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RESEARCH ARTICLE

BIOSORPTION OF REACTIVE BLACK B BY DRIED FUNGAL BIOMASS.

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Abstract

Biosorption of Reactive Black B (RBB) dye was attempted with dried biomass of *P. chrysosporium* MTCC 787, *Aspergillus* sp. WRF3 and *Trichoderma* sp. WG1. Effect of biosorbent dosage, pH and contact time on biosorption of RBB was assessed. All the three cultures were observed to have high potential for the removal of RBB even at low biosorbent dosage. Acidic pH was more suitable for biosorption of RBB from the solution and 7h contact time was needed for maximal removal of dye (>90%) from the dye solution at 3gL⁻¹ biosorbent dosage. Thus, these cultures can be useful in biosorptive removal of RBB containing wastewaters.

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Introduction:-

Among all synthetic dyes, azo dyes have found wide applications in the production of inks, food, cosmetics, leather, textile and paper industries (Almeida and Corso, 2014; Dellamtrice et al., 2017). For textile industries, it is estimated that about 15% of the dyes used are lost during synthesis, processing and application stages. Such dye containing wastewaters if released untreated could pose an environmental hazard, as several dyes are reported toxic and recalcitrant in nature. Diverse strategies used for the removal of dyes from effluents have been reported by diverse group of workers (Saratale et al., 2009; Verma & Madamwar 2003; Junnarkar et al., 2006). Among these, biosorption has been employed successfully in treating dye containing wastewaters.

The uptake or accumulation of chemicals by biomass has been termed biosorption (Tsezos and Bell, 1989; Hu 1992, 1996; Kumar et al., 1998). Dead bacteria, yeast and fungi have all been used for the purpose of decolorizing dye-containing effluents. Textile dyes vary greatly in their chemistries, and therefore their interactions with micro-organisms depend on the chemistry of a particular dye and the specific chemistry of the microbial biomass (Polman and Brekenridge, 1996). Depending on the dye and the species of micro-organism used different binding rates and capacities are observed. It can be said that certain dyes have a particular affinity for binding with microbial species.

Materials and Methods:-

Organisms:-

Phaenerochaete chrysosporium MTCC 787 was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. It was routinely subcultured on Malt Extract Agar (MEA, Himedia) medium and preserved at 4°C on MEA slants till further use. Fungal strains WRF3 and WG1 were isolated from the soil samples collected from Atmiya Campus, Rajkot; which were also routinely sub-cultured on MEA medium and preserved at 4°C on MEA slants.

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Bavendam's test:-

0.1 mL of fungal spore suspension, prepared in sterile distilled water containing Tween 80 (0.05% v/v) was spread on MEA medium supplemented with tannic acid (0.1%, w/v). The plates were incubated at 30°C and were observed for changes in the color of the medium.

Isolation and screening of white rot fungi:-

Soil samples collected from the garden at Atmiya Group of Institutions Campus (22° 17'11.6"N, 70°46'22.6" E), Rajkot, Gujarat, India; were suspended in sterile distilled water at the rate of 1% (w/v), from which 0.1 mL of the supernatant was spread on the Malt Extract Agar medium and incubated at 28°C. The fungal colonies were successively transferred to obtain pure cultures, which were then subjected to Bavendam's test, to assess their ability to bleach tannic acid containing medium (a test used to screen white rot fungi).

Biomass cultivation:-

Fungal cultures undertaken in this study viz. *P. chrysosporium* MTCC787, WRF3 and WG1 were subjected to biomass cultivation in 500 mL flask containing 300 mL Sabouraud's broth (HiMedia, India). Each flask was inoculated with 10% fungal spore suspension (prepared in sterile distilled water containing 0.05% v/v Tween 80, conidial count $1 \times 10^5 \text{ mL}^{-1}$) and were incubated at 28°C under shaking condition (100 rpm) for 7 days.

After 7d entire contents of the flasks were filtered out individually and fungal biomass of each culture was washed with distilled water, which was then subjected to drying in oven at 35°C. Dried biomass of each fungal culture was then finely ground using mortar and pestle and was used as biosorbent for the biosorptive removal of dye from the 50 ppm solution of Reactive Black B.

Effect of biosorbent dosage:-

To study the effect of sorbent dosage, varying concentration of dried biomass (0.2-5g/L) was added in 20 mL RBB solution (50ppm, pH 7) contained in 50 mL flask. All the flasks were placed on orbital shaker (100rpm) for 5h at 30°C. After 5h, the contents of the flask were filtered and decolorization rate was reported using the formula mentioned below. Experimental sets were run in triplicate.

$$\text{Decolorization rate} = C * \%D / 100 * t$$

Where, C= concentration of dye ($\text{mg L}^{-1} \text{ h}^{-1}$), %D = % Decolorization, t= time (h).

Effect of pH on biosorption of dye:-

Effect of pH was studied using 50 ppm RBB solution prepared in different buffers (0.1M), listed in Table 4.1. Biosorbent dosage was kept to 3g L^{-1} in each flask. For each pH, experimental sets were run in triplicates.

Table 1:- List of the buffers used to assess the effect of pH on biosorption of RBB by dried fungal biomass.

pH	0.1 M Buffer
2	Tartarate Buffer
3	Tartarate Buffer
4	Succinate lactate Buffer
5	Citrate Buffer
6	Phosphate Buffer
7	Phosphate Buffer
8	Phosphate Buffer
9	Carbonate Buffer
10	Carbonate Buffer
11	Carbonate Buffer

Effect of contact time on biosorption of dye:-

To study the effect of contact time, flasks were harvested at an interval of 1h till 9 h. Biosorbent dosage was kept at 3g L^{-1} in each flask. Experimental sets were run in triplicates.

Results and Discussion:-

Isolation and screening of white rot fungal cultures

A total of 16 different fungal cultures were isolated from the collected soil samples, on Malt Extract Agar medium. These were maintained on MEA slants at 4°C and were subjected to screening for ability to bleach tannic acid (a property of white rot fungi) through Bavendam's test. A total of 9 fungal isolates were able to bleach tannic acid containing Malt Extract Agar medium completely, however, fungal strains *Aspergillus* sp. WRF3 and *Trichoderma* sp. WG1 were reported to bleach the medium rapidly in 4d of incubation and hence these were assessed for the biosorptive removal of textile di-azo dye, Reactive Black B (C.I. Reactive Black 5) from dye solution.

Morphological Identification of WRF3 and WG1:-

Pure cultures of WRF3 and WG1 were morphologically identified as *Aspergillus* sp. and *Trichoderma* sp., respectively. SEM images of the fungal cultures *Aspergillus* sp. WRF3 and *Trichoderma* sp. WG1 are shown in Fig. 1a and 1b respectively. However, for convenience *Aspergillus* sp. WRF3 is referred as WRF3 and *Trichoderma* sp. WG1 is referred as WG1, further in this paper.

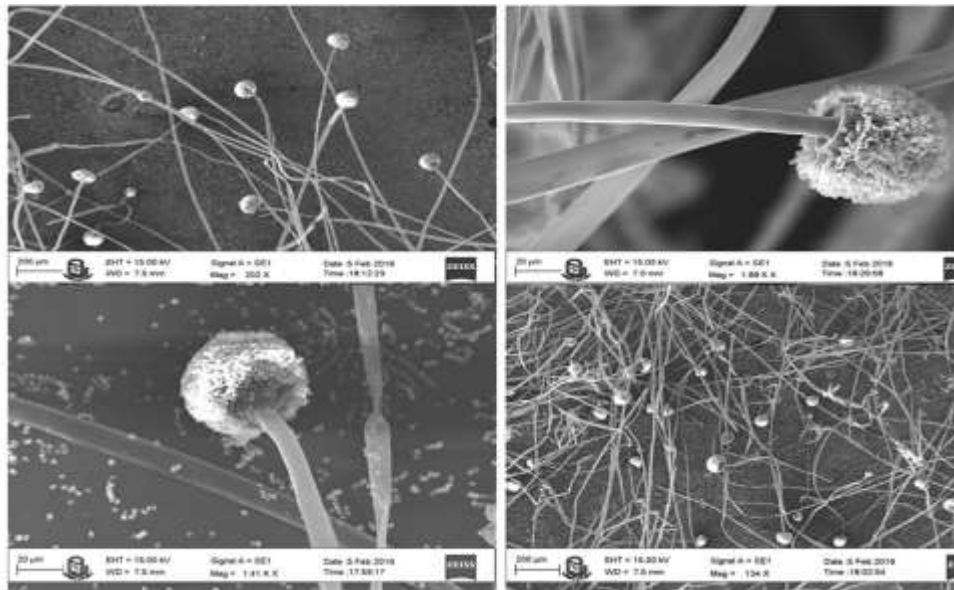


Fig. 1a:- Scanning Electron Micrographs of *Aspergillus* sp. WRF3 fungal culture.

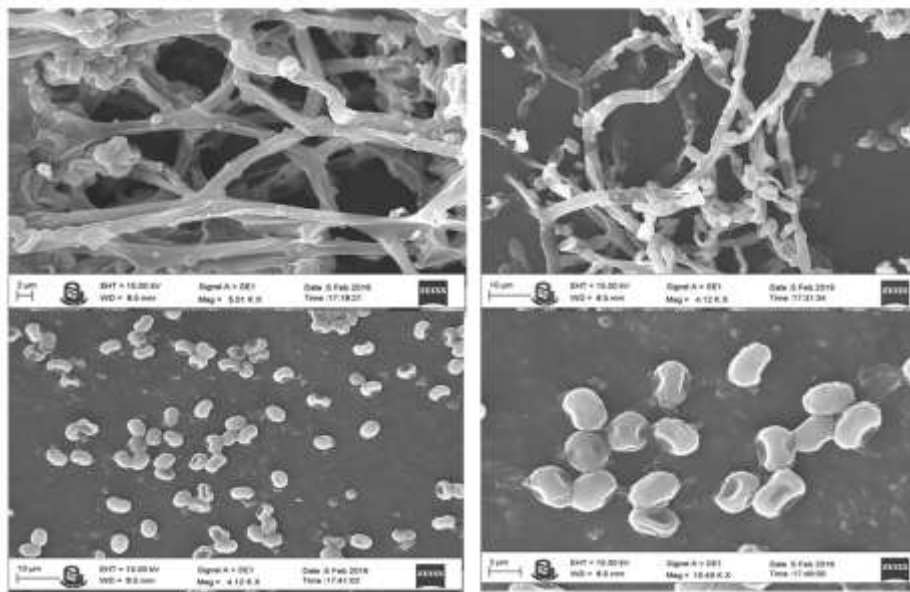


Fig. 1b:- Scanning Electron Micrographs of *Trichoderma* sp. WG1 fungal culture.

Biosorption of RBB by dried biomass of *P. chrysosporium*, WRF3 and WG1:-

Finely powdered dry biomass of fungal cultures *P. chrysosporium*, WRF3 and WG1 was individually tested as biosorbent for the biosorptive removal of RBB dye at 50 ppm concentration under shaking conditions. Different parameters viz. effect of biosorbent dosage, effect of pH on biosorption of RBB and effect of contact time on biosorption of RBB were tested.

Effect of biosorbent dosage on biosorptive removal of RBB:-

The effect of biosorbent dosage on decolorization rate of RBB under shaking conditions is shown in Fig. 2. Here, as biosorbent dosage increased, decolorization rate also increased. Increase in the decolorization rate may be attributed to the availability of more adsorption sites on biosorbent surface at higher dosage, for binding by the dye molecule. Similar observations were reported by Khalaf (2008) for biosorption of reactive dyes Synazol Red HF6BN and Synazol Yellow HF2GR by inactivated biomass of *Aspergillus niger* and *Spirogyra* sp. At higher biomass concentration, there is a very fast superficial biosorption onto the cell that produces a lower solute concentration in the solution than when cell concentration is lower (Donmez et al., 1999). There are marked differences in the structure and composition of the cell wall of fungi from diverse groups (Deacon, 2006). Gupta et al. (2000) suggested that the microbial biomass acts as an ion exchanger by virtue of reactive groups available on the cell surface. Different chemical groups of the fungal cell wall have been suggested as potential binding sites, such as carboxyl, amine, imidazol, phosphate, sulphhydryl, sulphate, hydroxyl groups and the lipid fraction according to the chemical class of the dye (Zhou and Banks, 1993; Aksu and Tezer, 2000; Fu and Viraraghavan, 2002).

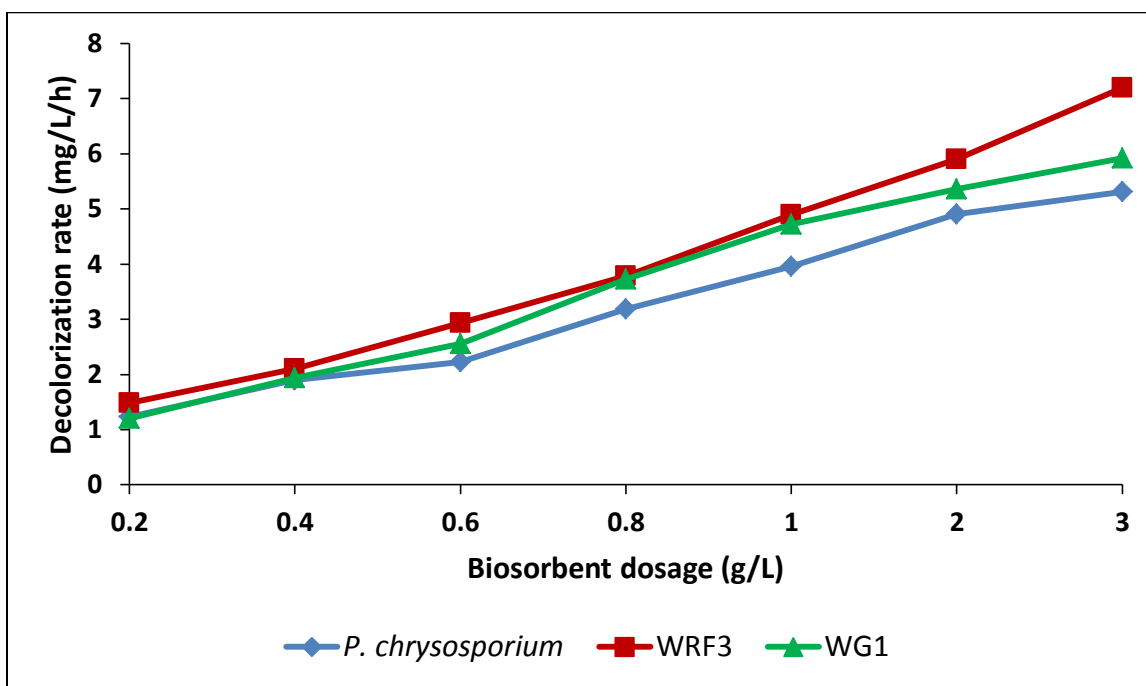


Fig. 2:- Effect of biosorbent dosage on biosorptive removal of RBB (50 ppm).

Effect of pH on biosorption of RBB by fungal biomass:-

Biosorption of RBB by inactivated biomass of fungal cultures *P. chrysosporium*, WRF3 and WG1 at different initial pH of RBB solution (50 ppm) is shown in the Fig. 3. Acidic pH was reported more suitable for the removal of dye from the dye solution as is evident from the maximum % decolorization in the acidic range. For *P. chrysosporium* and WRF3 biomass, at pH 4 maximum dye removal was observed (above 80%), while at pH 3 maximum biosorptive removal of RBB was achieved. And in all the three cases, as pH increased, it resulted in decreased biosorption of the dye.

In basic conditions, presence of excess OH^- competed with the anionic dyes for adsorption sites. For this reason, the biosorption amount of RBB decreased in basic conditions. While, acidic conditions could be favorable for the biosorption between the two dyes and the fungal biomass, because a significantly high electrostatic attraction could exist between the positively charged surface of the biosorbent under acidic conditions and the anionic dyes (Aksu

and Donmez, 2003). Maximum biosorption capacities in single system for AB 25 and AR 337 onto unmodified and CDAB-modified biosorbents were obtained at pH 2.0, and then decreased as the pH increased. When the pH of solution changed from 2.0 to 9.0, the biosorption capacities of unmodified biosorbent in single system for AB 25 decreased (Yang et al., 2011b). The biosorption capacity of *Penicillium* YW 01 increased from pH 1.0 to pH 3.0, and reached maximum at pH 3.0 (46.95 and 48.83 mg g⁻¹ for Acid Black and Congo Red, respectively), and then declined sharply with further increase in pH for both of the two dyes, indicating that the optimal pH for biosorption of *Penicillium* YW 01 is 3.0 for both of the two dyes under the experimental conditions (Yang et al., 2011a).

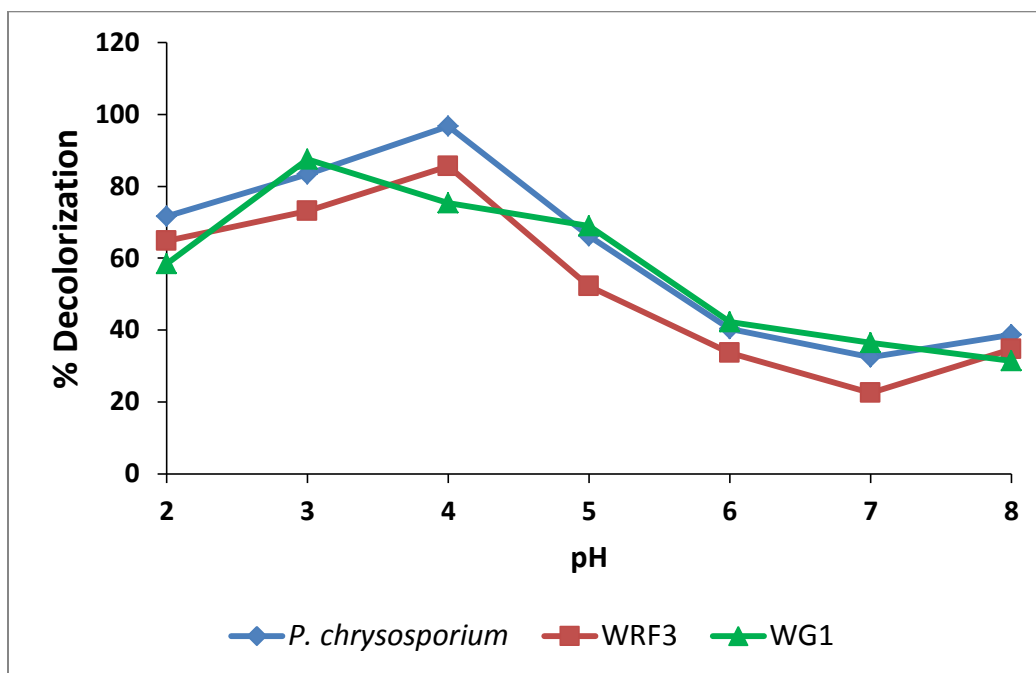


Fig. 3:- Effect of pH on RBB removal from the solution (50ppm) by the inactivated biomass of fungal cultures *P. chrysosporium*, WRF3 and WG1.

Effect of contact time on biosorption of RBB by fungal biomass:-

Fig. 4a-c illustrates the effect of contact time of biosorbent on removal of RBB. Here, with increase in the contact hours, % decolorization increased linearly and then became constant at longer period. This may be attributed to the complete removal of dye molecule from the solution as indicated by >90% decolorization by the fungal biomasses after 7h of contact time. Also, saturation of the adsorption sites by the dye molecule on the biosorbent surfaces has been reported for constant values of % biosorption or dye removal at longer contact hours (Ramkrishna and Viraraghavan, 1997; Won et al., 2006, Yang et al., 2011a & b).

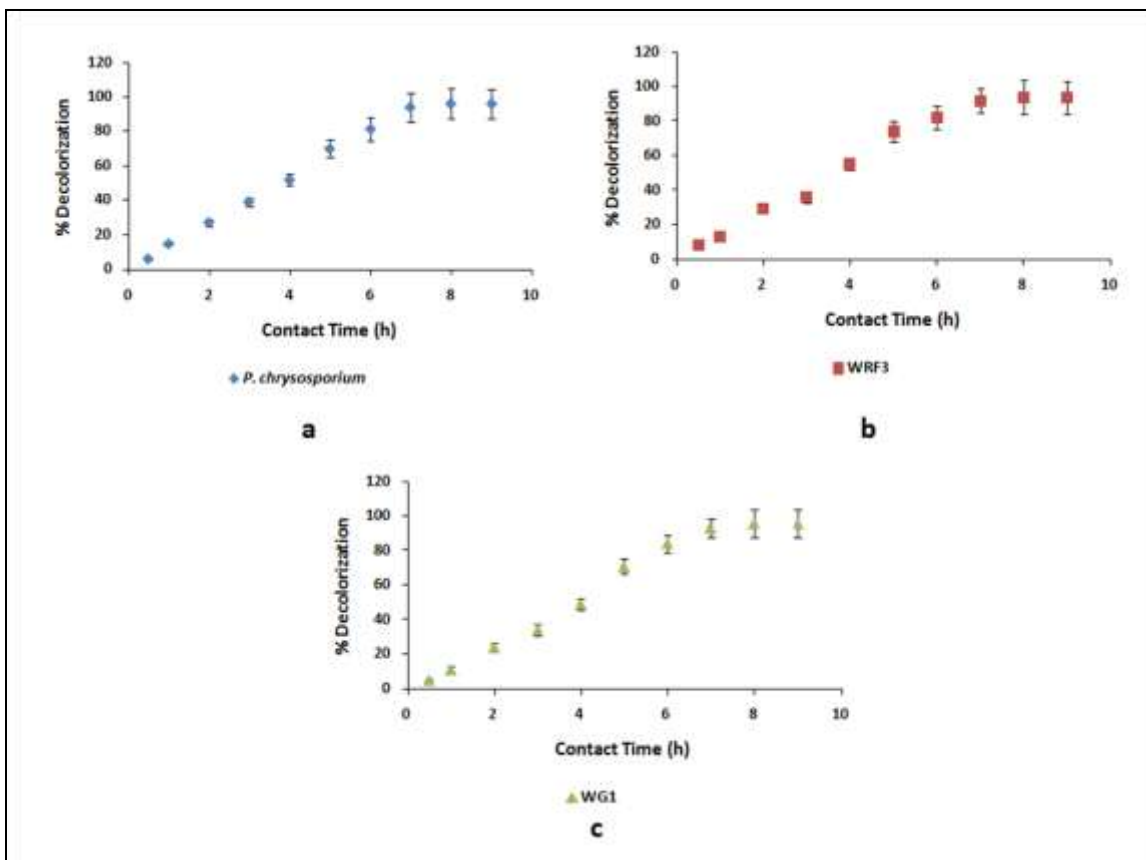


Fig. 4a-c:- Effect of contact time on biosorption of RBB by dried biomass of *P. chrysosporium* (a), WRF3 (b) and WG1 (c).

Conclusion:-

Three fungal cultures, *P. chrysosporium* MTCC 787, *Aspergillus* sp. WRF3 and *Trichoderma* sp. WG1, were studied for their ability to remove RBB from 50ppm dye solution under shaking conditions. All the three cultures were exhibited high potential for the removal of RBB even at low biosorbent dosage. Acidic pH was more suitable for biosorption of RBB from the solution and 7h contact time was needed for maximal removal of dye (>90%) from the dye solution at 3gL^{-1} biosorbent dosage. Thus, these cultures can be useful in biosorptive removal of RBB containing wastewaters. However, influence of heavy metals and other pollutants in the industrial effluents needs to be evaluated for successful application of these fungal cultures for biosorptive removal of RBB dye from RBB bearing wastewaters.

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