Journal of Science and Technology
ISSN: 2456-5660 Volume 7, Issue 01 (JAN-FEB 2022)

www.jst.org.in *DOI: https://doi.org/10.46243/jst.2022.v7.i02.pp01-10*

BATCH MODE BIOREMEDIATION STUDY ON REACTIVE BLUE-HER BY *Cladosporium oxysporum*

Saritha E[1,2], Swaminathan K[2], Gayathri S[1], Madhumitha R[1]

[1]*Department of Biotechnology, PSG College of Arts & Science, Coimbatore-641014, Tamil Nadu, India.*

[2] *Department of Microbial Biotechnology, Bharathiar University-641046, Tamil Nadu, India.*

***Corresponding author:** Dr. Saritha E, Assistant Professor, Department of Biotechnology, PSG College of Arts &Science, Email:sarithasenthikumar@gmail.com, Contact No: 8675310019

To Cite this Article
Saritha E, Swaminathan K, Gayathri S, Madhumitha R, ***"BATCH MODE BIOREMEDIATION STUDY ON REACTIVE BLUE-HER BY Cladosporium oxysporum"***, *Journal of Science and Technology, Vol. 07, Issue 02, Mar-Apr 2022.*

Article Info
Received: 06-02-2021 *Revised: 16-02-2021* *Accepted: 28-02-2022* *Published: 08-03-2022*

Abstract: The fungus, *Cladosporium oxysporum* Berk. and Curt. was screened for their ability to decolourize Reactive Blue HER in aqueous solutions and observed that had higher dye decolourization (99.81 %) achieved by live biomass, dead biomass. In batch mode (Agitated mode), the lower concentrations (10 mg/L) of Reactive Blue showed complete removal (100%) at an equilibrium time of 60 min for both live and autoclaved biomass. Dye adsorption isotherm, Langmuir model fitted well with R^2 values more than 0.9. R_L (equilibrium parameter) values (indicating type of isotherm) of live and autoclaved mycelia at different adsorbate concentrations were always less than one and more than zero thereby indicating favourable adsorption of dyes onto the adsorbent. With the increase of dosage, no re-stabilization phenomenon or removal reduction was observed. Highest adsorption was observed in autoclaved biomass, 48.31 mg/g. Agitated mode was found to be more efficient and easy to operate for the removal of dyes.

Key words: Langmuir model, Bioremediation, Azo dyes, textile dyes, Isotherms, Adsorption, *Cladosporium* sp.

1. INTRODUCTION

Many textile processing units in Tamilnadu use a number of unclassified chemicals that are likely to be from the Red list group which is said to be harmful and unhealthy[4]. Toxic compounds from dyeing effluent get into aquatic organisms, pass through food chain and ultimately reach man and cause various physiological disorders like hypertension, sporadic fever, renal damage, cramps and also skin cancer due to photosensitization and photo dynamic damage[6]. Dyes are chemically and photolytically stable and are visible in water even at low concentrations (>1mg/L). Textile wastewater

with dye content in the range of 10-200 mg/L are usually highly coloured and its discharge into lakes, rivers and other water bodies not only affects the aesthetic and transparency of water but it also decreases light transmission, soluble oxygen levels, gas dissolution and increased COD thus disturbing acquatic life[13]. The present study aims at developing a treatment strategy for dye industry effluent based on a fungal system.

II. MATERIALS AND METHODS

Isolation and maintenance of fungi

Soil samples were collected from dye industry effluent contaminated soil around the Common Effluent Treatment Plant (CEPT), Tirupur. The fungi were isolated by serial dilution technique[15] on Malt agar medium[12].

Adsorbate and Adsorbent

Various concentrations of working dye solutions were prepared which include 10, 20, 30, 40 and 50 mg/L. The live and autoclaved biomass of the selected fungus was used as adsorbent.

Preparation of live and autoclaved adsorbent

For the preparation of live and autoclaved adsorbent, 1mL (10^6 spores) of fungal spore suspension was inoculated into 100mL of sterile Czapek-Dox broth and incubated at 27±2 $^{\circ}$C for 5 days in an orbital shaker at 125 rpm. After the incubation period, the mycelium developed as pellets were separated by filtration through Whatmann No.1 filter paper and washed with generous amounts of deionized water. The autoclaved adsorbent was prepared by subjecting the live mycelium to autoclaving (a pretreatment) for 30 min at 121 $^{\circ}$C at 18 psi.

Batch mode treatment processes (Agitated mode)

Adsorption of Reactive Blue HER by the fungal mycelium was studied in agitated mode (stirred tank type). The effects of contact time, initial adsorbate concentration and adsorbent dosage on dye adsorption were studied.

a. Contact time

To determine the optimum contact time, 50 mL of adsorbate at various concentrations (10 to 50 mg/L of Reactive Blue HER was taken in 250 mL conical flasks. To these aqueous solutions, 1g of adsorbent was added and the flasks were agitated on a rotary shaker (150 rpm) at room temperature (27±2^{o}C). The flasks were withdrawn at predetermined time intervals. The adsorbent and adsorbate were separated by centrifugation at 3000 rpm for 20 min. A graph was drawn taking the contact time in minutes on the X-axis and per cent removal of adsorbate on the Y-axis. From the plot, the optimum contact time to obtain equilibrium in adsorbate adsorption was determined. Control experiments were carried out without adsorbent to estimate the adsorbate removal due to adsorption onto the walls of the flasks.

b. Adsorbent dosage

The adsorbate solutions were agitated with various dosages of adsorbent (0.5-2.5g/50mL) at 150 rpm at room temperature (27±2 °C) for the equilibrium period. After the equilibration period, the adsorbent and adsorbate were separated and the amount of adsorbate adsorbed was determined as stated above. A graph was plotted with adsorbent dosage verses percent adsorbate removal. The optimum adsorbent dosage for adsorbate removal was determined from the graph.

Langmuir isotherm

Adsorption isotherms, Langmuir was used to describe the adsorption data for a range of adsorbate concentrations. These isotherm relate adsorption density, q_e (uptake of adsorbate per unit weight of adsorbent) to equilibrium adsorbate concentration in the bulk fluid phase, C_e. This model assumes uniform energies of adsorption onto the surface without transmigration of adsorbate in the plane of the surface (Langmuir, 1918). The Langmuir isotherm is represented by the following equation:

$$C_e/q_e = 1/Q_o b + C/Q_o \quad \text{----------} \quad (1)$$

Where, C_e is the equilibrium concentration (mg/L)

q_e is the amount adsorbed at equilibrium time (mg/g)

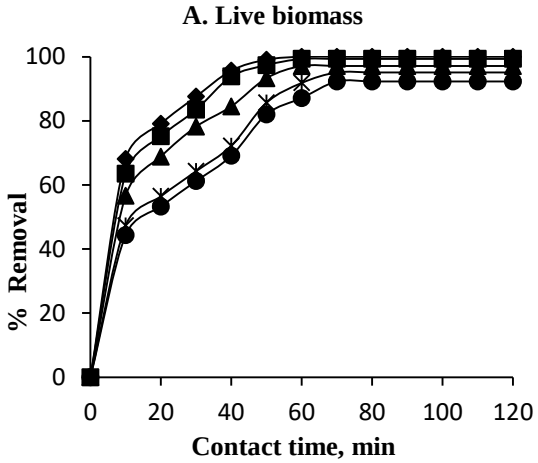
Q_o and b are Langmuir constants related to adsorption capacity (mg/g) and energy of adsorption (L/mg), respectively.

III. RESULTS

Effect of contact time and initial adsorbate concentration on adsorption of Reactive Blue HER

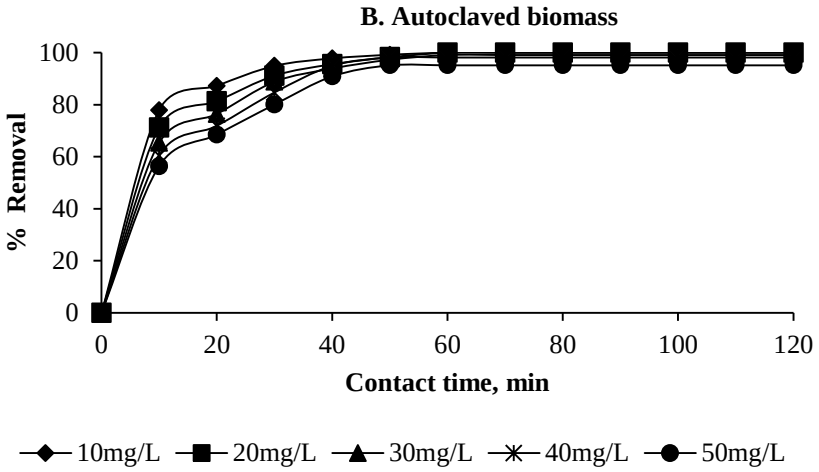
In agitation mode, time required for live biomass to reach adsorption equilibrium in 10 mg/L adsorbates was 60 min; whereas for autoclaved biomass it was 60 min for Reactive Blue HER. In this mode, Reactive Blue removal from aqueous solution by live biomass was in the range of 100 to 92.28% (10 to 50 mg/L) and in autoclaved biomass the removal was slightly in higher ranges (100 to 95.16%). Figure 1 revealed that increase in contact time increased the adsorbate uptake and remained constant after attaining equilibrium.

Fig. 1: Effect of contact time on removal of Reactive Blue HER dyes from aqueous solution by fungal biomass (Agitated mode)
(Adsorbent dosage: 1.0g/50mL; pH: 7.0; Temperature: 30^{o}C)


A. Live biomass
100
80
60
40
20
0
% Removal
0
20
40
60
80
100
120
Contact time, min

Journal of Science and Technology
ISSN: 2456-5660 Volume 7, Issue 01 (JAN-FEB 2022)

www.jst.org.in *DOI: https://doi.org/10.46243/jst.2022.v7.i02.pp01-10*



Effect of adsorbent dosage

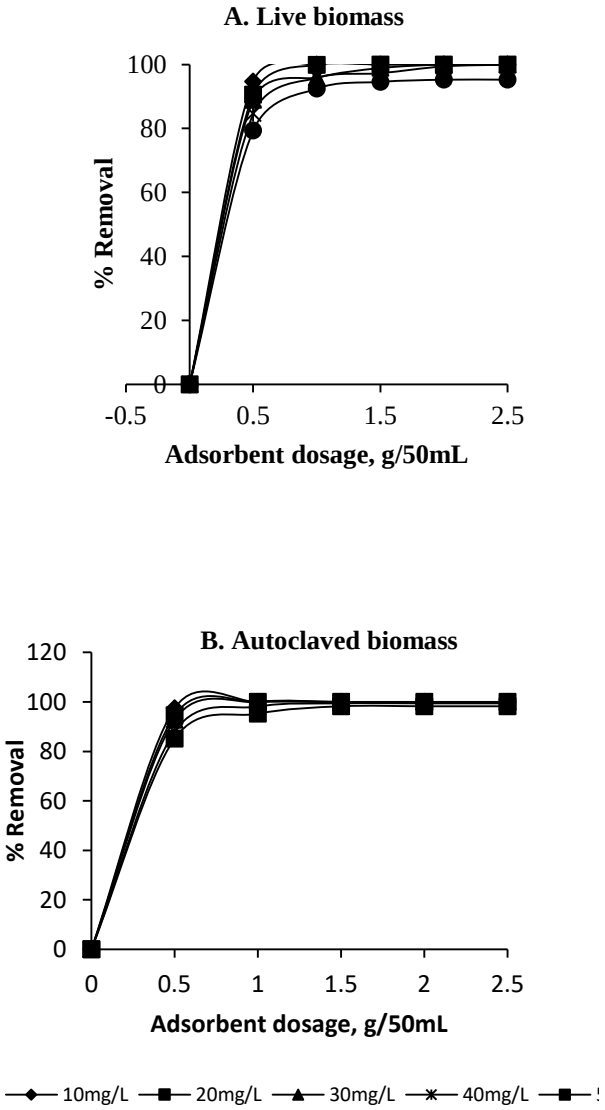
Dye solutions were treated with different dosages of adsorbent (0.5 to 2.5 g/50mL of adsorbate) for equilibrium time. The dosage level that yielded maximum dye removal was considered as optimum dosage. In agitation mode the optimum dosage for the removal of Reactive Blue was found to be 2.0g/50mL for live biomass whereas for autoclaved biomass it was 1.5g/50mL respectively (Figure 2). The removal rate of reactive dyes increased from 0.5 to 1.5g/50mL and then approached a plateau above 2.0g/50mL of adsorbent for all dyes. For aerated mode, the optimum adsorbent dosage was found to be 2.0g/50mL for both live and autoclaved biomass.

Fig.2: Effect of adsorbent dosage on removal of Reactive Blue HER by fungal biomass (Agitated mode)

(pH: 7.0; Temperature: 30°C)

Journal of Science and Technology
ISSN: 2456-5660 Volume 7, Issue 01 (JAN-FEB 2022)

www.jst.org.in *DOI: https://doi.org/10.46243/jst.2022.v7.i02.pp01-10*



Langmuir isotherm

In Langmuir plots for adsorption, the linear plots of C_e / q_e Vs C_e (Figure 3) confirm that the adsorption follows the Langmuir isotherm model. The Langmuir isotherm treats surface sites analogous to dissolve complexing ligands. It is derived by combining sorption equilibrium constant with a mass balance on the total number of adsorption sites. Dye adsorption isotherms fitted Langmuir model well with R^2 values more than 0.9. In Reactive blue removal, Q_o values not showed much difference in both live and autoclaved mycelium (Table 1). The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L[8], which is given by the equation

$$R_L = 1/1+bC_o \quad \text{----------} \quad (2)$$

Where, C_o is the initial dye concentration (mg/L)

b is the Langmuir constant (L/mg)

R_L indicates the shape of the isotherm as follows,

R_L	**Type of Isotherms**
R_L>1	Unfavorable
R_L= 1	Linear
0< R_L<1	Favourable
R_L= 0	Irreversible

R_L values of live and autoclaved mycelium at different adsorbate concentrations were always less than one and more than zero thereby indicating favourable adsorption of adsorbates onto the adsorbent (Table 2)

Fig. 3: Langmuir plot for removal of Reactive Blue HER from aqueous solution by fungal biomass (Agitated mode)

(Adsorbent dosage: 1.0g/50mL; pH: 7.0; Temperature: 30°C)

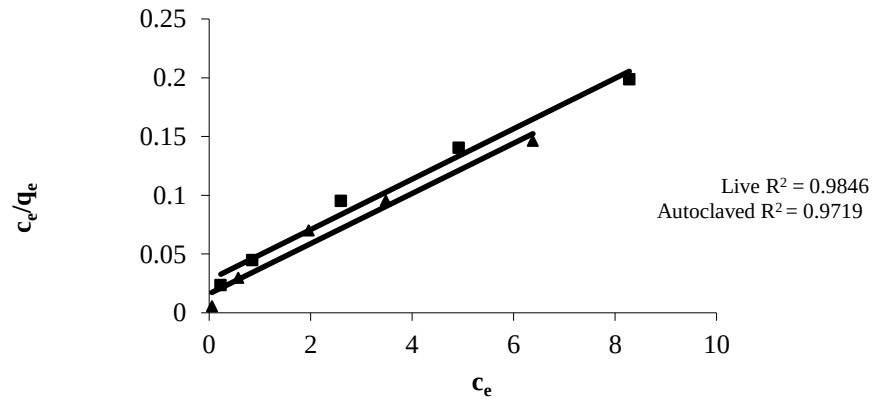


Table 1: Comparison of *Cladosporium oxysporum* adsorption capacity with other adsorbents

Adsorbent	**Dye**	**Q_0(mg/g)**	**References**
Flax shive	Reactive Red 228	14	Feng *et al*., 2012[5]
Bottom ash	Crystal Violet	3.06	Gandhimathi *et al*., 2013[7]
Algerian kaolin	Congo Red	5	Meroufel *et al*., 2013[11]
Live *C. oxysporum*	Reactive Blue	47.17	**The present study**
(Agitated mode)			
Autoclaved *C. oxysporum*	Reactive Blue	48.31	**The present study**
(Agitated mode)			

Table 2: Equilibrium parameter, R_L for Reactive Blue HER adsorption by fungal biomass

Process	Pretreatment			R_L		
		10mg/L	20mg/L	30mg/L	40mg/L	50mg/L
Agitated mode	Live	0.025	0.013	0.008	0.006	0.005
	Autoclaved	0.008	0.004	0.003	0.002	0.002

Adsorbent dosage: 1.0g/50mL; pH: 7.0; Temperature: 30ºC

IV.DISCUSSION

Equilibrium time and adsorbate uptake varied with adsorbates and adsorbent, which may be due to the difference in affinity of various adsorbents for adsorbates[14]. Ahalya reported that increase in agitation speed increases the uptake of adsorbates by the adsorbent, which was attributed to better contact at high agitation speed[1]. This shows that the movement of the adsorbent in the dye solution plays a major role in adsorption.

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 death or autoclaving can suffer rupture and denaturation of cell wall that can allow free access of cell wall binding sites. This could also be a reason for enhanced dye adsorption by autoclaved mycelium as observed in the present study. It has been reported that biosorption efficiency of biomass can be significantly enhanced by pretreatment methods such as autoclaving, dyeing and exposure to chemicals such as formaldehyde, acid, NaOH, $NaHCO_3$ and $CaCl_2$[2] .

Highest adsorption of reactive dyes was obtained with initial dye concentration of 10 mg/L, where as a slight decrease in dye removal was observed above the concentrations of 30 mg/L. This might be due to saturation of binding sites of biomass with high dye concentrations .

The adsorbent dosage in adsorption experiments is of important because it is closely related to the industrial application in wastewater treatment, in terms of efficiency and economy. The results revealed that increase in

adsorbent dosage increased the percent removal. It may be due to greater availability of surface area and exchangeable adsorption sites[9] .

The Langmuir isotherm is valid for adsorption of a solute from a liquid solution as monolayer adsorption on a surface containing a finite number of identical sites (Alkan and Onganer, 2000). In the present study it was observed that R_L values ranged between 0.002 to 0.238 for Reactive Blue, showing that the adsorption process employed is favourabe one.

V. CONCLUSION

Adsorption using fungal biomass emerged as an option for developing economic and eco-friendly wastewater treatment process. *Cladosporium oxysporum* was found to have good decolourization ability towards Reactive dye studied. Autoclaved biomass of *C.oxysporum* was found to be most effective in removal of dyes. Conventional treatment methods are not economical in the Indian context and so there is a need to develop low cost technologies for dye removal. As the fungus, *Cladosporium oxysporum* is easily cultivable and its utility as biosorbent for dyes will be economical, it can be viewed as a waste management strategy. Since the dye adsorbed on the surface of the fungal biomass can be easily desorbed, it can be reused as low grade dye for coloring carpets, glasses etc. and the biomass could be reused for several cycles for effluent treatment. Moreover, as this fugal biomass is not pathogenic to humans and is easily biodegradable it will avoid problem of sludge accumulation. Hence the process developed in the present study is recommended for decolourization of dye industry effluents prior to secondary and tertiary treatments; so that the cost of these advanced techniques will be very much reduced.

REFERENCES

[1]. Ahalya, N., R. D. Kanamadi and T. V. Ramachandra, 2005. Biosorption of chromium (VI) from aqueous solutions by the husk of Bengal gram (*Cicer arietinum*). Electron. J. Biotechnol., 8: 258-264.

[2]. Aksu, Z. and G. Dönmez, 2005. Combined effects of molasses sucrose and reactive dye on the growth and dye bioaccumulation properties of *Candida tropicalis*. Process. Biochem., 40: 2443-2454.

[3]. Alkan, M. M. and Y. Onganer, 2000. Adsorption of Methylene blue from aqueous solution onto perlite. Water Air soil pollut., 120: 229- 249.

[4]. Balakrishnan, M., S. Arul Antony, S. Gunasekaran and R. K. Natarajan, 2008. Impact of dyeing industrial effluents on the groundwater quality in Kancheepuram (India).

[5]. Feng, Y., F. Yang, Y. Wang, L. Ma, Y. Wu, P. G. Kerr and L.Yang, 2012. Basic dye adsorption onto an agro-based wastes material-sesame hull (*Sesamum indicum* L.). Bioresource Technol., 102(22): 10280-10285.Indian J. Sci. Technol., 1(7): 1-8. Butterworth, London, Pp. 240.

[6]. Gandhi, J., R. Dangi, J. C. Sharma, N. Verma and S. Bhardwaj, 2010. Photocatalytic bleaching of Malachite green and Brilliant green dyes using ZnS-CdS as semiconductor: A comparative study. Der Chemica Sinica, 1(3): 77-83.

[7]. Gandhimathi, R., S. Thanga Ramesh, V. Sindhu, and P. V. Nidheesh, 2013. Bottom ash adsorption of basic dyes from their binary aqueous solutions. Songklanakarin J. Sci. Technol., 35 (3): 339-347.

[8]. *Hall, K. R., L. C. Eagleton, A. Acrivos and T. Vermeulen, 1966. Pore and solid diffusion kinetics in fixed bed adsorption under constant pattern conditions. Ind. Eng. Chem. Fundan., 5: 212-219.

[9]. Kilic, M., E. Apaydin-Varol and A. E. Pütün, 2011. Adsorptive removal of phenol from aqueous solution activated carbon prepared from tobacco residues: Equilibrium, kinetics and thermodynamics. J. Hazard. Mater., 189(1): 397-403.

[10]. *Langmuir, I., 1918. The adsorption of gases on plane surfaces of glass, mica and platinum. J. Am. chem. Soc., 40: 1361.

[11]. Meroufel, B., O. Benail, M. Benyahia, Y. Benmoussa and M. A. Zenasni, 2013. Adsorptive removal of anionic dye from aqueous solutions by Algerian kaolin: Characteristics, isotherms, kinetic and thermodynamics studies. J. Mater. Environ. Sci., 4(3): 482-491.

[12]. Purvis, M. J., D. C. Collier and D. Walls, 1964. Laboratory techniques in botany.

[13]. Puvaneswari, N., J. Muthukrishnan and P. Gunasekaran, 2006. Toxicity assessment and microbial degradation of azo dyes. Indian J. Exp. Biol., 44: 618-626.

[14].Sathishkumar, M., A. R. Binupriya, D. Kavitha and S. E. Yun, 2006. Kinetic and isothermal studies on liquid-phase adsorption of 2, 4-Dichlorophenol by palm pith carbon. Bioresource Technol., 98(4): 866-873.

[15].*Waksman, S. A., 1922. A method of counting numbers of fungi in the soil. J. Bot., 7: 339-341.

* Originals not referred