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Surface modification of *Penicillium chrysogenum* mycelium for enhanced anionic dye removal

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Abstract

Dead *Penicillium chrysogenum* mycelium was used for the removal of three anionic dyes: acid orange 8 (AO8), acid blue 45 (AB45) and reactive orange 16 (RO16). The biomass surface was modified with polyethylenimine (PEI) and cross-linked with glutaraldehyde for enhanced anionic dye sorption. The sorption of dyes by both pristine and modified biomass was favored at lower pH, due to the electrostatic interactions between the dye anions and the protonated amine groups on the biomass. The effect of temperature over the range 30–70 °C on the sorption capacity was not significant. Compared to the pristine biomass, the maximum sorption capacity of the surface-modified biomass for AO8, AB45 and RO16 increased from 33, 18 and 25 mg/g, respectively to 352, 196 and 338 mg/g, respectively. Dye sorption using the pristine and modified biomass was well described by the Freundlich and Langmuir isotherm, respectively. FTIR analysis showed that PEI was grafted on the biomass surface via interactions with the amine groups on the pristine biomass. Regeneration of the PEI-modified biomass using AO8 as the adsorbate showed that the biomass could be used repeatedly up to seven cycles with negligible loss in dye removal efficiency.

Keywords: *Penicillium chrysogenum*; Anionic dyes; PEI-modified biomass; Regeneration

1. Introduction

Currently, there are over 100,000 dyes available commercially, and the estimated world annual production of dyes is 1×10^6 tonnes, with azo dyes constituting more than 50% of the total [1,2]. The use of dyes in diverse industries is substantial and, depending on the type of dye used, the extent of loss of dyes in the effluent typically ranges from 10% to 50% and 5% to 20% for reactive and acid dyes, respectively [2,3].

Synthetic dyes are mostly xenobiotic compounds which are difficult to treat. Although most dyes exhibit low toxicity, some dyes and their metabolites from anaerobic degradation are carcinogenic and/or mutagenic and may have acute and/or chronic effects [3,4]. In addition, effluent from dyestuff industries may be highly colored which is aesthetically unacceptable, apart from its deleterious effect in aquatic systems.

Although the most effective dye removal method to date is adsorption using activated carbon, its use is restricted due to high cost [5], and cheaper sources of adsorbents are being considered as alternatives. An adsorbent is considered as low cost if it is plentiful in nature or is produced as a waste material from another industry. Numerous studies involving the use of potential low-cost sorbents for the removal of dyes have been reported, with many involving the process of biosorption. For instance, dead bacteria biomass of *Streptomyces rimosus* and *Corynebacterium glutamicum* were used for the biosorption of basic dye and reactive dyes, respectively, and *Enteromorpha prolifera* (green seaweed) was used to remove acid dyes [6–10]. In addition to seaweed and bacteria biomass, another potential low-cost sorbent includes waste material such as fungal biomass from the fermentation industry which is abundant and requires disposal.

Both living and dead fungi have been shown to be capable of removing dyes due to the presence of various functional groups on the biomass [5,11]. However, dead cells offer several advantages over living cells. Firstly, for efficient dye removal using living fungal cells, the growth conditions (nutrients requirements, pH and temperature) of the fungi are extremely important. Use of dead fungal cells obviates the need for nutrients requirements as well as eliminates the problem of waste toxicity. In

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addition, dead fungal biomass can be stored easily and kept for prolonged periods. For instance, dead *Rhizopus arrhizus* biomass was used for the removal of reactive black (RB) 5, reactive orange (RO) 16 and gemazol turquise B blue-G with an uptake capacity of 588, 190 and 773 mg/g, respectively [5,12,13]. *Aspergillus niger* mycelia has been used for the removal of various acid, basic and direct dyes, with sorption capacities <20 mg/g [5]. Kumari et al. used biomass of *A. niger*, *Aspergillus japonica*, *Rhizopus nigricans*, *R. arrhizus* and *Saccharomyces cerevisiae* for the biosorption of various anionic reactive dyes, and reported sorption capacities in the range 112–204 mg/g biomass [14]. In a recent work by Ascen et al., dried *Penicillium restrictum* biomass was used for the biosorption of RB5 and an uptake of 142 mg/g biomass was reported [15].

Pretreatment of biomass has been shown to be effective in enhancing the sorption capacity for various dyes. For instance, pretreatment of *A. niger* using 0.1 M H_2SO_4 increased biosorption capacity from 6.63 to 13.83 mg/g for the removal of acid blue 29 [11]. Bayramoglu et al. treated the biomass of *Phanerocheate chrysosporium* using heat, acid and base to examine the effects of different treatment on the sorption of reactive blue 4 dye [16]. Acid and heat treatment of the fungal biomass enhanced dye sorption capacities [16]. In another study by Gallagher et al., a 7–15% increase in the sorption capacity was achieved by *Rhizopus oryzae* after pretreatment with NaOH and chitin/chitosan enrichment [17].

In this study, the fungal biomass of *Penicillium chrysogenum* was used for removing anionic dyes AO8, AB45 and RO16. *P. chrysogenum* is used in penicillin production and its biomass is generated as a fermentation waste product. Although the biomass, pretreated with PEI and cross-linked with glutaraldehyde, has been used for enhanced heavy metal sorption [18], to the best of our knowledge, its use in the removal of anionic dyes has not been reported in the literature.

2. Materials and methods

2.1. Materials

The fungus *P. chrysogenum* (No. 3.3890) was purchased from the China General Microbiological Culture Center, Beijing. Polyethylenimine (PEI), glutaraldehyde (50% aqueous solution), acid orange 8 (dye content: 65%), acid blue 45 (dye content: 50%) and reactive orange 16 (dye content: 50%) were purchased from Sigma–Aldrich Company. The molecular mass of AO8, AB45 and RO16 are 364.35, 474.33 and 617.54, respectively. The optimal wavelength λ_{max} for AO8, AB45 and RO16 are 490, 595 and 494 nm, respectively. The dye structures are shown in Fig. 1(a)–(c). Other chemicals were of reagent grade.

2.2. Biomass preparation and modification

P. chrysogenum was cultivated on 3.9% (w/v) potato dextrose agar in Petri dishes and incubated for 7 days at 30 °C [18]. Approximately 3.2×10^5 fungal spores were added into 100 mL of sterilized liquid medium in a 250 mL conical flask. The composition of the liquid medium (g/L in deionised water) was glucose (30); NH_4NO_3 (2); yeast extract (2); KH_2PO_4 (1); $MgSO_4{\cdot}7H_2O$ (0.5) and KCl (0.5) [18]. The flasks were agitated at 140 rpm and 30 °C for 3 days [18], after which spherical pellets of fungal biomass of uniform diameter (approximately 2–3 mm) were obtained. The pristine biomass was filtered and washed with copious amount of deionised water, after which the biomass was placed in a freezer overnight before undergoing the freeze-dry process.

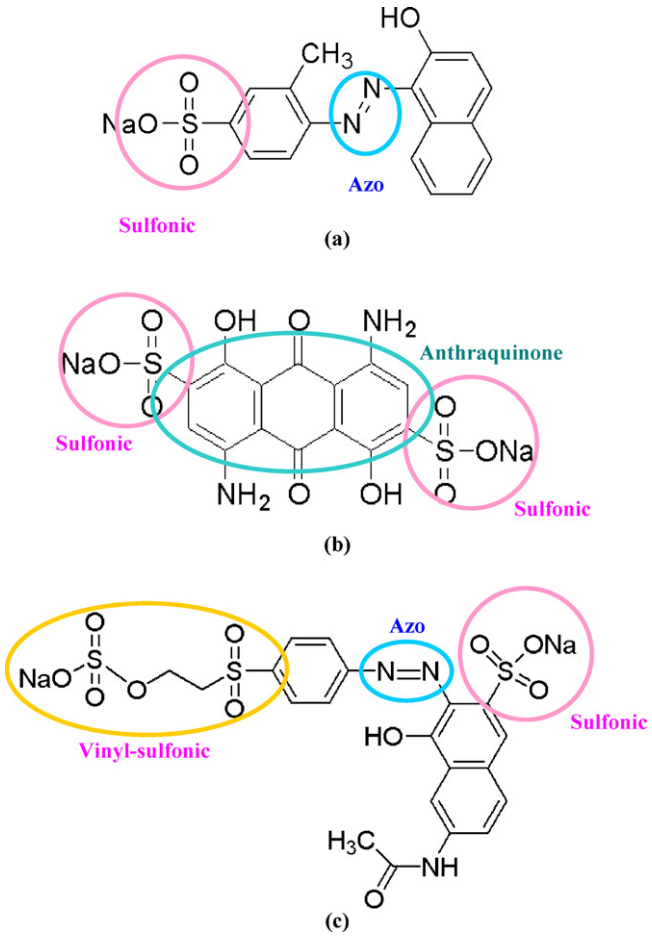

Fig. 1. Chemical structure of (a) acid orange 8 (AO8) dye, (b) acid blue 45 (AB45) dye and (c) reactive orange 16 (RO16) dye.

The freeze-dried fungal biomass was subjected to surface modification. 1.5 g of freeze-dried pristine biomass was added to 100 mL of 10% (w/v) PEI/methanol solution in a 250 mL conical flask. The flask is agitated at 140 rpm and 30 °C for 24 h. The biomass was subsequently filtered and washed with methanol to remove residual and unreacted PEI. The biomass was transferred into a 250 mL conical flask containing 100 mL of 1.0% (v/v) aqueous glutaraldehyde for cross-linking. The flask is agitated at 140 rpm and 30 °C for 20 min [18]. The modified biomass was washed with deionised water and freeze dried.

2.3. Sorption experiments

All sorption experiments were performed using 0.1 g of the pristine or modified biomass in 100 mL of 100 mg/L dye solution in 250 mL conical flask, agitated at 140 rpm and 30 °C,

unless otherwise stated. The various experiments were carried out separately with each dye. All experiments were conducted in duplicates. The resultant dye solution was filtered using Whatman® filter paper and 0.45 μm syringe filter before analysis using a Shimadzu UV-1601 UV–vis spectrophotometer at its optimal wavelength. The sorption capacity was calculated from the mass balance as follows

$$q_e = \frac{V(C_i - C_e)}{m} \quad (1)$$

where V is the volume of dye solution, C_i and C_e represent the initial and equilibrium dye concentrations and m is the mass of biomass added.

2.3.1. Sorption kinetics and equilibrium studies

Sorption kinetics experiments were performed over 8 h. Samples were removed at regular time intervals and analyzed using UV–vis spectroscopy. For the surface-modified biomass, the experiment was repeated using an initial dye concentration of 500 mg/L for 14 h. In the equilibrium study, the dye uptake was investigated over an initial concentration ranging from 100 to 600 mg/L after 24 h sorption.

2.3.2. pH and temperature studies

The desired solution pH was achieved using 0.1–1 M HCl and 0.1–1 M NaOH. The solution pH was monitored every 30 min interval for 6 h and maintained at ±0.1 of the desired pH. The resultant dye solution obtained from the repeated experimental run (both pristine and modified biomass) was analyzed for the total organic carbon (TOC) present using a Shimadzu TOC–VCSH total organic carbon analyzer. In the experiments on temperature effect (at 30 °C), the initial pH was between 6 and 8 and the experimental duration was 24 h. The experiment was repeated at 50 and 70 °C.

2.4. Regeneration experiments

The modified biomass after AO8 sorption was regenerated using 100 mL of 0.5 M HCl and agitated at 140 rpm and 30 °C for 2 h. The regenerated biomass was washed with deionised water before reuse for seven cycles. The initial solution pH was kept within ±0.1 of the initial pH of the first cycle.

2.5. Scanning electron microscope (SEM) analysis

The samples (pristine and modified biomass) were freeze dried and coated in a JEOL JFC-1300 Auto Fine Coater fitted with Pt target before SEM analysis.

2.6. Fourier transform infrared (FTIR) analysis

The samples (pristine and modified biomass, modified biomass after sorption) were washed thoroughly and freeze dried before the analysis. Each sample was placed on a gold mirror and determined using reflection mode in the wavenumber range of 700–4000 cm^{-1}.

3. Results and discussion

3.1. Sorption kinetics

The sorption kinetics of the three anionic dyes by the pristine and modified biomass are shown in Fig. 2(a)–(c). The initial solution pH is in the range of 6.5–7.8. Significantly higher uptake is seen for the modified biomass, where, following an initial rapid sorption, a more gradual increase occurred. The modified biomass has greater dye affinity compared to its pristine form since the initial rates of dye uptake (Table 1) for the former were higher. There were no significant shifts in λ_{max} after sorption.

For sorption using pristine biomass (Table 1), the initial rates of change in sorption capacity for all three dyes were comparable, i.e. 0.114, 0.094 and 0.091 mg g^{-1} min^{-1} for AO8, AB45 and RO16, respectively. As the number of amine groups avail-

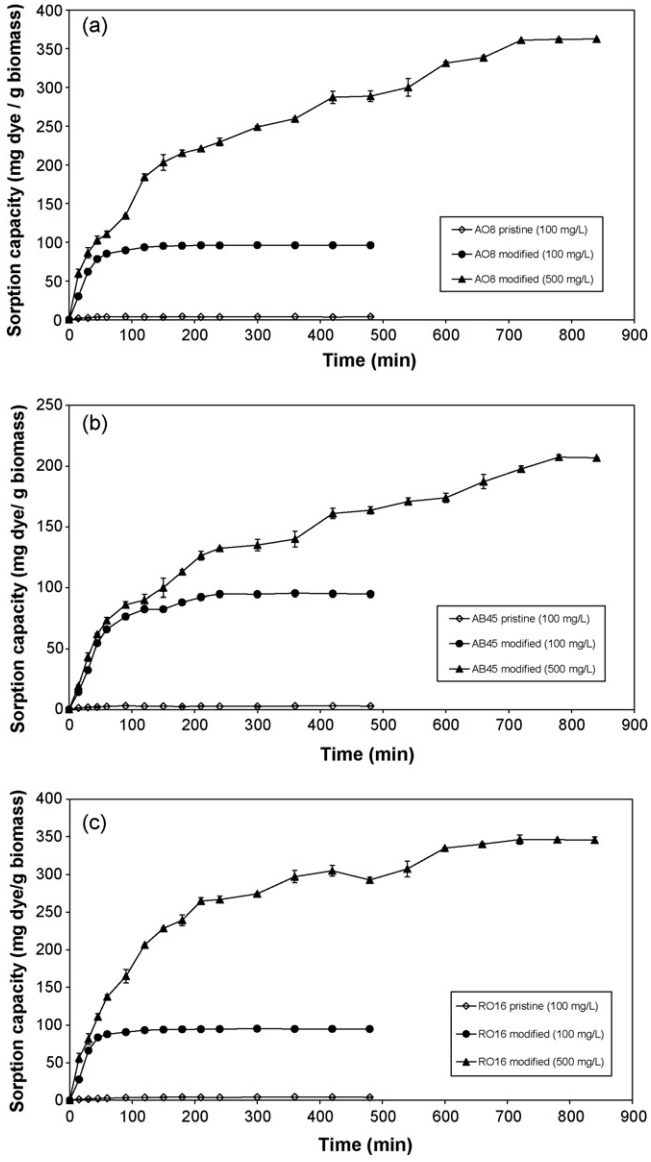

Fig. 2. Sorption kinetics of (a) AO8, (b) AB45 and (c) RO16 on the pristine and modified biomass (0.1 g biomass in 100 mL of 100 or 500 mg/L dye solution for 8 or 14 h, respectively at 140 rpm, 30 °C and initial pH of 6–8).

Table 1
Initial rates of change in sorption capacity

	Initial rate of change in sorption capacity (mg g^{-1} min^{-1})		
Initial dye concentration (mg/L)	100	100	500
Biomass used	pristine	modified	modified
Adsorbate—AO8	0.114	1.81	4.00
Adsorbate—AB45	0.094	1.06	1.56
Adsorbate—RO16	0.091	2.00	3.50

able for sorption on the pristine biomass is relatively limited, the dye uptake rate is perhaps more dependent on the nature of the adsorbent (i.e. number of available cationic sites for sorption) rather than that of the adsorbates (i.e. molecular mass and chemical structures). Hence, although the adsorbates used were different, the initial uptake rates were similar for all three dyes.

For sorption using modified biomass (Table 1), the rates of initial dye uptake were higher for the azo dyes, AO8 (1.81 mg g^{-1} min^{-1}) and RO16 (2.00 mg g^{-1} min^{-1}) as compared to AB45 (1.06 mg g^{-1} min^{-1}) which is an anthraquinone dye. In this case, the availability of amine groups on the adsorbent increased significantly after modification with PEI. As such, sorption using the modified biomass is more dependent on the nature of the dyes. The modified biomass showed a higher capacity affinity for AO8 and RO16 (azo dyes) than for AB45 (anthraquinone dye), possibly due to differences in dye structures and its molecular mass. The molecular mass of AO8, AB45 and RO16 are 364.35, 474.33 and 617.54, respectively.

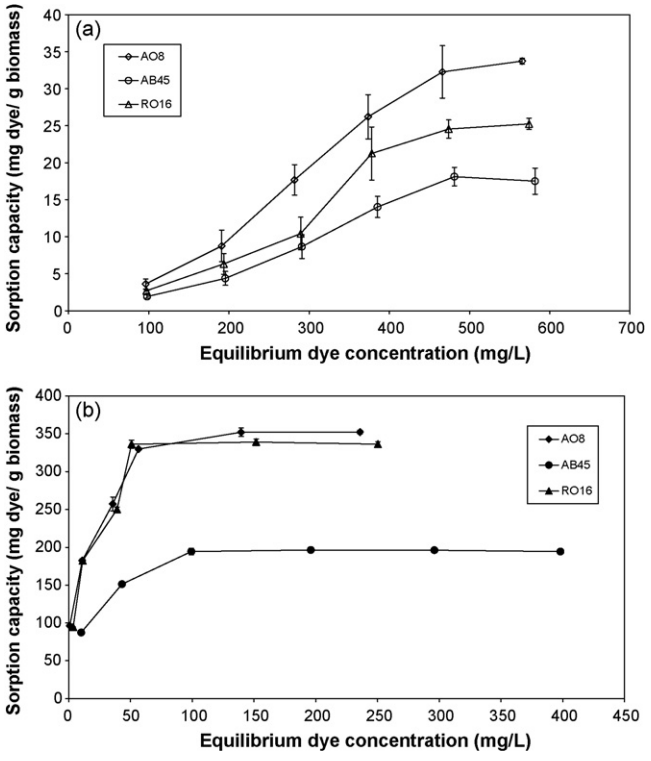

Fig. 3. Sorption isotherm of AO8, AB45 and RO16 on (a) pristine and (b) modified biomass (0.1 g biomass in 100 mL of dye solution of concentration in the range 100–600 mg/L for 24 h at 140 rpm, 30 °C and initial pH of 6–8).

The sorption behavior of anionic dyes is influenced by various factors including the molecular mass, dye dimensions, amount and position of the active functional groups (e.g. sulfonate and vinyl sulfonate) on the dye molecules [19]. For the acid dyes, AO8 has lower molecular mass compared to AB45 and therefore is preferentially adsorbed by the biomass. In addition, the fused aromatic ring system of AB45 renders the dye molecule bulky and introduces steric hindrance. Hence, AB45 is less preferentially adsorbed. Both the molecular size and dimensions for AO8 is more favorable for sorption as compared to AB45. This is consistent with the results from the initial rate of change in sorption capacity.

Although RO16 has larger molecular mass than AO8, the initial rate of sorption was higher for the former. This may be due to the difference in the dye functional groups. The removal of synthetic dyes in solution via sorption using suitable sorbents is similar to the process of dyeing textiles, since both involve the fixation of dye molecules on a substrate. In order to increase the degree of dye fixation, researchers have incorporated bifunctionality into dye molecules (an example being the vinylsulfonic groups which enhances fixation [20]). AO8 has a sulfonic group while RO16 has one sulfonic group and one vinylsulfonic group. The presence of a vinylsulfonic group in RO16 hence increases the degree of fixation between the dye and the modified biomass even though RO16 is less advantageous from the molecular size point of view.

3.2. Sorption equilibrium

The maximum sorption capacities of pristine biomass for AO8, AB45 and RO16 were 33, 18 and 25 mg/g, respectively (Fig. 3(a)) and that of the modified biomass were 352, 196 and 338 mg/g, respectively (Fig. 3(b)) at initial solution pH 6.5–7.8. These results, together with similar studies reported in the literature are tabulated in Table 2. O'Mahony et al. reported the use of *R. arrhizus* biomass for the sorption of RO16 with maximum dye uptake of 190 mg/g at pH 2 [12]. In the present study, the maximum dye uptake of pristine *P. chrysogenum* biomass for the same dye was lower at 25 mg/g. The experimental conditions for these two studies were comparable except for the solution pH. The former study used an acidic medium which is more favorable for sorption while in the present study, sorption experiments were conducted close to neutral pH. The significantly higher dye uptake by *R. arrhizus* biomass was possibility due to the presence of more protonated amine groups at lower pH which interacts with the RO16 dye anions [12]. On the other hand, the maximum RO16 dye uptake for the PEI-modified biomass in this study is

Table 2
Comparison of data reported in the literature and current study

Adsorbent	Dye	C_i (mg/L)	Amount of adsorbent (g/L)	Initial pH	Contact time (h)	Sorption capacity (mg/g)	Source
PEI-modified *Penicillium chrysogenum* biomass	AO8	500	1	6.8–7.6	24	352	Current Study
	AB45	500	1	6.8–7.6	24	196	
	RO16	500	1	6.8–7.6	24	338	
Pristine *P. chrysogenum* biomass	AO8	500	1	6.5–7.8	24	33	Current Study
	AB45	500	1	6.5–7.8	24	18	
	RO16	500	1	6.5–7.8	24	25	
Dead *Rhizopus arrhizus*	RO16	500	1	2	20	190	[12]
Corynebacterium glutamicum	RO16	4500[a]	10	1	20–24	187	[7]
Coconut shell-based powdered activated carbon (PAC)	RO16	–	Varying amounts	–	36	≈510	[19]

[a] Estimated initial concentration.

Table 3a
Freundlich model for sorption using pristine biomass

Isotherm	Model parameters	AO8	AB45	RO16
Freundlich	K	7.83E−03	3.82E−03	5.18E−03
	n	0.742	0.739	0.735
	R^2	0.983	0.980	0.974

338 mg/g which is higher than that using *R. arrhizus* biomass even with a less favorable solution pH for sorption. Won et al. used waste biomass of *C. glutamicum* for the sorption of RO16 [7]. The adsorbent dosage used was 10 times higher than the current study. Therefore, a direct comparison cannot be established. The dye uptake by coconut shell-based powdered activated carbon (PAC) is higher than PEI-modified biomass probably due to the larger specific surface area of PAC [21].

Various isotherms models including Langmuir, Freundlich, Tempkin, Dubinin–Radushkevich (D–R) and Redlich–Peterson (R–P) were used to represent the sorption equilibrium data of similar studies [22,23]. In the present study, the equilibrium data were modeled using these isotherms. For sorption using pristine biomass, Freundlich isotherm provided the best fit and the correlation coefficients (R^2) are 0.983, 0.980 and 0.974 for AO8, AB45 and RO16, respectively (Table 3a). Langmuir isotherm provided an excellent fit for sorption using the modified biomass and the R^2 values are 0.997, 0.999 and 0.998 for AO8, AB45 and RO16, respectively (Table 3b).

3.3. pH effect

Biosorption of the acid dyes, AO8 and AB45 using pristine biomass showed similar profiles at various pH (Fig. 4(a)); the maximum dye uptake occurred at pH 1 and decreased with pH, with significantly lower uptake between pH 6 and 11. An increase in uptake occurred at pH 12. For the resultant AO8 and RO16 solutions, λ_{max} remained in the range 490.5–493.5 and 488.5–494.5 nm, respectively. For AB45, λ_{max} remained in the range 595.0–597.5 nm from pH 1 to 11 but shifted to 620 nm at pH 12 which suggests that the decolorization observed may be due to changes in the structure of the dye at the solution pH.

Acid dye sorption by pristine biomass occurs via electrostatic interactions between the dye anions and the amine groups on

Table 3b
Langmuir model for sorption using modified biomass

Isotherm	Model parameters	AO8	AB45	RO16
Langmuir	q_m (mg/g)	365.1	201.1	352.7
	b (L/mg)	0.123	0.105	0.107
	R^2	0.997	0.999	0.998

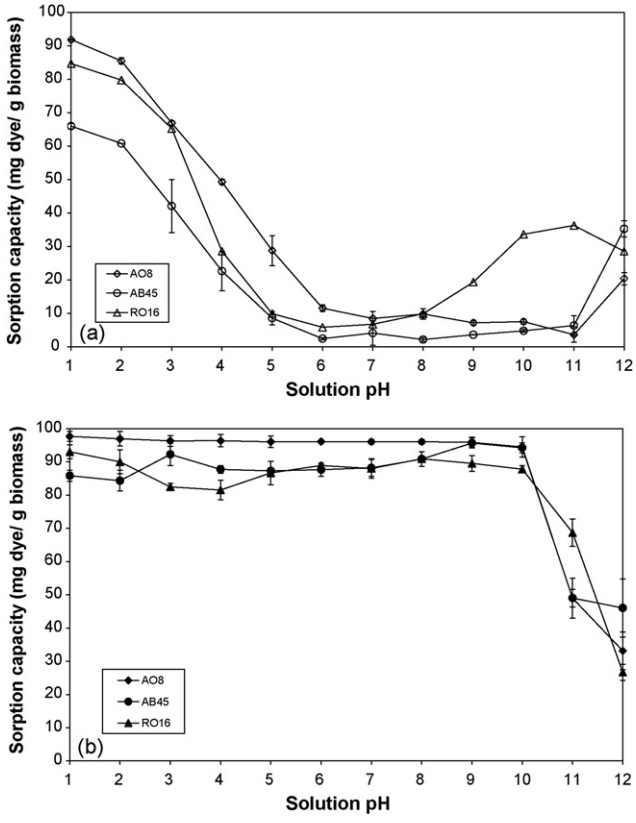

Fig. 4. Effect of pH on the sorption of AO8, AB45 and RO16 using (a) pristine and (b) modified biomass (0.1 g biomass in 100 mL of 100 mg/L dye solution for 6 h at 140 rpm, 30 °C; pH monitored every 30 min to maintain at ±0.1 of desired pH).

the pristine biomass. Deng and Ting (2005) reported that the main functional groups on the pristine biomass surface are amine and carboxylic acid groups [18]. The isoelectric point of most amino acids is about pH 6. Below the isoelectric point, the amine group is protonated while COOH exits in its undissociated form. The protonated amine groups provide cationic anchor points with which the dye anions interact via ionic bonding. Above the isoelectric point, the amine groups are not protonated and COOH dissociates to form COO^-, thereby resulting in lower dye uptake. Similar observations for acid dye sorption at various pH have been reported [24]. In contrast with acid dyes, it was found that while the sorption of reactive dye RO16 by the pristine biomass also decreased from pH 1 to 6, the sorption capacity increased from pH 7 to 11. At pH 12 however, the sorption capacity decreased.

The sorption of reactive dyes at pH below the isoelectric point occurs via ionic bonding. Above the isoelectric point, the interactions between the reactive anionic dyes and the biomass surface may be similar to the attachment of the reactive dyes to textile fibers in highly basic medium during textile dyeing. This suggests that in basic medium, there exist other functional groups on the dye anion which interact with the amine groups on the biomass via covalent bonding. This may explain the increase in RO16 sorption by the pristine biomass when the pH increased from 7 to 11.

O'Mahony et al. reported the use of *R. arrhizus* biomass for the sorption of RO16 with maximum dye uptake at pH 2 and less significant uptake for pH 4–10 [12]. However, in their study, an increase in sorption capacity at higher solution pH was not observed [12]. Won et al. used waste biomass of *C. glutamicum* for the sorption of RO16 and suggested that the higher dye uptake at lower pH was due to the presence of more binding sites ($-NH_3^+$) on the bacteria biomass [7]. In their study, beyond pH 9 a slight increase in sorption capacity occurred possibly due to the formation of precipitates which entrapped the dye molecules, and the formation of secondary amine groups at higher pH [7]. In another recent study by Won et al. waste biomass of *C. glutamicum* was used for the removal of another reactive dye, reactive black 5 and an increase in sorption capacity from pH 7 to 11 was reported [8].

The sorption of the three dyes by the modified biomass was significantly higher than the pristine biomass (Fig. 4(b)). Over the pH range 1–10, the dye uptake was >95 mg/g for AO8, 85–95 mg/g for AB45, and 82–92 mg/g for RO16. The sorption capacity however decreased drastically beyond pH 10. Dye sorption was not favored under strong alkaline condition as the amine groups were not protonated. It was also noted that λ_{max} of the resultant solutions shifted significantly (>10 nm) from the original λ_{max} at certain pH and the observed decolorization may be due to pH change (data not shown). The shift in λ_{max} may be due to the complex interactions (upon addition of alkali and acid) between the dye anions and the PEI-modified biomass.

The data obtained from TOC analysis are shown in Fig. 5(a)–(c). A comparison of the TOC of the dye solution after sorption with pristine and modified biomass shows that the latter results in dye solution with TOC that are 3.5, 2.2 and 2.2 times (average values obtained over the various pH) lower than the former for AO8, AB45 and RO16, respectively. Hence, the modified biomass was more stable than its pristine form. The TOC of the dye solution before sorption was 79.3, 57.7 and 48.3 mg/L for AO8, AB45 and RO16, respectively. It is noted, however, that in highly basic medium (above pH 10), a sharp increase in TOC in the dye solution occurred after sorption. This suggests that the modified biomass may be unstable at high pH.

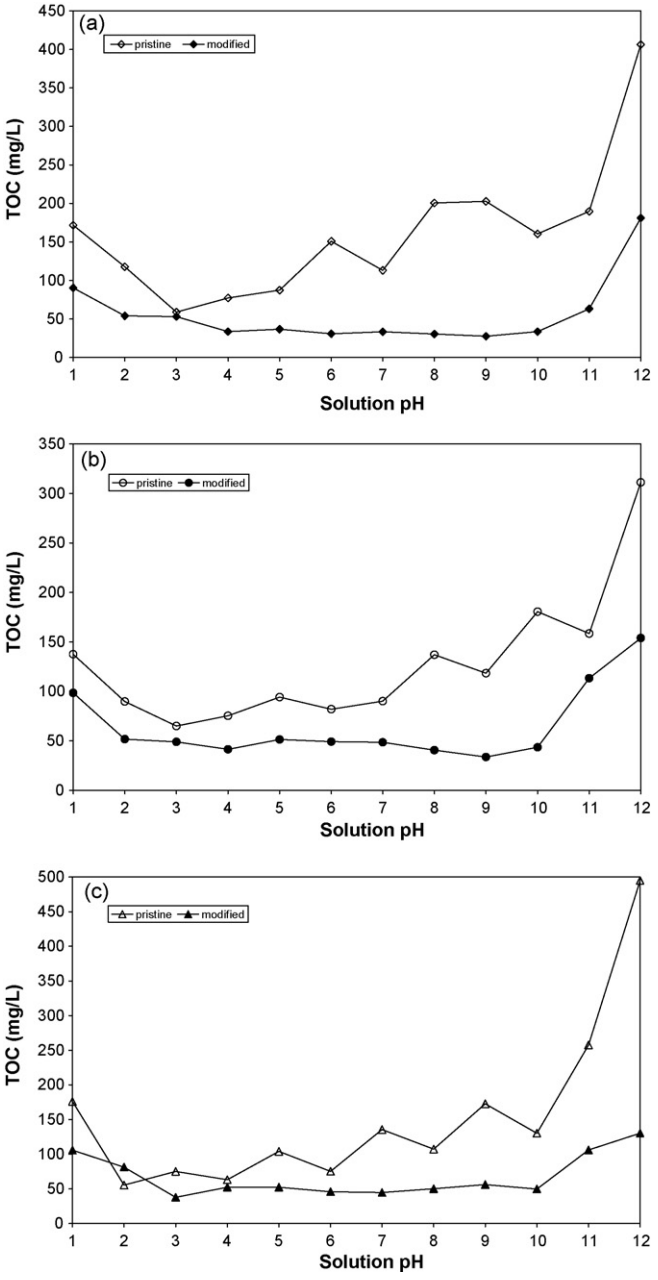

Fig. 5. Effect of pH on the TOC of resultant (a) AO8, (b) AB45 and (c) RO16 solutions after sorption with pristine and modified biomass.

3.4. Temperature effect

The final dye uptake by both the pristine and modified biomass was relatively constant over the temperature range 30–70 °C (Fig. 6). This is a useful property of the sorbent for

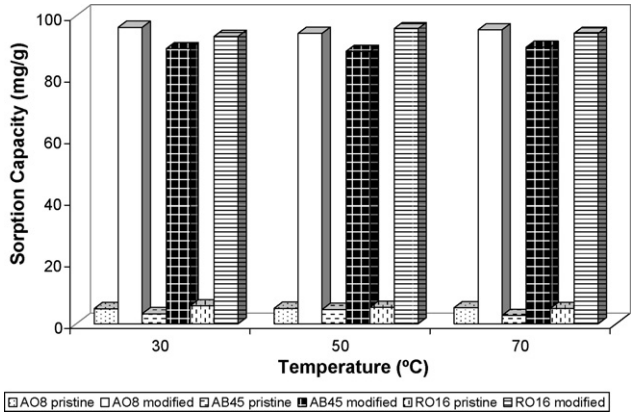

Fig. 6. Effect of temperature on the sorption of AO8, AB45 and RO16 on the pristine and modified biomass (0.1 g biomass in 100 mL of 100 mg/L dye solution for 24 h at 140 rpm, initial pH of 6–8).

use in wastewater treatment over a wide temperature range. No significant shift in λ_{max} was noted. The modified biomass was about 18, 25 and 17 times more effective than pristine biomass in the removal of AO8, AB45 and RO16 dyes, respectively over this temperature range.

3.5. Regeneration of modified biomass

One important consideration in the selection of a sorbent is its capacity for subsequent reuse after regeneration, with minimal reduction in sorption capacity. It was found that the modified biomass could be easily regenerated using 0.5 M HCl. The regenerated sorbent could be used repeatedly for up to seven cycles with negligible loss in its dye sorption capacity. Fig. 7 shows that the AO8 dye uptake for each cycle remained relatively constant at ±95 mg/g.

3.6. SEM analysis

The surface morphologies of the biomass were observed using SEM analysis. The hyphae diameters of the pristine and modified biomass are approximately 2 and 3 μm, respectively

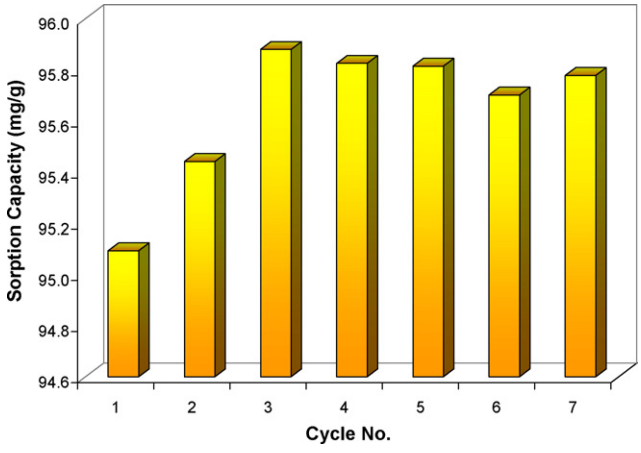

Fig. 7. Comparative sorption capacities of PEI-modified biomass for AO8 dye in seven successive sorption–regeneration cycles.

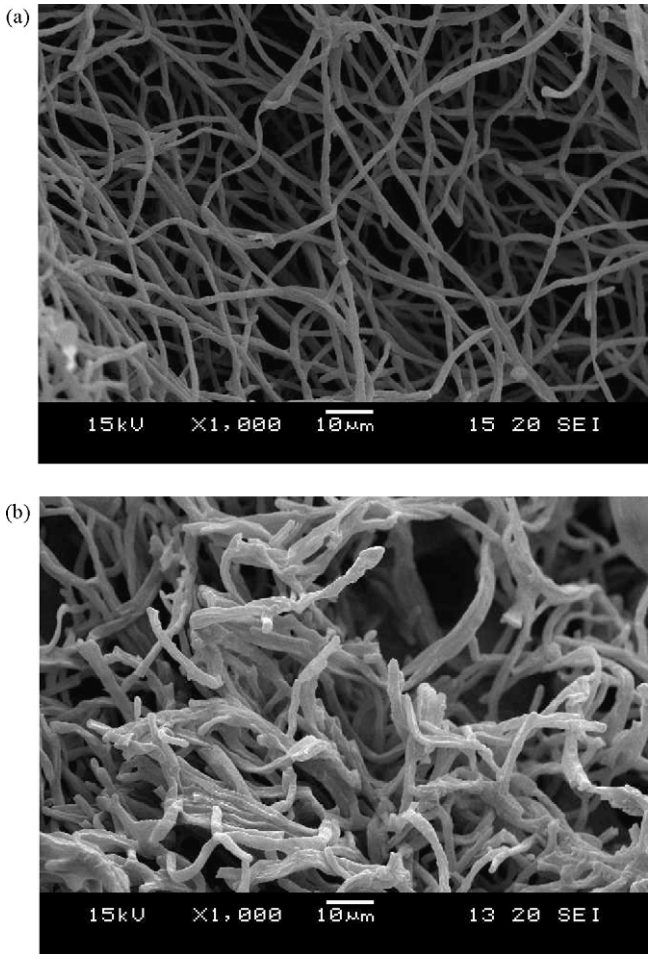

Fig. 8. SEM images of (a) pristine biomass and (b) PEI-modified biomass.

as (Fig. 8(a) and (b)). The increase in hyphae thickness was probably due to the cross-linking effects of glutaraldehyde.

3.7. FTIR analysis

For the spectrum obtained for pristine biomass (Fig. 9), the broad band at 3500–3200 cm^{-1} and the peaks at 1552–1379 cm^{-1} are indicative of the existence of amine group in the biomass. The broad band at 3500–3200 cm^{-1} is also the result of the overlapping of OH and NH functional groups. The peaks at wavenumbers 1675 and 1236 cm^{-1} corresponds to that of carboxyl group. These showed that the main functional groups on the pristine biomass are carboxyl and amine groups. These results correspond to those reported by Deng and Ting [18].

Comparing the band caused by OH and NH overlapping for pristine and modified biomass, the band is slightly broader and shifts towards lower wavenumber. This shows the presence of additional amine groups from PEI. The peak representative of carboxylic C=O shifts from 1675 to 1660 cm^{-1} which may be due to the overlapping of the C=O of carboxylic acid and the C=O of aldehydes (Fig. 9). This provides evidence of the presence of glutaraldehyde as the cross-linking agent.

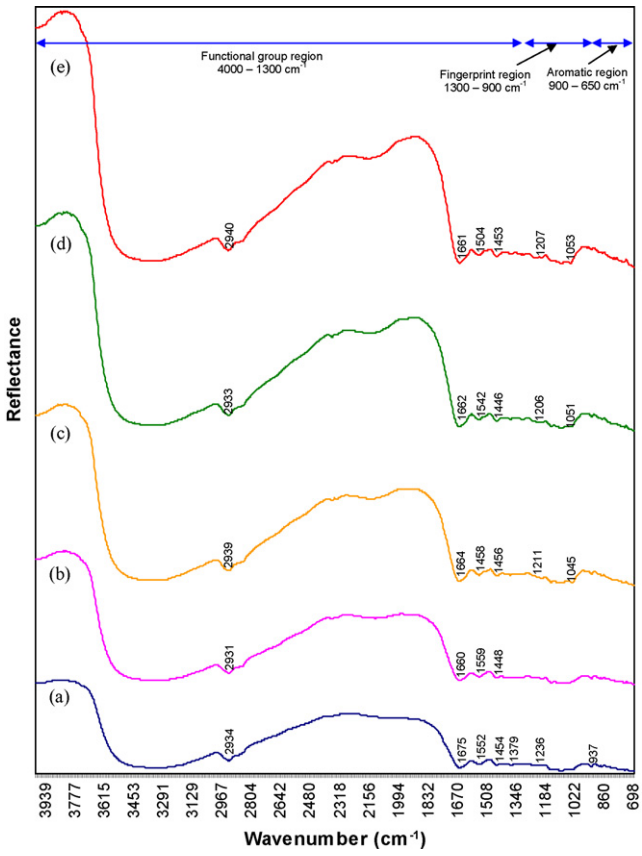

Fig. 9. FTIR spectra of (a) pristine biomass, (b) PEI-modified biomass, (c) PEI-modified biomass with AO8, (d) PEI-modified biomass with AB45 and (e) PEI-modified biomass with RO16.

In addition, the new peaks at 1211, 1206 and 1207 cm^{-1} is characteristic of S=O stretching. The structural formulae (Fig. 1 (a)–(c)) of the three dyes in this study show the presence of the sulfonic acid groups which has an S=O functional group. This provides evidence that the dyes molecules are indeed adsorbed onto the modified biomass surface.

4. Conclusion

Sorption of the three anionic dyes by both the pristine and modified *P. chrysogenum* mycelium was favored at lower pH. The uptake kinetics was rapid, followed by a slow gradual uptake. The rates of initial dye uptake were higher for the azo dyes (AO8 and RO16) as compared to the anthraquinone dye (AB45) which is due to the difference in molecular mass and chemical structures. It was found that the dye sorption was not sensitive to temperature for both the biomass. The maximum dye uptake of the pristine biomass for AO8, AB45 and RO16 were 33, 18 and 25 mg/g, respectively; this increased to 352, 196 and 338 mg/g, respectively when the biomass surface was modified with polyethylenimine (PEI) and cross-linked with glutaraldehyde. The sorption behaviors of the pristine and modified biomass are well described by the Freundlich and Langmuir isotherms, respectively. The modified biomass can be used repeatedly for up to seven cycles with negligible loss in dye removal efficiency. FTIR analysis showed that amine groups were involved in the surface modification. Results from this study show that the PEI-modified biomass exhibits potential as a low-cost adsorbent for dye removal.

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