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Removal of methylene blue from aqueous solutions onto *Bacillus subtilis:* determination of kinetic and equilibrium parameters

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ABSTRACT

In the present study, *Bacillus subtilis*, a Gram-positive bacteria, was used in dried biomass form as biosorbent for removal of methylene blue (MB) from aqueous solutions. Batch adsorption tests were performed at different contact times, temperatures, pH, adsorbent doses, and initial dye concentration. The adsorption isotherms are described by means of the Langmuir and Freundlich isotherms. It was found that the Langmuir equation fit better than the Freundlich equation. Maximum biosorption capacity was found to be 169.49, 178.57, and 181.82 mg g^{-1} at 298, 308, and 318 K, respectively. The adsorption kinetics of MB could be described by the pseudo-second-order reaction model. The activation energy of the biosorption (E_a) was determined as 11.30 kJ mol^{-1} at initial concentration of 50 mg L^{-1}. Free energy of adsorption (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) changes were calculated to predict the nature of adsorption. The estimated values for ΔG° were −21.22, −22.02, and −23.01 kJ mol^{-1} at 298, 308, and 318 K, respectively. The enthalpy changes and entropy of adsorption were 5.47 kJ mol^{-1} and 89.43 Jmol^{-1}, respectively. The experimental data obtained in the present study indicate that *B. subtilis* is a suitable and inexpensive adsorbent which can be used for dye removal in wastewater treatment processes.

Keywords: Biosorption; *Bacillus subtilis*; Methylene blue (MB); Thermodynamic parameters

1. Introduction

More and more dyes or pigments have been used to colour the final products in the industries, i.e. textiles, paper, plastics, cosmetics, rubber, leather, etc. [1]. Approximately 10–15% of dyes are released into the environment during manufacturing and usage. Since some of the dyes are harmful, dye-containing wastes pose an important environmental problem [2,3]. The presence of even very small amounts of dyes in water—less than 1 ppm for some dyes—is highly visible and undesirable [4]. Removal of such pollutants from aqueous solutions can be achieved by adsorption, precipitation or ion exchange. Adsorption on activated carbon and synthetic resins is the most popular method for removing cationic pollutants. Unfortunately, these adsorbents are high-cost materials [5].

The adsorption processes give the best results as they can be used to remove different types of colouring materials, providing an attractive treatment, especially if "low-cost" adsorbents are available [6]. The use of biomaterials for the treatment of wastewaters provides as an alternative method to the conventional treatment. These biosorbents have many advantages over conventional treatments; they are

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economic, nontoxic to environment and widespread [7–9]. These make the biosorption a practical and an economically feasible treatment process among its competitors [1]. A wide variety of microorganisms such as fungi, algae, and bacteria, either in their living or inactivated biomass form, has been investigated to remove dyes from wastewaters [10–13].

Methylene blue (MB) is a thiazine cationic dye and has widespread applications, which include colouring paper, temporary hair colourant, dyeing cottons, wood, wools, and coating for paper stock. It is also used in microbiology surgery, chemistry, diagnostics, and as a sensitizer in photo-oxidation of organic pollutants. Although it has low toxicity, it can cause some specific harmful effects in humans, such as heartbeat increase, vomiting, shocks, cyanosis, jaundice, and tissue necrosis [14–17]. Therefore, the treatment of effluent containing such dye is of interest due to its harmful impact on receiving waters.

The removal of dyes from aqueous solutions by the use of biological materials is termed bioadsorption. In this instance, biomass was used as an adsorbent to concentrate and remove MB dye from solution. Biomass adsorbent has a high potential due to its physico-chemical characteristics [18]. It is particularly suitable for the treatment of dilute MB wastewater, making the biosorption a promising alternative to conventional processes for the removal of MB [19,20].

In this study, MB cationic dye was selected as model compound in order to evaluate the capacity of *Bacillus subtilis* for its removal in batch system. For this aim, the effect of initial MB concentration, pH, temperature, contact time, and biosorbent dose were determined. The equilibrium, kinetic, and thermodynamic data of the adsorption process were then evaluated to study the adsorption mechanism of MB.

2. Materials and methods

2.1. Microorganism and growth conditions

B. subtilis, a Gram-positive saprophytic microorganism was incubated in 5-L nutrient broth growth medium on a rotary shaker at 120 rpm at 37 °C for 24 h. Then the cells were collected by centrifugation at 5.000 rpm for 10 min and then dried at 80 °C for 48 h. The powdered biomass was used as biosorbent after sieving to select the particle size of less than 200 μm.

2.2. Preparation of MB solution

MB (basic blue 9, C.I.52015; Chemical Formula, $C_{16}H_{18}ClN_3S.3H_2O$; and MW, 373.90 $gmol^{-1}$) was obtained from Sigma (Sigma-Aldrich Chemical Co., St. Louis, USA). Its chemical structure is shown in Scheme 1. Stock dye solution was prepared in distilled water as 1,000 $mg\,L^{-1}$ and the experimental solution was prepared by diluting the stock solution with distilled water when necessary.

2.3. Adsorption equilibrium experiments

MB was used as adsorbate to determine the adsorption potential of *B. subtilis*. The effect of initial MB concentration, contact time, biosorbent dose, pH, and temperature were studied. A weighted sample of biosorbent of 0.1 g was mixed with 50 and 100 $mg\,L^{-1}$ MB of the initial concentration at 150 rpm (Julabo SW 23, USA) for 60 min.

For other adsorption experiments, 100 mL of MB solution of 50 mg L^{-1} was shaken with a certain amount of biosorbent (0.1 g) at the desired temperature for 30 min of the equilibrium time.

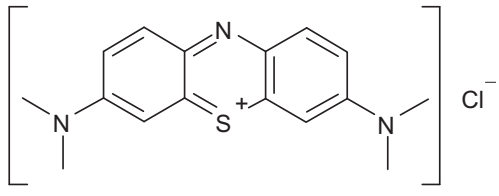
The concentrations of MB in the solution were measured on a spectrophotometer (UV–vis Shimadzu 1240, JAPAN) at 660 nm, before and after the adsorption process.

The amount of adsorbed MB per unit biosorbent (mg MB/g dry biosorbent) was calculated by the following equation:

$$q_e = (C_o - C_e)V/m \quad (1)$$

where q_e ($mg\,g^{-1}$) is the amount of adsorbed MB at equilibrium; C_o and C_e are the initial and equilibrium MB concentrations ($mg\,L^{-1}$), respectively; V is the volume of solution (L) and m is the amount of biomass (g). The equilibrium data were then fitted using two different isotherm equations, namely the Langmuir and Freundlich models.

In order to study the effect of pH, the solution was adjusted from pH 2.0 to 12.0, by adding 0.1 M HCl or NaOH solutions. The batch procedure was followed using an initial concentration of 50 $mg\,L^{-1}$. The solution pH was monitored using pH meter with a glass electrode (Mettler-Toledo, USA).



Scheme 1. The chemical structure of MB.

The MB adsorption experiments were performed in three replicates. For each set of data, standard statistical method was used to determine the mean value and standard deviation. Ninety-five percentage of confidence interval was calculated for each set of samples in order to determine the margin of error.

2.4. FTIR spectra

The autoclaved free cells MB were dried overnight at 80 °C. 0.01 g of the dried biomass was mixed with 0.1 g KBr and pressed into a tablet with the aid of a bench press. The resulting pellet was transparent and was used to test the surface functional groups by IR spectroscopy, where it was scanned between 4,000 and 400 cm^{-1} (Mattison 1000 FTIR).

3. Results and discussion

3.1. Effect of contact time and initial concentration

Contact time of adsorbate and adsorbent is of great importance in adsorption because it depends on the nature of the system used. The biosorption experiments were carried out for 0–60 min at 25, 35, and 45 °C in two different initial concentrations (50 and 100 mg L^{-1}). The results are given in Figs. 1(a) and 1 (b). It was found that 30 min of contact time was enough to reach the equilibrium beyond which there is no considerable increase in the biosorption capacity, and therefore it is fixed as the optimum contact time.

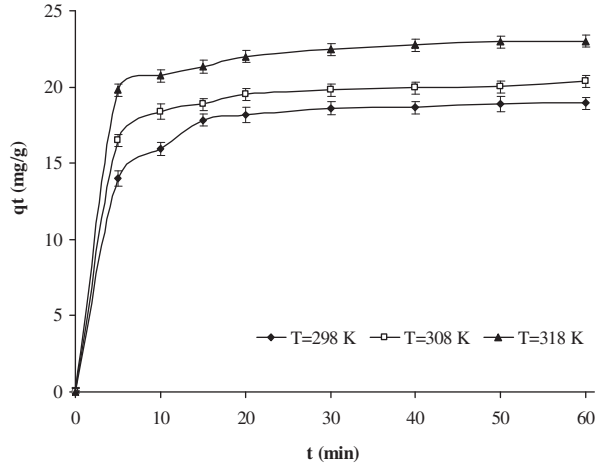


Fig. 1(a). Effect of contact time on the adsorption of MB on *B. subtilis* at different temperatures ($V = 100$ mL, $m = 0.1$ g, agitation speed = 150 rpm, initial concentration = 50 mg L^{-1}). The experiments and measurements were performed in three independently replicates. Confidence intervals of 95% were calculated for the data ($n = 3$) and error bars represent standard deviations.

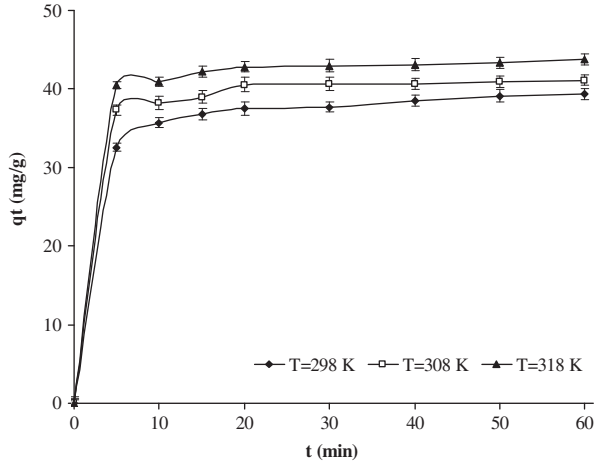


Fig. 1(b). Effect of contact time on the adsorption of MB on *B. subtilis* at different temperatures ($V = 100$ mL, $m = 0.1$ g, agitation speed = 150 rpm, initial concentration = 100 mg L^{-1}). The experiments and measurements were performed in three independently replicates. Confidence intervals of 95% were calculated for the data ($n = 3$) and error bars represent standard deviations.

The initial concentration of MB remarkably influenced the equilibrium uptake. It was observed that the amount of dye uptake increased with contact time and initial concentration at all the three temperatures. The increase of loading capacities is probably due to higher interaction between MB and biosorbent at higher initial concentration.

In order to investigate the adsorption mechanism, characteristic rate constants were determined by using a pseudo-first-order equation of Lagergren based on solid capacity [21] and pseudo-second-order equation based on solid-phase adsorption [22], respectively.

$$\log(q_{eq} - q_t) = \log q_{eq} - k_{pf}\ t/2.303 \quad (2)$$

where q_t is the amount adsorbed at time t (mg g^{-1}) and k_{pf} is the equilibrium rate constant of pseudo-first-order adsorption (min^{-1}).

The calculated q_{eq}, k_{pf}, and the corresponding linear correlation coefficient R^2 values are shown in Table 1. It was observed that the rate constant k_{pf} decreased with temperature. Lower R^2 values show no applicability of the pseudo-first-order model in predicting the kinetics of MB adsorption on *B. subtilis*.

Kinetic data were further analyzed with pseudo-second-order kinetic model. The different equation is as follows:

$$t/q_t = 1/k_{ps}q_{eq^2} + t/q_{eq} \quad (3)$$

where k_{ps} is the rate constant of pseudo-second-order adsorption ($gmol^{-1}min^{-1}$). If the second-order kinetics is applicable, the plot of t/q_t vs. t should show a linear relationship. The calculated q_{eq}, k_{ps}, and the corresponding linear correlation coefficient R^2 values are given in Table 1. The R^2 values are higher than 0.99, indicating the applicability and the second-order nature of the adsorption process of MB on *B. subtilis*. Similar phenomena have been observed in MB on wheat shells [23], adsorption of basic dyes onto pumice powder [24], and the removal of MB from perlit [25]. As seen in Table 1, both q_{eq} and k_{ps} values increase with temperature.

The activation energy for MB biosorption was also calculated from Arrhenius equation:

$$\ln k_{ps} = \ln A - E_a/RT \tag{4}$$

where k_{ps} is the pseudo-second-order rate constant ($g\,mg^{-1}\,min^{-1}$), A is the Arrhenius factor, E_a is the activation energy of adsorption ($kJ\,mol^{-1}$), R is the gas constant ($8.314\,J\,mol^{-1}\,K^{-1}$), and T is the temperature in K. E_a can be estimated from the slope of the plot ln k_{ps} vs. $1/T$ (data not shown). The magnitude of activation energy gives an idea about the type of adsorption, which is mainly physical or chemical. Low activation energies (5–50 $kJ\,mol^{-1}$) are characteristic of physical adsorption, while higher activation energies (60–800 $kJ\,mol^{-1}$) suggest the chemical adsorption nature [26].

The activation energy for biosorption of MB on *B. subtilis* was found to be 11.30 $kJ\,mol^{-1}$ at an initial concentration of 50 $mg\,L^{-1}$. From this value, it can be seen that the adsorption process was of a physical nature.

3.2. Adsorption isotherms

The relationship between the amount of dye adsorbed at constant temperature and its concentration in the equilibrium solution is called the adsorption isotherm. It is useful for estimating the total amount of adsorbent needed to adsorb a required amount of adsorbate from solution [27].

In this study, the equilibrium data were analyzed by the most commonly used models, Langmuir and Freundlich (Fig. 2). The Langmuir isotherm applies to adsorption on homogenous surfaces and is based on the assumption that maximum adsorption corresponds to a monolayer coverage on the adsorbent surface. For a single sorbate, Langmuir isotherm is given by the following equation [23]:

$$C_e/q_e = 1/bQ_m + C_e/Q_m \tag{5}$$

where q_e is the amount of adsorbate per gram of the biomass at equilibrium ($mg\,g^{-1}$), Q_m is the maximum amount of MB per unit weight of the biomass to form a complete monolayer on the surface bond ($mg\,g^{-1}$), and b is a constant related to the affinity of the binding sites (L mg^{-1}). Q_m and b are calculated from the slope and intercept of the straight lines of plot C_e/q_e vs. C_e.

The Freundlich is an empirical equation widely employed to describe solid-liquid adsorption, encompassing the surface heterogeneity. Freundlich model equation is as follows [28]:

$$q_e = kC_e^{1/n} \tag{6}$$

where k and n are the Freundlich's constants related to the capacity and intensity of the adsorbent.They can be determined from the linear plot of log q_e vs. log C_e.

The Langmuir and Freundlich adsorption parameters are presented in Table 2. These values for the correlation coefficient support the conclusion that the adsorption data follows the Langmuir model better.

3.3. Thermodynamic parameters

The thermodynamic parameters reflect the feasibility and spontaneous nature of the biosorption process.

Table 1
Adsorption kinetic parameters of MB on *B. subtilis* ($V = 100$ mL, $m = 0.1$ g, agitation speed = 150 rpm)

C_o ($mg\,L^{-1}$)	T (K)	Pseudo first order kinetic model			Pseudo second order kinetic model		
		q_e (mgg^{-1})	k_{pf} (min^{-1})	R^2	q_e ($mg\,g^{-1}$)	k_{ps} ($g\,mg^{-1}\,min^{-1}$)	R^2
50	298	13.69	0.132	0.9103	19.57	0.028	0.9998
	308	11.84	0.114	0.7600	20.70	0.036	0.9999
	318	6.572	0.045	0.7390	23.47	0.040	0.9999
100	298	19.24	0.105	0.8820	39.84	0.029	0.9998
	308	12.38	0.106	0.8690	41.49	0.032	0.9999
	318	16.19	0.117	0.7280	44.05	0.035	0.9999

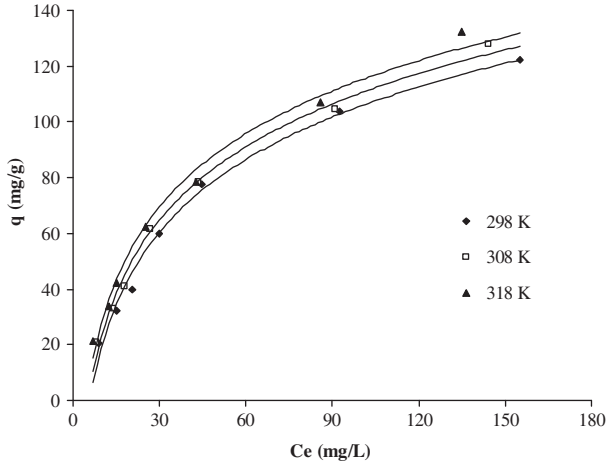


Fig. 2. Adsorption isotherms of MB on *B. subtilis* at different temperatures.

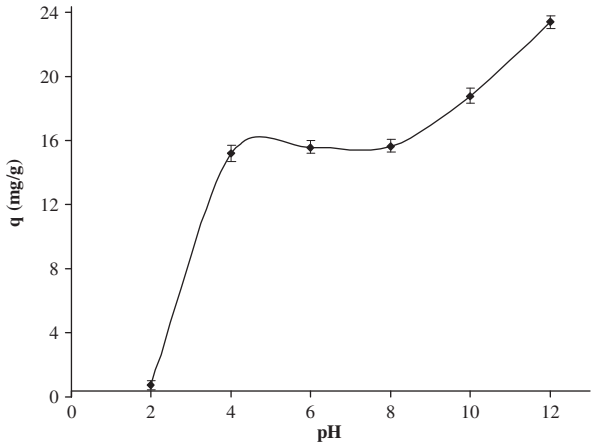


Fig. 3. Effect of pH on the adsorption of MB on *B. subtilis* ($V = 100$ ml, $m = 0.1$ g, agitation speed = 150 rpm, initial concentration = 50 mg L^{-1}). The experiments and measurements were performed in three independently replicates. Confidence intervals of 95% were calculated for the data ($n = 3$) and error bars represent standard deviations.

Table 2
Adsorption rate constants for MB on *B. subtilis* ($V = 100$ mL, $m = 0.1$ g, agitation speed = 150 rpm, $t = 30$ min)

T (K)	Freundlich constants			Langmuir constants		
	K (L g^{-1})	$1/n$	R^2	Q_m (mg g^{-1})	b (L mg^{-1})	R^2
298	5.901	0.633	0.9571	169.49	0.0164	0.9884
308	6.825	0.614	0.9572	178.57	0.1696	0.9916
318	7.549	0.604	0.9715	181.82	0.0188	0.9924

The parameters, such as free energy change (ΔG°), entropy change (ΔS°), and enthalpy change (ΔH°), can be estimated using equilibrium constants changing with temperature.

The values of standard Gibbs free energy change for the biosorption process were evaluated by using b values obtained from the Langmuir model at different temperatures and are represented in Table 3. The free energy change of the adsorption reaction is given by:

$$\Delta G^\circ = -RT \ln b \quad (7)$$

Table 3
Thermodynamic parameters for the adsorption of MB on *B. subtilis*

T (K)	ΔG^o (kJ mol^{-1})	ΔH^o (kJ mol^{-1})	ΔS^o (J $mol^{-1}K^{-1}$)
298	−21.22		
308	−22.02	+5.47	89.43
318	−23.01		

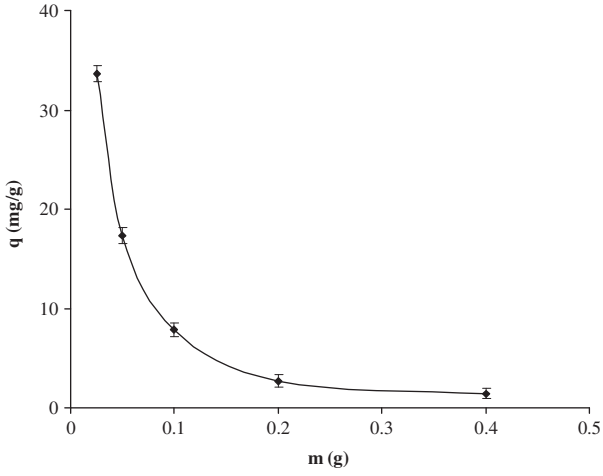


Fig. 4. Effect of adsorbent dose on initial adsorption rates ($V = 100$ mL, agitation speed = 150 rpm, temperature = 298 K, pH = 6.0). The experiments and measurements were performed in three independently replicates. Confidence intervals of 95% were calculated for the data ($n = 3$) and error bars represent standard deviations.

Table 4
FTIR absorption bands and corresponding groups

Frequency (cm^{-1})	Functional group
3325.66	–OH, –NH
2928.40	–CH
1547.62	$–COO^-$, -C=O
1397.19	$–COO^-$
1054.88	–C–O, –C–N

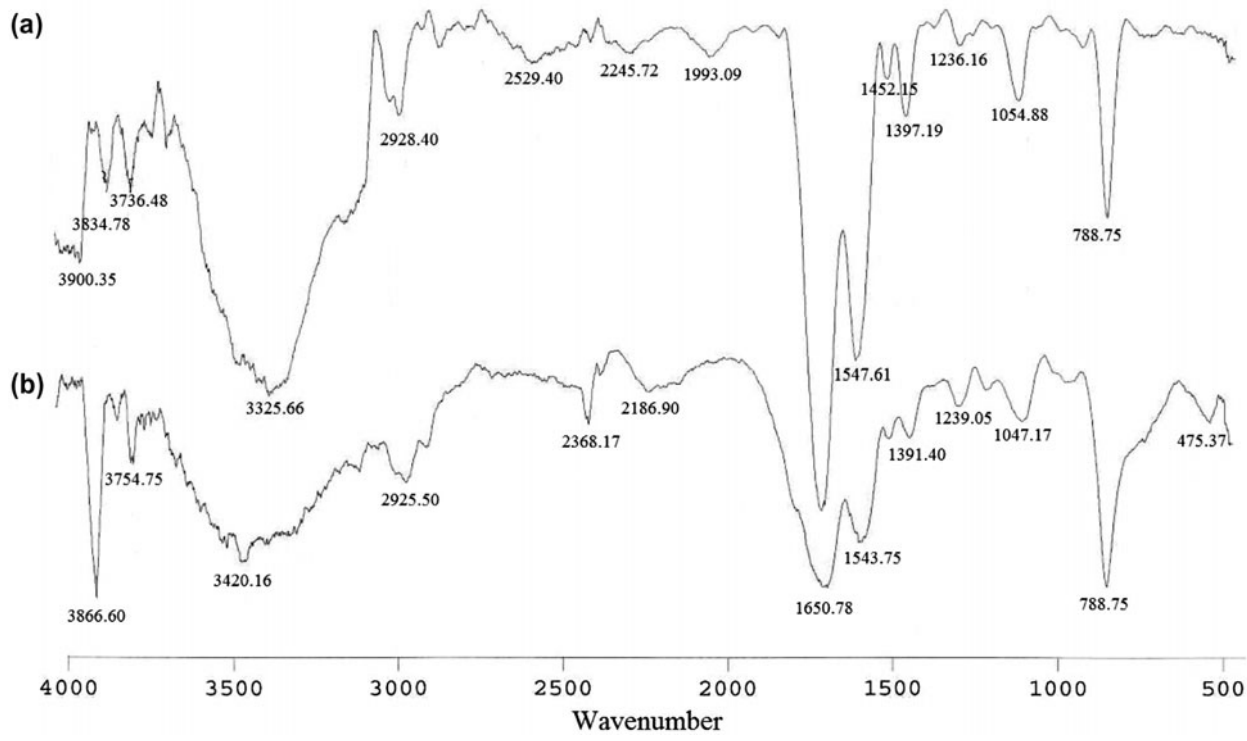


Fig. 5. FTIR spectra of *B. subtilis* (a) before MB was loaded and (b) after MB was loaded.

where R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature (K) and b states the Langmuir constant (L mg^{-1}).

As it is shown in Table 3, the negative value of ΔG° confirms the feasibility of the process and spontaneous nature of MB adsorption by the biosorbent.

The change of ΔG° and ln b with temperature can be represented as follows:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (8)$$

$$\ln b = \Delta S^\circ/R - \Delta H^\circ/RT \quad (9)$$

where values of ΔH° and ΔS° can be calculated from the slope and the intercept of the plot between ln b vs. $1/T$.

Using Eq. (9), standard enthalpy and entropy changes of sorption process were determined from the ln b − $1/T$ plots and are also represented in Table 3. The enthalpy changes of MB in *B. subtilis* were found to be +5.47 kJ mol^{-1}, while ΔS° was 89.43 J mol^{-1}. The positive value of ΔH° suggested the endothermic nature of adsorption [29]. This is also supported by the increase of the uptake capacity of biosorbent with temperature. The positive value of ΔS° reflects the affinity of MB and shows the increasing randomness at the solid/liquid interface during the sorption of MB on *B. subtilis* [30].

3.4. Effect of pH

pH is an important factor in adsorption and plays an important role in the whole process of dyes adsorption, particularly in adsorption capacity [31]. The effect of initial pH on MB uptake of *B. subtilis* was investigated between pH 2.0–12.0 at 50 mg L^{-1} MB concentration and 298 K. The maximum adsorption level was observed at pH 12.0 (23.05 mg g^{-1}) (Fig. 3). However, the MB removal was nearly constant in the pH range of 6.0–8.0. MB produces molecular cations in aqueous solutions. Reduced adsorption of MB at acidic pH reflects the presence of excess H^+ ions that compete with dye cations for the adsorption sites [32]. A similar trend was observed for adsorption of MB onto wheat shells [23]. The adsorption of MB on the *B. subtilis* is primarily influenced by the surface charge on the absorbent.

3.5. The effect of adsorbent dose

The effect of *B. subtilis* dose on MB adsorption was studied from 0.025 to 0.4 g L^{-1} at pH 6.0 and 298 K. As shown in Fig. 4, the adsorbed MB amount per unit biomass weight was decreased from 33.61 to 1.41 mg g^{-1} by increasing the biosorbent concentration from 0.025 to 0.4 g L^{-1}. Increase in biosorption with the biosorbent dose could be attributed to the increased surface area and the availability of more adsorption

sites. The decrease in adsorption capacity may be due to the decrease in solute transfer rate onto the adsorbent surface, i.e. the amount of solute adsorbed onto unit weight of adsorbent gets splitted with increasing biomass concentration [33].

3.6. FTIR spectral analysis

In order to find out which functional groups are responsible for the MB adsorption, FTIR analysis of MB loaded and unloaded *B. subtilis* biomass were taken in the range of 4,000–400 cm^{-1}, and the vibration frequencies and their corresponding groups are presented in Table 4. *B. subtilis* is a Gram (+) microorganism and its cell walls are made of large molecules (peptidoglycan) linked with teichoic acid and polysaccharides. These molecules possess functional groups of the type (–NH), carboxylate anions ($–COO^-$), hydroxy (–OH), and others, (–C–N–), (–C–O), (–C–H), and (–C=O), which present different affinity and can adsorb different molecules. As seen in Fig. 5, the adsorption peaks in the MB-treated *B. subtilis* is slightly lower than that of the native one. The analysis of the FTIR spectra showed the presence of functional groups (such as, carboxyl, amino, amide and hydroxy) that are able to interact with MB. It should be noted that the results obtained at this stage were considered sufficient to give an idea about the presence of functional groups on the *B. subtilis* cell surfaces.

4. Conclusion

The biosorption process of MB on *B. subtilis* could be best described by the pseudo-second-order kinetic model. Biosorption equilibrium was better described by the Langmuir isotherm than the Freundlich model. The interaction between MB and functional groups on the cell surface of the biomass was confirmed by FTIR analysis. Based on the results, the *B. subtilis* may be used as an inexpensive, selective, effective, and easily cultivable biosorbent for the removal of MB from aqueous solutions.

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