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Methylene Blue biosorption by *Rhizopus arrhizus*: Effect of SDS (sodium dodecylsulfate) surfactant on biosorption properties

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ABSTRACT

The biosorption of Methylene Blue, a cationic dye, onto dried *Rhizopus arrhizus*, a filamentous fungus, was examined in the absence and in the presence of increasing concentrations of sodium dodecylsulfate (SDS), an anionic surfactant. The fungus exhibited the maximum dye uptake at an initial pH value of 10 in the absence of surfactant. The addition of SDS did not change the initial pH of maximum dye uptake. Dye uptake by the fungus increased with increasing initial dye concentration up to 1100 mg l^{-1}. The presence of 1 mM surfactant in biosorption medium enhanced the dye removal dramatically. The Freundlich model better described the equilibrium dye uptake than the Langmuir model. According to the Langmuir model, the maximum dye uptake was determined as 370.3 mg dye g^{-1} of dried biomass in the absence of surfactant. When 1 mM (288.4 mg l^{-1}) SDS was added to the biosorption medium, this value raised to 1666.6 mg g^{-1} resulting in 4.5-fold increase in uptake capacity. The pseudo-second-order kinetic model described the biosorption kinetics accurately for all cases studied confirming that a chemisorption process controls the sorption rate.

1. Introduction

Among the different pollutants of aquatic ecosystem, dyes are a large and important group of chemicals. They are widely used in industries such as textiles, paper, rubber, plastics, cosmetics, etc., to colour their products. These dyes are invariably left in the industrial wastes and consequently discharged mostly to surface water resources. Dyes, even in low concentrations, are visually detected and meanwhile affect the aquatic life and food web. Besides the dyes, such effluents contain a number of other contaminants such as acids or alkalis, surfactants, dissolved and suspended solids, and other toxic compounds [1,2]. Since many organic dyes are harmful to human beings, the removal of colour from process or waste effluents becomes environmentally important. It is rather difficult to treat dye effluents because of their synthetic origins and their mainly aromatic structures, which are biologically non-degradable. Among several chemical and physical methods, adsorption process is one of the effective techniques that have been successfully employed for colour removal from wastewater. Many adsorbents have been tested to reduce dye concentrations from aqueous solutions. Activated carbon is considered as an effective but expensive adsorbent due to its high costs of manufacturing and regeneration. Therefore, a number of non-conventional sorbents have been tried for the treatment of wastewaters. Natural materials, biosorbents, and waste materials from industry and agriculture represent potentially more economical alternative sorbents [3–7].

Methylene Blue is a thiazine cationic dye and has widespread applications, which include colouring paper, temporary hair colourant, dyeing cottons, wools and coating for paper stock. It is also used in microbiology, surgery and diagnostics and as a sensitizer in photo-oxidation of organic pollutants. Although its low toxicity, it can cause some specific harmful effects in humans such as heartbeat increase, vomiting, shocks, cyanosis, jaundice and tissue necrosis [8–13].

Surfactants, which are known as surface-active matters, reduce surface tension of water and other liquids. Surfactants mainly do not exist in nature; they are manufactured by chemical reaction. The surface activity of surfactants derives from their amphiphilic structure that posses both hydrophilic and hydrophobic parts in one molecule. Surfactants are classified into four groups depending on the charge of the hydrophilic part: non-ionic (0), anionic (−), cationic (+) and zwitterionic (±). Surfactants have very common applications in science and industry not only in the primary processes such as the recovery and purification of raw materials in the mining and petroleum industries but also in more complex processes such as improving the quality of finished products such as paints, cosmetics, pharmaceuticals, and foods. Surface-active agents are also used in chemical processes, and other areas. Therefore, it is likely to meet with surfactants in waste streams resulted from both industries and municipalities. After the dyeing process,

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surfactants are also released into wastewaters in high amounts. The adsorption of dyes onto solid sorbents is influenced dramatically by the presence of surfactants. However, almost nothing is known about the role of the anionic surfactants in dye–surfactant aggregation and about dye adsorption at solid/solution interfaces [14–17].

Biosorption can be defined as sequestering of organic and inorganic species including metals, dyes and odour causing substances from aqueous solutions by using live or dead biomass or their derivatives. This biomass may be bacteria, fungi, algae, sludge from biological wastewater treatment plants, by-products from fermentation industries or seaweeds. Microbial cell surfaces carry various types of functional groups, which are responsible for the sequestration of hazardous materials from industrial effluents. The main attractions of biosorption are high selectivity and efficiency, cost effectiveness, good removal performance, possible regeneration at low cost and availability of known process equipment. Textile dyes vary greatly in their chemistries, and their interactions with microorganisms depend on the chemistry of a particular dye, type of biomass, its preparation and its specific surface properties and environmental conditions (pH, temperature, ionic strength, existence of competing organic or inorganic ligands in solution) [18–21]. Since fungal biomass is non-pathogenic to humans and animals and it can be produced cheaply by using simple fermentation techniques or obtained as a waste from various industrial fermentation processes, it is widely used for the biosorption of both anionic and cationic dyes. *Rhizopus arrhizus*, *A. niger*, *N. crassa*, and *P. chrysosporium* are some of the low cost fungal materials which have been used as sorbents for the anionic and cationic dyes [22–27].

Dried *R. arrhizus* was used as a low-cost biosorbent for removing the basic dye Methylene Blue from aqueous solution in this study. In the wastewaters from pigments, dyestuffs or textiles manufactures, anionic surfactants are always coexisted with dyes. For this reason, the effect of sodium dodecylsulfate (SDS), an anionic surfactant, on Methylene Blue biosorption onto fungus was examined in more detail. The Langmuir and Freundlich models describing the sorption of basic dye in the absence and in the presence of anionic surfactant SDS were applied to sorption data. Also the kinetics of the dye sorption for both cases were measured and modelled.

2. Materials and methods

2.1. Microorganism and growth conditions

The filamentous fungus *R. arrhizus* obtained from the US Department of Agriculture Culture Collection was used in this study. The microorganism was cultivated in liquid media using the shake flask method. The growth medium consisted of malt extract (17 g l^{-1}) and soya peptone (5.4 g l^{-1}). The pH of the medium was adjusted to 6.5–6.8 with dilute H_2SO_4 and NaOH solutions before autoclaving. Once inoculated, flasks were incubated on an orbital shaker at 100 rpm for 7 days at 25 °C.

2.2. Preparation of the microorganism and Methylene Blue and surfactant solutions for biosorption

After the growth period, the biomass was harvested from the medium and washed twice with distilled water, inactivated using 1% formaldehyde and then dried at 60 °C for 24 h. For the biosorption studies, a weighed amount of dried biomass was suspended in 100 ml of double-distilled water and homogenized in a homogenizer (Janke and Kunkel, IKA-Labortechnick, Ultra Turrax T25, Germany) at 8000 rpm for 20 min and then stored in the refrigerator. At the beginning of biosorption, 10 ml dried biomass suspension was contacted with 90 ml of solution containing a known concentration of Methylene Blue or a desired combination of Methylene Blue and SDS in an Erlenmeyer flask at a defined pH value. All the final solutions contained 1.0 g l^{-1} of biosorbent.

Methylene Blue [C.I.: 52030, chemical formula: $C_{16}H_{18}N_3OS$, MW: 333.6 g mol^{-1}] dye was supplied by Merck. A stock solution of Methylene Blue was prepared at 1.0 g l^{-1} concentration by dissolving weighed amount in double-distilled water. Stock surfactant solution was prepared from sodium dodecylsulfate (SDS) ($C_{12}H_{25}SO_4Na$, MW: 288.4 g mol^{-1}) supplied by Fluka, at 20 mM concentration by dissolving weighed amount in double-distilled water. The liquid media containing desired combinations of Methylene Blue and SDS were prepared by diluting stock solutions of dye and surfactant with distilled water and mixing them in the test medium. Before mixing with biosorbent solution, the initial pH of each test solution was adjusted to the required value with H_2SO_4 and NaOH solutions at different concentrations changing from 0.01 to 1 M. The ranges of initial concentrations of Methylene Blue and SDS prepared from stock solutions varied between 25 and 1100 mg l^{-1} and 0 and 20 mM, respectively.

2.3. Biosorption experiments

Sorption studies were conducted in a routine manner by the batch technique in 250 ml Erlenmeyer flasks containing 100 ml of dye or dye + SDS bearing synthetic solutions at desired level of each component at the beginning of the adsorption. The flasks were continuously agitated on a shaker at 150-rpm constant shaking rate for 1 day to ensure that equilibrium was reached. Samples (5 ml) were taken before and after mixing the biosorbent and single dye or the dye and SDS bearing solutions at definite time intervals. Before analysis the samples were centrifuged at 4000 rpm for 3 min and the supernatant fraction was analyzed for the remaining dye ions. Studies were performed at a constant temperature of 25 °C to be representative of environmentally relevant conditions. The experiments were also repeated with the flasks containing no biosorbent, but the dye and SDS at desired levels to observe any interaction between the dye and SDS. All the biosorption experiments were repeated twice to confirm the results. The data were the mean values of two replicated determinations.

The uptake of dye by unit mass of biosorbent at any time (q) was determined from Eq. (1):

$$q = \frac{C_o - C_{res}}{X} \quad (1)$$

where C_o is the initial Methylene Blue concentration (mg l^{-1}), C_{res} is the residual Methylene Blue concentration at any time (mg l^{-1}) and X is the sorbent concentration (g l^{-1}). C_{res} is equal to C_{eq} and q is equal to q_{eq} at equilibrium.

2.4. Analytical methods

Concentration of Methylene Blue dye in the solution (diluted properly) was determined spectrophotometrically by using a UV/VIS spectrophotometer (Labomed Inc., USA) with a matched pair of glass cuvettes having 1 cm optical lengths. The absorbance of the colour was measured at 663 nm where the maximum absorption peak exists. Calibration curve of absorbance versus concentration of the dye solution was plotted. To examine the effects of pH and SDS on the UV/VIS measurement of the dye and thus to avoid a misinterpretation of the spectrophotometric determinations, separate control experiments were performed to obtain a calibration curve for each case.

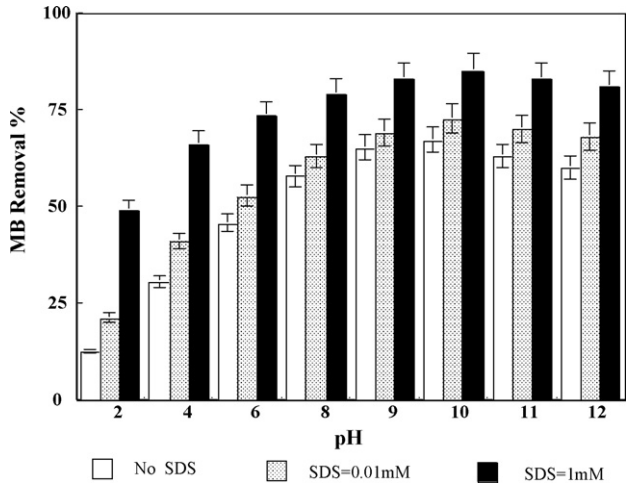

Fig. 1. Effect of initial pH on Methylene Blue biosorption yield in the absence and in the presence of 0.01 and 1 mM SDS concentrations (C_{oMB}: 50 mg l^{-1}, X: 1 g l^{-1}, T: 25 °C, agitation rate: 150 rpm).

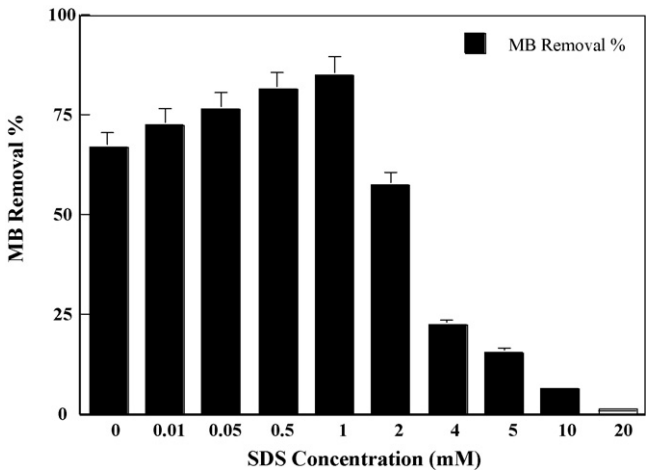

Fig. 2. Effect of initial SDS concentration on Methylene Blue biosorption yield (C_{oMB}: 50 mg l^{-1}, initial pH: 10; X: 1 g l^{-1}, T: 25 °C, and agitation rate: 150 rpm).

3. Results and discussion

3.1. Effect of initial pH on Methylene Blue biosorption in the absence and in the presence of 0.01 and 1 mM SDS

Initial pH and the presence of surfactant may have major effects on Methylene Blue biosorption. Fig. 1 shows the Methylene Blue uptake yield by dried *R. arrhizus* at various initial pH values ranging from 2 to 12 for the case of single dye biosorption. Evidently initial pH significantly affected the extent of biosorption of basic dye and the biosorption of Methylene Blue increased sharply with increasing pH. With changing the initial pH of dye solution from 2 to 10, the biosorption efficiency of dried fungus increased from 12.4 to 67.2%. The percentage dye removal diminished notably with further increasing in pH. This may be due to hydrolysis of functional groups on cell surface, which creates positively charged sites that reduce the biosorption of cationic dye ions. Moreover the solubilization of organic groups present on the surface of biosorbent might be caused a reduction in biosorption at pH values higher than 10 [29]. The effect of initial pH on Methylene Blue biosorption was also examined in 0.01 and 1 mM SDS containing media and the results given in Fig. 1 indicate that the presence of both 0.01 and 1 mM anionic surfactant SDS enhanced the removal of Methylene Blue. When 1 mM SDS surfactant was added to the biosorption medium, dye removal efficiency raised from 67.2 to 85.2%, causing a 26.8% increase in uptake capacity at the initial pH value of 10. In addition, in the presence of 1 mM SDS, the biosorption efficiency was greater than 50% at all pH values ranging from 2 to 12. As the maximum uptake was also observed at pH 10 in the presence of SDS, further studies were performed at this pH value.

The interaction between sorbate and sorbent is affected by the pH of an aqueous medium in two ways: firstly, since dyes are complex aromatic organic compounds having different functional groups and unsaturated bonds, they have different ionization potentials at different pH, resulting in the pH dependent net charge on dye molecules. Secondly, the surface of sorbent includes many functional groups, so the net charge on sorbent, which could be measured in the form of zeta potential or isoelectric point, is also pH dependent. Therefore, the interaction between dye molecules and fungal sorbent is basically a combined result of charges on dye molecules and the surface of sorbent. In the biosorption of Methylene Blue basic dye on dried *R. arrhizus*, the fungal biomass is usually charged negatively on its surface. Depending on pH, Methylene Blue (MB) can be present in aqueous solution in two forms: MB^+ and MBH^{2+}, protonated on the nitrogen in the heterocycle. Due to its very low pK_a value (<1), MB is mainly dissolved in unprotonated form of MB^+ at all pH values [30]. It is expected that negatively charged nitrogen-containing functional groups such as amines or imadazoles, which are the major biosorption sites for dye removal on the fungal surface, will favour the adsorption of positively charged dye cations due to electrostatic attraction that could be the primary mechanism at higher pH values. In addition, increasing solution pH will increase the number of hydroxyl groups, which thereby will increase the number of negatively charged sites and will strengthen the attraction between Methylene Blue and the biosorbent surface. As the biomass will have a net positive charge at pH values below the isoelectric point (<4.0) [19], biosorption of Methylene Blue will be reduced due to the change in the overall surface charge on the fungal cells. Moreover at lower pH values the availability of negatively charged adsorbent sites will be reduced due to the presence of excess H^+ ions competing with cationic Methylene Blue for adsorption sites [8,10,12,13].

Real textile dye effluents contain not only dyes but also surfactants. Although the existence of SDS surfactant in biosorption medium did not affect the optimum pH of biosorption, it influenced the Methylene Blue dye uptake dramatically. Moreover the effect of initial pH on the fungal biosorption capacity became insignificant with the addition of 1 mM SDS. Dried fungus removed 50% of Methylene Blue even at pH 2. In aqueous solution the formation of micellar species of different size and stability owing to the attractive forces between cationic dye and anionic surfactant increases the extent of biosorption. It is clear that the change of medium pH did not affect the formation of these species substantially. As surfactants affected the surface properties of sorbents, such as their surface charge or hydrophobicity/hydrophilicity, the addition of 1 mM SDS may have changed the surface and adsorption properties of fungal biomass and eliminated the serious effects of pH on Methylene Blue biosorption [14–16].

3.2. Effect of SDS concentration on Methylene Blue biosorption

In order to investigate the effect of SDS concentration on the biosorption of the Methylene Blue dye at pH 10, SDS concentration was varied between 0.01 (2.884 mg l^{-1}) and 20 mM (5768.0 mg l^{-1}) while initial Methylene Blue concentration was maintained at 50 mg l^{-1}. As seen from Fig. 2, with increasing the SDS concentration up to 1 mM, the removal efficiency of basic Methylene Blue dye enhanced from 67.2 to 85.2%. Further increasing in SDS concentration resulted in a significant decrease of dye uptake of 1.6%. Anionic

Table 1
Comparison of the pseudo-second-order kinetic constants obtained at different initial Methylene Blue concentrations in the absence and in the presence of increasing SDS concentrations (initial pH: 10; *X*: 1 g l^{-1}; *T*: 25 °C; and agitation rate: 150 rpm).

Surfactant concentration (mM)	C_{oMB} (mg l^{-1})	$q_{eq,exq}$ (mg l^{-1})	$k_{2,ad} \times 10^3$ (g mg^{-1} min^{-1})	$q_{eq,cal}$ (mg g^{-1})	R^2
0	22.8	17.2	29.56	17.3	1.000
	48.5	32.6	13.07	32.9	1.000
	108.2	63.2	7.78	63.9	1.000
	185.5	88.6	6.93	89.3	1.000
	315.4	127.5	6.14	127.8	1.000
	660.5	220.0	5.89	222.0	1.000
	1094.9	305.1	3.11	305.0	1.000
0.01	24.1	19.3	14.02	19.6	1.000
	49.3	35.8	6.67	36.3	1.000
	96.1	61.1	3.78	62.5	1.000
	199.4	106.1	1.53	107.4	1.000
	403.2	183.5	1.24	186.0	0.993
	600	264.2	0.89	267.6	0.998
	1103.4	364.1	0.85	370.4	0.999
1	25	22.5	6.55	23.1	1.000
	50	42.5	3.45	43.7	1.000
	100	86.2	1.88	88.5	0.992
	200	168.6	0.99	173.0	0.997
	400	315.7	0.54	325.0	0.993
	600	559.8	0.21	584.6	0.993
	1100	848.3	0.05	909.1	0.994

surfactants affect (in most cases) dramatically the sorption of basic (cationic) dyes. In general, at low SDS concentrations, the dye sorption increases with increasing surfactant concentration. To explain the sorption of ionic solutes in the presence of oppositely charged surfactants it is assumed that hydrophobic ion pairs, or slightly more complex associates or aggregates are formed between the cationic dye and anionic surfactant in solution with respect to the equilibrium between dye and dye–surfactant species in the aqueous medium. At low SDS concentrations the interaction of the dye with the fungal biomass is represented by the adsorption of "free (dissociated) dye cations" onto active sites of biosorbent in solution and by the adsorption of relatively small dye–SDS aggregates on some kinds of active sites or on non-polar parts of the sorbent matrix. Moreover, at low SDS concentrations surfactant monomers firstly adsorbed to hydrophobic sites on the biosorbent surface create additional negative charges, which increase dye biosorption electrostatically. Above critical micelle concentration (CMC), surfactant molecules form aggregates. Depending on the nature of the surfactant and solvent, the aggregates may form micelles, reverse micelles, micro-emulsion, vesicles, etc. Organic solutes tend to be solubilized in the hydrophobic core of the micelles. Ionic pollutants having opposite charge to that of the micellar surface are bound on its outer periphery. At higher SDS concentrations the dye sorption is suppressed steeply as a result of the complete micelle formation, desorption of dye from the biosorbent/water interface and incorporation the dye molecules into these micelles forming the water-soluble aggregates (complete solubilization in the free micelles) [31]. These explanations may not be sufficient, as several simultaneous and competitive mechanisms may be operating during the sorption process. Similar effects, such as sorption enhancement, were also observed in systems of oppositely charged surfactants that represent analogues to the basic dye–anionic surfactant system [14–17,28,31,32].

3.3. Effect of initial Methylene Blue concentration on Methylene Blue biosorption in the absence and in the presence of 0.01 and 1 mM SDS

The effect of the initial Methylene Blue concentration on the dye biosorption was examined in more detail in the following series of experiments, in which initial dye concentration was varied between 25 and 1100 mg l^{-1}, whereas the surfactant concentration was kept constant at 0, 0.01 or 1 mM concentration. Moreover the experiments were repeated without fungal biosorbent. The dependency of the dye sorption on the Methylene Blue concentration studied is shown in Table 1 and Fig. 3. As can be seen, in the absence of SDS, the uptake of Methylene Blue by dried *R. arrhizus* enhanced notably with increasing the initial dye concentration. On changing the initial Methylene Blue concentration from 25 to 1100 mg l^{-1}, the amount biosorbed increased from 17.2 to 305.1 mg g^{-1} due to the increase in the number of ions competing for the available binding sites on the biomass surface. Initial dye concentration also provided an important driving force to overcome all mass transfer resistances of the dye between the aqueous and solid phases. However, an increase in dye concentration exhibited an adverse effect on percent colour removal due to nearly complete coverage

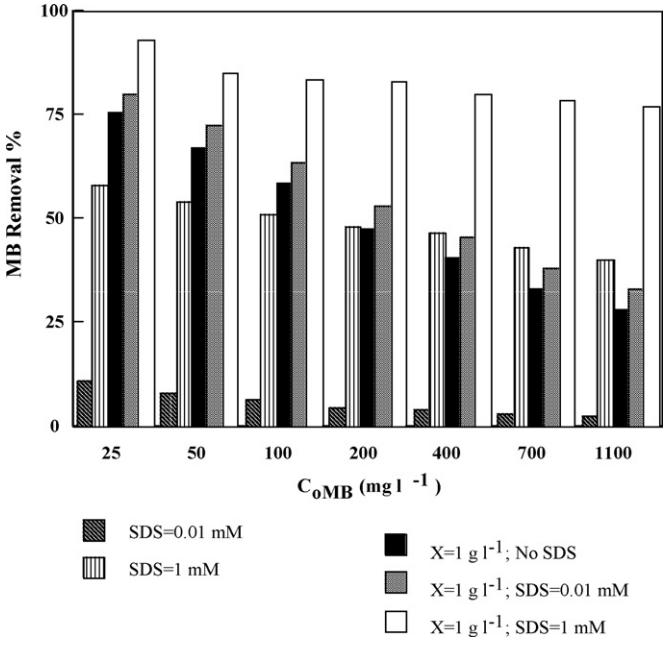

Fig. 3. Effects of 0.01 and 1 mM SDS concentrations and initial Methylene Blue concentration on Methylene Blue removal yield (initial pH: 10, *X*: 1 g l^{-1}, *T*: 25 °C, and agitation rate: 150 rpm).

of the binding sites of biosorbent at high dye concentrations. The removal efficiency of Methylene Blue decreased sharply from 75.4 to 27.9% with increasing the initial dye concentration from 25 to 1100 mg l^{-1}.

Table 1 and Fig. 3 also illustrate the effects of 0.01 and 1 mM SDS concentrations on the removal of Methylene Blue by dried *R. arrhizus* at different initial dye concentrations. As can be seen, while the presence of 0.01 mM surfactant affected only slightly the dye sorption, in the presence of 1 mM SDS, the adsorbed amount of the dye exceeded markedly the sorption capacity determined in the absence of SDS at each dye concentration studied. When studied with 25 and 1100 mg l^{-1} initial Methylene Blue concentrations, as surfactant concentration changed from 0 to 1 mM, the amount of dye adsorbed increased from 17.2 to 22.5 mg g^{-1} (1.31 times enhancement) and from 305.1 to 848.3 mg g^{-1} (2.78 times enhancement), respectively. Although the Methylene Blue dye removal efficiency lessened notably with the increasing Methylene Blue concentration, a significant enhancement in the percent dye removal was observed in the presence of anionic surfactant SDS. For instance, percent dye removal decreased from 93.0 to 77.0% as the initial MB concentration increased from 25 to 1100 mg l^{-1} with the addition of 1 mM SDS in the biosorption medium.

In the absence of fungal bisorbent, percent dye removals by 0.01 and 1 mM SDS at the changing initial Methylene Blue concentrations were also determined and presented in Fig. 3. While the addition of 0.01 mM SDS had an insignificant effect on the dye removal by SDS, the presence of 1 mM SDS exhibited a rather pronounced effect on the removal of oppositely charged cationic Methylene Blue dye at all the dye concentrations studied. At the lower surfactant concentration relatively small premicellar aggregates containing 3–4 molecules of SDS were formed in the presence of the oppositely charged dye, which resulted in a low dye removal while very complex dye–surfactant associates or aggregates formed at the higher surfactant concentration, which led to very high dye uptakes at all the dye concentrations tested. For 1100 mg l^{-1} initial Methylene Blue concentration, while the dye removal percents by the fungus and by SDS were determined as 27.9 and 40.3%, respectively, a 77.0% of total dye removal percent was obtained in 1 mM SDS containing biosorption medium indicating an 8.8% contribution of SDS on the fungal biosorption.

3.4. Biosorption kinetics of Methylene Blue in the absence and in the presence of 0.01 and 1 mM SDS

The sorption kinetics in a wastewater treatment is significant, as it provides valuable insights into the reaction pathways and the mechanism of a sorption reaction. The kinetics of Methylene Blue basic dye removal was measured to understand the dye adsorption behaviour of the fungal biosorbent *R. arrhizus* in the presence as well as in the absence of SDS. For this purpose biosorption capacity (q) was plotted as a function of time for 50 mg l^{-1} initial Methylene Blue concentration at 0, 0.01 and 1 mM surfactant levels (Fig. 4). The time and surfactant concentration dependent, single, smooth, and continuous biosorption curves were obtained. The extent of dye removal enhanced with increasing contact time for all cases studied. The presence of SDS influenced strongly the sorption of the examined basic dye and total sorbed amount of the dye increased in the presence of both surfactant concentrations. In the absence of surfactant, the amount of dye adsorbed on fungal sorbent was 32.6 mg g^{-1} at equilibrium. When 1 mM SDS was added to the biosorption medium, the equilibrium uptake of Methylene Blue raised to 42.5 mg g^{-1}. For the biosorption of Methylene Blue, the dye removal increased by 30.4%. The single biosorption of Methylene Blue onto dried fungus was almost instantaneous, 85.9% of total adsorbed amount was removed within first 10 min and an apparent equilibrium was reached within the first 4 h. Such a rapid

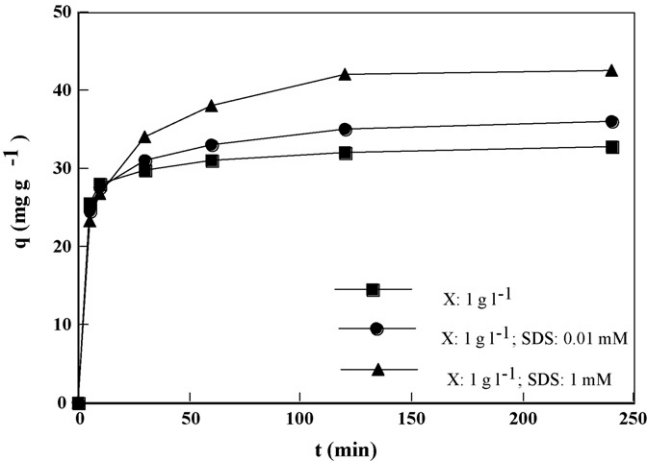

Fig. 4. Biosorption curves for Methylene Blue measured in the absence and in the presence of 0.01 and 1 mM SDS concentrations (initial pH: 10, C_{oMB}: 50 mg l^{-1}, X: 1 g l^{-1}, T: 25 °C, and agitation rate: 150 rpm).

uptake of dye by *R. arrhizus* indicates that this biosorbent has an affinity for the dye cations pointing towards physical adsorption and that the uptake of dye occurs predominantly by surface binding. Although the addition of 0.01 or 1 mM SDS did not affect the equilibrium time, it changed the adsorption profile of the dye. As seen, the adsorption of Methylene Blue onto fungal biomass in the presence of SDS increased gradually (62.8% of total adsorbed amount was removed within first 10 min) and then remained constant after 240 min equilibrium time. Such a slower biosorption process shows that external and internal diffusions of the dye and dye–surfactant species gained importance in adsorption rate control. Based on these results, the contact time was fixed at 4 h for the rest of the batch experiments to make sure that equilibrium was reached in all cases.

3.5. Application of pseudo-second-order kinetic model in the absence and in the presence of 0.01 and 1 mM SDS

Adsorption is a time-dependent process. In the removal of dyes from wastewater, it is necessary to know the rate of adsorption for process design, operation control and adsorbent evaluation. For this purpose simplified pseudo-second-order kinetic model was tested to fit the experimental data in the absence and in the presence of SDS surfactant. This model is the most commonly used to describe the sorption of dyes and basically include all steps of adsorption such as external film diffusion, adsorption, and internal particle diffusion, so it is a pseudo-model as pointed out by McKay and Ho [33]. Contrary to other well-established kinetic models, pseudo-second-order model also predicts the adsorption behaviour over the whole range of adsorption period and it is in agreement with the chemisorption mechanism being the rate-controlling step. Chemisorption (ion-exchange, electrostatic attractions) is commonly cited as the main mechanism for the adsorption of cationic species in basic conditions. The pseudo-second-order equation is based on the sorption capacity of the solid phase and is expressed as:

$$\frac{dq}{dt} = k_{2,ad}(q_{eq} - q)^2 \quad (2)$$

where $k_{2,ad}$ is the rate constant of second-order biosorption of Methylene Blue. After integration and applying the boundary conditions of $t = 0$ to $t = t$ and $q = 0$ to $q = q_{eq}$; the integrated form of Eq. (2) becomes a linear function and model parameters of q_{eq} and $k_{2,ad}$ can be estimated from the slope and intercept of the t/q against t plot.

Kinetic experiments were performed by varying the initial Methylene Blue concentration between 25 and 1100 mg l^{-1} and keeping the surfactant concentration constant between 0 and 1 mM for each experimental set. The values of rate constant ($k_{2,ad}$) and equilibrium uptake (q_{eq}) were determined from the plots of linearized form of the pseudo-second-order model at all Methylene Blue concentrations at 0, 0.01 and 1 mM SDS levels (data not shown) and are presented in Table 1 along with the corresponding linear regression coefficients. The results indicated that the second-order rate constants were affected by the initial dye concentration and the added concentrations of 0.01 and 1 mM SDS. The rate constants, diminished notably with both increasing Methylene Blue and SDS concentrations, may be attributed to dominant surface adsorption. The correlation coefficients obtained greater than 0.999 and the adequate fitting of theoretical and experimental q_{eq} values for all combinations suggest the applicability of second-order kinetic model in explaining the kinetics of Methylene Blue biosorption. The values of predicted equilibrium sorption capacities showed reasonably good agreement with the experimental equilibrium dye uptake values.

3.6. Application of equilibrium models in the absence of SDS

Adsorption isotherms are useful for selecting the most appropriate sorbent and also for predicting the performance of adsorption system. An adsorption isotherm is a mathematical expression that relates the amount of adsorbate at the interface (q_{eq}) to its equilibrium concentration in the aqueous phase (C_{eq}) at a constant temperature. Within the literature, the Langmuir [34] and the Freundlich [35] are the most frequently used two-parameter models to describe the sorption of dye ions on the biomass from the dye solution. Langmuir sorption model, which was originally developed to describe the gas–solid phase adsorption of activated carbon, has traditionally been used to quantify and to contrast the performance of different sorbents and the model serves to estimate the maximum uptake values where they cannot be reached in the experiments. The empirical Langmuir equation is valid for monolayer sorption onto a completely homogeneous surface with a finite number of binding sites and with negligible interaction between adsorbed molecules and is given by Eq. (3):

$$q_{eq} = \frac{Q^o b C_{eq}}{1 + bC_{eq}} \quad (3)$$

where parameters Q^o and b are the Langmuir constants related to maximum adsorption capacity and bonding energy of adsorption, respectively.

Unlike the Langmuir, the Freundlich isotherm model assumes neither homogeneous site energies nor limited levels of sorption. The Freundlich model is the earliest known empirical equation and is shown to be consistent with exponential distribution of active centres, characteristic of heterogeneous surfaces. It is expressed by the following equation:

$$q_{eq} = K_F C_{eq}^{1/n} \quad (4)$$

where K_F and n are the Freundlich constants characteristic on the system.

For the investigation of surfactant effect on Methylene Blue biosorption, the Langmuir, and Freundlich equations were applied to the equilibrium data obtained for single dye and dye–surfactant biosorption systems. The non-linearized adsorption isotherms of Methylene Blue in the absence and in the presence of 0.01 and 1 mM SDS are shown in Figs. 5 and 6 together with experimental points. The curvilinear relationship between the amount of Methylene Blue adsorbed per unit weight of biomass and the residual Methylene Blue concentration at equilibrium suggests that saturation of cell-binding sites occurred at the higher concentrations of this dye in the absence and in the presence of 0.01 mM SDS. The plots also indicated that the equilibrium uptake of Methylene Blue enhanced apparently by the addition of 0.01 and 1 mM SDS and the effect of 1 mM SDS anionic surfactant on Methylene Blue uptake was dominant.

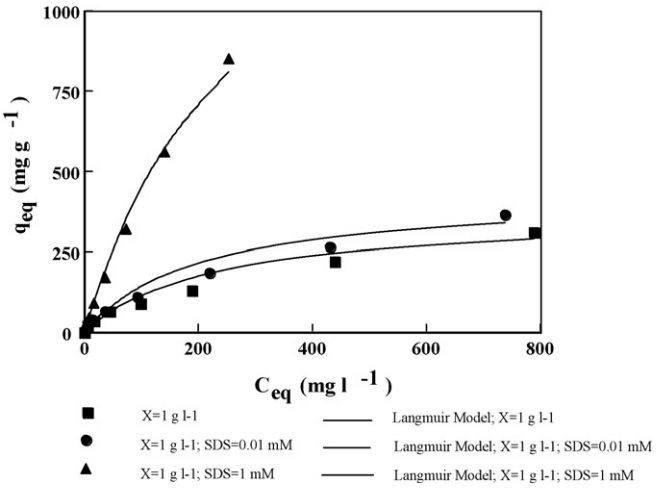

Fig. 5. Comparison of the experimental equilibrium data with the estimated Langmuir isotherms of Methylene Blue in the absence and in the presence of SDS (symbols represent experimental data and lines represent estimated adsorption isotherms).

The corresponding Langmuir and Freundlich parameters for Methylene Blue biosorption at two surfactant levels obtained by linear regression analysis were listed in Table 2. The applicability of the models was established from the regression correlation, R^2 and fitted curves. The results showed that the regression correlations for the Freundlich model are between 0.998 and 1.000 while those of the Langmuir model are between 0.839 and 0.923. This suggests a greater fit by the Freundlich model in comparison to the Langmuir model. Using the model parameters, equilibrium uptake values of Methylene Blue for each case were predicted from the related formulae and plotted. The data in Figs. 5 and 6 also confirmed that the Freundlich model closely predicted the equilibrium data, as evident from the overlapping of its model curves. These results suggest that dried *R. arrhizus* presents heterogeneous adsorption sites and that the interaction of the dye on fungal biomass, in the presence of high

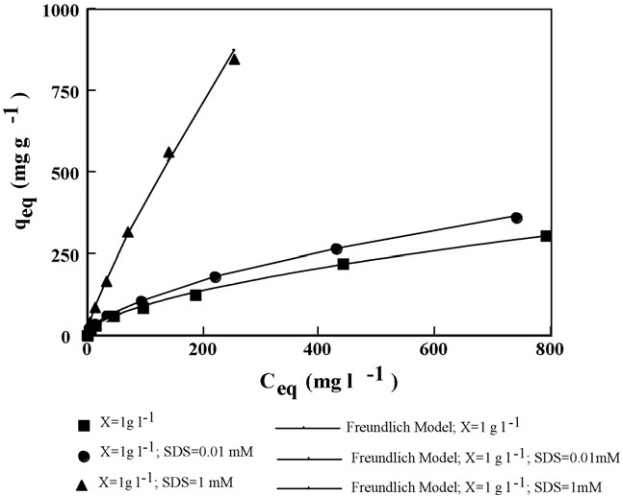

Fig. 6. Comparison of the experimental equilibrium data with the estimated Freundlich isotherms of Methylene Blue in the absence and in the presence of SDS (symbols represent experimental data and lines represent estimated adsorption isotherms).

Table 2
Effect of surfactant (SDS) concentration on the Freundlich and Langmuir constants of Methylene Blue biosorption (initial pH: 10; *X*: 1 g l^{-1}; *T*: 25 °C; and agitation rate: 150 rpm).

Surfactant concentration (mM)	Freundlich model		
	K_F [(mg g^{-1}) (mg l^{-1})$^{-1/n}$]	n	R^2
0	6.57	1.74	0.998
0.01	7.75	1.72	1.000
1	8.88	1.21	0.998
Surfactant concentration (mM)	**Langmuir model**		
	Q^o (mg g^{-1})	$b \times 10^3$ (l mg^{-1})	R^2
0	370.3	4.34	0.915
0.01	434.8	4.78	0.923
1	1666.6	3.68	0.839

SDS concentrations, occurs via the formation of complete dye–SDS aggregates.

Adsorption model constants, the values of which express the surface properties and affinity of the biosorbent, can be used to compare the Methylene Blue adsorptive capacity of dried *R. arrhizus* due to the SDS level chosen.

The Freundlich constant n is an empirical parameter that varies with the level of heterogeneity indicating the degree of non-linearity between Methylene Blue uptake capacity and unadsorbed Methylene Blue concentration and is related to the distribution of bonded ions on the sorbent surface. In general $n > 1$ illustrates that adsorbate is favourably adsorbed on an adsorbent and the higher the n value the stronger the adsorption intensity. In particular, the value of n, which is significantly higher than unity, indicated that Methylene Blue ions are favourably adsorbed by *R. arrhizus* at all SDS concentrations studied. The values of n also indicated that the Methylene Blue biosorption intensity was positively affected by the 0.01 and 1 mM SDS added into biosorption medium. The constant K_F, related to biosorption capacity, can be defined as a sorption coefficient, which represents the quantity of adsorbed Methylene Blue for a unit equilibrium concentration (i.e., $C_{eq} = 1$). The co-existence of SDS at its initial concentrations increased the K_F constant significantly. The highest K_F value was 6.57 in the absence of SDS and the value increased to 8.88 with the addition of 1 mM SDS, which was consistent with the experimental observation.

Table 2 also indicates that Langmuir model parameters (Q^o and b) of Methylene Blue biosorption were also highly dependent on the SDS added. While the Freundlich model does not describe the saturation behaviour of the biosorbent, Q^o represents the monolayer saturation at equilibrium or the total capacity of biosorbent for Methylene Blue dye. High Q^o values show a desirable high capacity of Methylene Blue binding. As seen from Table 2, dried *R. arrhizus* exhibited the maximum biosorption capacity (Q^o) at 1 mM SDS containing medium. The addition of 1 mM SDS enhanced dramatically the maximum Methylene Blue uptake capacity of biomass from 370.3 to 1666.6 mg g^{-1} compared to the single dye conditions. A high value of the other Langmuir parameter, b, indicates a steep desirable beginning of the isotherm which reflects the high affinity of the biosorbent for the sorbate(s). Its value is the reciprocal of the Methylene Blue concentration at which half of the saturation of the biosorbent is attained. The higher b values obtained in the presence of changing concentrations of SDS also indicated its positive effect on Methylene Blue biosorption.

Mono-component Freundlich model successfully predicted the sorption of Methylene Blue from both single and binary dye–SDS solutions. The isotherm parameters satisfactorily defined the experimental data and also provided useful information about the relative sorption capacity (K_F), maximum sorption capacity (Q^o), and nature and affinity of the biomass for Methylene Blue in single Methylene Blue and Methylene Blue–SDS binary systems.

4. Conclusion

In this study, the fungal biomass of *R. arrhizus* was used and evaluated as a possible biosorbent for the treatment of a cationic dye, Methylene Blue, from waters and anionic surfactant, SDS, bearing waters. The initial pH experiments revealed that neutral or basic conditions were required to optimize the Methylene Blue biosorption while initial Methylene Blue concentration also had an increasing effect on biosorption capacity up to 1100 mg l^{-1}. It was shown that Methylene Blue biosorption was influenced dramatically by the presence of SDS surfactant and Methylene Blue removal was enhanced 4.5 times with the addition of 1 mM SDS. It was seen that the adsorption equilibrium data fitted very well to the Freundlich model at all surfactant concentrations studied. It was decided that the biosorption kinetics of dye for each case was described well by pseudo-second-order kinetic model. The results indicated that dried fungal biomass of *R. arrhizus* can be used as an effective adsorbent for the removal of Methylene Blue from waters and the adsorption of the cationic dye can be supported with the addition of small amounts of anionic surfactant SDS. This is very important for potential applications of the fungus, because anionic surfactants are widely employed in industry and are commonly present in real wastewaters. This study indicated the effect of anionic SDS surfactant on the biosorption of single cationic dye. As the real dye effluents contain several other pollutants besides different kinds of dyes, microbial sorbent *R. arrhizus* should further be investigated for its efficiency for decolourization using real dye effluents from industries.

References

[1] E.A. Clarke, R. Anliker, Organic dyes and pigments Handbook of Environmental Chemistry. Anthropogenic Compounds. Part A, vol. 3, Springer, New York, 1980.
[2] H. Zollinger, Color Chemistry—Synthesis, Properties and Applications of Organic Dyes and Pigments, VCH, New York, 1987.
[3] S.S. Nawar, H.S. Doma, Removal of dyes from effluents using low-cost agricultural by-products, Sci. Total Environ. 79 (1989) 271–279.
[4] Y.M. Slokar, A.M. Le Marechal, Methods of decoloration of textile wastewaters, Dyes Pigments 37 (1997) 335–356.
[5] E. Forgacs, T. Cserhati, G. Oros, Removal of synthetic dyes from wastewaters: a review, Environ. Int. 30 (2004) 953–971.
[6] G. Crini, Non-conventional low-cost adsorbents for dye removal: a review, Biores. Technol. 97 (2006) 1061–1085.
[7] V.K. Gupta, Suhas, Application of low-cost adsorbents for dye removal—a review, J. Environ. Manage. 90 (2009) 2313–2342.
[8] N.S. Maurya, A.K. Mittal, P. Cornel, E. Rother, Biosorption of dyes using dead macro fungi: effect of dye structure, ionic strength and pH, Biores. Technol. 97 (2006) 512–521.
[9] N. Zaghbani, A. Hafiane, M. Dhahbi, Separation of Methylene Blue from aqueous solution by micellar enhanced ultrafiltration, Sep. Purif. Technol. 55 (2007) 117–124.
[10] V.J.P. Vilar, C.M.S. Botelho, R.A.R. Boaventura, Methylene blue adsorption by algal biomass based materials: biosorbents characterization and process behaviour, J. Hazard. Mater. 147 (2007) 120–132.
[11] X.S. Wang, Y. Zhou, Y. Jiang, C. Sun, The removal of basic dyes from aqueous solutions using agricultural by-products, J. Hazard. Mater. 157 (2008) 374–385.
[12] K. Vijayaraghavan, S.W. Won, J. Mao, Y.-S. Yun, Chemical modification of *Corynebacterium glutamicum* to improve methylene blue biosorption, Chem. Eng. J. 145 (2008) 1–6.
[13] A. Saeed, M. Iqbal, S.I. Zafar, Immobilization of *Trichoderma viride* for enhanced methylene blue biosorption: batch and column studies, J. Hazard. Mater. 168 (2009) 406–415.
[14] P. Janos, V. Smidova, Effects of surfactants on the adsorptive removal of basic dyes from water using an organomineral sorbent–iron humate, J. Colloid Interface Sci. 291 (2005) 19–27.
[15] A.R. Cestari, E.F.S. Vieira, G.S. Vieira, L.E. Almeida, Aggregation and adsorption of reactive dyes in the presence of an anionic surfactant on mesoporous aminopropyl silica, J. Colloid Interface Sci. 309 (2007) 402–411.
[16] B. Simoncic, M. Kert, Influence of the chemical structure of dyes and surfactants on their interactions in binary and ternary mixture, Dyes Pigments 76 (2008) 104–112.

[17] S. Paria, Surfactant-enhanced remediation of organic contaminated soil and water, Adv. Colloid Interface Sci. 138 (2008) 24–58.
[18] B. Volesky, Detoxification of metal-bearing effluents: biosorption for the next century, Hydrometallurgy 59 (2001) 203–216.
[19] T. O'Mahony, E. Guibal, J.M. Tobin, Reactive dye biosorption by *Rhizopus arrhizus* biomass, Enzyme Microb. Technol. 31 (2002) 456–463.
[20] Z. Aksu, Application of biosorption for the removal of organic pollutants: a review, Process. Biochem. 40 (2005) 997–1026.
[21] V. Prigione, G.C. Varese, L. Casieri, V.F. Marchisio, Biosorption of simulated dyed effluents by inactivated fungal biomasses, Biores. Technol. 99 (2008) 3559–3567.
[22] Z. Aksu, A.I. Tatli, O. Tunc, A comparative adsorption/biosorption study of Acid Blue 161: effect of temperature on equilibrium and kinetic parameters, Chem. Eng J. 142 (2008) 23–39.
[23] Y. Fu, T. Viraraghavan, Fungal decolorization of dye wastewater: a review, Biores. Technol. 79 (2001) 251–262.
[24] T. Akar, T.A. Demir, I. Kiran, A. Ozcan, A.S. Ozcan, S. Tunali, Biosorption potential of *Neurospora crassa* biomass for decolorization of Acid Red 57 (AR 57) dye, J. Chem. Technol. Biotechnol. 81 (2006) 1100–1106.
[25] G. Bayramoglu, G. Celik, M.Y. Arica, Biosorption of Reactive Blue 4 dye by native and treated fungus *Phanerocheate chrysosporium*: batch and continuous flow system, J. Hazard. Mater. 137 (2006) 1689–1697.
[26] K. Kumari, T.E. Abraham, Biosorption of anionic textile dyes by nonviable biomass of fungi and yeast, Biores. Technol. 98 (2007), pp. 1784–1718.
[27] P. Kaushik, A. Malik, Fungal dye decolourization: recent advances and future potential, Environ. Int. 35 (2009) 127–141.
[28] X. Jin, M.-Q. Jiang, X.-Q. Shan, Z.-G. Pei, Z. Chen, Adsorption of methylene blue and orange II onto unmodified and surfactant-modified zeolite, J. Colloid Interface Sci. 328 (2008) 243–247.
[29] O. Hamdaoui, Batch study of liquid-phase adsorption of methylene blue using cedar sawdust and crushed brick, J. Hazard. Mater. 135 (2006) 264–273.
[30] M. Havelcova, P. Kubat, I. Nmcova, Photophysical properties of thiazine dyes in aqueous solution and in micelles, Dyes Pigments 44 (1999) 49–54.
[31] M.K. Purkait, A. Maiti, S. DasGupta, S. De, Removal of congo red using activated carbon and its regeneration, J. Hazard. Mater. 145 (2007) 287–295.
[32] L. Zhu, J. Ma, Simultaneous removal of acid dye and cationic surfactant from water by bentonite in one-step process, Chem. Eng. J. 139 (2008) 503–509.
[33] G. McKay, Y.S. Ho, Pseudo-second order model for sorption processes, Process Biochem. 34 (1999) 451–465.
[34] I. Langmuir, The adsorption of gases on plane surfaces of glass, mica, and platinum, J. Am. Chem. Soc. 40 (1918) 1361–1368.
[35] H. Freundlich, Adsorption in solution, Phys. Chem. Soc. 40 (1906) 1361–1368.