

Reactive dye biosorption by *Rhizopus arrhizus* biomass

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Abstract

The biosorption of three commonly used reactive dyes, from aqueous solutions by oven-dried *Rhizopus arrhizus* biomass was studied in a batch system with respect to pH, initial dye concentration and initial metal ion concentration. The biomass exhibited maximum dye uptake at pH 2 due to its positively charged nature at acidic pH and the anionic nature of the reactive dyes. Reactive orange 16 dye was adsorbed most effectively to a maximum of approximately 200 mg/g. The presence of high levels of cadmium did not significantly impair the adsorption capacity of the biomass. Dye removal from a multicomponent solution of all three dyes was also achieved. *Rhizopus* biomass was found to exhibit superior removal properties than activated charcoal.

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1. Introduction

Synthetic dyes are used extensively by industries including dyehouses, paper printers and textile dyers. The effluents of these industries are highly coloured and disposal of these wastes into the environment can be extremely deleterious. Their presence in watercourses is aesthetically unacceptable and may be visible at concentrations as low as 1 ppm [1]. Moreover, they may also affect photosynthetic activity in aquatic systems by reducing light penetration [2]. More importantly, while most are considered relatively non-toxic, several commonly used dyes have been reported to be carcinogenic and mutagenic [3].

Consequently considerable research effort has been devoted to optimising colour removal from solution. Conventional techniques include chemical oxidation, ozonolysis, filtration, precipitation, sedimentation and adsorption. The most commonly used methods of dye removal involve a combination of methods. Physico-chemical methods have proved effective, and many commercial dye removal products take this form, for example Macrosorb[®]. Dyes bind to the Macrosorb[®] surface by an ion exchange mechanism and a floc is formed which can be readily separated by sedimentation or filtration.

Textile dyes are designed to be resistant to degradation or fading by chemicals and light. They must also be resilient

to both high temperatures and enzyme degradation resulting from detergent washing. For these reasons, biodegradation of dyes is typically a slow process. Reactive dyes, whose removal is examined here, are popular due to their bright colours, excellent colourfastness and ease of application. They differ from other dyes in that they bind to the textile fibre such as cotton via covalent bonds. They are especially difficult to displace from effluent and are removed to a maximum of approximately 30% and an average of only 10% by typical sludge systems. Additionally, fixing of these dyes is an extremely inefficient process with losses of up to 50% [4].

Although existing technologies such as ozonation and oxidation are efficient in reactive dye removal, both initial and operating costs are high. Anaerobic biological treatments of dyes are well-documented [5,6], although in the case of azo and some other dye types, complete mineralisation is difficult and the resulting aromatic amines may be toxic and carcinogenic [7]. Various two-stage treatment systems have been developed which achieve aerobic metabolisation of the products of an anaerobic decolourisation stage [8,9].

Adsorption techniques are seen as an economic alternative. Activated carbon has proved effective for dye removal but it is expensive. Less costly alternative sources of adsorption materials have been investigated, including peat, steel plant slag, bentonite clay and fly ash [10].

Many bacteria and fungi have shown value as potential adsorbents [11,12]. The uptake or accumulation of chemicals by microbial mass is termed biosorption and may involve a combination of active, metabolism-dependent and passive

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transport mechanisms. Binding to sites at or near the cell surface which exhibit chemical affinity for the adsorbate is generally non-metabolism dependent and so occurs for both viable and denatured microbial cells [2,13]. We have previously demonstrated the high metal binding capacity of the mould *Rhizopus arrhizus* which exceeds that of some commercial ion exchange resins [14,15]. However the potential of fungal biosorption processes to remove dye from effluent is relatively unknown.

The objectives of this study were to determine the dye uptake characteristics of *R. arrhizus* for a number of commercially used reactive dyes in single and in multicomponent solutions and to compare the findings with the characteristics of activated charcoal adsorbent. The effects of the presence of metal co-ions on dye uptake were also examined.

2. Materials and methods

2.1. Biomass preparation

R. arrhizus, strain CMI83711, was cultured in liquid medium comprising: bacteriological peptone, 10 g/l; sucrose, 20 g/l; KH_2PO_4 , 1 g/l; NaNO_3 , 1 g/l and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/l. Cultures were grown in 250 ml Erlenmeyer flasks at 25 °C on a rotating shaker (150 rpm) for 3 days. The micro-organism was maintained on solid medium obtained by adding 15 g/l No. 1 bacteriological agar (Oxoid, Hampshire, UK) to the above medium.

The biomass was separated from the broth by filtration through Whatman No. 1 paper and washed three times with de-ionised water. Biomass was oven dried (65 °C for 48 h) and the product was ground in a mortar and pestle before use. Sieving analysis showed the following particle size distribution: >600 μm : 8%; 600–250 μm : 49%; 250–150 μm : 18%; <150 μm : 24%.

2.2. Chemicals

The three dyes used in this study, whose structures are shown in Fig. 1, were anionic, reactive dyes and were obtained from Sigma–Aldrich chemical company (Dorset, UK). The dyes were:

- (i) Cibacron Brilliant Red 3B-A (reactive red), colour index (CI) 18105, Reactive Red 4, molecular weight: 995.23, UV wavelength max. (nm): 517;
- (ii) Remazol Brilliant Blue R, CI 61200, Reactive Blue 19, molecular weight: 626.56, UV wavelength max. (nm): 592; and
- (iii) Remazol Brilliant Orange 3R, CI 17757, Reactive Orange 16, molecular weight: 617.54, UV wavelength max. (nm): 494.

The multicomponent dye solutions referred to in this work consisted of equal concentrations of each of the three dyes, in solution. Cadmium solutions were prepared using

$\text{CdSO}_4 \cdot 8/3\text{H}_2\text{O}$, obtained from Riedel-de Haen (Seelze, Germany). Activated charcoal (untreated granular, 20–60 mesh) was obtained from Sigma-Aldrich (Dorset, UK).

2.3. Experimental procedure

2.3.1. Dye uptake

Dye uptake studies were conducted at room temperature and involved contacting 100 ml volumes of dye solution with 1 g biomass/l for 20 h on an orbital shaker at 150 rpm. All studies were carried out in duplicate and biomass-free controls were run concurrently. Following contact, the biomass was removed by centrifugation (4000 rpm, 5 min) and the dye concentration of the supernatant analysed. Dye concentrations were determined spectrophotometrically using a Beckman DU[®] 520 UV/VIS spectrophotometer (Fullerton, CA) at the dyes respective λ_{max} value.

2.3.2. Effects of pH on dye uptake

The optimal pH for dye removal by *R. arrhizus* biomass was determined by measuring uptake from 100 mg dye/l solutions over a range of pH values from 2 to 10. One molar HCl and 1 M NaOH were used for adjustment of the solution pH.

2.3.3. Dye adsorption isotherms

Dye uptake levels were determined from a series of solutions of initial dye concentration ranging from 0 to 500 mg/l at the optimal pH value determined as described above. Adsorption isotherms were constructed by plotting the resulting uptake levels (q) versus the remaining concentration in solution (C_f). The maximum uptake capacity of the dried *R. arrhizus* for each dye was estimated from the plateau value for each isotherm.

2.3.4. Dye and metal competition studies

Uptake competition between each dye and cadmium ions (Cd^{2+}) at pH 2 was also investigated. Uptake of both dye and Cd^{2+} from solutions containing 0–250 mg dye/l and 100 mg Cd^{2+} /l was determined. As above, following contact and centrifugation, the extent of dye removal was analysed spectrophotometrically. Cd^{2+} was analysed using a Perkin–Elmer 3100 atomic absorption spectrophotometer (AAS) equipped with an air/acetylene flame. Cd^{2+} concentrations were determined by reference to appropriate standard solutions. Three-dimensional plots, constructed using Matlab[®] Version 5, were used to represent the effect of cadmium on dye uptake of dyes.

2.3.5. Uptake from multicomponent dye solutions

Multicomponent dye solutions were prepared by addition of equal concentrations of each dye to give a final 'whole dye' concentration of between 0 and 450 mg/l. Adsorption studies were conducted as described above using both *Rhizopus* biomass and commercial activated charcoal which was selected as a comparison sorbent.

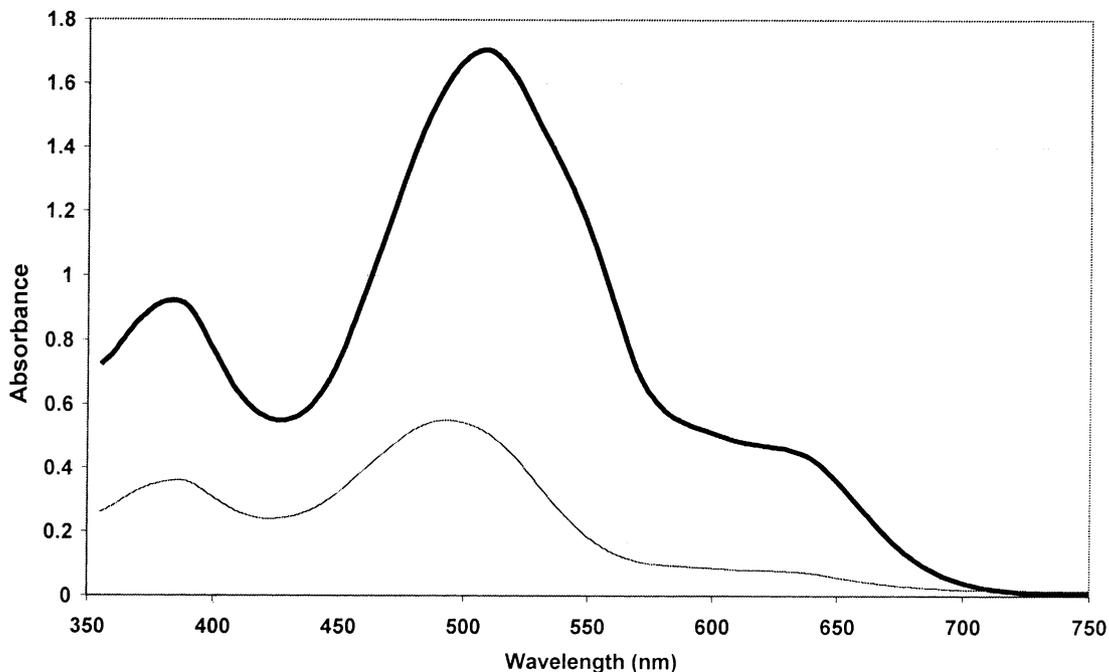


Fig. 2. Spectra of multicomponent dye solutions as plotted by scanning spectrophotometer, before (bold) and after (faint) contact with *Rhizopus* biomass (initial dye concentration of 150 mg/l).

influenced by the surface charge that in turn is influenced by the solution pH. With diminishing pH increasing numbers of weak base groups in the biomass become protonated and acquire a net positive charge [16]. These charged sites become available for binding anionic groups such as the reactive dyes used in this study.

The variation in uptake capacity of the *Rhizopus* biomass across the pH range may be explained in terms of its effective isoelectric point. At pH values below the isoelectric point, the biomass will have a net positive charge as outlined above. It is expected that nitrogen-containing functional groups in the biomass will be protonated at acidic

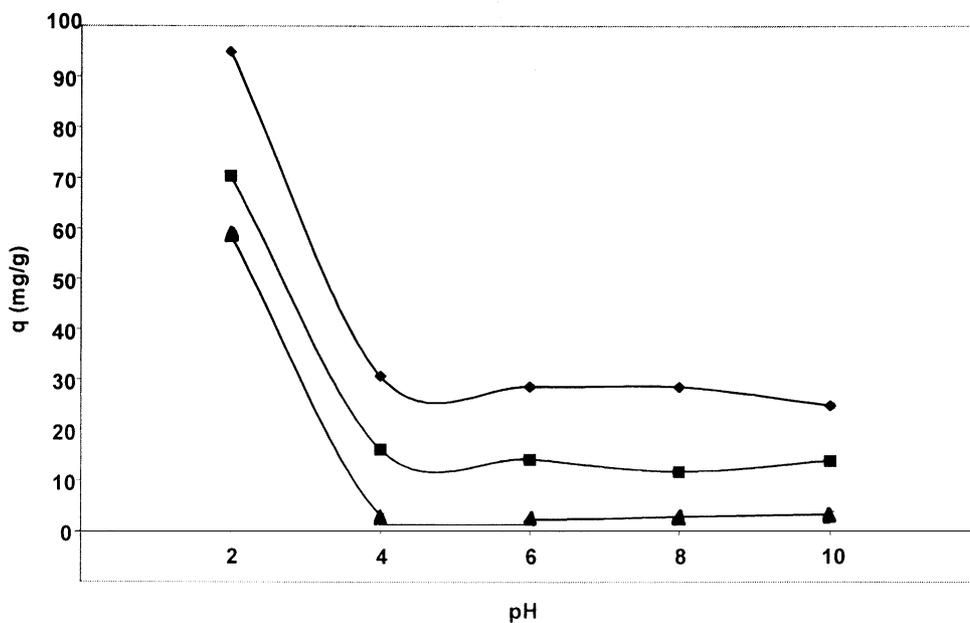


Fig. 3. Uptake of Cibacron Red (◆), Remazol Blue (■) and Remazol Orange (▲) from initial concentration of 100 mg dye/l by *Rhizopus* biomass between pH 2–10.

pH values. For example, most common amino acids have isoelectric points in the pH range 5–6 [17]. Uptake of anionic molybdate and vanadate species by *R. arrhizus* has suggested that the biomass has an effective isoelectric point below pH 5.5 [16]. However, the results presented here suggest an isoelectric point in the region of pH 4. In comparison, uptake of anionic metal complexes to dried *Chiorella vulgaris* increased at low pH and has been ascribed to electrostatic binding to positively charged groups, such as amines or imadazoles [18]. Also, fungal binding of the reactive dye Remazol Black B has shown maximum values in the range pH 1–2 with a sharp drop off at higher values [2].

3.2. Dye adsorption isotherms

Uptake of each of the dyes increased with increasing solution concentration (Fig. 4). Remazol Blue, which exhibited the lowest uptake (maximum value ca. 90 mg dye/g biomass), reached quasi-saturation at the relatively low equilibrium concentration of ca. 40 mg dye/l. In contrast, Remazol Orange was taken up to levels of ca. 190 mg dye/g biomass and did not appear to reach saturation over the concentration range involved. Cibacron Red exhibited the steepest initial isotherm slope (which is a measure of the sorbent–solute affinity) and an eventual maximum uptake level of 150 mg dye/g biosorbent although saturation levels were clearly not attained.

When compared on a more appropriate molar basis, Remazol Orange uptake was greatest with a maximum value of ca. 0.31 mmol/g biomass, while Remazol Blue and Cibacron

Red uptake levels were similar at ca. 0.14 and 0.15 mmol/g biomass respectively. Moreover, the solution concentrations were up to ca. 0.2 mM for Cibacron Red as compared to 0.3 mM for each of the other dyes. These molar uptake values are consistent with uptake of metal ions by *R. arrhizus* biomass [16]. Molybdate and vanadate anions were bound to maximum levels of 0.38 and 0.45 mmol/g biomass respectively while uptake of divalent cations was in the range 0.1–0.5 mM/g at equilibrium concentrations similar to this work [16]. In that work uptake saturation was not observed at solution concentrations below 1 mM which is consistent with the shape of the isotherms in Fig. 4. Here maximum concentrations in the range 250–500 mg/l were chosen as representative of industrial effluent levels [19].

The present uptake values are comparable with dye binding by other biosorbent materials. Linseed cake bound Basic Blue dye to levels of 150–200 mg/g biosorbent although neither Acid Blue or Reactive Red dyes were taken up [20]. The sorption pH was not reported. At pH 2 uptake of the reactive dye Remazol Black by *R. arrhizus* biomass was between 130 and 180 mg/g biosorbent at concentrations corresponding to this work [2]. In both studies biosorption levels increased with increasing solution concentrations until at least 800 mg/l.

The present results also compare favourably with dye binding to activated carbon, which is used in most commercial adsorption systems. At final solution concentrations of 200 mg/l, the activated carbon Filtasorb-400 (F-400) biosorbed Remazol Reactive Red and Reactive Black to levels of 250 and 280 mg/g sorbent respectively [21].

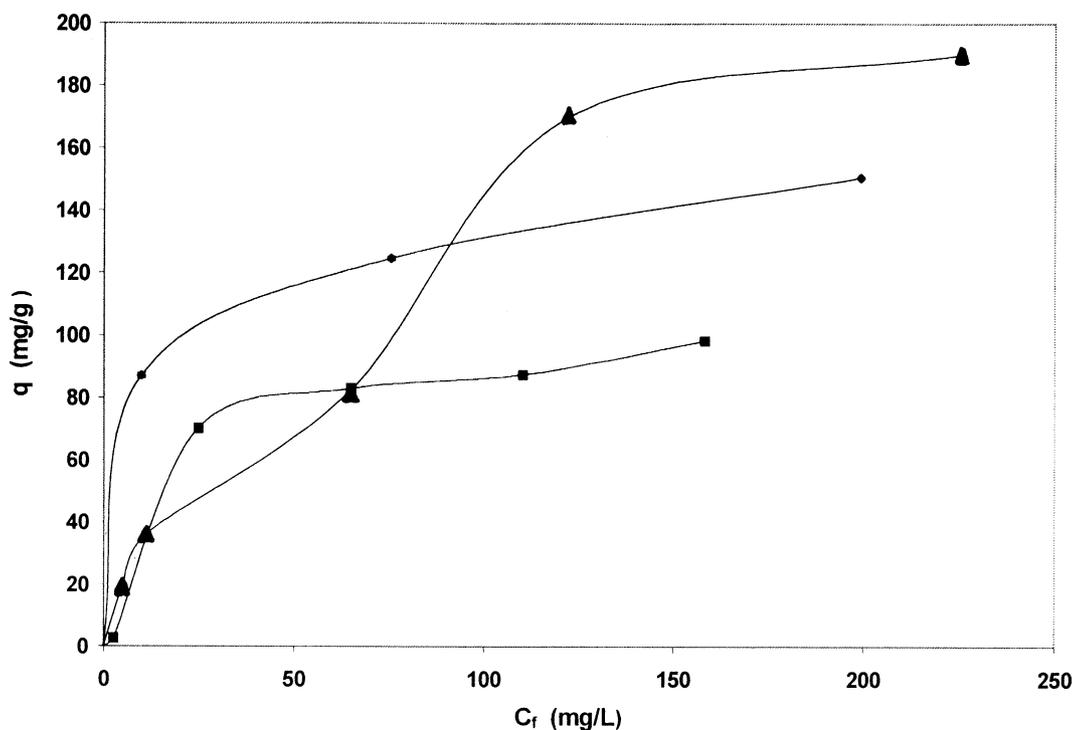


Fig. 4. Dye adsorption isotherms to *Rhizopus* biomass: Cibacron Red (◆), Remazol Blue (■) and Remazol Orange (▲).

No marked increase in uptake was evident with increasing solution concentrations. In contrast, uptake of Reactive Yellow was greater with a maximum sorption of 680 mg/g sorbent at a final solution concentration of only 100 mg/l [21].

3.3. Dye/metal competition

Uptake of each of the three dyes was diminished by the presence of 100 mg Cd²⁺/l. For Cibacron Red, the maximum reduction was of the order of 20 mg/g biosorbent which represents 12.5% of maximum dye adsorption levels (Fig. 5). Reductions in uptake of Remazol Blue and Remazol Orange were similar (data not shown). At the same time uptake of cadmium was low and reached a maximum of 5.5 mg Cd²⁺/g biosorbent.

The competition between Cd²⁺ and dye may be represented as a 3-D sorption surface (Fig. 6). By adding another concentration axis (for final cadmium concentration), the conventional sorption–isotherm curves are transformed into a three-dimensional representation. This approach has been used to show the effects of Ca²⁺ on Cd²⁺ biosorption by *Sargassum* biomass [22] and similarly to illustrate the influence of iron on algal biosorption of cadmium [23].

The decrease in Cibacron Red uptake with increasing Cd²⁺ levels and the low Cd²⁺ removal (reflected in the small decrease from the initial concentration of 100 mg/l) are clearly illustrated (Fig. 6). In comparison, Cd²⁺ uptake levels of 30 mg/g biosorbent have previously been reported

for *Rhizopus* biomass at pH 4 [16]. However, reduced uptake would be expected here as a result of competition from H⁺ ions at the lower pH.

At pH 2 the presence of cationic species, even at relatively high concentrations, does not significantly inhibit the dye removal by *R. arrhizus*. This is of significance for potential applications as textile mill effluents may contain a variety of cationic metal species [24] including cadmium, copper, lead, mercury, nickel and lead [25].

3.4. Uptake of multicomponent dye solutions

In order to simulate dyehouse or textile mill effluent multicomponent dye solutions were prepared with equal concentrations of the three dyes and a maximum total dye concentration of 450 mg/l. There was negligible difference between dye removal determined by single peak height analysis at λ_{\max} and that determined by total peak area. Nonetheless total peak areas were used in calculating colour removal as these represent the whole adsorption range and are likely to be the basis for regulatory effluent standards in the future.

Uptake of dye from multicomponent solution by *Rhizopus* biomass at pH 2 and by activated charcoal at pH 2 and 5 increased with increasing solution concentration (Fig. 7). Maximum *Rhizopus* biosorption levels were 140 mg/l at a final solution concentration of 300 mg/l. It is noteworthy that this value approximates to the mean of the maximum uptake values from single dye solutions suggesting a direct

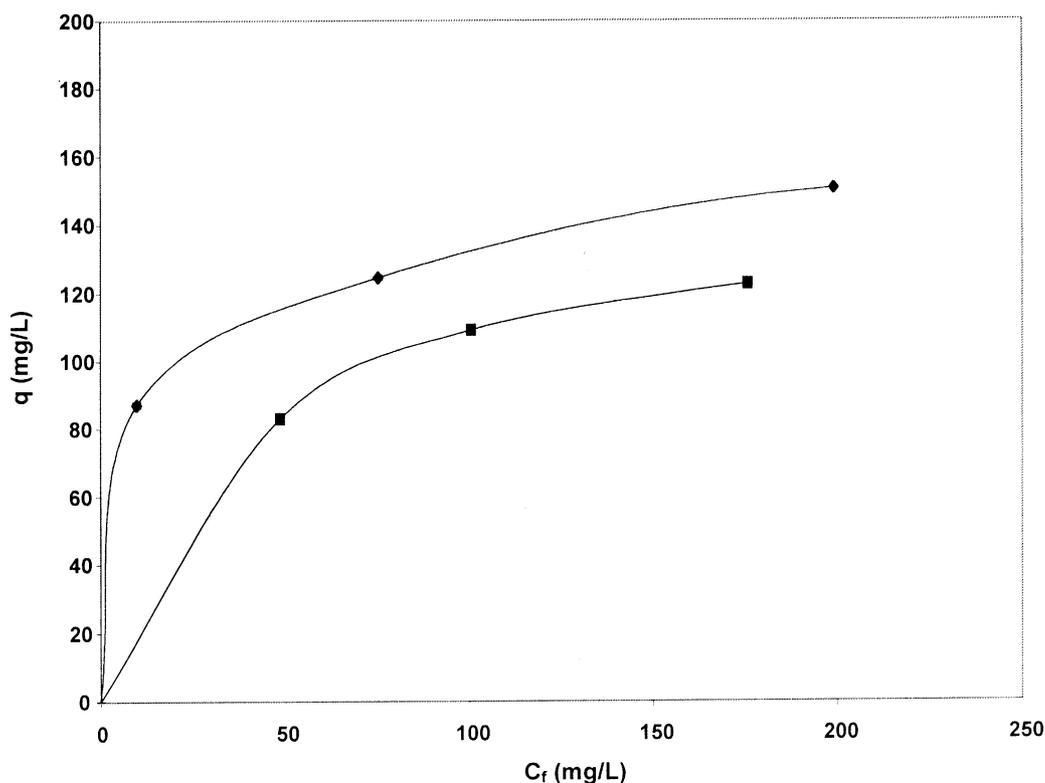


Fig. 5. Effect of 100 mg Cd²⁺/l on uptake (q) of Cibacron Red by *Rhizopus* biomass: dye (◆) and dye plus Cd²⁺ (■).

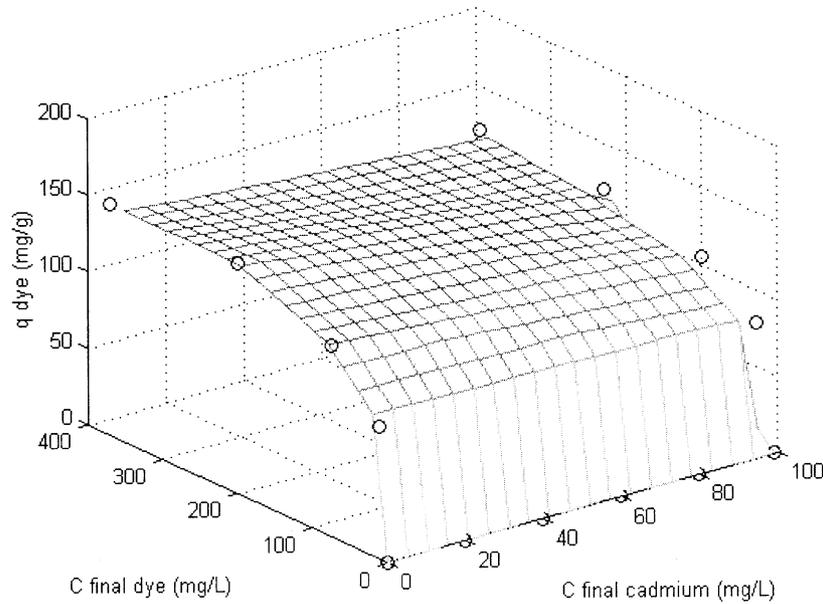


Fig. 6. Sorption surface showing effects of $100 \text{ mg Cd}^{2+}/\text{l}$ on the uptake (q) of Cibacron Red by *Rhizopus* biomass. Experimental data points (○).

competition mechanism and that no dye is preferentially bound. Equally, the fact that each of the peak heights was reduced by a similar percentage (Fig. 2) following biosorption supports the view that binding was non-preferential.

The influence of pH on dye uptake by activated charcoal (Fig. 7) was less than expected on the basis of the marked pH influence on dye uptake by *Rhizopus* biomass (Fig. 3). The results indicate that few additional binding sites are present at pH 2 as compared to pH 5. This is in contrast to the behaviour of *Rhizopus* biomass and points to an isoelectric point in excess of 5. In previ-

ous studies isoelectric point values ranging from 5.7 to 7.2 have been reported for various activated carbon types [21,26,27].

The fact that *Rhizopus* biomass adsorbed more dye than the activated carbon may be attributed in some degree to differing particle size distributions. Sieving analysis showed that approximately 11, 82, 6 and 1% of the activated carbon had particle diameters in the 1000–600, 600–250, 250–150 μm and <150 μm ranges respectively. Though similar to the size distribution of the *Rhizopus* biomass cited in Section 2.1, the lower percentages of the smaller size ranges result in a

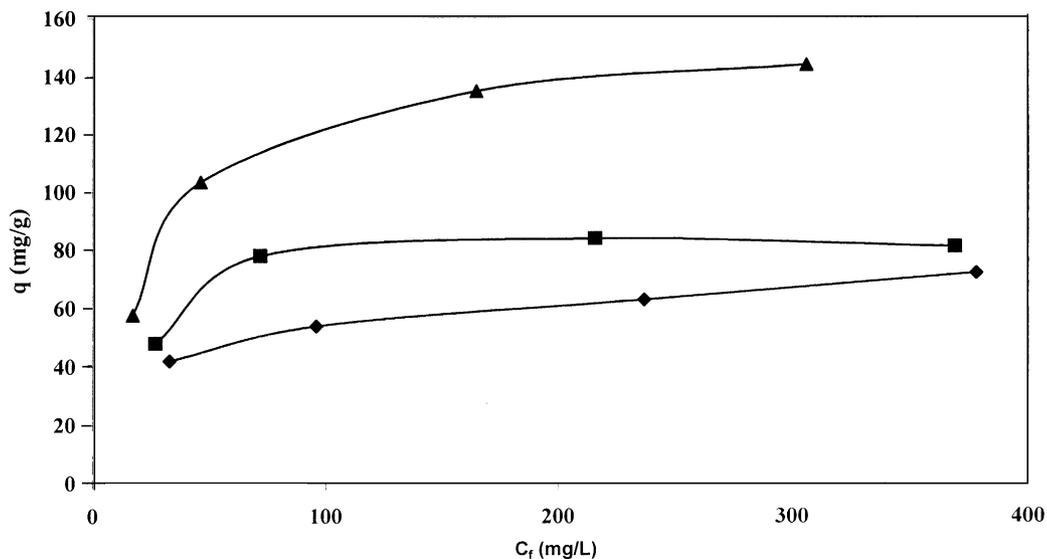


Fig. 7. Comparison of dye uptake (q) from multicomponent solutions by *Rhizopus* and activated charcoal adsorbents. *Rhizopus* biomass at pH 2 (▲); activated charcoal at pH 2 (■) and activated charcoal at pH 5 (◆).

mean particle diameter approximately 30% greater than that of the *Rhizopus* biomass.

Activated carbon is a material that is widely used on account of its adsorption properties. These are based on an intricate porous structure and a large surface area that is predominantly within the micropores [28]. In keeping with the present study, a marked decrease in dye uptake levels with increasing activated carbon (F-400) particle size has recently been reported [21], suggesting that the large molecular size of the dyes may prevent penetration to the particle interior and result in adsorption only near or on the carbon surface. The present uptake values are lower than those of that work, however the maximum dye solution concentrations are some three-fold lower here. Also, the maximum activated charcoal particle size here was in the 1000–600 μm range which is on average approximately 25% larger than the mean of the largest size range used in the F-400 study. Taking these factors into account it is interesting to note that the maximum uptake levels exhibited here by *Rhizopus* biomass (ca. 190 mg dye/g) are comparable with the uptake values of ca. 250 mg/g for reactive black and reactive red dyes on F-400 activated carbon.

These results suggest that a dye uptake process mediated by *Rhizopus* biomass has potential for large-scale treatment of textile mill discharges. The rapid kinetics of biosorption of metal ions [29] as well as dye [2] suggest the feasibility of continuous flow stirred tank or column reactor configurations. Indeed, such applications would involve simply the substitution of conventional adsorbent materials by biosorbent. However, the strong pH dependence of the dye biosorption process will remain a limiting factor and is likely to restrict the range of applications.

4. Conclusions

The present work demonstrates that *R. arrhizus* biomass has good dye adsorption potential exceeding that of activated charcoal at low pH values. Moreover, dye uptake values are in line with previously reported results for F-400 activated carbon under comparable conditions. Dye removal from multicomponent solution approximates mean uptake values of the constituent dyes indicating a non-preferential binding mechanism. The presence of cadmium ions at concentrations of 100 mg/l does not appreciably diminish dye uptake and negligible cadmium binding occurs at pH 2. Uptake is strongly pH dependent and decreases markedly above pH 3.

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