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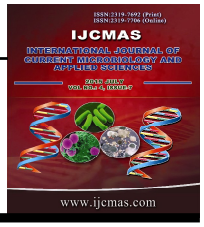

Original Research Article

Cunninghamella elegans UCP 542 as an Alternative Biosorbent: Effects of pH, Pre-Treatment and Dye Concentration on Reactive Black B Removal

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Keywords

Cunninghamella elegans, Reactive Black, Biosorbents, Pretreatment

A B S T R A C T

This study was carried out to evaluate biosorbents obtained by *Cunninghamella elegans* biomass with different physico-chemical treatments and its efficiency to remove Reactive Black (B) from aqueous solutions. The pH, dye concentration and morphology were evaluated, and then, compared to activated charcoal. The pH was a crucial parameter in the process. Biomass treated with NaCl, NaOH, $NaHCO_3$ and autoclaved determined highest percentages of decolourisation and removal efficiency of the dye. The morphologic analysis revealed changes induced by the treatments. The assays showed that the biosorbents of *C. elegans* biomass were capable to remove reactive dyes like Reactive Black (B).

Introduction

A combination of environmental pressures and the need for low cost process makes the treatment of textile industry wastewater represents business opportunities associated with the scientific challenge. Among the compounds present in the effluent, the dyes containing azo groups are the most important class of substances which promote color. These contaminants can affect the transparency of the water, the solubility of gases in receiving bodies and change the processes of photosynthesis. These molecules exhibit high toxicity, and are mostly considered carcinogenic and mutagenic (Aksu, 2005; Al-Degs *et al.*, 2007).

In this sense, through environmental biotechnology, there are numerous studies that indicate wide variety of microorganisms

as fundamental tools in environmental remediation of aromatic compounds, heavy metals, polycyclic aromatic hydrocarbons, oil and dyes. The main incentives for industrial-scale biosorption are high selectivity, efficiency and effectiveness of removal, the possibility of regeneration, low cost and availability of equipment with conventional costs (Anjaneya *et al.*, 2009).

Azo dyes are characterized by one or more groups -N=N- attached to aromatic systems. Reactive dyes have this name due to its ability to form covalent bonds with fiber. They can be used for dyeing cellulosic fibers with good dyeing characteristics, strength and chemical stability. The azo dye Reactive Black (B) represents one of the most dyes used in the textile industry today (Anjaneya *et al.*, 2009; Aksu and Balibek, 2010).

Microbial surfaces have different functional groups, which are responsible for the kidnapping of hazardous compounds present in industrial effluents. *Cunninghamella elegans* is a filamentous fungus which has been used as a model for microbial metabolism of xenobiotics in mammals (Abourashed *et al.*, 1999; Aksu, 2005) as well for the biodegradation of chemical environmentally relevant. Their biomass can be produced from low cost manner, using simple fermentation techniques or obtained as a waste of many industrial fermentation processes and can be widely used for biosorption of anionic and cationic dyes (Ambrósio and Campos-Takaki, 2004; Aksu and Balibek, 2010).

Based on the ability of biosorption of *C. elegans* UCP 542 in the removal of dyes, already reported, the present study aimed to investigate the influence of physical-chemical treatment, dye concentration and pH on the potential of fungi as biosorbent, low cost removal of reactive commercial Reactive Black B in aqueous solution.

Materials and Methods

Microorganism

The strain of *Cunninghamella elegans* (UCP 542) was obtained from the Culture Collection of the Nucleus of Research in Environmental Sciences and Biotecnology – Catholic University of Pernambuco – Brazil and registered in the World Federation Culture Collection (WFCC). The strain was maintained in Synthetic Medium for Mucoralean (SMM) at 5°C.

Growth and production of biomass

The isolate was inoculated in SMM medium (Synthetic Medium for Mucoralean - Hesseltine and Anderson (1957) Solid, pH 5.3, temperature 28°C. A pre-inoculum was made containing 10^5 spores per ml, with 50 ml of this solution was inoculated in 500 ml of liquid medium SMM. Cultures were incubated at 28°C on an orbital shaker at 150 rpm shaken for 15 days for biomass production.

Preparation of Reactive Black B solution

A stock solution of the azo dye Remazol Black (B) was prepared at a concentration of 100 mg/L. Dilutions were made for the respective final concentrations to 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L.

Preparation of biomass for removal tests

A portion of the washed mass was used as live biosorbent (Type I - control) to remove the dye from the solution. The rest of the produced biomass was pretreated with six different methods as follows:

(I) 0.1M H_2SO_4, (II) 0.2M HCl, (III) 0.1M NaOH (IV) 0.1M $NaHCO_3$, (V) 0.1M NaCl, (VI) Autoclaving at 121°C and 18 PSI.

The biomass after each chemical pretreatment was washed until the pH of the washing solution reach a neutral range. Finally, the biomass was filtered and dried at temperature of 50°C ± 5°C for 2 h. After this time the masses were used as biological sorbents for the removal and discoloration assays using 200 mg of living biomass and treated.

Determination of pH effect and dye concentration

Studies were conducted to determine the influence of pH and dye concentration in the removal process. Aqueous solutions of the same initial concentration of Remazol Black (B) dye (30 mg/L) were prepared and adjusted to pH 3, 4, 5, 6, 7 and 8 by addition of hydrochloric acid (1N HCl) or sodium hydroxide (1N NaOH) were added 125mL of the dye solution in 250mL Erlenmeyer flask, which was placed in contact with 0.2 g of biosorbent subjected to chemical and physical treatments under controlled temperature of 28°C in a shaker (Marconi MA-184) to 150 rpm for a period of 18 hours. The same process was performed at different initial dye concentrations (5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L) with the pH set at 3. The material was filtered separating the biomass for structural analysis and the filtrate was analyzed for residual dye concentration by UV/VIS spectrophotometry with the wavelength of 600 nm. All the experimental tests were carried out in triplicate. Through the absorbance readings of each supernatant solution, was estimated the percentage of discoloration by biomass. From this preliminary observation, was established a mathematical relation between the discoloration and biosorption (Cripps *et al.*, 1990), widely used in studies of discoloration of azo dyes recently, through which it was possible to calculate the percentage of discoloration of the samples after the treatments (Kalme *et al.*, 2006; Maurya *et al.* 2006).

Determination of discoloration percentage, removal efficiency and relation of dye concentration adsorbed by biomass

The filtrate was analyzed for residual dye concentration to determine the discoloration percentage, and the mass of dye adsorbed to biomass was calculated by the equation:

Discoloration percentage (Discoloration %)

$$Discoloration\ \% = \frac{(ABSi - ABSf)}{ABSi}\ 100 \quad (Eq.\ 1)$$

ABSi – Initial absorbance
ABSf – Final absorbance
Removal Efficiency (R)

$$R = \frac{(Co - Cf)}{Co}\ 100 \quad (Eq.\ 2)$$

Co – Initial concentration
Cf – Final concentration
Dye adsorbed by Biomass (q)

$$q = \frac{(Co - Cf)}{M}\ V \quad (Eq.\ 3)$$

Co – Initial concentration M –adsorbent mass
Cf – Final concentration V - Solution volume

Activated charcoal adsorption assay

The powdered activated charcoal – PAC, used in the study was supplied by Sigma Aldrich inc. The powder was used in a comparative study conducted at 150 rpm at a temperature of 28°C for 18 hours. A mass of 0.2 g AC corresponding was added in Erlenmeyer flask containing 125 ml of dye solution at concentrations of 5 mg/L, 10mg/L, 20mg/L, 30mg/L, 40mg/L, 50mg/L was used with values corresponding pH from 3 to 8. All tests were performed in

triplicate, the final dye concentrations were determined by spectrophotometric reading at a wavelength of 600 nm using a UV/VIS spectrophotometer.

Ultrastructural and morphological analysis

The samples collected at intervals quoted are subject to routine method described by De Souza, (2000) using SEM JEOL 5600LV scanning electron microscopy. For the morphological analysis of samples isolated subjected to various treatments will be deposited directly on the surface of glass slides and were viewed under a microscope NIKON Model Alphaphot 2 Y52.

Results and Discussion

pH effect and biosorption kinetcs

Figures 1 and 2 show the results of the kinetic tests for the influence of pH in the different treatments biomass of *Cunninghamella elegans* UCP 542.

The analysis of the graph shows that the discoloration reduces with increasing of pH for all the treatments. Biomass treated with H_2SO_4 showed the lowest percentage of reactive black in relation to all other biomass used - at pH 3 getting a percentage of discoloration dye 73.49% and a percentage of removal efficiency of 64.5% as the best results obtained with the treatment of biomass NaCl at pH 3 achieved the best percentage of discoloration and efficiency with 98.16% and 97.17% respectively, it is noteworthy treatment with autoclaved biomass that even in alkaline pHs got percentage of discoloration of 90.91% and as a percentage of removal efficiency of 83.2% at pH 8.0. It was also observed that the percentage decrease is dependent on the treatment applied. The pH has a significant impact on the efficiency of dye removal by biomass. The efficiency also decreases with increasing pH of the solution.

Keum *et al.* (2006) analyzed the removal of the indigo carmine dye as an adsorbent using dead fungal biomass of *Aspergillus niger* in nature and could observe that the adsorption kinetics for biomass is favored in acidic range around from 4.0 to 5.0. Vijayaraghavan and Yun (2007) reported that extremely acidic conditions are favor for the biosorption of reactive dyes and also important for biosorption by microbial biomass. The pH of a dye solution plays an important role in the biosorption process, especially in the ability of reactive dye biosorption yeast (Zhang *et al.,* 1995; Umbuzeiro *et al.,* 2005; Srinivasan and Viraraghavan, 2010). Changes in pH can affect the adsorption process through dissociation of functional groups present in the active sites of the adsorbent (Rakhsaee *et al.,* 2006; Sharma *et al.,* 2007; Won and Yun, 2008). There is an increase in the attractive electrostatic force between the biosorbent and dye.

Clearly, the use of biomass from *C. elegans* both the percentage of discoloration and the removal efficiency increases consistently with decreasing pH. Such behavior can be explained on the basis of the biosorbent surface charges. The ability to lower biosorption under alkaline conditions could be attributed mainly to the increased number of negative charges on the surface of the biomass, which can result in electrostatic repulsion between the dye molecule and the adsorbent. The presence of excess OH-ions would compete with the anionic dyes to reduce the number of positively charged sites on the surface of the mold when the pH increases. A similar trend was observed for the biosorption of different dyes by fungus *Trametes versicolor, Phanerochaete*

chrysosporium, *Aspergillus niger*, *Penicillium* sp., *Gonoderma reesinaceu* and *Rhizupus arrhizus* (Cerniglia and Gibson, 1977; Cerniglia, 1997; Cha *et al.*, 2001; Binupriya *et al.*, 2008).

The data obtained in this study support the literature regarding the effect of pH. The pH is the most important to be evaluated in the removal process parameter, being a parameter that directly affects the active sites of the sorbent (dissociation reactions and protonation), which are strongly influenced by affecting the availability of the dye removal. However, data concerning the effect of pH on the process of removal of biomass by Reactive Black by *C. elegans* are the first.

Effect of pre-treatment in potential removal of Reactive Black B by *Cunninghamella elegans* UCP 542

Figures 3, 4 and 5 show the results obtained for determining the effect of pretreatment of the potential removal Reactive Black (B) dye:

Thus, there has been a sorption capacity (Figure 5) of 14.97 mg/g for the living biomass values corresponding to 13.98 mg/g, 14.55 mg/g, 18.33 mg/g, 17.95 mg/g, 18.42 mg/g and 17.95 mg/g for samples subjected to chemical treatment with H_2SO_4, HCl, NaOH, $NaHCO_3$, NaCl and physical treatment by autoclaving, respectively.

Comparing the effects of acid and alkali treatment, it was noted that pre-treatment of biomass from *C. elegans* UCP 542, with NaOH induced higher percentages of discoloration and higher removal efficiency of adsorptive power comparative to the biomass exposed to HCl and H_2SO_4. Such treatments have been studied due to the ability of these solutions to catalyze hydrolysis processes or simply acting in protonation and dissociation of functional groups. As expected, treatment of the biomass of *C. elegans* with a solution of H_2SO_4 and HCl resulted in a greater loss of removal efficiency, probably due to the protonation of important functional groups sorption, giving a positive character on the surface of the biomass.

Several research groups have shown that the efficiency of biosorption of fungal biomass can be significantly enhanced by pretreatment methods such as autoclaving, drying, and exposure to chemicals such as formaldehyde, acid, NaOH, $NaHCO_3$ and $CaCl_2$. Therefore, the biosorption capacities can be improved by up to 90 % (Chen *et al.*, 2003; Erdem and Ozverdi, 2006). Since the 90s that pretreatment processes for biomass of fungi are used to improve the sorptive capacity for dyes.

Uzun (2006) reported that chitosan acts as an excellent adsorbent for dyes. Others studies show the kinetics of adsorption of indigo carmine dye into dead fungus *Aspergillus niger* pretreated biomass pretreated with NaOH solutions followed by H_2SO_4, and linking the data found for the kinetic assay of differently treated biomasses could note that the acid treatment of the biomass, even if applied after basic treatment was able to potentiate the adsorption, with removal of 96% of the dye. According to the authors chemically modified biomass showed better results and higher adsorption kinetics of untreated biomass (Moody *et al.*, 2002; Lisowska and Dlugonski, 2003; Mall *et al.*, 2006; Parshetti *et al.*, 2006; Kumaran and Dharani, 2011).

Pre-treatment of biomass with salts exhibited a positive influence on the sorptive capacity of *C. elegans*. The assay was performed considering the relevance of the

ionic medium on sorption processes. The increase in biosorption observed for biomass treated with $NaHCO_3$ could be explained based on the fact that the bicarbonate ion, HCO_3 - can provide protons or accept protons in water. The protons in turn, can neutralize the negative charges on the surface of fungal biomass and the change of the surface charge it negatively charged positively. Meanwhile, changes in charge density could also affect adsorption affinity for particular dyes.

Pretreatment of biomass from *C. elegans* with NaCl resulted in increased sorptive capacity of the living biomass ratio. Increasing the ionic strength of the solution can lead to greater sorption to act as a colloid, allowing rapprochement between biosorbent and dye, favoring connections between them.

Comparing the effects of acid and alkali treatment, it was noted that pre- treatment of biomass from *C. elegans* UCP 542, with NaOH induced higher percentages of discoloration and higher removal efficiency of adsorptive power in the biomass to biomass ratio exposed to HCl and H_2SO_4. Such treatments have been studied due to the ability of these solutions to catalyze hydrolysis processes or simply acting in protonation and dissociation of functional groups. As expected, treatment of the biomass of *C. elegans* with a solution of H_2SO_4 and HCl resulted in a greater loss of removal efficiency, probably due to the protonation of important functional groups sorption, giving a positive character on the surface of the biomass.

The autoclaving process disclosed in *C. elegans* increased relative to the living biomass sorptive capacity. This result is due to increased biomass sorptive ability to induce an increase in surface area and porosity of the biosorbent. *Aspergillus niger* biomass autoclaved showed an increase in sorptive capacity of 1.17 mg/g to 18.54 mg/g, when tested 50 mg/g Acid Blue 29, and increased 6.63 mg/g to 13.83 mg/g for dye basic blue 9. A similar result was observed for *Rhizopus arrhizus* by Zhou and Banks (1991, 1993).

The autoclaving process disclosed in *C. elegans* increased relative to the living biomass sorptive capacity. This result is due to increased biomass sorptive ability to induce an increase in surface area and porosity of the biosorbent. *Aspergillus niger* biomass autoclaved showed an increase in sorptive capacity of 1.17 mg/g to 18.54 mg/g, when tested 50 mg/g Acid Blue 29, and increased 6.63 mg/g to 13.83 mg/g for dye basic blue 9 (Fu and Viraraghavan, 2001; Khalaf, 2008; El-Zaher and Eman, 2010).

Effect of initial concentration of Remazol Black B on the removal process

Tests on the effects induced by the initial concentration of dye in the removal process was conducted using a mass fixed biosorbent (0.2 g) and varying the concentration of Reactive Black (B) to 50 mg/L. The results related to the effect of the initial concentration of the Reactive black (B) in the removal process tests are shown in table 1.

Under the same conditions, the concentration of the Reactive Black (B) solution is higher, the active binding sites are surrounded by more coloring, the process being carried out more efficiently, or more dye is bound. The sorption capacities have increased in all tested biosorbents with increasing dye concentration. An increase in dye concentration induces, however, reduction in the percentage of color removal due to the coverage of binding sites on the surface of

biosorbents in high concentrations of dye for all tested biosorbents.

Ultrastructural and morphological analysis

The results obtained by analysis with light microscopy are shown in figure 6. Scanning Electronic Microscopy analysis of the control sample, living biomass, allows us to observe the presence of hyaline, thin and homogeneous surface (A) hyphae. The sample exposed to the dye exhibits the presence of amorphous material in the structure of hyphal (B). Moreover, the figure shows that the mycelium biomass of all subjected to pretreatment with hyphae exhibit variable transparency, irregular shape, variation in branching pattern and an uneven surface in relation to the control samples. Furthermore, the presence of biomass in color after exposure to the dye solution, indicating that the major mechanism in the removal process is biosorption. It is noteworthy that the changes are also related to the type of treatment applied. There are few studies on the morphology of microbial biomass after dye removal processes. Roncero and Duran (1985) using fluorescence microscopy verified that changes in cell wall structure of microorganisms, as well as the emergence of a deposition layer on the cell surface stemmed from exposure to calcofluor. Bacterial cell surface changes color and appearance were also observed after bleaching of textile dyes. Park *et al.* (2007) identified changes in the mycelium of the fungus *Funalia trogii* cultivation in response to the presence of the reactive black 5. However, comparisons are not possible considering the metabolic activity of the fungus. The data obtained for the ultrastructural analysis of biosorbents produced as well as the structures after exposure to reactive black B are shown in figure 7.

We notice that the living biomass is represented by mycelium with moderate electrondensity, homogeneous surface and hyphae with homogeneous diameter (A). All biomass underwent pretreatment exhibited low electrondensity compared to control sample. Pretreated biomass with HCl (C) shows heterogeneous mycelium shortened hyphae with irregular surface. Biomass pretreated with H_2SO_4 (E) displays with amorphous hyphae elongated surface. Pretreated biomass with NaOH (L) presents highly heterogeneous, shortened and degraded. Biomass pretreated with NaCl (I) shows hyphae elongated and collapsed. In pretreated biomass with $NaHCO_3$ (K) hyphae elongated, distorted with low electrondensity deposits are observed. Biomass subjected to autoclaving show distorted and degraded with granular surface. The samples submitted to the different treatments are shown compressed and shows the presence of amorphous material between the hyphae, possibly this material is adhered to the dye mycelium (B, D, F, H, J, L and N). The presence of this amorphous material gives rise to clusters of mycelium in the samples.

Moreover, the figure we noticed that the structure - smoother for living biomass appears to be more irregular, the pre - treated samples, suggesting that the modification process also operates in the external structure of the sorbent, contributing to an increase in the area surface and hence to the sorption process. The modified material has a rougher morphology of the material in nature, which can also contribute to increased efficiency of the sorption process.

Although many works are related to dye biosorption by fungal biomass, few are the studies that evaluate the fine structure of biosorbents produced.

Some studies evaluated the surface modification of biosorbents undergo pretreatments after exposure to dyes and pretreatments. It is observed that after the autoclave and oven drying the mycelium of *Aspergillus niger* presented pro-zone and abundant with long and cross hyphae. The electron micrographs of biomass treated with acid (0.1M H_2SO_4 and 0.2M HCl) show mycelium has large voids and pores between the hyphae, are therefore able to positively influence the kinetics of biosorption since they can increase the surface contacting the biomass with the dye molecule (Fu and Viraraghavan, 2002).

The same authors determined that treats with 0.1M NaOH resulted in mycelium with minor roughness. On the other hand, treatment with 0.1M $NaHCO_3$ resulted in a very bonded mycelium for a single slots and recesses with little mass. After treatment with 0.1M NaCl, the fungal mycelium proved swollen, however, coalesced, forming a continuous and concavities. Some of the cited changes are observed in the present study.

Eichlerová *et al.* (2007) demonstrated the effects of Reactive Brilliant Blue ® on the ultrastructure and morphology of the fungus *Dichomitus squalens* in culture medium. The results obtained in this study are similar to those obtained by the authors, although they have not been obtained in metabolic conditions.

Comparative study of removal of Reactive Black B by activated charcoal

The influence of pH on the percentage of discoloration and removal efficiencies is shown in figures 8 and 9. It is demonstrate that the change in pH also has effects on the adsorption by activated charcoal reactive black. As the pH becomes alkaline is reduced in percentage. The values remain relatively constant for the pH between 3 and 6. These data differ from that observed in *C. elegans* biomass, since the pH increased significantly reduces the percentage of discoloration and the removal efficiency. The data allow us to observe that the values vary around 90% for all concentrations tested.

The table 2 presents the results for the tests of determining the mass of Reactive Black (B) adsorbed by activated charcoal as a function of dye concentration (mg/g).

Activated charcoal has been used successfully as an adsorbent for the removal of dye effluents. This time, a comparative assay of the effect of pH and dye concentration was conducted to determine the discoloration percentage and removal efficiency.

Similar behavior was described in the work of Al-Degs *et al.* (2007), in which three different reactive dyes Blue 2, Red 4 and Yellow 2 were adsorbed into activated charcoal. According to these authors, if electrostatic interaction were the only mechanism of adsorption of dyes, the maximum removal capacity would be in a pH between 6 and 8. In this pH band the surface of the activated charcoal is loaded. It is also possible that the activated charcoal form hydrogen bonds with the dye molecules.

The data obtained of the kinetics of activated charcoal show the adsorption process it reactive black dye, with a total mass of adsorbed dye of 18.65 mg/g.

Table.1 Effect of initial concentration of Reactive Black B sorptive capacity of the biomass of *Cunninghamella elegans* UCP 542 (mg/g)

Reactive Black B (mg/L)	Untreated*	H_2SO_4*	HCl*	NaOH*	$NaHCO_3$*	NaCl*	Autoclaved*
5	3.65	4.93	5.0	4.85	4.48	4.85	4.63
10	8.72	9.85	9.78	9.70	8.19	9.48	9.93
20	17.96	18.12	18.49	19.85	18.80	19.55	18.27
30	23.96	22.37	23.28	29.33	28.72	29.48	28.72
40	33.13	25.80	33.51	38.65	38.19	38.87	38.04
50	37.30	35.80	36.90	42.37	44.41	47.06	48.12

pH 3.0; 0.2 g/L; 28ºC; 150rpm. * mg of reactive Black B/g biomass

Table.2 Concentration of dye adsorbed by the activated charcoal as a function of the initial concentration of Reactive Black (mg/g) and pH

	Reactive Black B concentrations (mg/L)					
pH	5	10	20	30	40	50
3	4.55	9.78	19.7	29.85	39.7	49.93
4	4.4	9.85	19.55	29.55	39.78	49.78
5	4.25	9.02	19.78	29.93	39.78	48.72
6	4.17	7.36	17.29	29.7	37.66	44.49
7	4.25	7.04	13.28	24.72	35.17	45.85
8	4.25	7.24	13.13	22.15	33.28	42.07

Figure.1 Influence of pH on the discoloration process by biomass of *Cunninghamella elegans* UCP 542 subjected to different treatments. Conditions: 30mg/L Reactive Black (B), biomass 0.2 g/L, 28 ºC, 150 rpm

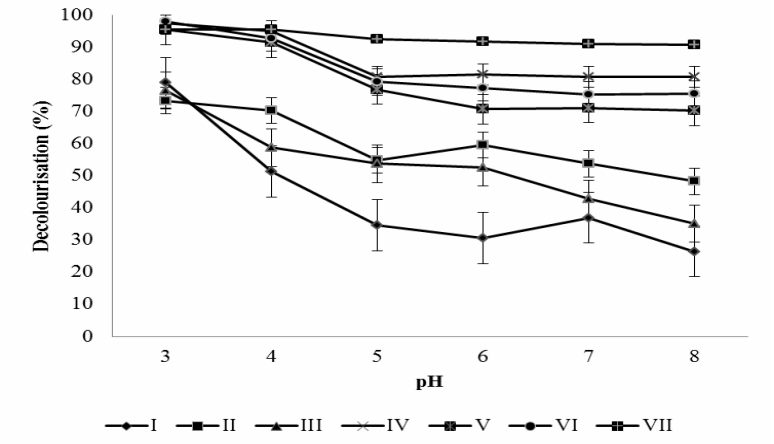


Figure.2 Influence of pH on removal efficiency for biomass *Cunninghamella elegans* UCP 542, subjected to different treatments. Conditions: 30mg/L Reactive Black (B), biomass 0.2g/L, 28ºC, 150 rpm

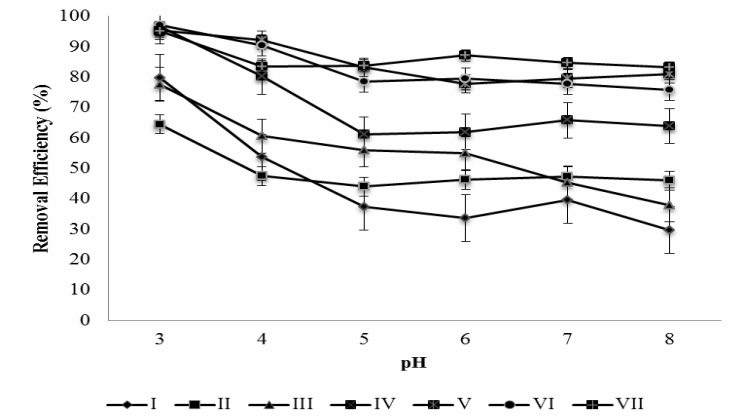


Figure.3 Percentage of discoloration of reactive black B by biomass of*Cunninghamella elegans* UCP 542. Conditions: 30mg/L reactive black B; pH 3; Biomass 0.2g/L; 28 ºC; 150 rpm

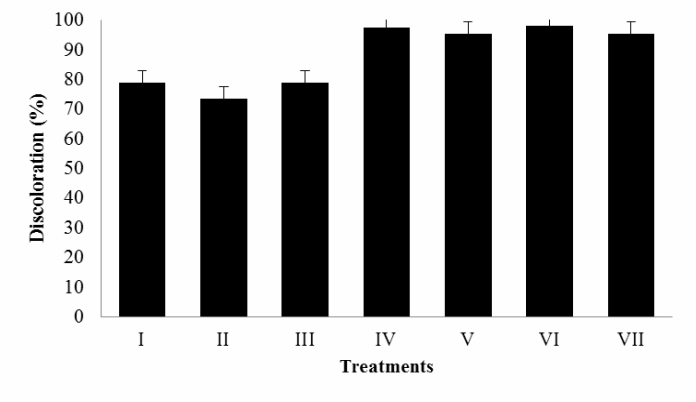


Figure.4 Removal efficiency reactive Black B by biomass of *Cunninghamella elegans* UCP 542, subjected to different treatments. Conditions: 30mg/L reactive black B; pH 3; Biomass 0.2g/L; 28 ºC; 150 rpm

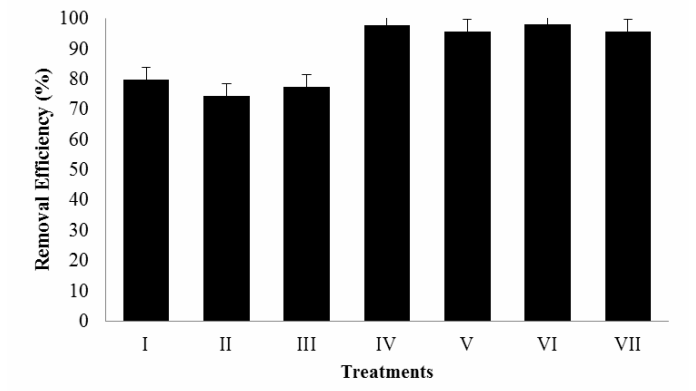


Figure.5 Percentage of the concentration of reactive black B adsorbed by biomass of *Cunninghamella elegans* UCP 542, subjected to different treatments. Conditions: 30mg/L reactive black B; pH 3; Biomass 0,2g/L; 28 ºC; 150 rpm

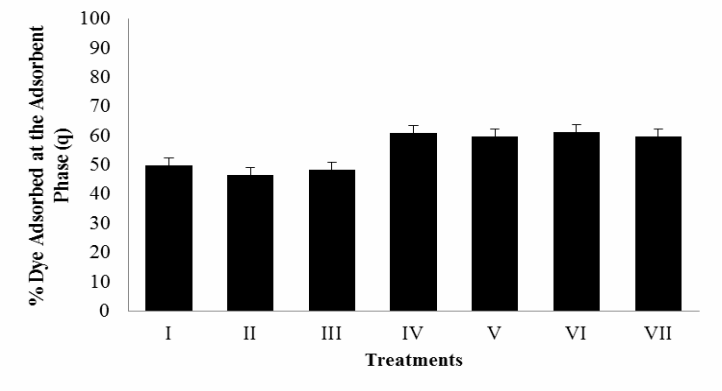
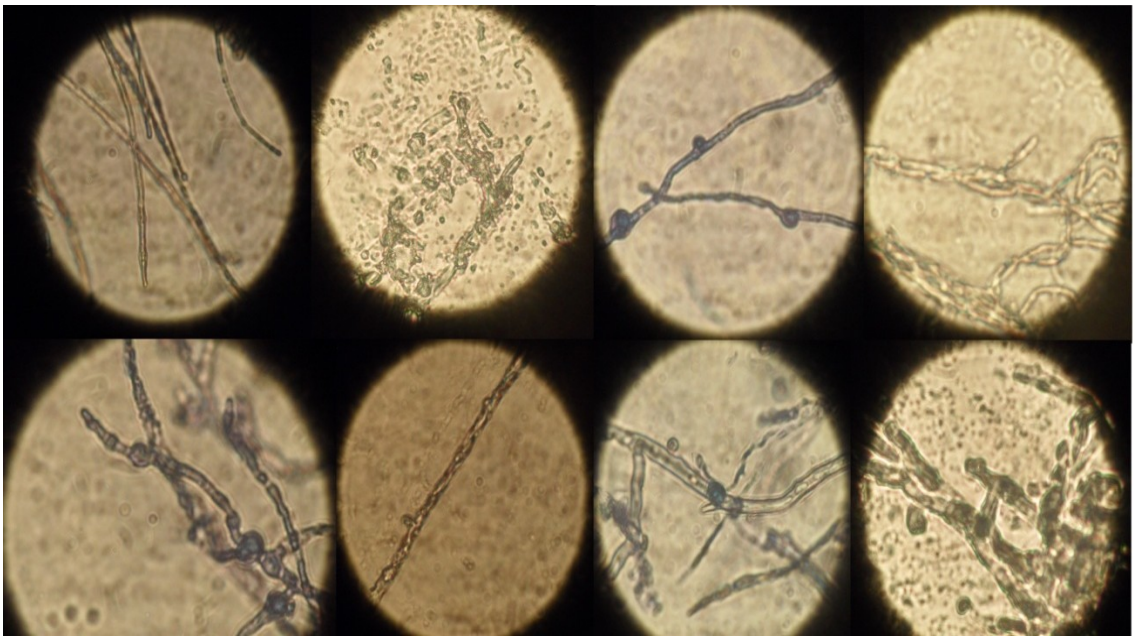


Figure.6 Micrographs of *Cunninghamella elegans*UCP 542. (A)-Untreated biomass (I); (B)-I exposed to Reactive black (RB); (C)-II; (D)-II + RB; (E) – III; (F) – III + RB; (G) – IV; (H) – IV + RB; (I) – V; (J) – V +RB; (K) – VI; (L) – VI + RB; (M) – VII; (N) – VII + RB. X 40. Conditions: pH 3; 0,2g/L; 28 ºC; 150 rpm



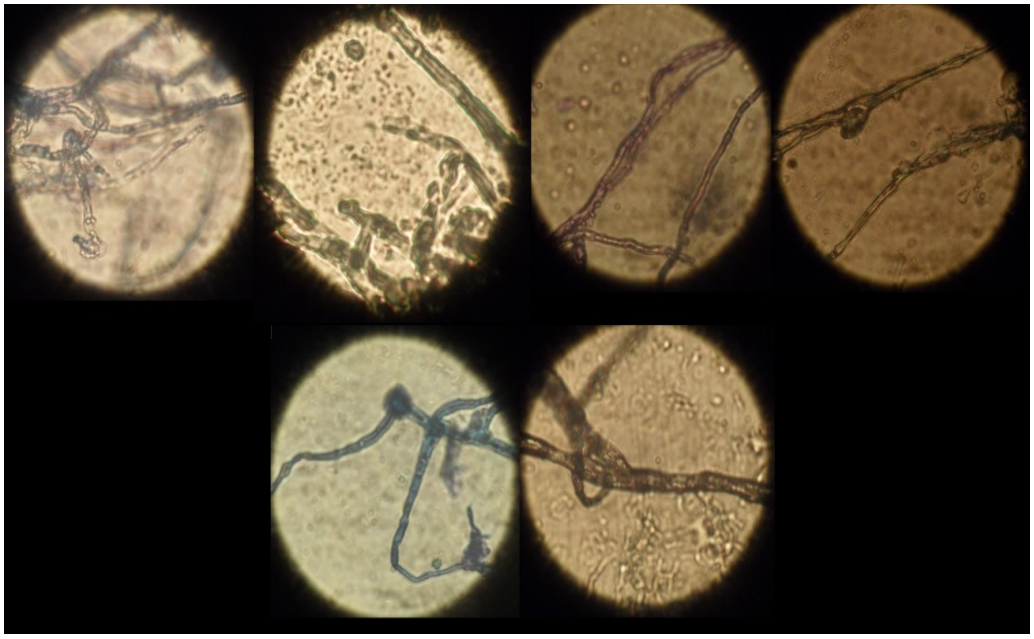


Figure.7 SEM of *Cunninghamella elegans* UCP 542. Treatments: (A) - Untreated biomass (I); (B)-I exposed to reactive Black (RB); (C)-II; (D)-II + RB; (E) – III; (F) – III + RB; (G) – IV; (H) – IV + RB; (I) – V; (J) – V + RB; (K) – VI; (L) – VI + RB; (M) – VII; (N) – VII + RB. X 1.000. Conditions: pH 3; 0.2g/L; 28 ºC; 150 rpm

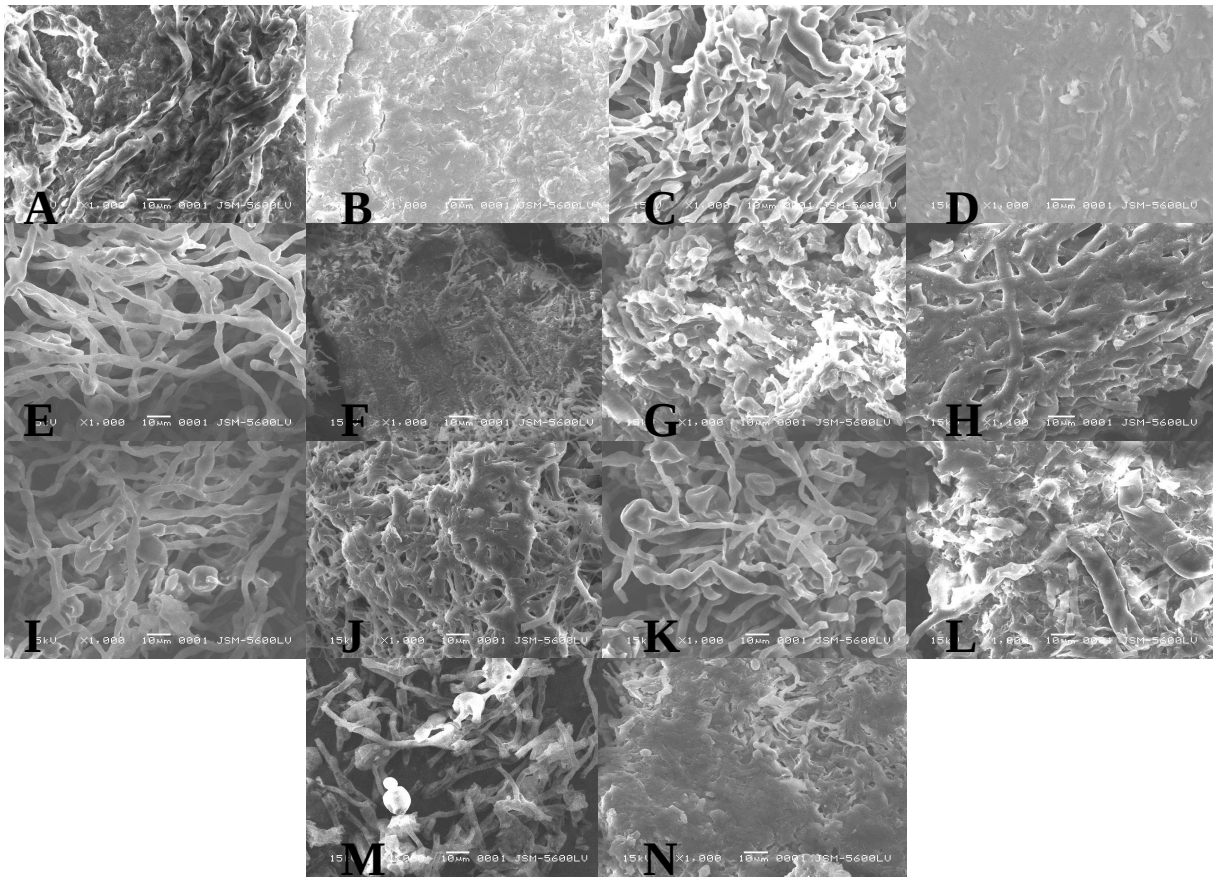


Figure.8 Effects of pH on discoloration percentage by activated charcoal (charcoal mass = 0.2 g/L, 30 mg/L of Reactive Black, 28°C, 150 rpm)

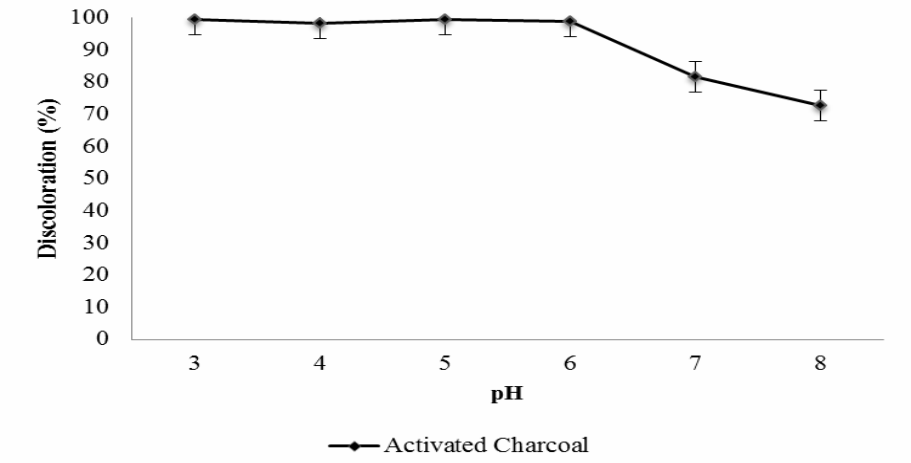
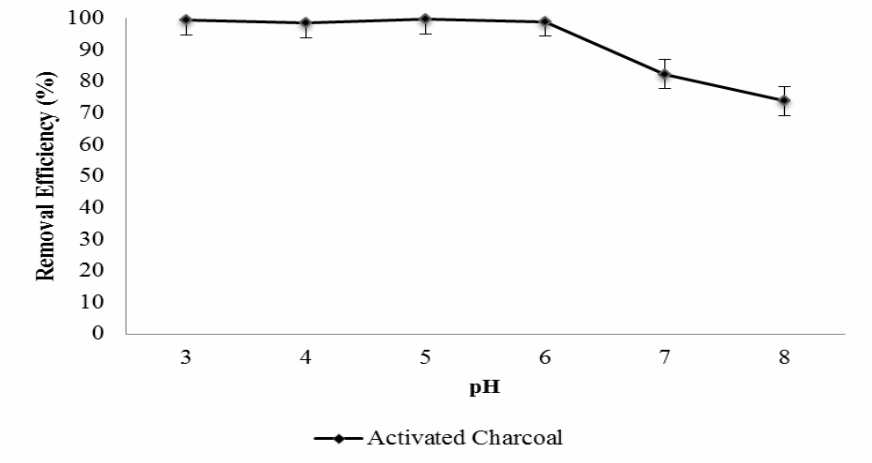


Figure.9 Effects of pH on the removal efficiency percentage by activated charcoal (charcoal mass = 0.2 g/L, 30 mg/L of Reactive Black, 28°C, 150 rpm)



Therefore, evaluating the ability of removing the dye, we have tested the biosorbents: biomass autoclaved, treated with NaCl, NaOH and $NaHCO_3$ to possess similar efficiency to traditionally adsorbent used in large textile industry.

According to the obtained results it was concluded that *Cunninghamella elegans* UCP 542 biomass showed high adsorption capacity for Reactive Black (B) dye. The change in pH induces variation in the ability of removing in all tested biosorbents. Higher removal capabilities, discoloration and efficiency were determined at low pH values. Pretreatments, chemical and physical, positively influenced the process of removing the dye in the different conditions tested. The kinetic experiments showed increasing sorption capacities for living samples <H_2SO_4 <HCl <$NaHCO_3$ and Autoclaved <NaOH <NaCl. The morphological and ultrastrutural analysis revealed changes in the appearance of biomass from *C. elegans* UCP 542, suggesting changes in the hyphal surface, increasing its power Reactive Black binding, indicating that the treatment exposes binding

sites in differentially treated biomass. During this process, the pH was monitored and it was found that the acid treatment left the dye solution at acidic pH (between 3 and 5), indicating the release of protons from the solution biomass for checking the effect contrary to the basic treatment and saline. The physical and chemical interactions, dye-biosorbent and the acid-base properties of the surface of biosorbents have an essential role in the mechanism of adsorption. The relation of the dye sorption capacity (mg) per mass (g) of biosorbents produced was efficient, being equivalent to the kinetic results obtained using activated carbon, indicating that biosorption by *C. elegans* a promising process for the removal of reactive dyes from aqueous effluents. Whereas a real effluent contains many components, and many types of dyes, the biosorbents produced should be investigated to test their efficiency discoloration using an effluent from one of the processing steps in the industry.

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