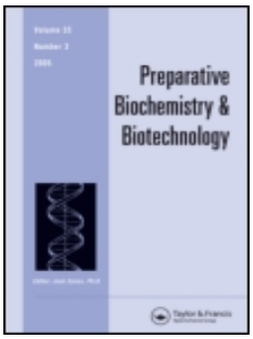

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DETERMINATION OF METHYLENE BLUE BIOSORPTION BY *Rhizopus arrhizus* IN THE PRESENCE OF SURFACTANTS WITH DIFFERENT CHEMICAL STRUCTURES

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□ *Methylene blue (MB) biosorption properties of* Rhizopus arrhizus *were investigated in the presence of surfactants. The effects of cationic and anionic surfactants on MB removal by dead biomass ($1\,g\,L^{-1}$) were determined. MB removal was tested as a function of initial pH (2–12), contact time (5–1440 min), and dye ($37.4–944.7\,mg\,L^{-1}$) and surfactant (0–10 m*M*) concentrations. The opposite charged anionic surfactant dodecylbenzenesulfonic acid sodium salt (DBS) enhanced sorption of cationic MB by biomass dramatically. Maximum biosorption capacity was $471.5\,mg\,g^{-1}$ at pH 8 with 0.5 m*M *DBS at $944.7\,mg\,L^{-1}$ MB concentration. The surfactant-stimulated fungal decolorization method may provide a highly efficient, inexpensive, and time-saving procedure in biological wastewater treatment technologies.*

Keywords anionic surfactant, biosorption, cationic surfactant, methylene blue, *R. arrhizus*

INTRODUCTION

The cationic dye methylene blue (MB) is extensively used for dyeing cotton, wool, and silk in the textile industry. Extensive use of MB results in its frequent detection in wastewater streams. Although MB is not very toxic, it causes harmful effects such as irritation to the gastrointestinal tract, nausea, vomiting, and diarrhea in humans and animals.[1] It is reported that MB is a model compound for removing colored bodies from aqueous solutions due to its strong adsorption onto solids.[2] The adsorption characteristics of MB dyes on various expensive adsorbents, such as activated carbon (AC), silica, clay, industrial solid wastes, and others, have been extensively investigated.[3] Recently, fungal biomasses have been suggested as inexpensive biosorbents for removal of various contaminants such as

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textile dyes.[4,5] Removal of pollutants like dyes from aqueous solutions by inactive dead biomasses is called biosorption and represents the passive interactions of the cell wall with pollutants.[6] Dye binding sites are localized in the cell surface.[7] There are few reports in the literature on using surfactants in the dye treatment systems by dead fungal biomass.

Surfactants are amphiphilic molecules that have both hydrophilic and hydrophobic parts.[8] The head part of the molecule is hydrophilic and the tail part is hydrophobic. The head can be a charged or uncharged polar group. According to the nature of the head groups, the surfactants are classified into anionic, cationic, nonionic, and zwittterionic (amphoteric).[9] Surfactants are used as leveling agents in dyeing stuff.[10] Recently, the usage of surfactants in dye removal from wastewater treatment has gained importance.[11,12] Surfactants are also used as levelling, dispersing, and wetting agents for improving the dyeing process in the textile industry.[13] Therefore, the textile industry wastewater also contains surfactants with dyes.

It is important to improve low-cost wastewater treatment technologies.[14] The goal of this study was to determine the removal of MB by inexpensive bioadsorbent (dead *Rhizopus arrhizus* fungal biomass) in the presence of surfactant with different chemical structures such as alkyltrimethylammonium bromide (ATAB), hexadecyltrimethylammonium bromide (HTAB), cetylpyridinium chloride (CPC), dodecyl trimethyl ammonium bromide (DTAB), and dodecylbenzenesulfonic acid sodium salt (DBS). Considering the literature on the subject, this is the first report that investigates the effect of cationic and anionic surfactants on biosorption of MB by *R. arrhizus* biomass and the usage of surfactants with dead microorganisms in treating industrial wastewaters containing textile dyes.

EXPERIMENTAL

Microorganism and Growth Conditions

The fungus *R. arrhizus* was used in the current study. The microorganism was obtained from the U.S. Department of Agriculture Culture Collection. The composition of the growth medium was malt extract ($17\,g\,L^{-1}$) and peptone ($5.4\,g\,L^{-1}$) dissolved in deionized water. The initial pH of the medium was adjusted to 6.5. The pure cultures (*R. arrhizus*) were inoculated into 250-mL Erlenmeyer flasks containing 100 mL of growth medium and cultivated in an orbital shaker (New Brunswick Scientific Innova 4230, USA) at an agitation rate of 100 rpm for 7 days and 25°C.[6]

Biosorbent Preparation

At the end of 7 days of incubation, the fungus was harvested and washed with distilled water, treated with 1% formaldehyde, and dried at

60°C until a constant weight of biomass was obtained. The homogenized dried biomass was used for biosorption studies as mentioned in our previous work.[6] For the biosorption experiments 10 mL dried biomass suspension was added to the 90 mL of solution with the desired amount of dye and surfactant solution in an Erlenmeyer flask at a defined pH value. All experiment series performed with the final solutions contained 1.0 g L^{-1} of biosorbent.

Dye and Surfactant Solution Preparation

MB dye was obtained from Merck and stock solution was prepared as 1000 mg L^{-1} in distilled water. The chemical structure of MB is given in Figure 1. The working solutions of MB were prepared by diluting the stock solution to the desired concentrations.

ATAB, HTAB, CPC, and DTAB surfactants were used as cationic surfactants and DBS was used as an anionic surfactant. The chemical structures of surfactants are given in Figure 1. The figures were drawn by the Chemosis program. All of the surfactants were supplied by Fluka and each of them was prepared in 20 m*M* concentration as a stock solution.

Desired amounts of dye and surfactant solutions were added to the Erlenmayer flasks at known initial pH values for the biosorption experiments.

Biosorption Studies at Batch Scale

All of the biosorption experiments were performed in Erlenmayer flasks containing 100 mL working solution with desired amounts of dye and surfactant. The experiments were conducted at 150 rpm for 24 hr at 25°C. For analysis, 3-mL samples were taken at definite times from the working solution that contains microorganism, dye, and surfactant. The samples were centrifuged (Hettich EBA12 model centrifuge) at 4000 rpm for 3 min, and supernatant was used for dye analysis after appropriate dilutions.

The percentage biosorption of dye was calculated from

$$\text{Methylene blue biosorption (MBB\%)} = (C_o - C_f)/C_o \times 100 \quad (1)$$

The uptake of dye by unit mass of biosorbent at any time (q_{m-} mg g^{-1}) was determined from

$$q_m = C_o - C_f/X_m \quad (2)$$

where C_o is the initial MB concentration (mg L^{-1}), C_f is the final methylene blue concentration at any time (mg L^{-1}), and X_m is the sorbent concentration (g L^{-1}). All the experiments were carried out at least twice.

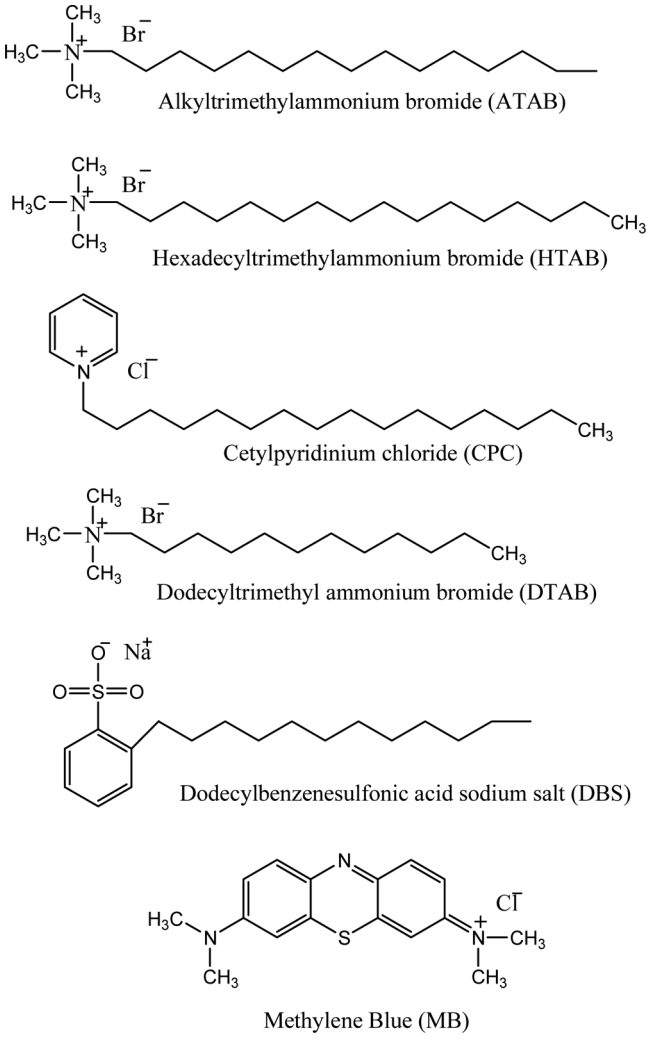

FIGURE 1 The chemical structure of MB and surfactants.

Analytical Methods

MB concentration in the supernatant was determined spectrofotometrically (Shimadzu ultraviolet [UV] 2001 model spectrophotometer). The concentration of MB was determined by measuring the absorbance at 663 nm.

RESULTS AND DISCUSSION

The effects of surfactants on MB removal by dead *R. arrhizus* biomass were tested as a function of initial pH, contact time, dye, and surfactant concentrations.

Effect of Cationic and Anionic Surfactants on MB Biosorption

The effect of different surfactants such as cationic (ATAB, HTAB, CPCM, DTAB) and anionic (DBS) ones on 68.9 mg L^{-1} MB dye biosorption properties of fungal biosorbent was examined in the presence of 0.5 m*M* surfactants and absence of surfactant at pH 8 after 24 hr of incubation. There wasn't a significant change in the dye uptake of fungus with cationic surfactants and without surfactant (Figure 2). Maximum dye biosorption by fungal biosorbent occured in the presence of anionic DBS surfactant as 95.1% in 24 hr (Figure 2). MB is a cationic dye.[6] It was observed that surfactants enhanced dye biosorption of fungus, and the opposite charged surfactant (anionic DBS) with dye (cationic MB) performed the best enhancement.

Effect of Contact Time on MB Biosorption in the Presence of DBS

The effect of contact time on 68.9 mg L^{-1} dye uptake of fungus was determined in the absence and presence of 0.5 m*M* DBS. The sorption of MB by fungal biosorbent increased rapidly. The removal rate of MB was 67.7% without surfactant and 75.1% with DBS within first 5 min, and then desorption of a little amount of MB dye from fungal biomass was observed after 240 min in the absence of DBS (Figure 3). The dye sorption of fungal biomass increased after 240 min in the presence of 0.5 m*M* DBS (Figure 3) and remained constant after 1440 min equilibrium time. Based on these results, the contact time was fixed at 24 hr for the rest of the batch experiments to make sure that equilibrium was reached in all cases in the

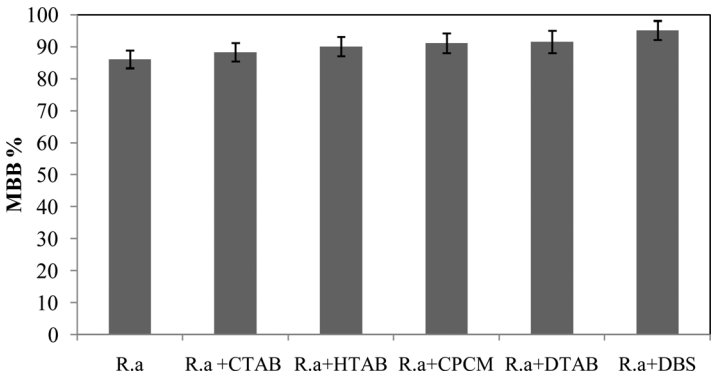

FIGURE 2 The effect of surfactants on MB Biosorption (MBB%) by *R. arrhizus*. R.a: *R. arrhizus*; ATAB: alkyltrimethylammonium bromide; HTAB: hexadecyltrimethylammonium bromide; CPCM: cetylpyridinium chloride; DTAB: dodecyltrimethylammonium bromide; DBS: dodecylbenzenesulfonic acid sodium salt; Co MB: 68.9 mg L^{-1}; Co surfactant: 0.5 m*M*; pH:8; T: 25°C; agitation rate: 150 rpm; 24 hr.

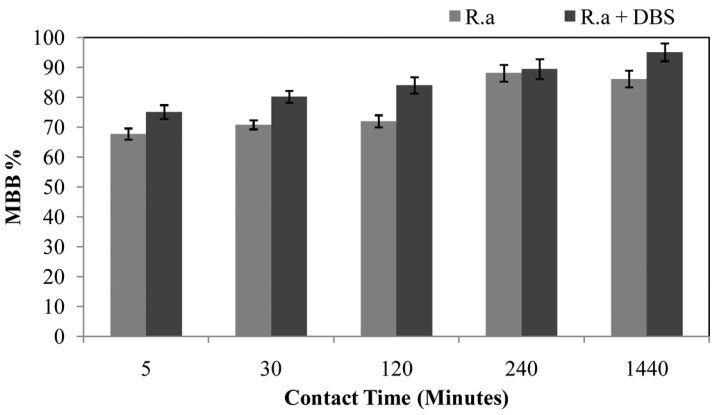

FIGURE 3 The effect of contact time on MB biosorption (MBB%) by *R. arrhizus*. R.a: *R. arrhizus* DBS: dodecylbenzenesulfonic acid sodium salt; Co MB: 68.9 mg L^{-1}; Co surfactant: 0.5 m*M*; pH: 8; T: 25°C; agitation rate: 150 rpm.

presence of DBS. Desorption of dye wasn't observed in the presence of DBS. Surfactants are used as dye fixing agents in the dyeing textile fiber process due to electrostatical interactions between oppositely charged dye, surfactant, and the surface of fiber.[13] It was assumed that DBS fixed MB dye on fungal biosorbent surface by means of electrostatical interactions.

Effect of pH on MB Biosorption

The effect of pH on dye (68.9 mg L^{-1}) sorption of fungus in the presence of 0.5 m*M* DBS was determined at pH 2, 4, 6, 8, 10, and 12. Maximum MB removal occured at pH 8 (Figure 4). Initial pH of medium had a

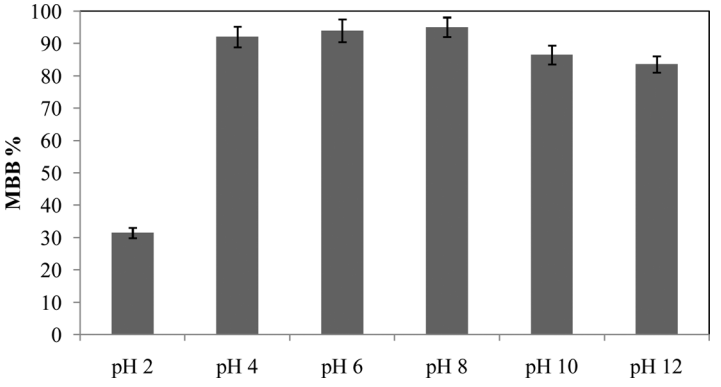

FIGURE 4 The effect of initial pH on MB Biosorption (MBB%) by *R. arrhizus* in the presence of DBS. DBS: dodecylbenzenesulfonic acid sodium salt; Co MB: 68.9 mg L^{-1}; Co DBS: 0.5 m*M*; T: 25°C; agitation rate: 150 rpm.

significant effect on dye removal because of the chemical properties of dyes and fungal surface.[15] The fungal cell wall was the primary site of sorption, and decolorization was related to both protonation or deprotonation of the functional groups of the biopolymers on the biomass surface and the ionization potential of complex dye molecules in solution.[15] The cell walls contained cation exchange groups with high pKa, which enabled a cell wall to be a natural cation exchanger, depending on the environmental conditions such as pH.[7] The surface charge of each fungal biomass is predominantly negative at high pH values.[16] The isoelectric point of *R. arrhizus* was shown as below pH 5.5.[17] The surface charge of *R. arrhizus* was negative at pH 8, and cationic MB dye removal was maximum at pH 8 because of electrostatic interactions between the dye and fungal surface. Gül and Dönmez[18] have reported that positively charged cationic DTAB was linked to negatively charged fungal surface and anionic Remozol blue dye at pH 6. In this study, negatively charged DBS enhanced the biosorption of positively charged cationic MB dye by fungal surface at pH 8.

Effect of DBS Concentration on MB Biosorption

To examine the effect of DBS concentration on the biosorption of the MB dye at pH 8, DBS concentration was varied as 0, 0.05, 0.5, 1, 5, and 10 m*M* while initial MB concentration was maintained at 68.9 mg L^{-1}. As seen from Figure 5, with increasing the DBS concentration up to 0.5 m*M*, the removal efficiency of basic methylene blue dye was enhanced from 82 to 95.1%. Further increase in DBS concentration resulted in a significant decrease of dye removal. Aksu et al. (2010) reported that anionic sodium

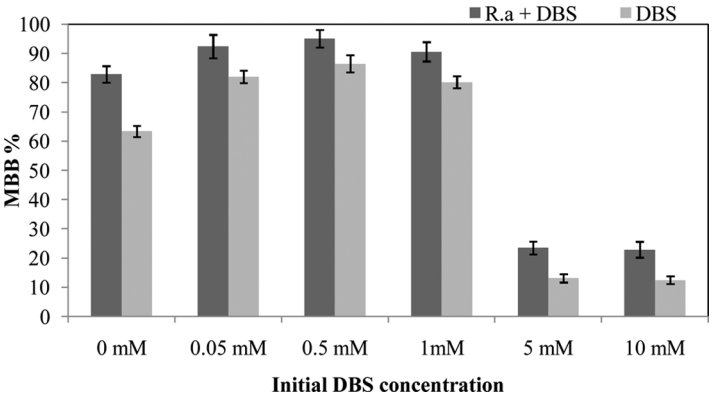

FIGURE 5 The difference of MB Biosorption (MBB%) in the medium with *R. arrhizus* and increasing surfactant concentrations (R.a + DBS) and in the medium only contains increasing surfactant concentrations without *R. arrhizus*. DBS: dodecylbenzenesulfonic acid sodium salt; Co MB: 68.9 mg L^{-1}; Co DBS: 0.5 m*M*; T: 25°C; agitation rate: 150 rpm.

TABLE 1 Effect of Initial MB Concentration on MB Biosorption (MBB%) and Maximum Specific Dye Uptake (q_m) by *R. arrhizus* in the Presence and Absence of Surfactant

CoMB ($mg\,L^{-1}$)		MBB (%)		q_m (mgg^{-1})	
Without DBS	With DBS	Without DBS	With DBS	Without DBS	With DBS
21.8	37.4	85.3	81.8	18.6	30.6
58.8	68.9	76.7	95.3	45.1	65.6
91	126.5	76.8	95.1	69.9	120.3
185.5	204.9	63.6	96.04	118	196.8
315.4	322.6	49.9	97.4	157.5	314.1
1094.9	944.7	15.2	49.9	166.1	471.5

Note. DBS: dodecylbenzenesulfonic acid sodium salt; Co DBS: 0.5 m*M*; T: 25°C; agitation rate: 150 rpm.

dodecyl sulfate (SDS) surfactant affected dramatically the sorption of basic (cationic) dyes.[6] At low SDS concentrations, the dye sorption increases with increasing surfactant concentration. 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 of the dye molecules into these micelles forming the water-soluble aggregates.[19] Similar effects, such as dye removal enhancement, were also observed in systems of oppositely charged surfactants and dyes at low surfactant concentrations.[6,18,19]

Effect of MB Concentration on MB Biosorption

The effect of MB concentration on biosorption was examined at different dye concentrations, such as 21.8, 58.8, 91, 185.5, 315.4, and 1094.9 $mg\,L^{-1}$ in the absence of surfactant and 37.4, 68.9, 126.5, 204.9, 322.6, and 944.7 $mg\,L^{-1}$ in the presence of surfactant. Increase of MB concentration from 37.4 to 322.6 $mg\,L^{-1}$ raised decolorization of dye from 81.8% to 97.4% in the presence of DBS, but at 944.7 $mg\,L^{-1}$ dye concentration dye biosorption by fungus was decreased (Table 1). On the other hand, dye biosorption by fungus was decreased with increasing dye concentration in the absence of DBS. DBS enhanced decolorization of dye in high dye concentrations. Maximum specific dye uptake (q_m) occured at 944.7 $mg\,L^{-1}$ MB concentration in the presence of DBS at 471.5 $mg\,g^{-1}$ (Table 1).

CONCLUSIONS

In this study, the effect of cationic and anionic surfactants on cationic MB dye biosorption properties of *R. arrhizus* fungal biomass was investigated. The oppositely charged anionic surfactant DBS enhanced sorption

of cationic MB by fungal biomass dramatically. The effects of pH, contact time, dye, and surfactant concentrations on dye biosorption by fungus were examined at batch-scale levels. Maximum biosorption uptake capacity was $471.5\,mg\,g^{-1}$ at pH 8 with $944.7\,mg\,L^{-1}$ MB and 0.5 mM DBS concentrations in 24 hr.

The results indicated that anionic surfactant DBS can be used as a potential inducer to remove MB from waters by dried *R. arrhizus* fungal biomass. Surfactants are present in real wastewater with synthetic dyes because they are commonly used in industrial applications. The results of this study recommend using surfactants in biological wastewater treatment technologies.

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