

Removal of Dyes from Textile Wastewater Using Treated *Aspergillus tamarii* Biomass in Batch and Column Reactor

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Abstract: Dyes used in textile industries have a complex chemical structure; chemical and biological stability making them resistant to degradation process. They pose great threats to aquatic life and environmental safety and affect human health severely even in very small amounts when they discharged in water streams without treatments. There are many methods for decolorizing wastewater but they lack the ability to deal with all types of dyes. Recently, dye biosorption through biological means has gained momentum over other chemical and physical means as these are cheap and can be applied to a wide range of dyes. This investigation focused on the enhancement of the biosorption capability of *Aspergillus tamarii* biomass using combined chemical and physical methods; The optimum treatment was immobilization of *Aspergillus tamarii* biomass with gamma irradiation dose 10 kGy and 5% formaldehyde on loofa sponge in batch and packed bed column reactor; The residual dye concentration was 16.29 mg/l in batch system after 12 hrs and 22.5 mg/l in reactor mode using real textile dye effluent from one of textile dye facilities in Shobra El Khema, Cairo, Egypt, composed of three different dyes at concentration of 150 mg/l in both experiments. The experimental data fit both Langmuir and Freundlich adsorption isotherm.

Key words: Biosorption • Waste Treatment • Immobilization • Gamma irradiation • Packed Bed Bioreactors

INTRODUCTION

Synthetic dyes are released into the environment through industrial effluents which are hazardous to ecological systems and public health. Due to these concerns, the removal of dyes from wastewater has received considerable attention. Treatment of dye containing effluents is currently based on a variety of physicochemical procedures which are usually inefficient, costly and little adaptable to a wide range of dyes. These inadequacies of the currently used procedures have led to studies on alternative methods that may be applied more efficiently and effectively. Several investigations have been focused on biosorption of dyes by microorganisms such as algae, fungi, bacteria and yeast. Fungal biomass, among these, has been projected as an efficient and inexpensive sorbent that can be produced at low-cost, as it is available as a waste from various industrial processes [1]. Free cells are not suitable for use in a column reactor due to their low density and size. They tend to plug the

bed, resulting in large drops in pressure. Support matrices are suitable for biomass immobilization includes alginate, polyacrylamide, polyvinyl alcohol, polysulfone, silica gel; cellulose and glutaraldehyde have been used for the purpose. Due to their closed embedding structures, the immobilization matrices based on these polymeric gels, however, may cause mass transfer resistance resulting in restricted dye diffusion. Their use is further limited by their insufficient mechanical strength and the lack of open spaces to accommodate active cell growth resulting in their rupture and cell release into the growth medium. These problems were overcome by the application of fibrous network of loofa sponge (LS) and papaya wood in a novel procedure of fungal hyphae immobilization. The biostructural matrix of these plant materials has extensive surface area, depressions and cavities making it ideally suited for immobilization of fungal hyphae. Whereas fungal biomass immobilized on loofa sponge has been used for the removal of heavy metals; its application for dye sorption is limited to only one preliminary report

[2]. The potential of this study is the usage of real dye effluent from one of the textile facilities and the combined treatment that have been made on the fungal biomass. This investigation was aimed to develop an economic, biocompatible and easy handled biosorbent that can adsorb the dye system from the wastewater of the textile factories.

MATERIALS AND METHODS

Materials: All chemicals are of analytical grade provided by Sigma, USA. Dye effluent was obtained from one of the textile facilities in Shobra El Khema, Cairo, Egypt. It contains [sodium sulphate 50 g/l, Sodium carbonate 20 g/l, mixture of Remazol Reactive Blue, Remazol Reactive Yellow and Remazol Reactive Red 150 mg/l, pH 13.0]; Textile dye effluent was measured spectrophotometrically at 620 nm according to Data sheet of the dye. Fungal isolate used in this research was *Aspergillus tamaraii*. It was isolated and identified in the National Center for Radiation Research and Technology, Cairo, Egypt (NCRRT). The culture was kept on potato dextrose agar (PDA) at 4°C.

Immobilization: Two immobilizing materials were used, loofa sponge (LS); obtained from matured dried fruit of *loofa egyptica* and polyurethane foam; obtained from the local market. The LS and polyurethane foam were cut into discs of approx 2.4-2.5 cm and 2-3 mm thickness, soaked in boiling water for 30 mint and washed with distilled water several times. The LS and polyurethane discs were dried at 70°C and stored in desiccator till further use. Pre-weighted discs (0.5 g) were transferred to 250 ml Erlenmeyer flasks containing 100 ml of malt extract broth then autoclaved and on cooling inoculated with one loopful of *Asp. tamaraii* spores in each flask at 28°C under shaking (150 rpm) for 2 days. Similar procedure was repeated two other times; one without the discs, for the production of free biomass and the other without the fungal spores for the production of the control discs. The incubated cultures were harvested as *Asp. tamaraii* free biomass and *Asp. tamaraii* immobilized on loofa and polyurethane sponge discs. Dry weight of *Asp. tamaraii* biomass entrapped within the discs was determined as the weight difference of discs before and after *A. tamaraii* biomass immobilization procedure.

Effect of Pretreatment: After the incubation period was finished the discs were harvested, divided into two

equally weight samples the first one was subjected to autoclaving using Autester-E Pselecta autoclave at 121°C/1.1 bars for 30 mints then kept the discs in a cool, dry place for the next treatment. The second half of the discs were transferred to Egypt Mega-Gamma I unit supplied by Atomic Energy of India Ltd. at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt, subjected to doses 0.5, 1, 2, 3, 5, 7.5, 10, 15 and 20 kGy. The unit was furnished with a ⁶⁰Co gamma source. The dose rate during all the experiments was 1 kGy/h. After the treatment were completed, the irradiated / autoclaved immobilized biomasses were transferred as equal weight samples to 250 ml Erlenmeyer flasks containing 100 ml of 5% formaldehyde and boiled for 15 mint under reflux, the biomass was filtered and washed thoroughly with distilled water several times. The modified fungal biomasses were transferred into 250 ml conical flasks and brought into contact with 100 ml of wastewater solution with initial dye concentration of 150 mg/l. The initial pH of the solutions was adjusted at 2.00±0.1 by 1N hydrochloric acid; the flasks were agitated for 12 hrs at 150 rpm in rotatory shaker in ambient temperature. Samples were taken from the filtrate at the end of incubation period for the assay of residual dye concentration using SHIMADZU PC-1601 UV-VIS. spectrophotometer.

Effect of Initial pH of the Dye Solution on Dye Sorption:

The initial pH of the aqueous solution of dyes were adjusted at 2, 3, 5, 7, 9 and 11 before mixing with the biomass using 1N HCl. Fixed weight of the immobilized biomass was mixed with dye solution (150 mg/l) at the specified pH for 12 hrs at 150 rpm in a rotatory shaker. At the end of incubation period samples were taken for analysis using UV/VIS spectrophotometer.

Equilibrium Sorption Studies: Fixed weights of biomasses were transferred into 250 ml conical flasks and brought into contact with 100 ml of dye solution with predetermined initial dye concentrations (50, 100, 200, 300, 400 and 500 mg/l). The initial pH of the solutions was adjusted to 2.00 ± 0.1 by the addition of 1N Hydrochloric acid. The flasks were sealed and agitated for 12 hrs at 150 rpm in a thermostatic shaker until equilibrium was reached. At time $t=0$ and equilibrium, the dye concentrations of the solutions were measured by UV/VIS spectrophotometer.

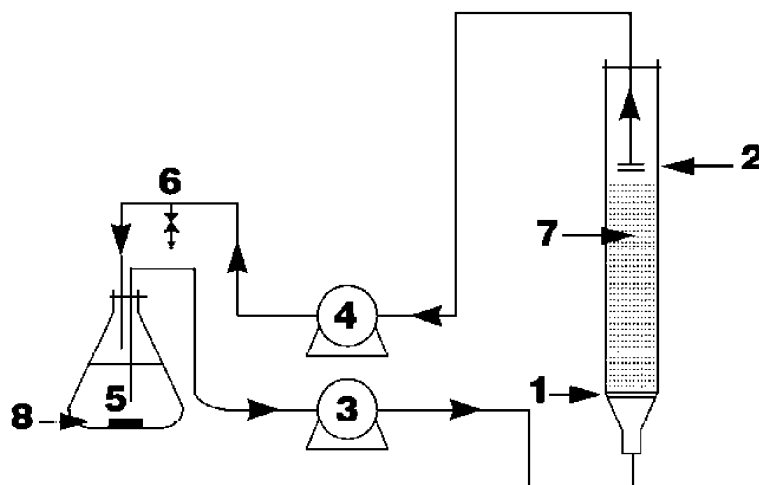


Fig. 1: Packed bed bioreactor diagram. (1) Sieve plate support. (2) Glass column (3 and 4) peristaltic pumps (5) Reservoir (6) Sampling valve (7) Matrix (8) Magnetic stirrer

Discoloration of Textile Dye Effluent Using Packed Bed Column Reactor:

A glass column of 2.06 cm in radius and 46 cm in height as shown in Fig. 1 was packed with 160 gm discs of freshly prepared immobilized *Asp. tamarii* biomass treated with 10 kGy absorbed dose and 5% formaldehyde solution. The pH of the textile dye effluent was adjusted by 1N HCl to pH 2 then pumped upwards through the column at a flow rate of 30 ml/min. Samples were collected at regular intervals from the effluent to measure residual dye concentration. As the bed was saturated, the dye loading was terminated and the bed was eluted with 0.1N HCl solution to recover the loaded dye. The regenerated bed was washed thoroughly with deionized water before use in the next cycle.

Statistical Analysis: Statistical analyses were carried out using Microsoft spread sheets and Sigma plot v.10.0 software.

RESULTS AND DISCUSSION

Effect of Immobilization, pH and Pre-Rreatment:

The differences in adsorption capability between free untreated fungal biomass and the immobilized untreated fungal biomass on both loofa and polyurethane sponge are illustrated in Table 1, the immobilized biomass on loofa sponge gave the lowest residual dye concentration

(52.2 mg/l) comparing with free untreated biomass and immobilized biomass on polyurethane sponge. The dye removal increases by the combined treatments been made on the fungal biomass. The fungal biomass pretreated with irradiation dose 10 kGy and with formaldehyde solution (5%) gave the lowest residual dye concentration 16.29 mg/l (Table 2). The pre-treatment of biomasses, either with autoclaving or gamma irradiation plus formaldehyde (5%), increased the adsorption capacity of the biomass to adsorb dyes but the treatment with radiation gave lower residual dye concentration than with autoclaving (Table 2).

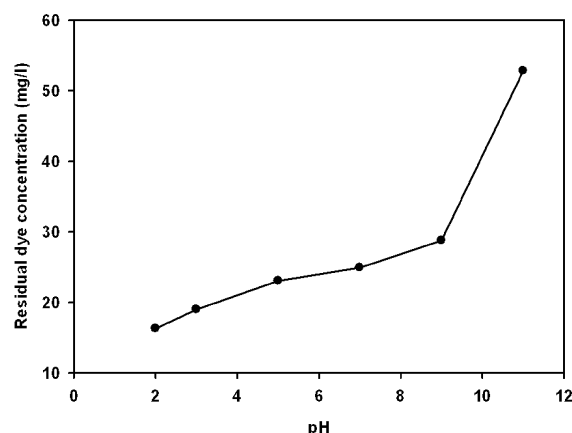
The free microbial cells are basically small particles, with low density, poor mechanical strength and little rigidity, which may come up with the solid-liquid separation problems, possible biomass swelling, inability to regenerate/reuse and development of high pressure drop in the column mode in real application. Excessive hydrostatic pressures are required to generate suitable flow rates in a fixed or expanded bed reactor. High pressures can cause disintegration of free biomass. These problems can be avoided by the use of immobilized cell systems. The immobilization of the biomass in solid structures would create a biosorbent material with the right size, mechanical strength, rigidity and porosity which are necessary for use in practical processes [3].

Table 1: Comparison between free and immobilized fungal biomass to adsorb dye

Item	Free untreated fungal biomass	Polyurethane sponge disc	Loofa disc	Immobilized untreated fungal biomass on sponge	Immobilized untreated fungal biomass on loofa
Residual dye concentration (mg/l)	90	127	125.55	54.3	52.2

Table 2: The difference in the effect of autoclaving and radiation on biosorption of dyes

Item	Immobilized autoclaved <i>Asp. tamarii</i> treated with 5% formaldehyde	Immobilized irradiated (10 KGy) <i>Asp. Tamarii</i> treated with 5% formaldehyde
Residual dye concentration (mg/l)	21.56	16.29

Fig. 2: Effect of pH on the biosorption of textile dye effluent using immobilized pretreated *Asp. tamarii*

The common supporting materials do not solve these problems in a sufficient way. Hence, it was necessary to use new supporting materials like loofa. The biosorption process involves mainly cell surface sequestration; the modification of cell wall can greatly alter the binding of dye molecules. Several methods have been employed for cell wall modification of microbial cells in order to enhance the dye binding capacity of biomass and to elucidate the mechanism of biosorption. Due to the important role of cell wall for dye biosorption by nonviable cells, dye biosorption may be enhanced by heat or chemical modification or by radiation. Thus, degraded cells would offer a larger available surface area and expose the intracellular components and more surface binding sites because of the destruction of the cell membranes [4]. It was found that the adsorption capacity of dyes by fungal biomass was increased after the autoclaving process than it in the case of living biomass. It could be attributed to that autoclaving process could disrupt the biomass structure and expose the potential binding sites for dyes biosorption and that will lead to an increase of surface area, an increase in porosity of the cell wall and expose latent sites, consequently increasing the dye adsorption.

The effect of ionizing radiation on the surface charges of the biomass can be explained on the basis of formation of more electrostatic charges on the surface of the biomass which will change the overall surface charge and modification of binding sites thus the formation of

electrostatic bonds between the biomass surface and the dye molecules [5]. The high results of the combined treatment could be attributed to the cross linking that was made between the free active groups on the irradiated cell wall and the formaldehyde leading to an increase of the adsorption area several times making some kind of network that was capable of capturing the dye molecules in their pores [6]. The effect of the initial pH of the dye solution was illustrated in Fig. 2. The adsorption capability increases with the decrease of the pH values and vice versa showing least residual dye concentration at pH 2 with residual concentration of 16.29 mg/l. Solution pH influences both the cell surface charges and the dye chemistry in water. The reactive dyes release colored dye anions in solution. The cell wall matrix of the fungi contains different functional groups such as carboxyl, hydroxyl, sulphate and other charged groups which are created by their complex heteropolysaccharides and lipid components. At lower pH values, the biomass will have a net positive charge. It is expected that nitrogen containing functional groups such as amines or imadaoles, in the biomass will also be protonated at acidic pH values. Higher uptakes obtained at lower pH values may be due to the electrostatic attractions between these negatively charged dye anions and positively charged cell surface. The reduction in adsorption capacity of dyes on fungal biomass with increasing pH could be attributed to change in surface characteristics and charge. As the pH of the system increases, the number of negatively charged sites increases and the number of positively charged sites decreases. A negatively charged surface site on the sorbent does not favor the adsorption of dye anions due to the electrostatic repulsion [7, 8, 9].

Adsorption Kinetics

Langmuir Isotherm: Langmuir equation is based on the assumption of a structurally homogeneous adsorbent where all sorption sites are identical and energetically equivalent. Theoretically, the sorbent has a finite capacity for the sorbet. Therefore, a saturation value is reached beyond which no further sorption can take place [10]. The saturated or monolayer (as $Ct \rightarrow \infty$) capacity can be represented by the expression:

$$q_e = K_L C_e / (1 + a_L C_e) \quad (1)$$

where q_e is solid phase sorbet concentration at equilibrium (mg/g), C_e is aqueous phase sorbate concentration at equilibrium (mg/l); K_L and a_L are Langmuir isotherm constants. Therefore, a plot of C_e/q_e versus C_e gives a straight line of slope a_L/K_L and intercept $1/K_L$, where K_L/a_L gives the theoretical monolayer saturation capacity, Q_0 . The Langmuir equation was applicable to homogeneous sorption where the sorption of each sorbate molecule onto the surface has equal sorption activation energy. Therefore, a linear expression of Langmuir equation is:

$$C_e/q_e = 1/K_L + a_L/K_L C_e \quad (2)$$

The sorption data were analyzed according to the linear form equation (2) of the Langmuir isotherm. The plots of specific sorption C_e/q_e against the equilibrium concentration, the isotherms of all three dyes were found to be linear over the whole concentration range studies and the correlation coefficients, were extremely high as shown in Table 3.

Freundlich Isotherm: The Freundlich equation is an empirical equation employed to describe heterogeneous systems, in which it is characterized by the heterogeneity factor $1/n$. Hence, the empirical equation can be written:

$$q_e = K_f C_e^{1/n} \quad (3)$$

where q_e is solid phase sorbate concentration in equilibrium (mg/g), C_e is liquid phase sorbate concentration in equilibrium (mg/l), K_f is Freundlich constant and $1/n$ is the heterogeneity factor. A linear form of the Freundlich expression can be obtained by taking logarithms of Equation (3).

$$\ln q_e = \ln K_f + 1/n \ln C_e \quad (4)$$

Therefore, a plot of $\ln q_e$ versus $\ln C_e$ enables the constant K_f and exponent $1/n$ to be determined for approaching of the adsorption on an "amorphous" surface. The amount adsorbed material is the summation of adsorption on all sites. The Freundlich isotherm describes reversible adsorption and is not restricted to the formation of the monolayer. The Freundlich equation predicts that the dye concentrations on the adsorbent will increase so long as there is an increase in the dye concentration in the liquid as shown in Table 4.

Table 3: Langmuir isotherms

Langmuir isotherm	Q_0	al	R^2
	329.32	0.058	0.990

Table 4: Freundlich isotherm

Freundlich Isotherm	K_f	n	R^2
	44.46	2.60	0.991

Packed Bed Reactor Studies: The performance of biosorbents in closed system dye biosorption process is often an important factor in assessing the feasibility of a biosorbent in real-time practical applications. For this purpose, discs were packed in a column bioreactor and the dye effluent solution was passed through the column in an upward direction at the flow rate of 30 ml/min.

$$\text{Dilution rate: } D = f/v \quad (5)$$

where D is the dilution rate which gave the indication of how often the reactor has to be filled per hour with the fluid flowing through it, f is the flow rate and v is the working volume which can be calculated from the equation (6):

$$\text{Working volume} = \pi r^2 h \quad (6)$$

where r^2 is the square of the radius of the column, h is the height of the column.

$$\text{Residence time } (\tau_m): \tau_m = 1/D \quad (7)$$

Residence time is the average duration of time that the flowing fluid spends in the reactor. The residence time was 20.47 min, which mean that the dye effluent solution will be in contact with the discs for 20.47 min. In this study the system mode is closed and recycled to make sure of maximum contact time between the matrix and the dye effluent to obtain high adsorption pattern as shown in Fig. 4. The dye loading curve showed an excellent pattern, before the breakthrough point at 120 min. The reactor can treat 5 liters of textile dye effluent in 120 min before reaching equilibrium. Desorption study made to determine the number of cycles the matrix could be used. After ending the first cycle, the matrix was soaked in 0.1N HCl and left for 60 min on rotatory shaker, then the matrix was washed with deionized water and the results of five cycles are shown in Fig. 3.

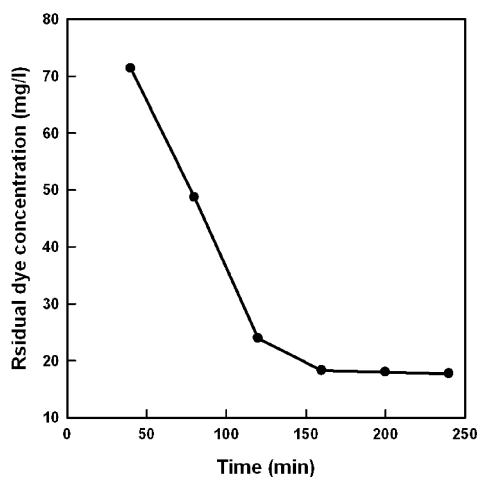


Fig. 3: Residual dye concentration in the packed bed column reactor against time.

CONCLUSION

This study reports the potential of using several modifications on the same biomass to increase its ability for adsorption of the dyes. In the future we hope to scale up the reactor capacity to reach the maximum volume for the industrial use.

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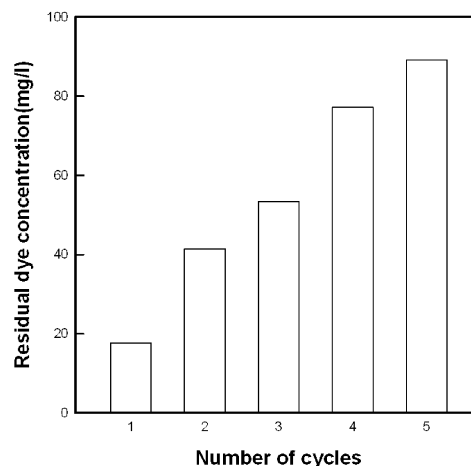


Fig. 4: Residual dye concentration difference after 5 cycles in the packed bed column reactor.

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