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Aspergillus niger is able to decolourize sepia ink contained in saline industrial wastewaters

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ABSTRACT

An isolated fungi *Aspergillus niger* was found to be an effective decolourizing agent for wastewaters containing ink of sepia under aerobic conditions. It was found that decolourization of sepia ink by *A. niger* biomass includes two important processes: biosorption and biodegradation. Results showed that the entire black colour was found to be strongly bioadsorbed to the settling spherical fungal biomass pellets of *A. niger*. An optimisation of decolourization conditions using *A. niger* was quite beneficial for colour removal. The study revealed that maximum biosorption using *A. niger* biomass was obtained after 24 h of culture in liquid synthetic media (LSM) containing glucose as carbon source (1 g/L), mineral elements, sepia ink (0.5 g/L) and pH between 4.0 and 5.0. The process of decolourization is concomitant with the growth phase of the fungus and has a necessary requirement for a biodegradable substrate such as glucose. The results showed the capacities of *A. niger* biomass to degrade 3 g/L sepia ink containing in LSM in 96 h in optimal conditions and colour removal reached 96%.

Keywords: Aspergillus niger; Treatment; Decolourization; Bisorption; Biodegradtion

1. Introduction — Decolourization of wastewaters

Currently there is considerable interest in the problem of colour in water bodies despite the fact that some of this colour has a natural origins. Besides the problem of colour, there is concern that some colorants are either toxic or can be modified biologically to become toxic or carcinogenic compounds [1,2]. There are no universal useful methods available for decolourization of wastewaters because of the complex and very varied chemical structures of these compounds [3]. Besides, few of the currently used biological treatment methods can be successfully employed [4]. Colour can be removed from wastewater by chemical and physical methods such as adsorption, coagulation, flocculation, oxidation, filtration and electrochemical methods [2–5]. However, these methods are quite expensive and have operational problems. Adsorption seems to be the most efficient tested colour removal method [5,6]. The most commonly used agent adsorbent for adsorption is powdered activated carbon, which is expensive [7]. Recent reports indicated the possibility of using low-cost adsorbents like zeolite, bentonite, fly ash, slag instead of powdered activated carbon for colour removal from wastewater [1–7]. In recent years, a number of studies have focused on some microorganisms which are able to biodegrade and biosorb colorants in wastewaters. A wide variety of microorganisms such as bacteria, fungi and algae are capable of decolourizing a wide range of

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colorants [2,6,8]. The ability of fungi to degrade a wide range of synthetic chemicals, many of which are resistant to biodegradation, has been reported [9,10].

Fungi have been extensively studied due to their extensive variety and availability. As it has already been emphasized, fungi were characterized by their ability to degrade lignin and cellulose [11]. Their enzyme capacities provide them with the potential to colonize different media. Some fungi, especially white-rot fungi, appear to have some potential for bioremediation applications due to their non-specific system for depolymerisation and mineralization of the complex and recalcitrant polymer of lignin. Besides white-rot fungi there is a variety of fungi such as *Aspergillus niger* [6,12–15], *Rhizopus arrhizus* [16], *Coriolus hirsutus* [17] and *Rhizomucor pusillus* [18] which can also decolorize and/or biosorb divers colorants.

Wastewaters generated by marine-products processing factories are characterized by high concentrations of nitrogen ions, organic matter and salt constituents (>30 g/L) and also presented a black colour. Studies showed that sepia ink is composed of melanin, natural colored polymers [19,20].

Melanins are often poorly insoluble in alkali and generally insoluble in water, aqueous acids, and common organic solvents [21,22]. Although, it must be emphasized that the overall structure is not known, most melanin appear to be an irregular light-absorbing polymer containing indoles and other intermediate products derived from oxidation of tyrosine [23].

These characteristics are of environmental concern and legislation is becoming stricter in an effort to control the impact of wastewater on the environment. Gharsallah et al. [24] and Khannous et al. [25] showed the possibility to treat saline industrial wastewaters using acclimated halophilic bacteria. The COD removal obtained was 90% in fixed bed reactor and 92% in activated sludge reactor. However, they reported that biofilm kept a black colour at the end of the treatment.

These wastewaters can not be decolourized by conventional methods such as activated sludge processes because melanins are resistant to bacterial attack. Furthermore, coloured wastewaters discharged into natural water bodies often cause a coloration problem with respect to the water environment [17]. Hence, it seems imperative to develop an effective method for reducing the colour.

The fungi are unique among microorganisms in that they secrete a variety of extracellular enzymes. The role of fungi in the degradation of complex carbon compounds such as starch, cellulose, pectin, lignin, lignocellulose, inulin, xylan, araban etc., is well known.

Aspergillus niger was known to be the microorganism which is the most efficient to remove colour from wastewaters. It was previously used for single cell protein production, possesses extracellular pectin hydrolyzing enzymes, anthocyanins and polyphenols. Moreover, *A. niger* metabolizes many simple phenolic compounds identified in oil mill waste (OMW). It was used to remove zinc and copper from polluted water by biosorption [26]. Meanwhile, *A. niger* has been used to remove colour by biodegradation [6]. However, it was not yet needed to remove sepia ink from wastewaters.

In the present study, investigations were carried out using a two- prong approach. The first approach involves biosorption of sepia ink by the most spread saprophytic fungus in the terrestrial environment *Aspergillus niger*. The second approach aimed to study the ability of *A. niger* to decolourizing black effluent by degradation.

2. Materials and methods

2.1. Organisms

A strain of *Aspergillus niger* was isolated from acclimated culture which prepared using effluent generated by a Tunisian marine products factory (SOCEPA). Identification was effected according to its microscopic morphology and culture conditions as reported by Raper and Fennel [27]. *Aspergillus* was maintained on potato dextrose agarose from Difco laboratories (PDA). Culture was incubated 5 days at 25°C.

2.2. Preparation of spore inoculation

Preparation of spore solution was effected as described previously [14]. 10 ml of sterile physiologic water (9 g/L NaCl) are added into Petri dishes containing *A. niger* which was in actively growing. Using a transfer needle, the surface of the culture was scraped gently to bring spore into suspension. The suspension was then poured into sterile flask and shaken for 10 min to disperse the spore. The spore concentration was measured by direct microscopic counts using hematimetric cell. 1 ml aliquot of a spore suspension (containing 43×10^5 spores) was used to inoculate 1 L Erlenmeyer conical flasks, each containing 100 ml sterile liquid media.

2.3. Ink of sepia (sepiomelanin)

The ink was collected from ink sac of cuttlefish during their wash within the industry and dried overnight at 80°C.

2.4. Cultures media

2.4.1. Agar plate screening

The agar plate screening was performed using glass Petri dishes containing 10 ml of the solid synthetic medium (SSM) contains 5 g/L glucose; 1 g/L K_2HPO_4; 0.5 g/L $MgSO_47H_2O$; 0.5 g/L KCl; 10 mg/L $FeSO_47H_2O$; 1 mg/L $ZnSO_47H_2O$; 1 mg/L MnSO47H2O; 1 mg/L $CuSO_47H_2O$; 34 g/L NaCl; 0.5 g/L sepia ink and 20 g/L agar. The pH of the media was adjusted to 4.0 before autoclaving. Each plate was inoculated with one plug obtained from the actively growing mycelium of each test fungus incubated

4 days at 25°C. The radial growth and the zone of colour change were measured every two to three days. The melanin containing plates were examined for the visual disappearance of colour from the plates.

2.5. Decolourization by biosorption

2.5.1. Fungal biomass preparation

Biomass of *A. niger* was prepared according to Naoyuki et al. [17]. 1 ml aliquot of spore suspension was used to inoculate 1 L Erlenmeyer conical flasks, each containing 0.1 L sterile GPY liquid medium containing 10 g/L glucose; 3.0 g/L peptone; 2.0 g/L yeast extract; 1.0 g/L K_2HPO_4 and 0.5 g/L $MnSO_47H_2O$. pH was adjusted to 4.0 prior autoclaving. Culture was incubated for 1 day at room temperature on a reciprocal shaker (150 rpm). This cultivation produced pellets of about 3 mm diameter.

For the decolourization test by biosorption, the pellets were harvested after 24 h of growth by filtering. The harvested fungal pellicles were washed with generous amounts of deionised water. The biomass was transferred into 100 ml of liquid synthetic media (LSM) in 1 L Erlenmeyer conical flasks. LSM contains 5 g/L glucose; 1.0 g/L K_2HPO_4; 0.5 g/L $MgSO_47H_2O$; 0.5 g/L KCl; 10.0 mg/L $FeSO_47H_2O$; 1.0 mg/L $ZnSO_47H_2O$; 1.0 mg/L $MnSO_47H_2O$; 1.0 mg/L $CuSO_47H_2O$; 34.0 g/L NaCl and 0.5 g/L sepia ink. The pH of the media was adjusted to 4.0 before autoclaving.

2.5.2. Dry biomass preparation

The mycelia were harvested after 24 h of growth on GPY medium by filtering and dried for 1 h at 80°C.

2.5.3. Dead biomass preparation

The mycelia were harvested after 24 h of growth on GPY medium by filtering and autoclaved for 20 min at 120°C [28].

2.6. Decolourization by degradation

1 ml aliquot of spore suspension was used to inoculate 1 L Erlenmeyer conical flasks, each containing 0.1 L sterile liquid synthetic media (LSM) and then incubated at 25°C on a reciprocal shaker (150 rpm). For the determination of dry weight, the mycelia pellets were filtered and washed twice with distilled water and dry weight was determined using tarred foil cups dried to constant weight at 80°C.

2.7. Analytical methods

COD was carried out on the supernatant and determined as described by Ross Kneichtel [29]. The pH was measured using pH meter MP 220.

2.8. Decolourization essay

The culture was harvested and the pellets washed by filtration. The supernatants were employed for measurement of absorbance at 600 nm (Spectrophotometer UV-Visible Cecil CE 2021).

2.9. Experimental design

In order to optimise the medium composition (LSM) for the studies of the biosorption and the degradation processes using *A. niger* biomass, various carbon sources and nitrogen sources, pH, sepia ink concentration were investigated. All experiences cited in results are duplicated and results values presented are the average.

3. Results and discussion

3.1. Agar-plate screening for decolourization of sepia ink

The initial evaluation of decolourization of sepia ink by *Aspergillus niger* was made using solid synthetic medium (SSM) and decolourization was assessed as the disappearance of colour during cultivation. *A. niger* showed a decolourization of black melanin within 7 days. Other strains of fungi were tested and *A. niger* showed that it was the only fungus able to grow in SSM. Melanin would be toxic for the other strains of fungi. Luigi Rosso et al. [30] showed the toxicity of melanin-free of *Sepia officinalis* to a variety of cell lines. We choose *A. niger* for further investigation because of its ability efficiently to remove colour from synthetics solutions.

3.2. Adsorption of colour using A. niger biomass

Aspergillus niger biomass obtained previously by cultivation in GPY was transferred sterilely in liquid synthetic media (LSM) containing sepia ink. Results show that *A. niger* biomass was able to adsorb total sepiomelanin present in LSM during experiments, the maximum colour removal was obtaining after 24 h of culture which indicates that the mechanisms of adsorption was very fast. COD removal obtained was 74%. Biosorption mechanism might also play an important role in the wastewater treatment. Miranda et al. [31] observed that the percentage of adsorbed colour was in the range of 10–25 in the study on *A. niger*. During experiments using biomass, we observed a total colour removal after 24 h of culture (Table 1).This fungal strain exhibited also a strong ability (70%) to decolourize media containing heat treatment liquor (HTL) [15]. With dry biomass there was no decolourization even after 3 days of culture (Table 1). The biosorption using dead biomass was also tested and the maximum of COD reduction obtained was 14% and the medium kept the black colour. Tatarko and Bumpus [32], used both living and autoclaved cultures of *Phanerochaete crysosporium* to decolourize cango red and observed that the autoclaved cells had a higher colour removal (90%) than the living

Table 1
Biosorption of ink using A. niger biomass after culture during 24 h

	Wet biomass	Dry biomass	Dead biomass
pH_i	6	6	6
pH_f	3.29	—	4.85
COD removal (%)	77	—	14
Colour removal (%)	94	—	10

cells (70%). Mou et al. [33] showed an equally effective in decolourizing the dye solution with 94.9% colour removal vs. 96.5% for wet living cells. These different results might have been due to the fungal growth conditions or to the chemistry of different dyes.

Living cells have a wide variety of decolourization mechanisms. Their performance has a close relation with the operating conditions such as the concentration in effluent, pH and temperature. They require nutrients, as well as cultural maintenance [34].

3.2.1. Optimisation of A. niger *biomass adsorption conditions*

It is necessary to create an optimal environment favourable to fungal adsorption and thus make the fungi possess the maximum ability to decolorize ink of sepia in wastewater. Fungi are mostly growing in a medium with dyes or dye wastewaters to develop biosorbent containing living cells. The medium is mainly composed of carbon source, nitrogen source and other nutrients.

3.2.2. Effect of glucose on A. niger *biomass biosorption capacities*

Zhang et al. [35] reported that carbon sources present in LSM can have an effective role on effluent decolourization by fungi and glucose was a good carbon source. Fig. 1 shows the effect of glucose (5 g/L) on biomass and COD removal, respectively from synthetic liquid medium by biosorption process using biomass of A. niger. Biosorption of sepia ink decreased to a significant extent. The absence of glucose in the synthetic medium reduced the growth of fungal biomass from 0.27 g to 0.18 g, thus decreasing COD removal from 76 to 31%.

3.2.3. Effect of concentration of glucose on A. niger *biomass biosorption capacities*

A. niger biosorption capacities were tested at different concentrations of glucose (1, 2, 3, 4 and 5 g/L). Results are presented in Fig. 2. We observed that biomass biosorption capacities were similar to different concentrations used. 1 g/L would be an effective and economical concentration for decolourization, since a maximum (78%) COD removal was obtained at this concentration. Consequently, this concentration of glucose was used for ink biosorption in further studies.

3.2.4. Effect of concentration of mineral elements on A. niger *biomass biosorption capacities*

Fig. 3 shows the effect of mineral concentration on the biosorption capacity of *A. niger* biomass. The results indicated that the ionic strength is an important factor for the biosorption process. The maximum COD removal was observed on M1 medium which contained mineral elements. This result confirms previous findings by Yuzhu and Viraraghavan [28] and Zhou and Banks [16].

3.2.5. Effect of pH on A. niger *biomass biosorption capacities*

The effect of pH on the fungal biosorption capacities was tested in LSM containing glucose (1 g/L); mineral elements and sepia ink (0.5 g/L). Fig. 4 shows the changes in adsorption capacities of *A. niger* biomass with change in initial pH. The maximum sepia ink adsorption was obtained between initial pH 4.0 and 5.0. The observed values correlated with the results obtained by Yuzhu and Virarghavan [28]. However, the biosorption capacities

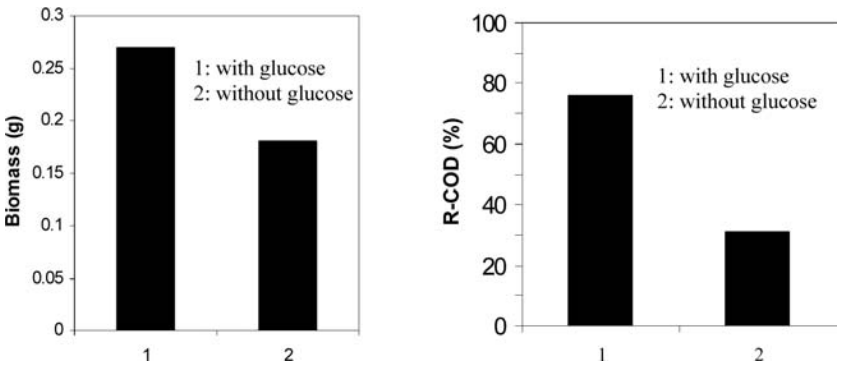

Fig. 1. Effect of glucose on the fungal biosorption capacities.

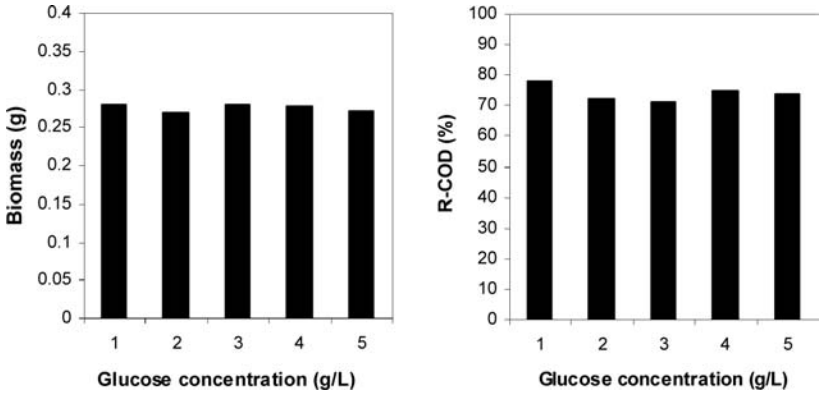

Fig. 2. Effect of glucose concentration on the fungal biosorption capacities.

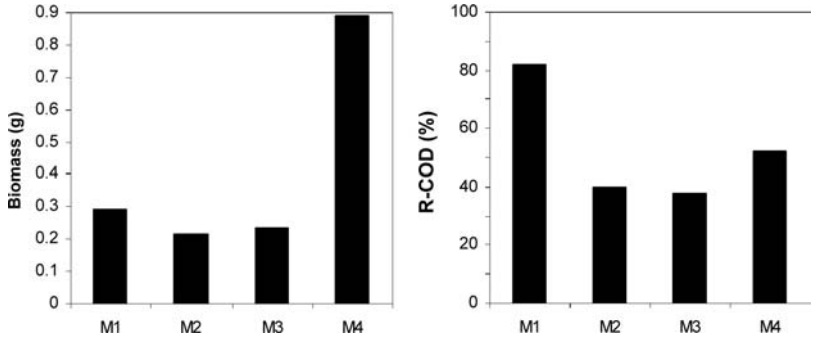

Fig. 3. Effect of minerals salts on the fungal biosorption capacities. M1: Distilled water (1 L); K_2HPO_4 (1 g/L); $MgSO_47H_2O$ (0.5 g/L); KCl (0.5 g/L); $FeSO_47H_2O$ (10 mg/L); $ZnSO_47H_2O$ (1 mg/L); $MnSO_47H_2O$ (1 mg/L); $CuSO_47H_2O$ (1 mg/L); NaCl (34 g/L); glucose (1 g/L); ink of sepia (0.5 g/L); pH 6. M2: Tap water (1 L); glucose (1 g/L); ink of sepia (0.5 g/L); pH 6. M3: Distilled water (1 L); glucose (1 g/L); ink of sepia (0.5 g/L); pH 6. M4: ASW (1 L); glucose (1 g/L); ink of sepia (0.5 g/L); pH 6.

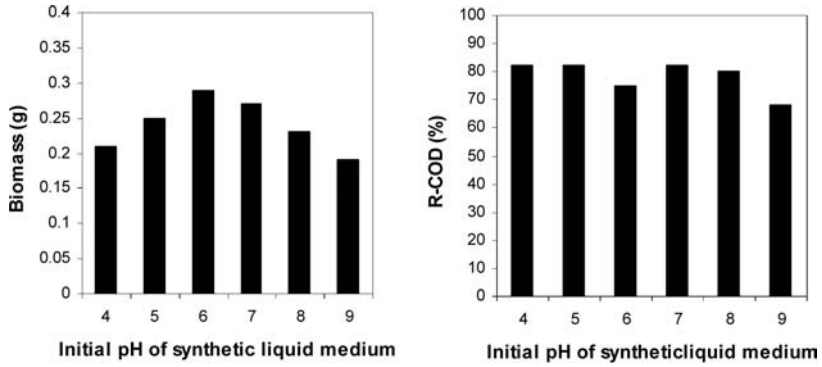

Fig. 4. Effect of initial pH on the fungal biosorption capacities.

decreased at pH 8.0 and 9.0 and no biosorption occurred. This indicated a possible competition between proton of the active sites in the fungal biomass surface and the dye.

3.2.6. *Kinetic studies of* A. niger *biomass biosorption*

The adsorption kinetics of *A. niger* biomass at different times of incubation is shown in Fig. 5. This study was conducted in LSM [distilled water (1 L); K_2HPO_4 (1 g/L); $MgSO_47H_2O$ (0.5 g/L); KCl (0.5 g/L); $FeSO_47H_2O$ (10 mg/L); $ZnSO_47H_2O$ (1 mg/L); MnSO47H2O (1 mg/L); $CuSO_47H_2O$ (1 mg/L); NaCl (34 g/L); glucose (1 g/L); ink of sepia (0.5 g/L); pH 4.0]. The result indicated maximal decolourization of culture reached within 24 h. This result suggests that incubation at 24 h would be sufficient for total decolourization. These results differ with previ-

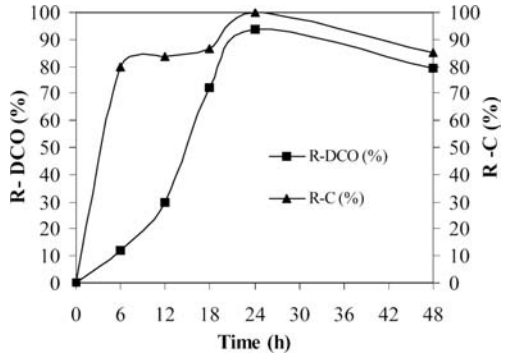

Fig. 5. Kinetic studies of fungal biosorption.

ous findings. Yuzhu and Viraghavan [28] found that A. niger biomass can biosorb Basic Blue 9 at 30 h of incubation. Jasper and Penninckx [9] found that mycelia of P. crysosporium can adsorb 60 % of the colour from Kraft bleach plant effluent after 24 h. These differences can be explained by the fact that a fungus capable of decolorizing one dye may have different capacities for the other dyes, because, dyes have different molecular structures. Besides, dye concentration affects the efficiency of colour removal.

3.3. *Decolourization by degradation using* Aspergillus niger

The ability of *A. niger* to degrade ink of sepia present in LSM was tested and the result showed the appearance of pellets of A. niger (in LMS) after 24 h of culture incubation at 30°C. The colour was removed gradually. Total colour removal was observed after 4 days. This result showed that *A. niger* biomasses were able to totally degrade sepia ink. This is in live with previous studies which stipulated that fungi produce different enzymes to mineralize synthetic dyes. Miranda et al. [31] found that *A. niger* is able to biodegrade and adsorb molasses from wastewaters at 57% of colour removal in 3 or 4 days. Yuzhu and Virarghavan [7] reported that enzymes secreted by fungi depend on dye structure. Recently, Bulter et al. [36] reported that melanin can occur as a result of the indirect action of some fungal enzymes, rather than by direct melaninase activity such as that of manganese peroxydase. A number of fungi, such as *Aspergillus niger* can excrete this enzyme.

In the decolourization process by degradation using fungi more than one factor can play a role. These can be classified into two groups. One may be related to fungal growth conditions, the other may be related to the characteristics of the dye solution or wastewaters.

3.3.1. *Fungal growth conditions*

Different fungus groups possess different abilities to decolorize dyes. It is necessary to create a favourable environment to facilitate the optimum growth and thus make the fungus possess the maximum ability to decolorize dyes in wastewaters.

3.3.2. *Optimisation of carbon sources*

Fig. 6 shows the effects of variation of carbon sources on *A. niger* growth and capacities to degrade sepia ink. In relation to them, Fig. 7 shows the colour removal percentage of different carbon sources (5 g/L). The results showed that glucose, galactose and wheat flour were the good carbon substrates for *A. niger* growth. However, the highest colour removal was obtained with maltose but the culture kept a yellow colour after incubation. The colour removal percentage obtained in the presence of glucose was 94 % wiles is good as well.

This result confirms previous findings. Indeed, Zhang et al. [35] studied the carbon sources as effective co-substrate on decolourization by fungus and reported that glucose, starch, maltose and cellobiose were good carbon sources while sucrose, lactose, xylan, xylose, methanol and glyoxal were poor carbon sources.

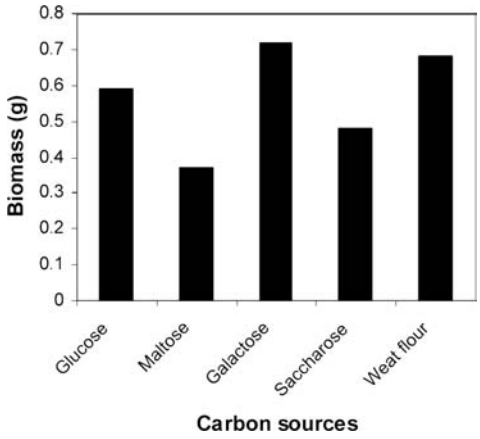

Fig. 6. Effect of carbon sources on *A. niger* growth.

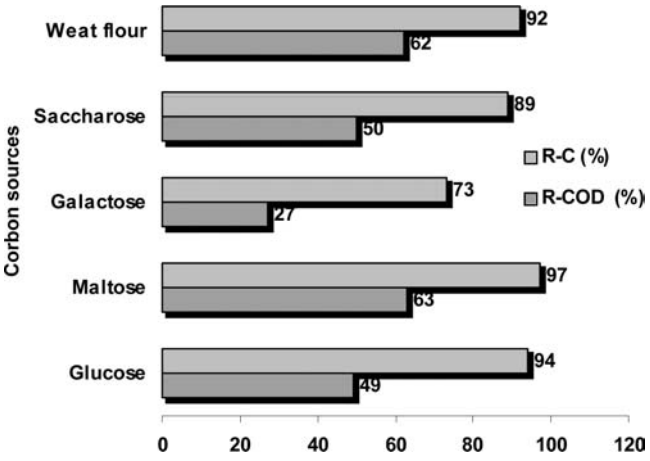

Fig. 7. Effect of carbon sources on colour removal and COD removal by degradation using *A. niger.*

3.3.3. Effect of glucose concentration on degradation capacities of A. niger *biomass*

Different concentrations of glucose were tested (1, 2, 3, 4 and 5 g/L). Fig. 8 shows that biomass of *A. niger* increased with the increase of glucose concentration. Higher percentage of COD removal was obtained (48%) by adding 5 g/L of glucose (Fig. 9). This value also yielded 94 % of colour removal. At the same time, this glucose concentration yielded the highest biomass production 0.6 g (Fig. 8). It seems that 5 g/L glucose were the best carbon concentration retained for the purpose study.

3.3.4. Optimisation of nitrogen sources

In the investigation of the effect of various nitrogen sources on degradation capacities of *A. niger* (Fig. 10), yeast extract was found to be the most promising one. In the case of biomass growth, yeast extract seemed to be suitable as well (Fig. 11), but in the case of COD removal, $(NH_4)_2SO_4$ showed a maximum of 84% of COD removal. The nitrogen source was an important factor for *A. niger* growth and colour removal. Biomass growth decreased from 0.51 g to 0.18 g in the absence of yeast extract and there was no decolourization of the medium. This result confirmed observations by Hamdi et al. [14] who found that yeast extract had a positive effect on *A. niger* decolourization of olive mill wastewater (OMW).

3.3.5. Effect of yeast extracts concentration

The effects of various concentrations of yeast extract (1, 2, 3, 4 and 5 g/L) on the growth biomass and decolourization capacities of A. niger are shown in Figs. 12 and 13, respectively. At 4 g/L, yeast extract showed the

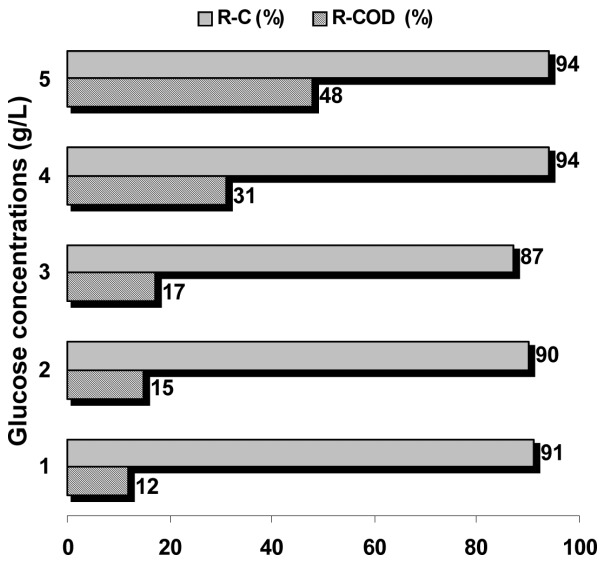

Fig. 8. Effect of glucose concentration on colour removal and DCO removal by degradation using *A. niger.*

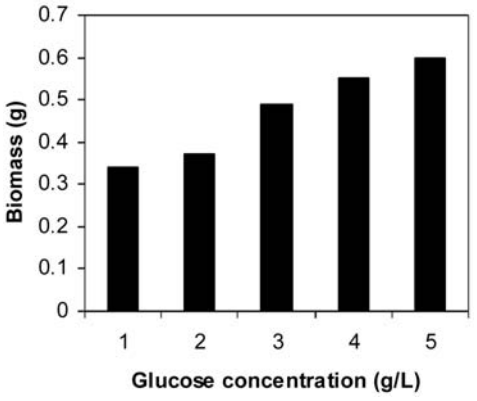

Fig. 9. Effect of glucose concentration on *A. niger* growth.

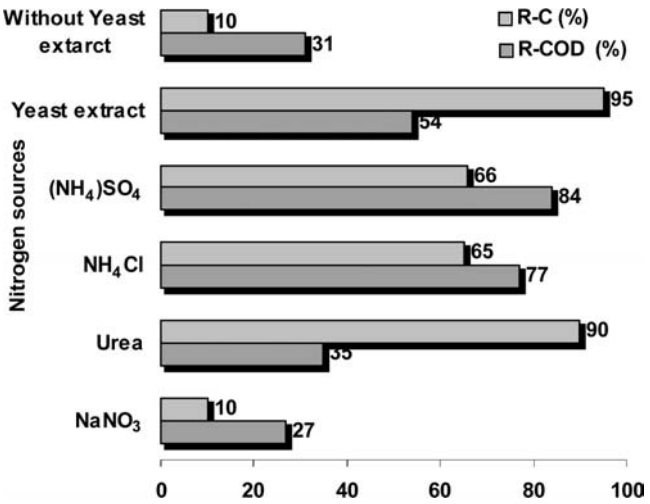

Fig. 10. Effect of nitrogen sources on colour removal and DCO removal by degradation using *A. niger*.

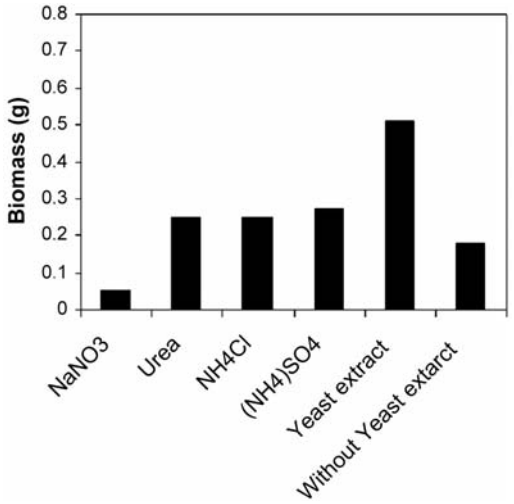

Fig. 11. Effect of nitrogen sources on *A. niger* growth.

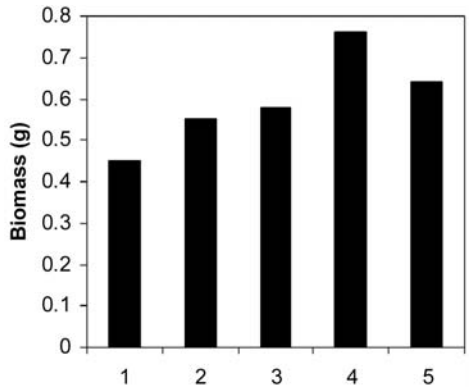

Fig. 12. Effect of yeast extract concentration on *A. niger* growth.

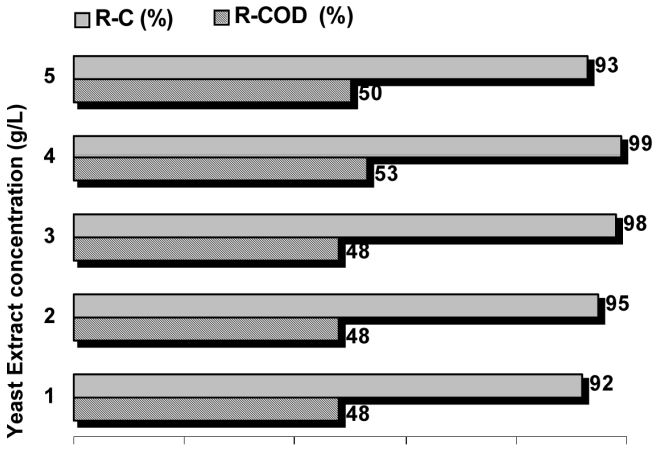

Fig. 13. Effect of yeast extract concentration on colour removal and COD removal by degradation using *A. niger* biomass.

best biomass growth of 0.76 g, the highest colour removal of 99% and the best COD removal of 53%.

3.3.6. Effect of sepia ink concentration

Sepia ink concentration also affected the efficiency of colour removal. This natural dye had a negative effect on biomass growth of *A. niger* in LSM. For the determination of the threshold ink toxicity, different concentrations were tested (0.5, 1, 1.5, 2, 2.5, 3.0, 3.5 g/L) and the results obtained are shown in Figs. 14 and 15. The increase of ink concentration had a considerable effect on the colour of biomass obtained in the end of the culture. At concentrations higher than 3 g/L, pellets of *A. niger* presented a black colour. This was in live with findings by Zhang et al. [35] observed that the colour removal efficiency decreased with an increase in the concentration of the cotton bleaching effluent. Mou et al. [33] reported that high dye concentration resulted in low colour removal.

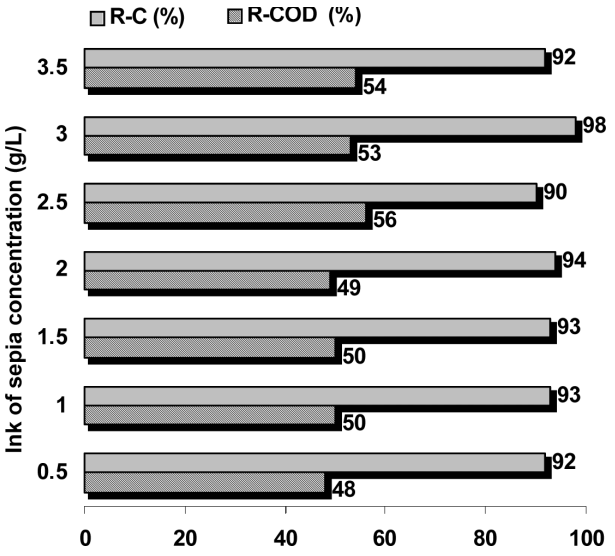

Fig. 14. Effect of ink of sepia concentration on colour removal and COD removal by degradation using *A. niger* biomass.

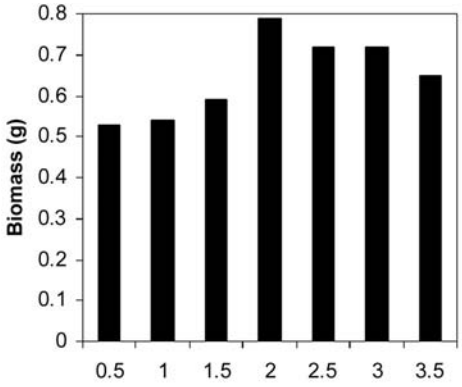

Fig. 15. Effect of ink of sepia concentration on using *A. niger* growth.

3.3.7. Effect of size of spore inoculation

Fig. 16 illustrates the effects of spore inoculation size on the growth of *A. niger* in LSM containing 3 g/L sepia ink. Results show the optimal spore's number can be seen between 4.3 106 and 6.45 106 spores/ml. At higher numbers of spores, the biomass decreased to 0.32 g and COD removal to 20% (Fig. 17).

3.3.8. Effect of initial pH

The effect of pH on the degradation capacities of *A. niger* was studied. Results are presented in Fig. 18. The maximum decolourization of the medium using *A. niger* biomass was observed at pH between 4.0 and 5.0. This result was in agreement with values obtained by Zhang et al. [35] and Miranda et al. [31].

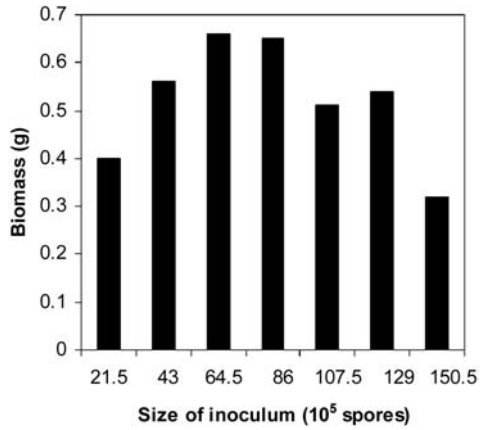

Fig. 16. Effect of size of inoculum on *A. niger* growth.

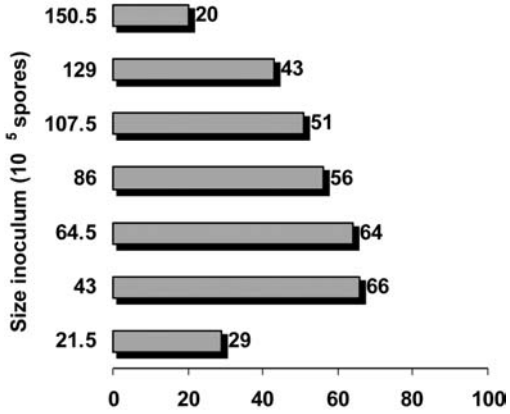

Fig. 17. Effect of size of inoculum on COD removal by degradation using *A. niger* biomass.

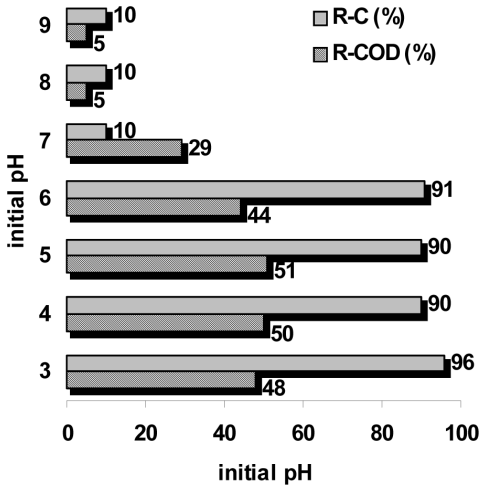

Fig. 18. Effect of initial pH on colour removal and COD removal by degradation using *A. niger* biomass.

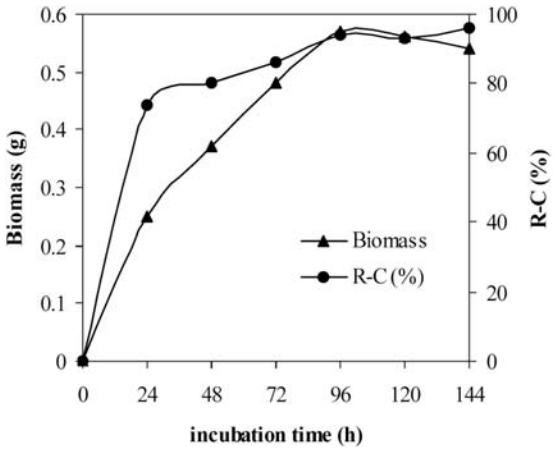

Fig. 19. Kinetic of ink degradation by *A. niger*.

3.3.9. *Kinetic study of ink degradation by* A. niger *biomass*

Fig. 19 shows the kinetic of ink degradation by *A. niger* biomass in LSM containing 5 g/L glucose; 4 g/L yeast extract; 3 g/L sepia ink; pH 4. The highest biomass was produced and the greatest COD removal was obtained after 96 h of culture incubation.

4. Conclusion

In this study, investigations were focused on the ability of *Aspergillus niger* to decolorize sepia ink contained in culture media. The results showed that this fungus presents a double capacity to remove colour from wastewaters: adsorption and biodegradation. An optimisation of the mechanism adsorption of sepia ink was study and the results showed that *A. niger* biomasses was found to be effective in removing sepia ink from aqueous solution. It is a promising biosorbent for dye removal from coloured wastewaters. An optimisation of the degradation mechanism of sepia ink was also study and the results shows that this process was depended on medium culture composition. Colour can be removed only after 96 h.

5. Recommendations

It would be very interesting to test a full-scale application of *A. niger* as a decolourizing agent at industrial level. One of its most attractive benefits would be the efficiency and the reduction of the treatment experiences.

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Abbreviations

A. niger	—	Aspergillus niger
COD	—	Chemical oxygen demand
GPY	—	Glucose peptone yeast
LSM	—	Liquid synthetic medium
pH_f	—	Final pH
pH_i	—	Initial pH
R-C	—	Removal of colour, %
R-COD	—	Removal of COD, %
SSM	—	Solid synthetic medium

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