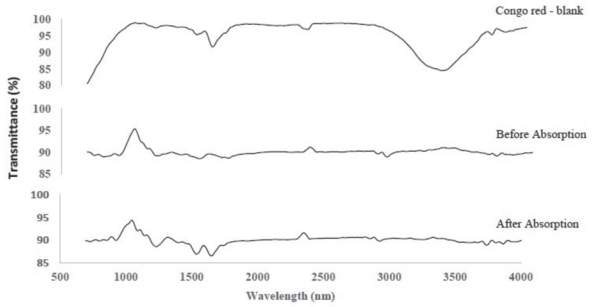


Fig. 2. Comparative FT-IR spectra of Congo red and red seaweed (before and after adsorption of dye)

Scanning Electron Microscopy analysis

SEM characterization of *K. Alvarezii*, before and after adsorption, showed morphological changes in surface texture in response to dye adsorption. the surface of the biomass found to be rough and porous before adsorption (Fig. 3a), while after adsorption of dye, smoother layer on the surface of adsorbent was observed (Fig.3b) due to precipitation of dye ions around the adsorbent cells.

Effects of pH

The initial pH of the adsorbate plays a significant role in maintaining the surface charge of the adsorbent, ionization of the adsorbate and dissociation of functional groups on the active sites of the adsorbent (Banerjee and Chattopadhyaya, 2017). The outcomes showed that the removal of CR dye (50 mg/L) increased with increasing pH from 5 to 9 by using *K. alvarezii* biomass (1g per100 m/L) after 72 hrs of sorption (Fig. 4b). The equilibrium sorption capacity was lowest at pH 5 (33 mg/g) and maximum adsorption of the dye was achieved at pH 8 (45 mg/g). The presence of various ligands such as

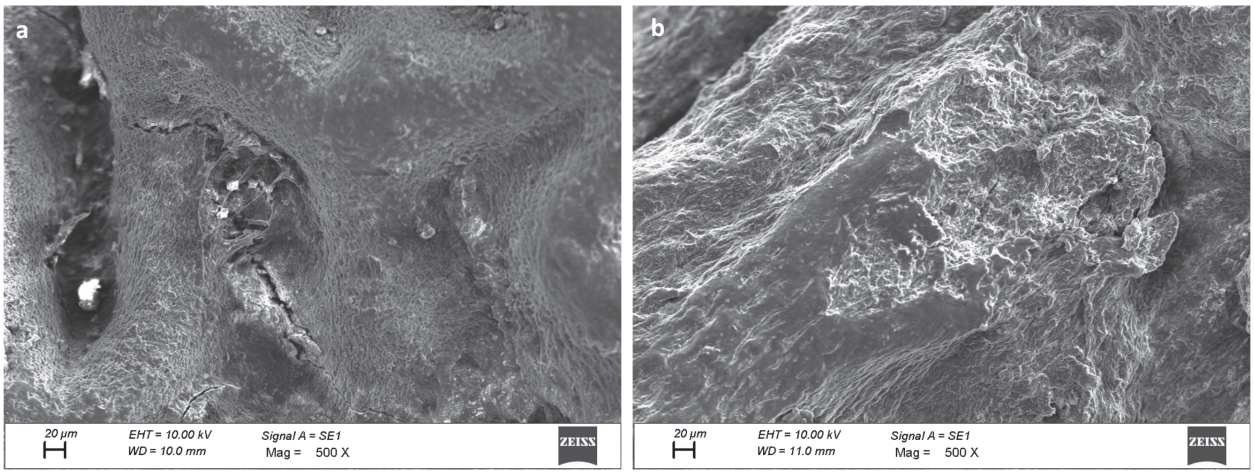
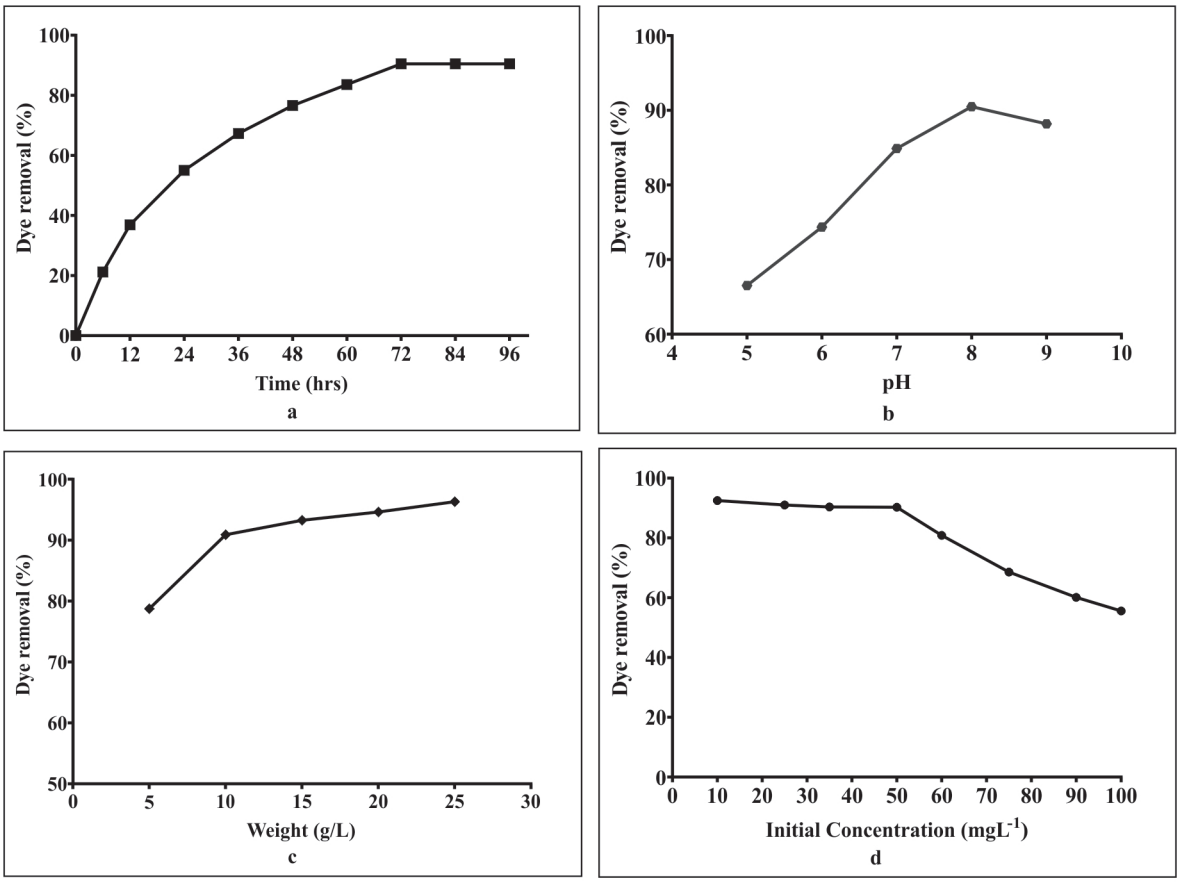


Fig. 3. Scanning electron microscopy of the surface of *K. Alvarezii* before (a) and after (b) Congo red dye treatment

carboxyl, phosphate and amino group in the ionic state contributes to the reaction with dye ions (Banerjee and Chattopadhyaya, 2017). At lower pH, competition between H^+ ions with dye ions for the adsorption sites, may lead to inhibition of dye adsorption (Annadurai *et al.*, 2002). Similar results were observed for Congo red adsorption while using cellulose based waste (Annadurai *et al.*, 2002), bottom ash and de-oiled soya (Mittal *et al.*, 2009) and peanut shell (Abbas *et al.*, 2012).

Effects of contact time

A given amount of adsorbent has the fixed number of active sites for the adsorbate molecules. Hence, initial concentration of the adsorbate solution is one of the deciding factors of dye removal (Banerjee and Chattopadhyaya, 2017). Fig. 4a shows the graph plotted between the dye removal efficiency (%) versus time, t. The time variation plot postulates that the adsorption of dye is rapid in initial phases, but while approaches equilibrium, it slows down. The removal of dye augmented quickly during the first 36 hrs (67%), then increased moderately up to 64 hrs (83%) and finally reached an equilibrium (90.8%) after 72 hrs. The change in the rate of adsorption might be due to fact that initial stage all the adsorbent sites are vacant, and the adsorbate concentration gradient is very high. Later, the lower adsorption rate is due to a decrease in the number of vacant sites and dye concentrations on the adsorbent and adsorbate, respectively (Dawood and Sen, 2012). The decreased adsorption rate, particularly, toward the end of the experiments, indicates the possible monolayer formation of Congo red on the adsorbent surface. This may be attributed to the lack of available active sites required for further uptake after attaining the equilibrium (Mall *et al.*, 2005; Pathania *et al.*, 2017). This suggests that after equilibrium is attained, further treatment does not provide more removal. In batch adsorption, the rate of removal of the adsorbate from aqueous solutions is controlled mainly by the transport of dye molecules from the surrounding sites to the interior sites of the adsorbent particles (Banerjee and



(a) Contact time (b) pH (c) Adsorbent dosage (d) Initial dye concentration

Fig. 4. Effects of different parameters on dye removal by *K.Alvarezii*

Chattopadhyaya, 2017).

Effects of sorbent dose

The influence of adsorbent dosage of algal biomass (5– 25 g/L) on the uptake of the dye from the aqueous solution was examined. The removal efficiency of dye increased sharply with an increase in the adsorbent dosage (Fig. 4c). The observed increment in removal efficiency with the increase in seaweed dose could be due to an increase in the number of possible functional groups and surface area of the seaweed biomass (Chaker *et al.,* 2007). On the other hand, the CR uptake decreases while increasing the seaweed dosage. For example, the CR uptake capacity decreased from 39.37 to 9.63 mg/g, when the biomass dosage increased from 5 to 25 mg/L. At low sorbent dosages, the available dye molecules are higher than the number of binding sites, hence sorptional uptake is higher. In contrary, at high bio-sorbent dosages, the available dye molecules are insufficient to cover all the exchangeable sites on the seaweed leads to low dye uptake (Namasivayam and Kavitha, 2002; Garg *et al.,* 2004; Jegan *et al.,* 2016). This result was consistent with studies by Vijayaraghavan *et al.,* (2015), Angelova *et al.,* (2016) and Jegan *et al.,* (2016) on biosorption dyes onto marine macroalgae. Comparing the percent removal efficiency and sorption uptake values, algal dosage of 10 g/L was selected as optimum for further experiments.

Effects of dye concentration

The impact of different dye concentrations (10-100 mg/L) on the removal of Congo red dye at the optimum conditions of pH (8.0), weight of biomass (1.0 g/100 mL) and contact time (72 hrs) is illustrated in Fig. 4d. The results showed that the removal efficiency of dye by study adsorbent decreased with increasing dye concentration. For instance, the highest removal efficiency (92.48%) was recorded at the concentration 10 ppm, which is slowly reduced to 90.24% at 50 ppm. Beyond 50 ppm concentration, rapid decrease in the percent dye removal was detected. In contrast, the equilibrium adsorption capacity was found to be increased with an increase in Congo red concentration from 10 to 50 mg/L. At low concentrations the number of freely available active sites on the adsorbent surface for the dye molecules is high. However, beyond optimal concentration (50 mg/L), no significant changes in adsorption capacity were observed due to the lack of active sites required for the adsorption of dye molecules at the higher concentrations (Pathania *et al.,* 2017). Several investigators reported the same trend in their adsorption studies using various bio-sorbents.

Isotherm Studies

The obtained experimental data was tested on two different isotherms viz. Langmuir and Freundlich isotherms (Eq. (3) and (4)) to attain the in-depth mechanism for uptake of dye during adsorption process. The results were assessed based on the coefficients of determination (R^2) and chi square test (χ^2) and SSE functions as per formulas given by Ayawei *et al.,* (2017). Isotherms obtained for adsorption of CR by biomass were presented from Fig. 5 (a and b). As shown in Table 1, the Langmuir isotherm data revealed that maximum adsorption capacity (q_m) of *K. alvarezii* for Congo red dye was 69.04 mg/g. Whereas, affinity with the binding site (b) is 0.22 l/mg. The Langmuir constant (b) was used to show the affinity of adsorbent to adsorbate through R_L (Eq. 4). R_L indicates that the shape of the isotherm may be favourable ($0 < R_L < 1$), unfavourable ($R_L > 1$), linear ($R_L = 1$), or irreversible

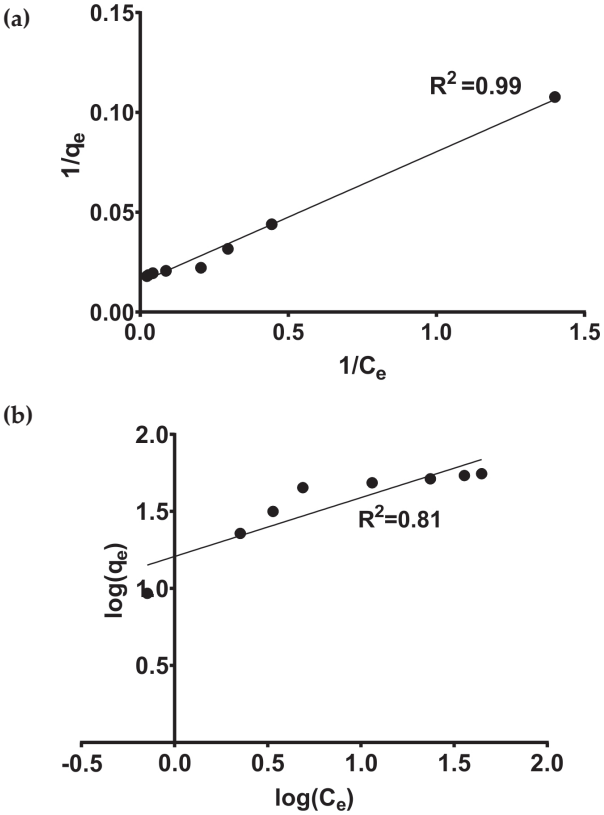


Fig. 5. Isotherm model for CR dye adsorption on *K. alvarezii* biomass
(a) Langmuir isotherm (b) Freundlich isotherm

(R_L= 0) (Omar *et al.*, 2018). The value of R_L for *K. alvarezii* is 0.11 clarifying that the removal process of Congo red dye by using algal biomass is favourable (Table 1).

Table 1. Langmuir and Freundlich isotherm constants for adsorption of CR dye

Langmuir adsorption isotherm	q_m (mg/g)	69.04
	b (L/mg)	0.22
	R^2	0.99
	R_L	0.11
	χ^2	4.80
	SSE %	1.93
Freundlich adsorption isotherm	K_F	3.34
	n	2.62
	R^2	0.81
	χ^2	188.56
	SSE %	11.52

The Freundlich isotherm showed that the plot of log q_e versus log C_e gives a straight line with a slope of 1/n and intercept of ln K_F for adsorption by using algae (Fig. 5b). The value of n and K_F were found as 2.62 and 3.34, respectively (Table 1). On comparing isotherm models studied, Langmuir isotherm described the adsorption of CR onto marine algal biomass better than the Freundlich isotherm with higher R^2 values highlighted in Table 1. This suggests the monolayer coverage of the surface of adsorbent by CR molecules and after that no further adsorption takes place. The monolayer adsorption was also reported for the CR adsorption onto red mud (Namasivayam and Arasi, 1997), activated coir pith (Namasivayam and Kavitha, 2002), and coal based activated carbon (Lorenc-Grabowska and Gryglewicz, 2007). Thus, based on R^2, χ^2 and SSE statistics (Table 1) Langmuir model efficiently elucidated the adsorption mechanism of Congo red dye onto *K. alvarezii*.

Kinetics Studies

To visualize the adsorption mechanism involved during the removal of CR dye, three kinetic models was used to fit the experimental data, namely, Pseudo first-order and Pseudo-second-order and Elovich models. The best-fit model was selected based on both linear regression correlation coefficient (R^2) and the calculated q_e values.

Values of k_1 and q_e were calculated from the plots of log (q_e-q_t) vs t for different concentrations of the dye and listed in Table 2. The linear plots of log (qe-q_t) vs t show a good agreement of experimental data with the first-order kinetic model for different initial dye concentrations (Fig. 6a). The correlation coefficients for the first- order kinetic model is greater than 0.99 (Table 2). Also, the calculated q_e

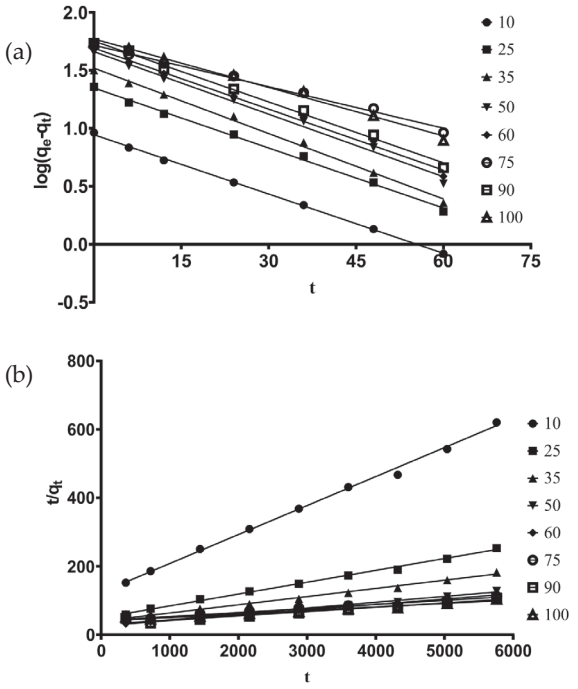


Fig. 6. Kinetics plot for the adsorption of CR dye by *K. alvarezii*

(a) Pseudo first-order model (b) Pseudo second-order model

Table 2. Kinetic rate constants related to the biosorption of Congo red on *Kappaphycus alvarezii*

Kinetic models and its parameters	
q_e exp (mg/g)	39.79
Pseudo-first order kinetic	
q_e cal (mg/g)	40.84
k_1 (min^{-1})	38×10^{-3}
R^2	0.99
χ^2	0.45
SSE %	0.58
Pseudo-second order kinetics	
q_e cal (mg/g)	59.77
k_2 (g/mg/min)	1.53×10^{-5}
h (mg/g/min)	26×10^{-3}
R^2	0.98
χ^2	92.87
SSE %	8.63
Elovich	
q_e cal (mg/g)	30.64
α *(mg/g/min)*	28.3×10^{-3}
β *(g/mg)*	29×10^{-2}
R^2	0.75
χ^2	18.14
SSE %	3.34

value agreed with the experimental data. The second- order rate constant, k_2 and q_e were calculated from the intercept and slope of the plots of t/q_t vs t (Fig. 6b). The correlation coefficients were lower than the first order kinetic model values. Also, the large difference between the experimental q_e values with the calculated ones, shows that the adsorption of dye onto study algae is not a second-order reaction, whereas belongs to the first- order kinetic model.

The constant of the Elovich equation for the same experimental data were obtained from the slope and intercept of the plot of q_t against ln(t). In this case, poor linear relationship was obtained between CR adsorbed, q_t and ln(t) with a correlation coefficient of 0.747 (Table 2). The correlation coefficients of Elovich model are lower than those of the pseudo first and second-order equations. The Elovich equation does not predict any definite mechanism, hence the Elovich plot is not shown here.

CONCLUSION

The present study shows that the *K. alvarezii* is an effective adsorbent for the removal of Congo Red from aqueous solution. Optimal conditions required for the effective removal of dye stuff was demonstrated. Pollutant removal efficiency of algae mainly depends on the pH and the contact time. The value of mathematical constants of the Langmuir isotherm favours the monolayer adsorption process while the pseudo first order kinetics model explained its mechanism. Integrated pollutant and dye removal using algal biomass may establish a cost-effective treatment of textile wastewater. Hence, algal biomass as a feedstock has huge potential to produce liquid and gaseous biofuels by transesterification and anaerobic digestion for bioenergy options, respectively. Since, the raw material (marine algae) is freely available in large quantities, the current treatment method seems to be economically efficient.

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