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18. Biodecolorization of Reactive Blue 19 Dye from Effluents by Sorption on *Aspergillus brasiliensis* Fungal Biomass

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Abstract: Water quality resources in the current context of excessive pollution, are in constant decline. This involves a much better control over the water pollution phenomenon. Thus, strategies are needed to maintain effluent control. This is equally important for the biocenoses of the aquatic environment, so that optimal conditions can be created for organisms living in water and, last but not least, those who benefit from water resources. Dyes represent the main sources of water contamination responsible for the continuous pollution of the environment. The potential of the hyphal network characteristic of fungal morphology represent an alternate to the conventional treatment methods. The main advantages of biosorption are environmental friendly, cost effective, pollutants high selectivity and good removal efficiency. The research was conducted at laboratory scale using biosorbents derived from starter culture *Aspergillus brasiliensis* **(**ATCC 16404) of fungal biomass and their capacity to removed Reactive Blue 19 Dye (CAS no. 2580-78-1) was evaluated. The influence of process variables such as pH, temperature, exposure time and initial dye concentration had its purpose to highlight their effects in sorption process. The initial biosorbent system parameters were had values of pH = 3.0 and temperature value beginning from 20°C. The equilibrium data fitted well with Langmuir and Freundlich sorption isotherms and their corresponding sorption parameters such as KF and KL. Furthermore, the temperature and pH of these effluents is important, because have the ability to control the biosorption process and ultimately affect their efficiency. The high biosorption performance has been reached for *Aspergillus brasiliensis* ATCC 16404 biomass at high pH values in range of 6.0 – 7.0 and low temperature T=20°C. For *Aspergillus brasiliensis* ATCC 16404 biosorbent, maximum removal efficiency of 78.67% was obtained, already proven to be very effective in dye biosorption. Finally, the results revealed that this biomass can be considered an exceptionally versatile material and excellent biosorbent.
Keywords: biosorbent, dyes, fungal, effluents, *Aspergillus brasiliensis.*

INTRODUCTION

The dyes have environmental impact due to their toxic and reactive properties. Used in various industrial activities such as textile, plastic, cosmetic, paints, rubber, paper, these are most harmful to the environment due to the high discharge volume and diversity in their chemical composition. (Nwabanne and Mordi, 2009) One of the largest producers of effluents charged with dyes is the textile industry. (Marimuthu et al., 2013) The wastewater from textile is also bind with water-soluble and fast dispersible organic compounds, having high polluted potential. At all this, it also contributes amounts of dye lost during the dying process. (Venkata and Karthikeyan, 2004) Textile effluents are highly colored and discharging them into an emissary without prior treatment inevitably affects the equilibrium of aquatic ecosystems. (Novotny et al., 2006; Khalid et al., 2009) Last but not least, color affects the life of aquatic plants, by increasing turbidity and thus, inhibits sunlight penetration, reducing photosynthetic ability. (Sivaraj et al., 2001) Concerning to the environmental impact, it is also worth mentioning the risk induced in human health, due to their carcinogenic and mutagenic potential. (Guaratini and Zanoni, 2000)

Depending on the chemical structure, textile dyes are classified in multiple classes (azo, anthraquinone, phthalocyanine, xanthene, nitroso, nitro, etc.) in accordance with different classes of use (i.e.acid, base, disperse, reactive, vat, etc.). (Venkata and Karthikeyan, 2004) Azo dyes are especially recalcitrant due to their xenobiotic nature and manifest considerable resistance to decolorization treatments. (Hua et al., 2013)

Conventional treatments, such as physicochemical methods: adsorption on activated carbon, electrocoagulation, flocculation, froth flotation, ion exchange, membrane filtration, ozonation, and reverse osmosis are used for decolorization of the effluents containing textile dyes. (Ogugbue and Sawidis, 2011) These are generally inefficient, more expensive, and with less applicability because of the chemical stability of these pollutants. (Forgacs et al., 2004) Effluents containing these compounds resist many types of treatments due to their molecular complexity.
For that reason, alternative treatments, such as biodegradation, are still being studied widely to solve the problems caused by these substances, on their harmful effects on the environment and organisms. (Almeida and Corso, 2014)

Biosorption process represents the adhesion of a chemical structure to the surface or pores of a biological substrate. The biosorbents such as chitin, bacteria, yeasts, filamentous fungi or algae are characterized by a large variety of functional groups that form complexes with dye molecules, and thus allow their subsequent removal from the effluent system. (Crini, 2006)
Biodegradation is a technique that biodegradation is a technique involving different types of microorganisms, which by their metabolism are able to convert complex chemical molecules into simple molecules. (Solís et al., 2012) Microorganisms are commonly found in polluted environments and can easily adapt to different sources of carbon and nitrogen and thus obtain the energy required for growth and reproduction.

However, the microbial decolorization and degradation of dyes has gained considerable interest for researchers because it is inexpensive, environmental-friendly and produces less amount of sludge. (Carvalho et al., 2008; Parshetti et al., 2006)
Filamentous fungi have been widely studied for use in biosorption and biodegradation of dyes. One of the main morphological features of filamentous fungi is the hyphae network, which makes them an excellent biomaterial for the absorption, adsorption and filtration of the dyes from the water.
This paper proposes the use fungal biomass of *Aspergillus brasiliensis* ATCC 16404 to remove Reactive Blue 19 dye from wastewater and assessing its capacity under different temperature, pH, volume and concentration conditions.

MATERIALS AND METHODS

Reactive Blue 19 dye, no. CAS 2580-78-1 with the similar name of Remazol Brilliant Blue R was provided by Sigma-Aldrich Company. The chemical formula of this dye is $C_{22}H_{16}N_2Na_2O_{11}S_3$ with molecular weight of 626.54g/mol, as can be shown in figure 1.

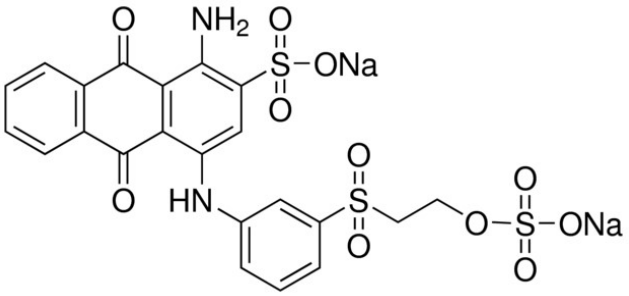

Figure1. Molecular structure of Reactive Blue 19 dye

In this study it works as a substance to be absorbed. Distilled water was used to prepare all the solutions and reagents. The experimental dye solutions were prepared by diluting the Reactive Blue

19 dye stock solution, without further purification, in accurate doses to obtain different initial concentrations varying from 2 to 10 mg/L.
Biosorbents was prepared at laboratory scale using stock culture collection of *Aspergillus brasiliensis* ATCC 16404 made available by Liofilchem Company from Italy. To develop fungal biomass, a Czapek - Dox liquid growth medium was prepared. The composition of this medium is detailed in Table 1.

Table 1. Czapek - Dox liquid growth medium for fungal culture

Composition	Quantity for 1 liter
Sucrose	30 g
$NaNO_3$	3 g
KCl	0.5 g
$MgSO_4 \cdot {}_7H_2O$	0.5 g
$FeSO_4 \cdot {}_7H_2O$	0.01 g
K_2HPO_4	1 g

The pH of medium was adjust at 6,0 ± 0,2. Inoculation has been achieved from stock culture in 100 mL Erlenmeyer flasks, contains liquid grown medium for 7 days. To obtained filamentous biomass, whose hyphae and conidia can be seen in the microscope images of Figures 2 and 3.

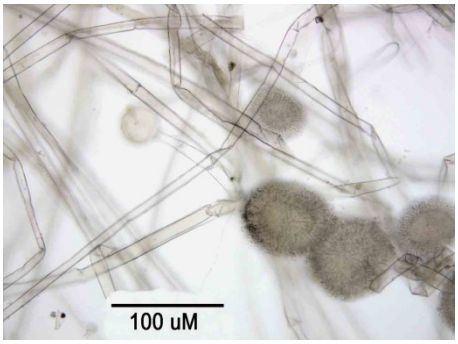

Figure 2. Microscopic view of *Aspergillus brasiliensis* hyphae

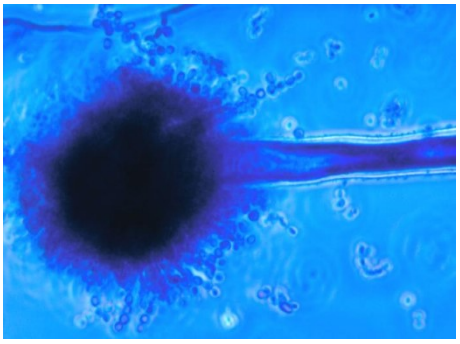

Figure 3. Conidia head of *Aspergillus brasiliensis*

Biomass has developed, previously, the contents of each flask were filtered through the Whatman filter paper, inactivated by autoclaved at 121 °C for 20 minutes and after that was dried.
Sorption system was made up of: 300 mL shaking flasks, each containing 100 mL of working dye solutions at different concentrations and pH values. Afterwards, each flask received an amount of biosorbent in range of 1 - 5 g/L inactivated fungal biomass and put at orbital shaking. For accuracy of results and to avoid volume changes in dye solutions, the flasks have been hermetically sealed.
The system variables were taken into account, at five different levels as follows: biosorbent quantity (1 - 5 g/L), temperature at 20 – 40 °C, pH values of the initial solutions in range of 3.0 - 7.0, initial dye concentrations in solutions from 2 to 10 mg/L, shaking mode correlated with contact times during 30 - 240 minutes.
Biosorption studies were performed dynamically by orbital shaking using GFL 3031 device, being endowed with thermostat and temperature control mode. The pH of the solutions was determined with the HI 98129 multimeter from Hanna Instruments and adjusted with 0.1 M HCl or NaOH. The dye concentrations were determined spectrophotometrically, from the supernatant of the solutions studied at an absorbance value of 665 nm.

The biosorption system capacity was estimated by dye removal, calculating R% (removal) from following equation:

$$R = \left(1 - \frac{C_e}{C_0}\right) \times 100 \quad (1)$$

The equilibrium of biosorption performance was determined by the next equation:

$$q_e = \frac{(C_0 - C_e)\ V}{w\ 1000} \quad (2)$$

where,
q_e - the equilibrium sorption capacity, expressed in mg of dye adsorbed /g of biosorbent;
C_0 and C_e - the initial and final dye concentrations in the working solutions expressed in mg/L;
V - the volume of the solution (mL)
m - the weight of the dried biosorbent (g).

RESULTS AND DISCUSSIONS

Taking into account the effect of biosorbent dose on the biosorption process, in range of studied values 1 to 5 mg/L, it can be noticed that with increasing the dose of biosorbent increases the dye removal percentage, as this means a larger contact surface, which will obviously take over a larger amount of dye. According to the figure 4, at the biosorbent dose of 1 respective 2 mg/L, the biosorption process decreases in intensity after the first 120 minutes due to the faster saturation of the sorbent compared to the doses of 4 and 5 mg/L at which the sorption process after 120 minutes keep on.

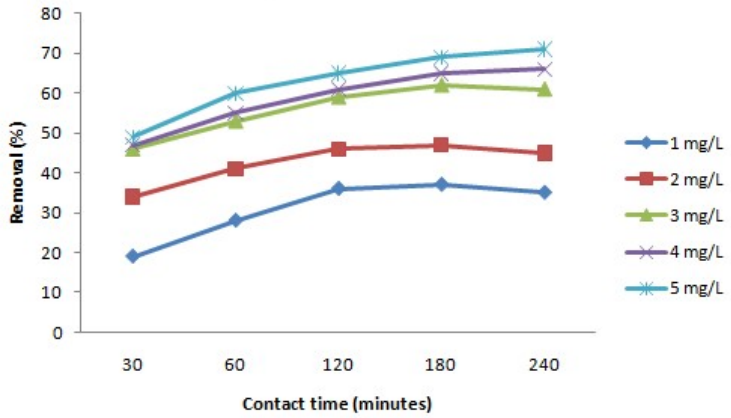

Figure 4. Effect of fungal biomass quantity in biosorption process

The effect of initial dye concentrations expressed in mg of dye/g of biosorbent, indicates that the removal of dyes depends on the concentration of the colored solution. It is therefore, when increasing the concentration of colored solution from 2 to 10 mg/L, the amount of biosorbed (*qe*) increases from 0.9 to 3.9 mg/g after 240 minutes of process in dynamic mode (figure 5).

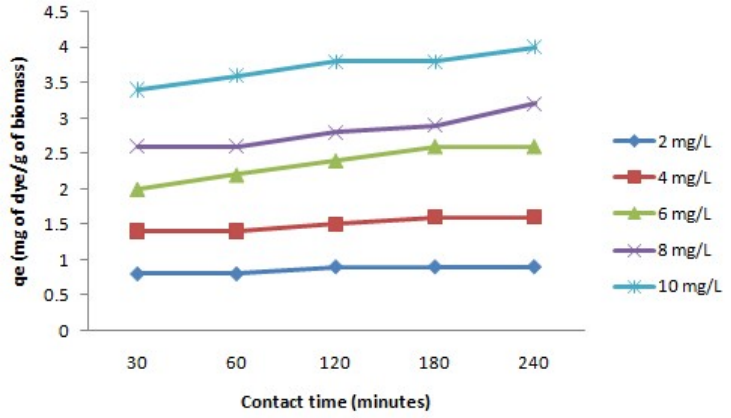

Figure 5. Effect of initial dye concentration in biosorption process

A very important parameter that can influence the biosorption process is temperature. As the temperature was higher (35 - 40 °C) it was found that the yields were lower than the bottom temperature range (20 - 30 °C). It can be mentioned, (figure 6) at temperatures of 35 respective 40 °C, after a contact time of 180 minutes the sorption process decreases. The higher the temperature, removal efficiency (%) is lower, because the sorption is an exothermic process. It is finally found that the maximum efficiency of the biosorbtion process is recorded at an ambient temperature of 20 - 25 ° C. Moreover the efficiency of the process is higher after 30 minutes of contacting time at 20 °C, but at the end of the process the yields are equal.

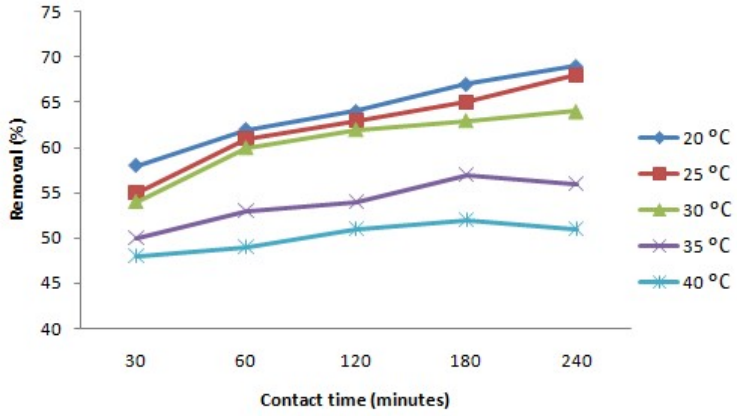

Figure 6. Efficiency of biosorption process and removal percentage at different temperatures

In close correlation with the temperature of the sorption process is also the pH of the working colored solutions. It ranged from acid to neutral (3.0 to 7.0). In this range, the sorption process dynamics varied on three levels, according to figure 7. At the pH value of 3, the process recorded a very low efficiency, tends almost to 0. At pH values of 4 and 5, the biosorption percentages are relatively similar with an insignificant increase to pH 5 from pH 4 during 240 minutes. While at pH values of 6 and 7, higher sorption yields than more acidic pH are recorded and the process are keep up after 180 minutes.

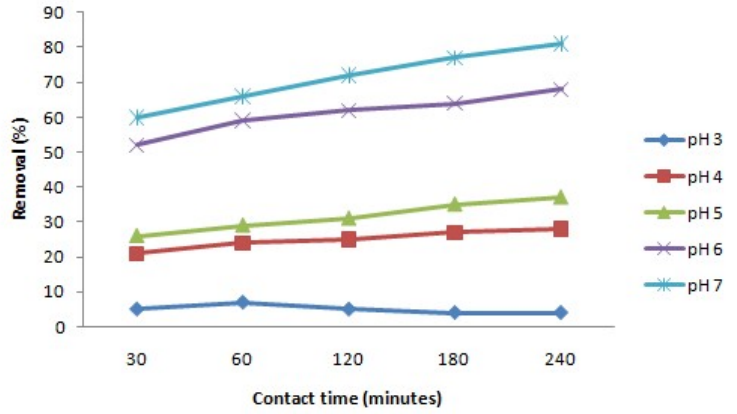

Figure 7. Efficiency of biosorption process and removal percentage at different pH values

To be able to highlight the shaking effect in the biosorption process two types of samples were prepared under the same conditions, with the specification as one of them was placed in the orbital shaker and the other one has been studied in static mode. As can be seen in figure 8, the dynamic regime facilitates the biosorption process of the dye. Dyestuff particles adhere more quickly to the biosorbent by being entrained by the shaking process. Thereby, net yields higher than static mode can be obtained. However, intensity of the sorption process in the dynamic mode diminishes after 120 minutes, because the biosorbent saturates.
In the static regime, the saturation of the biosorbent occurs in a longer time, the yields are lower, the biosorption process is maintained even after 180 minutes.

Maximum removal efficiency of 78.67% was obtained in dynamic mode after 240 minutes.

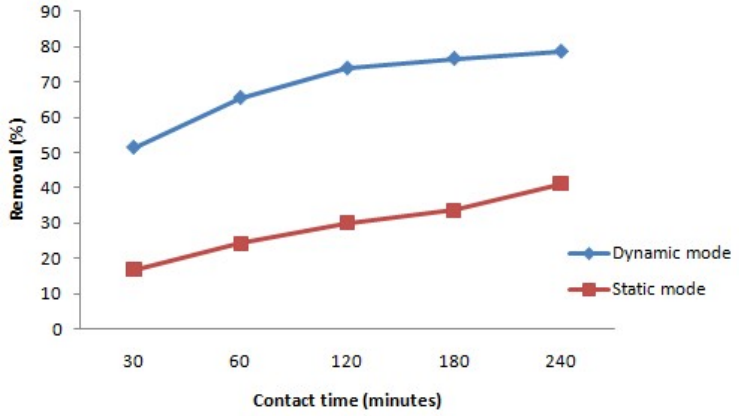

Figure 8. Contact time and shaking influence in biosorption process

For the equilibrium modeling was used mathematical expressions to calculate sorption isotherms. In this regard, the Langmuir and Freundlich expression and their corresponding parameters were given by the equations (Freundlich and Heller, 1939; Langmuir, 1916):

Langmuir: $$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \quad (3)$$

Freundlich: $$q_e = K_F \; C_e^{\frac{1}{n}} \quad (4)$$

The linear forms of the Langmuir and Freundlich equations can be expressed as follows:

$$\frac{C_e}{q_e} = \frac{1}{q_m} C_e + \frac{1}{K_L q_m} \quad (5)$$

$$\ln(q_e) = \ln(K_F) + \frac{1}{n} \ln(C_e) \quad (6)$$

In table 2 can be seen the calculated Langmuir and Freundlich constants at 20, 25, 30, 35 and 40 °C for biosorbent. Results indicates that biosorption of Reactive Blue 19 dye by *Aspergillus brasiliensis* depends on the Langmuir isotherm at different parameters with a regression coefficient in range of 0.905 - 0.971. The essential characteristic of a Langmuir isotherm is expressed by a balance parameter K_L, and for the values obtained the isotherm is considered favorable. The maximum adsorption capacity *Qmax* recorded 15.7 mg/g.

Table 2. Biosorption isotherms constants for Reactive Blue 19 dye onto *Aspergillus brasiliensis* inactivated biomass at different temperatures

Temperature (^{0}C)	Langmuir			Freundlich		
	Qmax (mg/g)	K_L (L/mg)	R^2	n	K_F	R^2
20	15.7	0.18	0.971	1.47	3.43	0.952
25	15.5	0.17	0.969	1.48	3.21	0.941
30	12.3	0.14	0.934	1.53	2.67	0.927
35	11.8	0.12	0.918	1.67	2.14	0.905
40	10.1	0.10	0.905	1.73	1.98	0.892

The adsorption obeys not only to the Langmuir model but also the Freundlich model. A regression coefficient situated in range of 0.892 up to 0.952 is obtained with the Freundlich isotherm. The values of K_F and n are 1.47 – 1.73 mg/g and 1.98 – 3.43 respectively. In this case the adsorption is favorable, because n values are situated between 1 and 10.

The above mentioned indicates that the surface of the biomass is heterogeneous and also the biosorption process takes place locally with a different affinity to the dyestuff particles.

CONCLUSIONS

This study has focused to evaluate the biosorption of the azo dye Reactive Blue 19 in solutions with the biomaterials from filamentous fungi, *Aspergillus brasiliensis* (ATCC 16404), in line with the search for novel techniques for the wastewater treatment.

It was highlighted that the sorption behavior of Reactive Blue 19 on the *Aspergillus brasiliensis* (ATCC 16404) biosorbent could be described by the Langmuir and Freundlich models.
Biosorption capacity is highly dependent on initial dye concentration, pH, temperature and sorbent dose. When increase the initial concentration of colored solution, the amount of biosorbate (mg/g) increases and with increasing the dose of biosorbent increases dye removal percentage. Maximum removal efficiency of 78.67 % was reached for *Aspergillus brasiliensis* (ATCC 16404) inactivate fungal biomass, after 240 minutes in dynamic mode.

The results indicate that biosorption decreases with increase in temperature, because the sorption is an exothermic process. Moreover, the efficiency was increased at pH 7.0, whereas it was diminished at pH 4.0 and at pH 3.0 it has a very low yield.
The biosorption process has a higher efficiency in the first 120 minutes, after which it diminishes.
Based on the present findings, this biomass can be considered an excellent and exceptionally versatile biosorbent material.

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