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REMOVAL OF INDIGO CARMINE FROM AN AQUEOUS SOLUTION BY FUNGUS *PLEUROTUS OSTREATUS*

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Abstract: The role of fungi in the treatment of wastewater has been extensively researched. Many genera of fungi have been employed for the dye decolourization either in living or dead form. In this study, the removal of an acidic dye, Indigo Carmine (IC), from an aqueous solution by biosorption on dead fungus, *Pleurotus ostreatus*, was investigated. The effects of contact time, initial dye concentration, amount of dead biomass, agitation rate and initial pH on dye removal have been determined. Experimental results show that an increase in the amount of dead biomass positively affected the dye removal. The highest removal was obtained at 150–200 rpm. Slightly lower removing activities were found at lower agitation rates. The dye adsorption efficiency was not affected by pH except minor variation in the pH of 2–8. Color removal was observed to occur rapidly within 60 minutes. The removal of dye by dead biomass of *P. ostreatus* was clearly dependent on the initial dye concentration of the solution. Dye removal was reduced from 93% to 64% as concentration was increased from 50 to 500 mg/L Indigo Carmine. This study showed that it was possible to remove textile dyes by dead biomass of *P. ostreatus*.

INTRODUCTION

Textile industries generate large volumes of wastewater. The removal of dye from textile effluents is one of the most significant environmental problems. Color is one of the greatest contaminants in wastewater, as it is highly visible and undesirable and also reduces light penetration and photosythesis. In general, dyes have complex chemical structures and are resistant to biological degradation. There are various methods available for the removal of dyes, including membrane-separation, electrochemical, flocculation-coagulation, reverse osmosis, ozone oxidation, biological treatments, sorption, etc. [12, 20].

Due to low biodegradability of dyes, a conventional biological wastewater treatment process is not very efficient in treating a dye wastewater. It is usually treated by physical – or chemical – treatment processes [3]. Adsorption on activated carbon has been found to be an effective process for dye removal from dye wastewater, but it is too expensive. Low-cost adsorbents developed to replace activated carbon generally have low adsorption

capacities [8]. Therefore, there is a need to find new, economical, easily available and highly effective adsorbents and techniques.

Biosorption can be defined as sequestering of organic and inorganic species including metals, dyes and odor causing substances using live or dead biomass or their derivatives. This biomass may be bacteria, fungi, algae, sludge from biological wastewater treatment plants, byproducts from fermentation industries or seaweeds. In this process, adsorbents are biological materials, and the removal mechanism is mainly sorption [18]. Most of the studies concentrated on living fungi for biodegradation and biosorption of the dyes. There are a few studies on dye removal using dead fungal biomass. 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 [6, 7]. 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 addition, dead fungal biomass can be stored easily and kept for prolonged periods [17]. Apart from these factors, researchers have also reported that dead biomass is more effective in adsorbing various pollutants than live biomass [2, 13, 21].

Indigo is one of the oldest and most important dyes, mainly used in the dyeing of clothes (blue jeans) and other blue denim. This dye in water is transformed into more soluble products before industrial application. Its reaction with sulfuric acid yields indigo carmine (Acid Blue 74, Fig. 1), a common dye also used as food coloring, as indicator in analytical chemistry, and as a microscopic stain in biology [1]. However, indigo carmine is considered a highly toxic indigoid class of dye and its touch can cause skin and eye irritations to human beings. It can also cause permanent injury to cornea and conjunctiva [12].

In this study, dead biomass of Pleurotus ostreatus was used as a biosorbent to remove the Indigo Carmine (IC). The aim of this research was to develop effective adsorbents for dye-removal technology. So, the effect of various operating parameters on dye removal such as initial pH, dye concentration and adsorbent amount were studied.

MATERIALS AND METHODS

Dye solution

The dye stock solution was prepared by dissolving accurately weighed dye in distilled water to the concentration of 500 mg/L. The experimental solutions were obtained by diluting the dye stock solutions in accurate proportions to different initial concentrations.

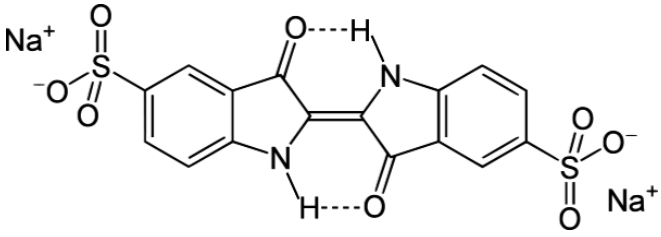

Fig. 1. Chemical structure of Indigo Carmine

Fungal biomass preparation

The white rot fungus *Pleurotus ostreatus* was cultured at 30°C on slant Sabouraud Dextrose Agar (SDA). After 1 week, mycelial suspensions were prepared and used for the cultivation of inoculum. Mycelial suspensions were transferred into 250-mL flasks with 100 mL Sabouraud Dextrose Broth (SDB). Conidial suspension was transferred into a 250-ml flask with 100 mL Stock basal medium (SBM). Growth medium consisted of (in g/L of distilled water); KH_2PO_4, 0.2; $CaCl_2 \cdot 2H_2O$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.05; $NH_4H_2PO_4$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.035; Glucose, 2; Yeast Extract, 1; Sabouraud dextrose broth, 5. Fungal pellets were formed after 2–5 days under 30°C and 150 rpm agitation. When pellet diameters became 3 to 5 mm, the pellets were harvested. After washing, the pellets were dried at 30°C for 24 h and powdered in a pestle and blender. The powdered biomass (particle size less than 100 μm) referred as "dead biomass". Dead biomass was used in dye removal studies.

Assay

Different amounts of dead biomass were transferred to 100 mL flask containing 20 mg/L of dye containing distilled water. The effect of agitation was investigated at different agitation rates, namely 0, 50, 100, 150, 200 and 250 rpm. The effect of the amount of dead biomass was investigated at different amounts, namely 0.1, 0.2 and 0.5 g/20 mL and the effect of contact time was tested at 30, 60, 90, 120, 180 minute. In order to study the effect of pH on dye removal, the pH of the solutions was varied from 2 to 8, by adding 0.1M NaOH or 0.1M HCl solutions. The effect of the initial dye concentration on the removal was studied between 50–500 mg/L at 30°C. Samples were taken at different time intervals. Dye concentration was determined with an UV visible spectrophotometer at their visible maxima (610 nm). The absorbance of the supernatant was estimated to determine the residual concentration. Residual dye concentration was determined using absorbance values measured before and after the treatment with spectrophotometer at their visible maxima. All the biosorption experiments were run in triplicate. Controls without the adsorbent were also run.

RESULTS AND DISCUSSION

Effect of adsorbent amount on decolorization

The dye removal of Indigo Carmine (IC) by dead biomass of *P. ostreatus* was studied by changing the quantity of dead biomass (0.1, 0.2, and 0.5 g/20 mL) in test solution. The initial dye concentration, temperature and time were 50 mg/L, 30°C, 60 and 120 minutes, respectively. Unless otherwise stated, experiments were conducted without adjusting the pH of the dye solution (Figure 2). As shown in Fig. 2, the percent decolorization was increased with adsorbent dose. 0.2 g/20 mL of dead biomass was selected as the sorbent dose in the further experiments. The removal efficiency of different amount of biomass was in order 0.2 g = 0.5 g > 0.1 g. The use of a smaller sorbent dose may be a more economically viable option.

Increase in the dye removal with adsorbent dose can be attributed to increased adsorbent surface area and availability of more adsorption site [4]. Similar results were reported by Asma *et al.* [2] for decolorization of Astrazone Blue by dead biomass of *Phanerochaete chrysosporium*. It was possible to increase the decolorization of Malachite

Green by increasing the adsorbent dose of the agro-industry waste [9]. Similar results were reported by Gong *et al.* [10] and Gupta *et al.* [11] for decolorization of Quinoline yellow and three anionic dyes, respectively. Also, the similar study by Kahraman and Yalcin [14] demonstrated that removal of Astrazone Blue and Red was dependent on the agro-industrial waste adsorbent dose.

Effect of initial pH on decolorization

The effect of initial pH on the dye removal capacity of dead biomass (*P. ostreatus*) was investigated in the pH range 2.0−8.0 To study the effect of pH on decolorization capacity of dead biomass, the experiments were carried out at 50 mg/L initial dye concentration with 0.2 g 20/ mL adsorbent mass (100 mesh) at 30°C for 60 and 120 minute time and 150 rpm (Figure 3). As shown in Fig. 3 the dye adsorption efficiency was not affected by pH except minor variations in pH of 2−8.

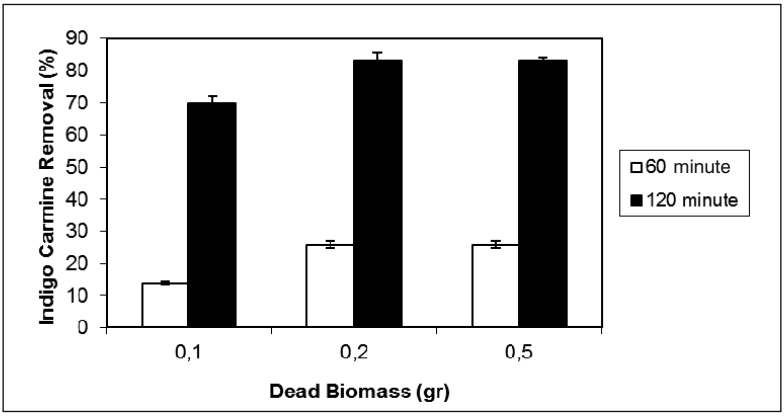

Fig. 2. The effect of dead biomass amount of *Pleurotus ostreatus* on the Indigo Carmine dye removal. (The initial dye concentration and temperature – 50 mg/L, 30°C, respectively)

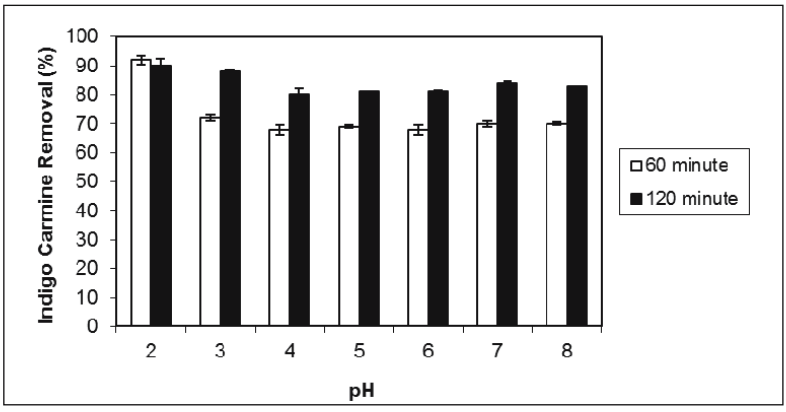

Fig. 3. The effect of pH on the Indigo Carmine dye removal. (The experiments were carried out at 50 mg/L initial dye concentration with 0.2 g 20/mL adsorbent mass (100 mesh) at 30°C and 150 rpm)

There are two possible mechanisms for the effect of pH on adsorption of dyes on any adsorbent: (a) electrostatic interaction between the protonated groups of carbon and acidic dye, and (b) the chemical reaction between the adsorbate and the adsorbent [19]. Dye molecules may interact with the functional groups on dead biomass via extremely complicated pathways. Also, there may be weak electrostatic interaction between the dye molecules and the electron-deficient sites on the surface of the dead biomass. However, for this study, the extent of IC adsorption onto dead biomass remained constant in the pH range of 4.0−8.0. This may be attributed to a second mechanism of weak electrostatic interaction [5, 16].

Removal of indigo carmine by dead biomass at different time intervals

In this part of study, dead biomass of P. ostreatus was tested for Indigo Carmine removal capacity at different time intervals. To study the effect of time on decolorization capacity of dead biomass, the experiments were carried out at 50 mg/L initial dye concentration with 0.2 g/20 mL adsorbent mass (100 mesh) at 30°C, pH 2.0 and 150 rpm. Color removal was observed to occur rapidly within 60 min (Table 1). Dye decolorization was high at the beginning of adsorption (within the initial 60 min) and after this period, the concentration of adsorbed dyes did not significantly change.

Effect of agitation on the removal of Indigo Carmine by dead biomass of P. ostreatus

The effect of agitation rate on Indigo Carmine removing was studied in the range of 0–250 rpm. To study the effect of the agitation rate on decolorization capacity of dead biomass, the experiments were carried out at 50 mg/L initial dye concentration with 0.2 g/20 mL adsorbent mass (100 mesh) at 30°C for 60 minute time and pH 2.0. Dead biomass showed high dye removal at all of the agitation rates (Table 1). As shown in Table 1, the optimal agitation values for decolorization were around 150–250 rpm. Slightly lower activities were found at lower agitation rates. At lower agitation speeds, the biomass particle agglomerated. Knapp *et al.* [15] reported that the decolorization of Orange II was 45% after 23-h incubation in static conditions and 98% in agitated conditions. High decolorization yield in agitated conditions offers many advantages over static cultivation for development of practical processes [22].

Table 1. Effect of Time and Agitation rate on the Removal (%) of Indigo Carmine by Dead Biomass of *Pleurotus ostreatus*

Time (Minute)	Indigo Carmine Removal (%) ± Sd	Agitation Rate (rpm)	Indigo Carmine Removal (%) ± Sd
30	88 ± 0,57	Static	92 ± 0,57
60	93 ± 0,57	50	93 ± 0,57
90	94 ± 0,00	100	94 ± 1,00
120	94 ± 0,57	150	95 ± 0,57
180	95 ± 1,00	200	93 ± 2,51
		250	95 ± 2,08

Sd: Standard deviation

Effect of initial dye concentration on decolorization

The influence of the initial concentration of dye in solutions on removal of Indigo Carmine was studied. The experiments were carried out at fixed adsorbent dose (0.2 g/20 mL) in the test solution, 30°C temperature, pH 2.0, fixed agitation (150 rpm) for 60 minute time at different initial concentrations of dye (50, 100, 200, 300, 400, 500 mg/L). The removal of dye by dead biomass of *P. ostreatus* was clearly dependent on the initial dye concentration of the solution (Figure 4). Dye removal was reduced from 93% to 64% as concentration was increased from 50 to 500 mg/L Indigo Carmine. Generally, the amount of dye in the industrial textile wastewaters is lower than 500 mg/L (approximately 10–50 mg/L). Therefore, these results are quite significant in terms of prevention of environmental pollution.

CONCLUSIONS

During the last decade, more attention has been focused on the development of new treatment technologies that lead to complete decomposition of dye molecules. In the present study, dead biomass of *P. ostreatus* was applied successfully for the sorption of Indigo Carmine. Operating conditions (dye concentration, pH and temperature) may negatively affect the decolorization potential of living cells. However, when compared with live biomass, dead biomass has many advantages. Dead biomass may be stored or used for extended periods and operation is easy and regeneration is simple. Dead biomass can be obtained from industrial sources as a waste product. Therefore, it can be used as cheap and effective biosorbent. The results obtained in this paper for the mechanisms involved in dye removal can be considered as a fundamental step for the representation of the experimental behavior and for development of process design.

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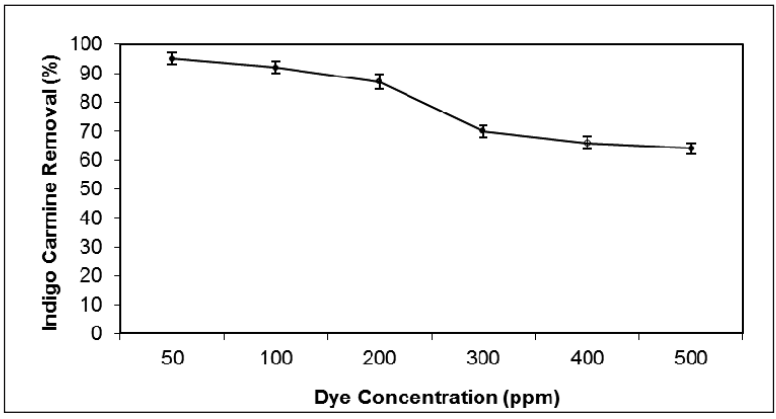

Fig. 4. The effect of initial dye concentration (mg/L) on the removal of Indigo Carmine by dead biomass of *Pleurotus ostreatus*. The experiments were carried out at fixed adsorbent dose (0.2 g/20 mL) in the test solution, 30°C temperature, pH 2.0, fixed agitation (150 rpm) for 60 minute time

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