Bull. Environ. Contam. Toxicol. (2005) 75:1046–1053

DOI: 10.1007/s00128-005-0855-0

Bulletin of Environmental Contamination and Toxicology

Comparison of the Efficacy of Live and Autoclaved Mycelium of *Trichoderma harzianum* on the Removal of Trypan Blue

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Received: 15 May 2005/Accepted: 16 August 2005

Dyes are one of the major pollutants of the industries like textile, dye manufacturing, paper and pulp mills, electroplating, food and chemical etc. Approximately a half of all known dyes are azo dyes, making them the largest group of synthetic colourants (Selvam et al. 2003). Azo dyes are considered to be toxic to the aquatic biota and are reported to be carcinogenic to humans. The azo linkage present in the dye molecule form carcinogenic break down products. Azo dyes are widely used in textile industries. The complicated structure of azo dyes, makes the treatment process difficult. During the past two decades, several physico-chemical decolorization techniques have been reported, few however, have been accepted by the textile industries. However, these technologies are usually inefficient in terms of high cost, fails to remove the color and inapplicability to a wide variety of dyes (Ibrahim et al. 1996). Biosorption by microorganisms has been found as a useful method for treating dye effluents at a greater extent. Adsorption on microbial cell surface is the primary mechanism of decolorization (Knapp et al. 1995).

Bacteria such as *Pseudomonas cepacia* 13NA and *Bacillus subtilis* IFO-13719 are capable of dye decolorization (Ogawa and Yatome 1990; Yatome et al. 1991). Since, fungal biomass can be produced cheaply and obtained as a waste from various industrial fermentation processes (Kapoor and Viraraghavan 1995), it is widely utilized for dye decolorization studies. Wang and Yu (1998) reported the adsorption of acid green 27, acid violet 7 and indigo caramine dyes on live and dead mycelia of *Trametes Versicolor*. The present study deals with removal of trypan blue by live and autoclaved *Trichoderma harzianum* (Subramaniam, 1983) biomass in batch mode operation.

MATERIALS AND METHODS

A stock solution of 1000 mg/L of trypan blue dye solution was prepared by dissolving 1.0 g of dye in double distilled water and made up to 1000 mL. From the stock solution, various concentrations of dye solutions such as 10,20,30,40 and 50 mg/L, were prepared. The wavelength maximum absorption (λ max) 450 nm was set as a monitoring wavelength for the analysis of various dye concentrations spectrophotometrically (Shimadzu, UV-Vis, Japan).

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For the preparation of live biomass, 1.0 mL (10^6 spores) of *Trichoderma harzianum* spore suspension was inoculated into Czapek-Dox broth in 250 mL Erlenmeyer flasks and incubated at room temperature (27° ± 3°C) for five days in an orbital shaker at 125 rpm. At the end of fifth day, the mycelium developed as pellets were separated by filtration through Whatman No.1 filter paper. Then the biomass was washed with generous amount of deionized water until free from the media components. A portion of washed pellets was autoclaved at 15 lbs for 15 min, to obtain "autoclaved biomass". Both the live and autoclaved biomass were used as biosorbents in this study. To determine the adsorption efficiency of the biosorbents, 1.0 g of the biosorbent was added to 50mL of dye sample and agitated in a rotary shaker at 125 rpm for predetermined time at 30°C. The adsorbate and biosorbent were separated by centrifugation at 10,000 rpm for 10 min. The dye remaining in the adsorbate was quantified spectrophotometrically. The study was carried out with different dosages of biosorbent ranging from 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0g/50 mL, at predetermined equilibrium time.

The effect of pH was studied with and without adsorbent in order to determine whether the dye has been adsorbed or precipitated by changing the pH of the aqueous solution. The experiment was carried out with different pH ranges from 2.0-10 using HCl (1.0 N) and NaOH (1.0 N) with optimum dosage of adsorbent for predetermined time at 30°C. The Langmuir, Freundlich and Lagergren isotherms were determined from the data obtained in the batch mode studies. Control experiments were carried out in absence of the biosorbent in order to find out whether there is any adsorption on the container walls. No adsorption onto the container walls was observed.

RESULTS AND DISCUSSION

In general, uptake of dye increased with increase in contact time, but remained constant after an equilibrium time. The optimum equilibrium time required for the removal of trypan blue by live and autoclaved biomass were 90 min and 75 min, respectively. The difference in equilibrium time required for autoclaved and live biomass was due to differences in affinity of adsorbent for dye. The study revealed that comparatively autoclaved mycelium was more efficient in dye adsorption. Mou et al. (1991) reported that autoclaved *Ganoderma* spp and *Myrothecum* spp cells were effective in adsorption of orange II, 10 B (Blue) and RS (Red) dyes. The data also suggests that the removal of dye increased rapidly in the beginning and then declined slowly until the equilibrium. This may be due to prevalence of strong attractive forces between the dye molecules and the sorbent and fast diffusion of dyes into the intraparticle matrix to attain rapid equilibrium.

The study revealed that, adsorbent dosage of 1.0 g/50 mL was the optimum dosage for dye removal. Consequently, this biosorbent dosage was selected for further studies. Availability of more surface area of the adsorbent could be the reason for increase in adsorbent dosage (Kadirvelu et al. 2000).

The pH of the solution has a significant influence on the adsorption of the released charged groups onto the adsorbent surface (Ramakrishnan and Viraraghavan 1997). The optimum pH was found to be 8.0 for live and autoclaved biomass. The results showed that increase in solution pH increased the precipitation of dye and decrease in pH results in less precipitation in the absence of adsorbent. The optimum pH for color removal is often at a neutral pH value or a slightly alkaline pH value. (Pearce et al. 2003). But in the presence of adsorbent, dye removal was higher than precipitation and the removal was observed even at low pH levels. Adsorption equilibrium data, express the relationship between mass of adsorbate, adsorbed per unit weight of adsorbent. The data obtained from the batch mode studies helps in the determination of optimum conditions for the removal of trypan blue were adsorbent dosage of 1.0g/50 mL, pH of 8.0 and contact time of 90 min for live and 75 min for autoclaved mycelium.

The data were analyzed for the applicability of Langmuir, Freundlich and Lagergren isotherms. These isotherms are used to describe the adsorption kinetics for a range of adsorbate concentrations. Adsorption isotherms represent the liquid phase equilibrium concentration of adsorbate and also used to design data for adsorption system (Walker et al. 2001). The Langmuir model assumes uniform energies of adsorption onto the surface and no transmigration of adsorbate in the plane of surface (Langmuir, 1918). The linearized form of the Langmuir isotherm was used to calculate the maximum adsorption capacity of the adsorbent. The Langmuir isotherm is represented by the following equation.

$$C_e/q_e = 1/Q_0b + C_e/Q_0 \qquad (1)$$

Where q_e is the amount of dye adsorbed per unit of adsorbent, C_e is the concentration of dye remaining in solution at equilibrium; Q_0 is the maximum adsorption capacity and b is the Langmuir constant. The Langmuir plots for adsorption, follows the typical Langmuir pattern as shown by the linear transformation of the experimental data (Fig.1). Langmuir constants, Q_0 and b were determined from the slope and intercept of the plots respectively.

When comparing the Q_0 and b values, there was not much difference in adsorption of live and autoclaved mycelium; the Q_0 values were 2.32 and 2.33 respectively. Walker and Weatherley (2001) reported that the Q_0 values for the adsorption of tectilon blue 4 R – 01 and tectilon red 2 B onto bone char brimac were 0.2740 and 0.758 respectively. The Q_0 values for the adsorption of remazol turquoise blue and crystal violet onto activated charcoal (30°C) and neem sawdust at 45°C were 0.12 and 1.99 (mg/g) respectively. When compared to the above reported adsorbents the fungal biomass used in the present study showed high Q_0 values, revealing their efficiency in dye removal. The b values of live and autoclaved biomass were 4.11 and 4.13 respectively.

The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L (Hall et al. 1966), which is given by the equation.

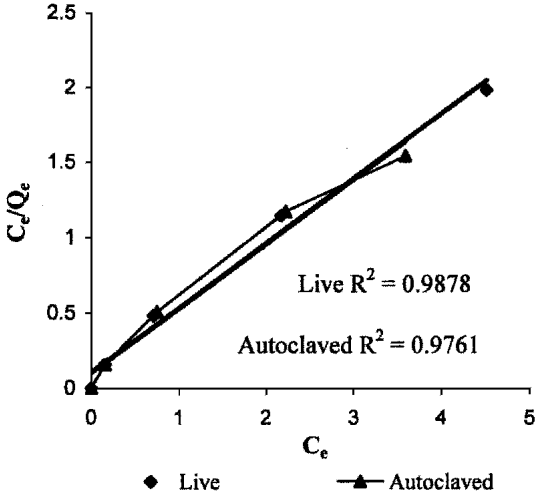

Figure 1. Langmuir plots for dye removal

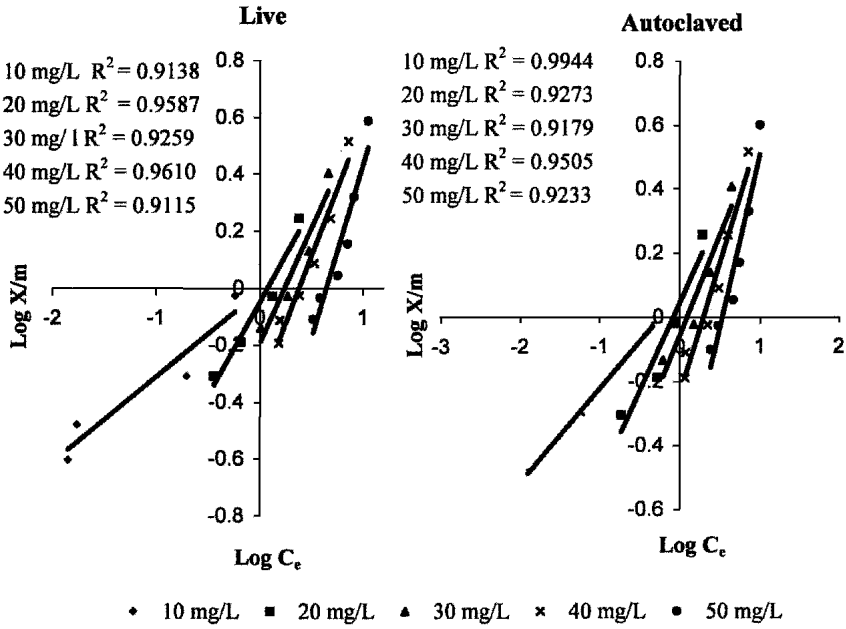

Figure 2. Freundlich plots for dye removal

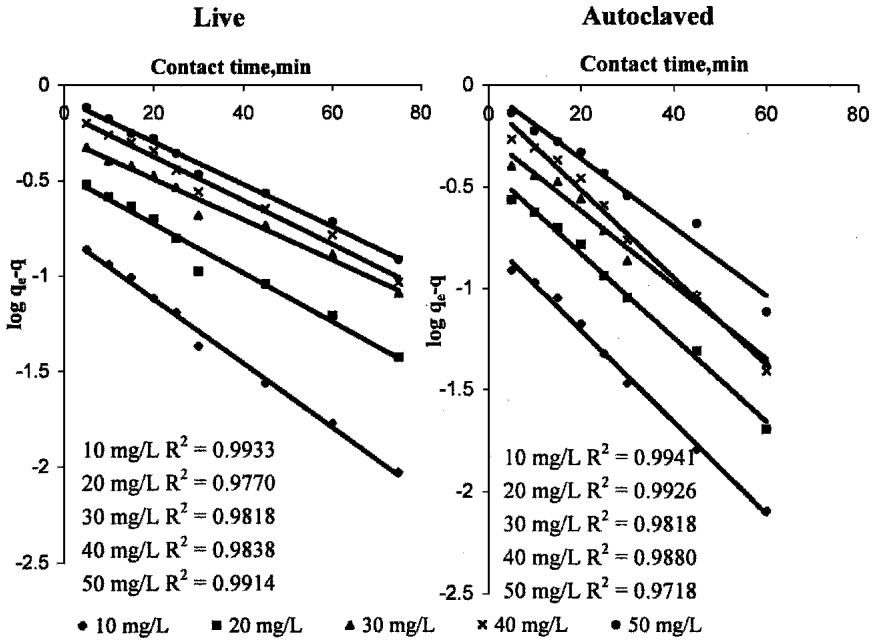

Figure 3. Lagergren plots for dye removal

Table 1. Freundlich and Lagergren constants for removal of trypan blue

Dye Concentration (mg/L)	Freundlich				Lagergren	
	Live		Autoclaved		Live	Autoclaved
	k_f [mg/g (L/mg)n]	n	k_f [mg/g (L/mg)n]	n	k_{ad} (L/min)	k_{ad} (L/min)
10	0.972	3.325	1.169	3.408	0.038	0.052
20	0.888	1.514	1.105	1.805	0.029	0.047
30	0.636	1.232	0.874	1.606	0.024	0.041
40	0.437	1.06	0.567	1.213	0.026	0.049
50	0.164	0.824	0.267	0.93	0.025	0.038

$$R_L = 1 / 1 + b C_o \qquad (2)$$

Where, C_o is the initial dye concentration (mg/L), b is the Langmuir constant (L/mg), and R_L is the shape of the isotherm. R_L values of live and autoclaved mycelium at different adsorbate concentrations were always less than one and more than zero (0.004 for 50 mg/L to 0.023 for 10 mg/L dye concentration) indicating favorable adsorption of adsorbates onto the adsorbent.

Freundlich equation can be derived by assuming that, free energy of adsorption decreases logarithmically as adsorption density increases. The Freundlich equation is

$$\text{Log } X/m = \text{Log } k_f + {}^{1/n} \log C_e \qquad (3)$$

Where, C_e is the equilibrium concentration (mg/L), X/m is the amount adsorbed at equilibrium time (mg/g), k_f and n are Freundlich adsorption isotherm constants, n gives an indication of favorability and k_f [mg/g/(L/mg)^n], the capacity of the adsorbent (Pollards et al. 1991). This Freundlich isotherm is applied for the adsorption of dye by the live and autoclaved biomasses. Linear plots of log C_e Vs log X/m show that the adsorption of dyes onto the biosorbents follows the Freundlich isotherm model (Fig.2). The values of n and k_f were calculated from the slope and intercept, and are presented in Table 1. Ramakrishna and Viraraghavan (1997) reported n values for adsorption of acid blue 29 onto bentonite and slag as 0.333 and 0.279 and k_f values as 4.31×10^{-6} and 3.7×10^{-6} respectively. Ghosh and Bhattacharya (2001) reported n and k_f values for the adsorption of methylene blue and safranine onto rice husk carbon at 30° C as 0.35 and 0.10 and k_f values as 57.54 and 204.17 respectively.

The rate constant of adsorption was determined from the following first order rate expression.

$$\log (q_e - q) = \log q_e - k_{ad} / 2.303 \times t \qquad (4)$$

Where, q and q_e are amounts of dye adsorbed (mg/g) at time, t (min) and at equilibrium respectively. k_{ad}, is the rate constant for adsorption (1 /min).The straight line plots of log (qe – q) Vs time (t) for different dye concentrations indicate the applicability of the above equation (Fig.3). Values of k_{ad} were calculated from the slope of linear plots and are given in Table 1. The k_{ad} values obtained in the present study are comparable with the k_{ad} values reported by Jain et al (1999) and Khattri and Singh (2000).

Among industrial wastewaters, dye wastewater from textile and dyestuff industries is one of the most difficult to treat. Conventional treatment technologies are not economical and ecofriendly in the context of growing countries and so there is a need to develop low cost technologies for effective dye removal. Physical and chemical methods used for removal of dyes, i.e. chemical transformation, incineration, photocatalysis, ozonation, precipitation or reverse osmosis are effective but rather costly (Banat et al. 1996; De Moraes et al. 2000).

The potential usage of dead fungal biomass as biosorbents has received a significant importance because the biomass is obtained cheaply as a by-product from several fermentation processes and produces less sludge (Volesky et al. 1993). Dead fungal biomass of *Aspergillus niger* was found to be effective in removing congo red from an aqueous solution (Yuzhu Fu et al. 2002). When compared to live biomass, the autoclaved biomass was found to be more effective. Cell surfaces are mainly anionic due to the presence of ionized groups such as carboxylate, hydroxyl and phosphate in various cell wall polymers. Cells, when subjected to autoclaving can suffer rupture and denaturation of cell wall that can allow free access of cell wall binding sites (Hughes and Poole 1989). This could be the reason for enhanced dye adsorption by autoclaved biomass as observed in the present investigation. Compared to live fungal cells, dead fungal biomass possesses various other advantages such as absence of nutrient needs and ease of regeneration (Gadd, 1990).

The present study revealed that the autoclaved fungal biomass could be effectively used as a biosorbent for the removal of trypan blue from aqueous solution, at the same time it can be exploited for the treatment of wastewater effluents generated from various industries. This biological treatment process would be a promising cost effective and eco-friendly approach in treating dye industry effluents.

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