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Decolourization of Textile Dyes by *Aspergillus lentulus*

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Abstract—*Increasing industrialization has gained a global concern about the introduction of several hazardous chemicals into the environment in the form of effluents and wastewaters. Textile industry is one such emerging industry, which releases toxic effluents containing high amount of dyes, mordants and other auxiliaries. High concentration of these chemicals has created an ironical situation for the environment as well as human health. Hence, development of any process for the decolourization of such dyes is on highest priority among the scientific society. In the present study, bioaccumulation and biosorption of various Reactive and Vat dyes using a fungal strain Aspergillus lentulus were investigated. The results indicated that the biosorption by pre-grown fungal biomass was highly efficient in removing VAT Novatic Grey (100%), Reactive Remazol Red (98.47%), Reactive Yellow (91.55%) and Indanthrane Blue (99.28%) within 4 hours of exposure at initial dye concentration of 100mg/L. Least biosorption after 4 hours (55.33%) was observed for dye Reactive S Blue. The bioaccumulation studies revealed 75.9 to 100%removal of all the dyes after 40 hours of growth. Interestingly, Vat dyes were completely removed in 16 hours, while Reactive dyes took 40 hours for significant removal. None of the dyes exhibited inhibition of fungal growth. These results indicate that the fungus Aspergillus lentulus has a very good potential for treatment of industrial wastewater containing widely synthetic dyes.*

Keywords*: A. lentulus, Biosorption, Bioaccumulation, Vat dyes, Reactive dyes.*

1. INTRODUCTION

Environmental pollution can be attributed to the release of contaminants and toxic chemicals from various industries. The textile dyeing is one of the oldest process in which use of several hazardous dyes for coloring fibres and clothes has been practiced till date. Since last few decades, the extensively growing textile industries have impacted the environmental safety and human health. Several types of dyes like azo dyes, metal containing dyes, reactive dyes and Vat dyes are widely used in the textile industry. Wastewater obtained from various printing and dyeing units of textile and various other industries are often rich in residues of reactive dyes and other chemicals and requires appropriate removal before being released into the environment [1]. A high concentration of different synthetic dyes present in effluent are mostly toxic, mutagenic and carcinogenic [2]. Moreover, these effluents inhibit photosynthesis of algae and aquatic plants, cause reoxygenation problems and potential toxicity in water bodies [3], [4]. Synthetic dyes are hard to decolourize due to their chemical structure and resistance to light, water and other chemicals.

Various effluent treatment strategies based on different physio-chemical methods have been developed in the past [5], [6] but these techniques are less effective and expensive. Different studies on biological methods for dye removal have gained significance due to their low cost and eco-friendly nature. Fungi are considered a better biological agent for dye removal techniques because of its better capacity to adapt to environmental factors due to the production of large variety of extracellular proteins, organic acids and other metabolites [7]–[9]. Different strains of *Aspergillus* sp. have been employed in recent studies for removal of various synthetic dyes from industrial wastewater [10].

The aim of this study was the removal of various Reactive and Vat dyes from dye solutions at varied concentrations using a fungal strain *Aspergillus lentulus* at batch scale. The methods employed for the dye removal process were biosorption and bioaccumulation using live fungal biomass and spore suspension, respectively. This study is important in its kind as it uses a biological source for decolourization of dyes, which is a eco-friendly approach to overcome the toxic effects of textile pollution.

2. MATERIALS AND METHODS

2.1 Test organism and growing conditions

The experiments on dye removal were performed with a fungal strain *Aspergillus lentulus* FJ172995 which has been

successfully established for efficient dye removal especially Azo dyes in the previous studies [10]. The fungal isolate was maintained on slants of Potato Dextrose Agar. Freshly revived cultures were used for all the experiments.

2.2 Dyes and chemicals

The dyes used in the experiment were VAT Novatic Grey, Reactive Remazol Red, Reactive Yellow, Reactive S Blue and VAT Indanthrane Blue. Absorption maxima of each dye were estimated by scanning the dye solution over the visible range (400 – 700 nm) and are tabulated in Table 1. The stock solutions of 1000 mg/L were prepared for each dye in distilled water. All the chemicals used were of analytical grade.

Table 1: Dyes and its absorption maxima (λ_{max}. nm) used in the study

S. No.	Dyes	Class	Absorption Maxima (λmax) (nm)
1.	Novatic Grey	Vat	610
2.	Remazol Red	Reactive	520
3.	Yellow	Reactive	420
4.	S Blue	Reactive	610
5.	Indanthrane Blue	Vat	660

2.3 Biosorption of dyes by *A. lentulus*

Removal of dyes by pre-cultivated biomass under growth non-supportive conditions has been referred to as Biosorption. To cultivate the fungal biomass, sterile Potato Dextrose Broth (100 ml in each flask) was inoculated with spore suspension (10^7 spore/mL) of *A. lentulus* and incubated at 150 rpm and 30°C for 24 h. The grown fungal biomass (6 ±0.5 g/L) was added to 100ml of dye solutions at varied initial concentrations: 25, 50 & 100 mg/L (prepared in distilled water) and agitated at 150 rpm and 30°C. Samples were withdrawn from the flasks at regular intervals of 30 min for 4 hours and centrifuged for 12 min at 10,000 rpm. The supernatants of all samples were then estimated for absorbance at their absorbance maxima by Elisa plate reader (Biotek Eon C). Control (without biomass) for each set was run simultaneously.

Dye mixture was also prepared by mixing all five dyes in equal concentration (initial concentrations were taken to be 50 mg/L& 100 mg/L for bioaccumulation experiments and 25 mg/L & 50 mg/L for biosorption experiments). Removal efficiency of the fungal isolate for this mixture was tested by bioaccumulation as well as biosorption mode separately. Two different absorption maxima were obtained for the dye mixtures at 540 nm and 610 nm respectively; hence, the dye mixtures were scanned for absorbance at both these absorption maxima.

2.4 Bioaccumulation of dyes by *A. lentulus*

The dye removal efficiency in growing mode by *A. lentulus* were tested for various dyes (initial concentration 50 mg/L and 100 mg/L) using sterilized Potato Dextrose Broth (PDB) as media. Sterilized PDB amended with respective dye as well as dye mixtures in different concentrations was inoculated with spore suspension of *A. lentulus* (10^7 spore/mL) aseptically and incubated in the orbital shaker at 30°C and 150 rpm. Samples were withdrawn at regular intervals (2 hours) and were centrifuged at 10,000 rpm for 12 min. Later absorbance was measured at the absorbance maxima of the respective dye. Control (without inoculum) for each set was run simultaneously Resultant dried biomass was measured at the end of the experiment and the results were expressed as concentration of dye removed per gram biomass.

2.5 Analytical techniques

The concentration of test dye was determined by measuring absorbance of samples through spectrophotometer (Elisa plate reader). A calibration plot between concentration and absorbance maxima of the respective dye were used for determination of dye concentration. Dye removal (%) from the solutions was calculated using the following equation (Eq. 1):

$$Dye\ removal\ (\%) = \frac{(A^0 - A^t)}{A^0} \times 100 \quad (1)$$

Where A^0 is the initial absorbance, A^t is the absorbance at incubation time, t. Test samples were analysed for absorbance after centrifuging the samples at 10,000rpm for 12 min.

Dried biomass at the end of each experiment was collected and dye uptake efficiency calculated as the concentration of dye removed per gram biomass (mg/g dried biomass). The dye uptake efficiency in percentage can be calculated by the following formula (Eq. 2):

$$Dye\ uptake\ (\%) = \frac{(C^0 - C^f)}{C^0} \times 100 \quad (2)$$

Where C^0 and C^f are initial and final concentrations, respectively.

3. RESULTS AND DISCUSSIONS

3.1 Biosorption of dyes using *A. lentulus*

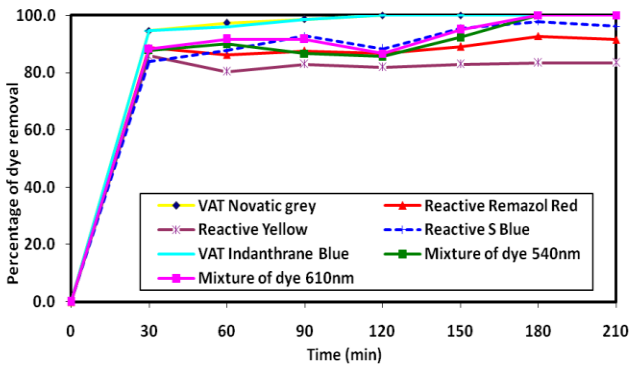
Vat dyes and Reactive dyes at a concentration of 25, 50 & 100 mg/L were used for biosorption experiments using *A. lentulus* (Fig. 1 A-C). Vat dyes showed better removal efficiency at all tested concentrations as compared to reactive dyes. At lower concentrations (25 mg/L & 50 mg/L), Vat dyes i.e. VAT Novatic Grey and Vat Indanthrane Blue, were almost completely removed (more than 95-99%) in the first 30-60 minutes of incubation. As the concentration was increased upto 100 mg/L, a gradual removal was obtained with respect to time and complete removal was obtained after 150 minutes. This was possibly due to an increase in dye concentration in the solution while the amount of biomass used for all batches remained constant.

On the other hand, among the reactive dyes, the most efficient removal by fungus was observed for Reactive Yellow, where 98.4 % dye decolourization was estimated after 240 minutes at

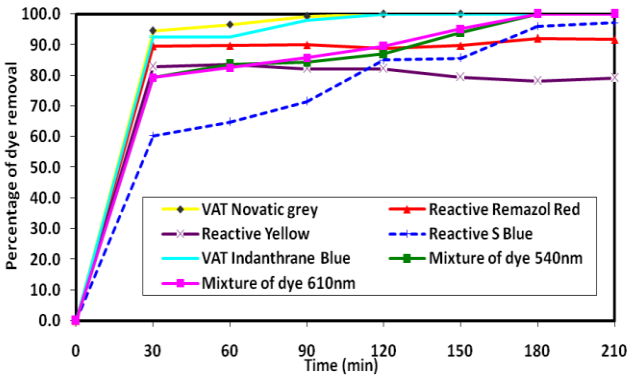
100 mg/L initial concentration. Rest of the 2 reactive dyes i.e. Reactive Remazol Red and Reactive S Blue showed removal upto 92% in lower concentrations like 25 mg/L and 50 mg/L after 210 minutes but a limitation beyond 74.2% and 50.9%, repectively in removing both these dyes at higher concentration (100 mg/L) was observed even after 4 hours (240 minutes) of incubation. The fungal strain showed least dye removal efficiency for Reactive S Blue, for which only 55.3% removal was observed by the end of 18th hour.

Earlier study on *A. lentulus* by the author has also shown its potential in the efficient dye removal for different dyes. The fungus could significantly removal anionic (96.7–94.3 %) and cationic (35.4–90.9 %) dyes in 24 h [11]. Study on Reactive Green 19 dye removal [12], showed similar removal pattern by non-viable fungal biomass of *Rhizopus nigricans.* The percentage removal decreased from 85% to 30% as the concentration was increased from 50 mg/L to 200mg/L. The reason behind this may be due to the saturation of all the binding sites present on fungal biomass.

A mixture of all Vat dyes and Reactive dyes was also tested for its decolourization. The aim behind this experiment was to expose the fungus with a dye mixture similar to the actual condition of effluent in the textile industry. The dyes from the mixture was removed upto 90% within 30 minutes of experiment at concentration of 25 mg/L but at 50 mg/L complete removal was observed only after 150 minutes.



A.



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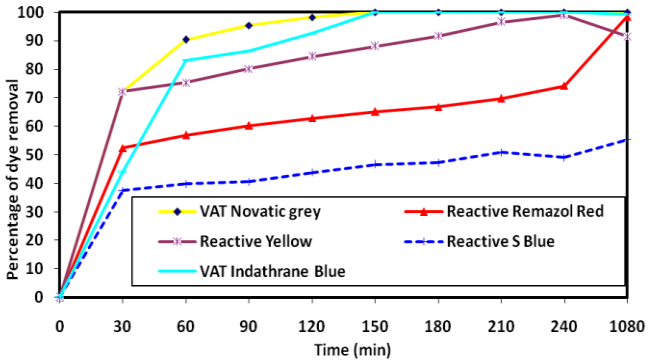

C.

Fig. 1: Percentage reduction of various dyes vs time using the biosorption method at initial dye concentration A.) 25 mg/L; B.) 50 mg/L; C.) 100 mg/L

Removal efficiencies of anionic and cationic dye mixtures have also been studied in *Aspergillus lentulus* earlier in the lab [11]. But it was observed that removal efficiency decreased in case of dye mixtures as compared to pure dyes. No such difference in biosorption capacity was observed in the present study.

3.2 Bioaccumulation of dyes by *A. lentulus*

The bioaccumulation experiments showed that at the concentration of 50 mg/L of Vat dyes, complete removal by fungus was observed by the end of 16th hour. Slow and gradual removal was observed in the case of reactive dyes. Reactive Remazol Red, Reactive Yellow & Reactive S Blue could be removed up to 93.6%, 94.26% & 98.2% respectively by the end of 40th hour when the initial dye concentration was 50 mg/L. A mixture containing all the five dyes in an equal concentration of 50 mg/L was also removed completely after 40 hours (Fig. 2A).. No dye decolourization was observed in control flask without inoculums (Controls).

At concentrations of 100 mg/L, both the Vat dyes were removed completely by the end of 16th hour. Even Reactive S Blue dye was removed completely by the end of 40th hour but Yellow and Remazol Red could be removed only up to 75.9 % & 89.2% respectively. Dye mixture with 100 mg/L initial concentration of all five dyes was also decolourized completely by the end of 40th hour (Fig. 2B).

Earlier experiments of bioaccumulation in the lab showed that *A. lentulus* could efficiently remove more than 98-99% of 100 mg/L cationic and anionic dyes after 48 hours of spore inoculation [11] and azo dyes after 72 hours [13]. Similar decolourization of Reactive Blue has been reported by *Aspergillus* sp. within 3 days[14]. But the strain in the present study took much less time to completely remove all the tested dyes (18 hours for Vat dyes, 40 hours for reactive dyes). Hence, this study bears significance for the dye decolourization and this fungal strain has a high potential to decolourize the textile effluent.

At the end of the experiment, the biomass was harvested and measured to estimate specific dye removal per unit fungal biomass (Table 2). At low concentration of dye (50 mg/L) containing media the yield of biomass was higher than that obtained from the high concentration of dye (100 mg/L) in supplemented media. However, the specific dye removal was observed in both systems ranging from 6.83 to 10.83 mg/g of biomass at dye concentration 50 mg/L. At 100 mg/L, highest specific removal was obtained in mixture of dyes and VAT Indanthrane Blue (21.87 and 22.01, respectively), whereas for rest pure dyes it was ranging from 10.88 to 19.94.

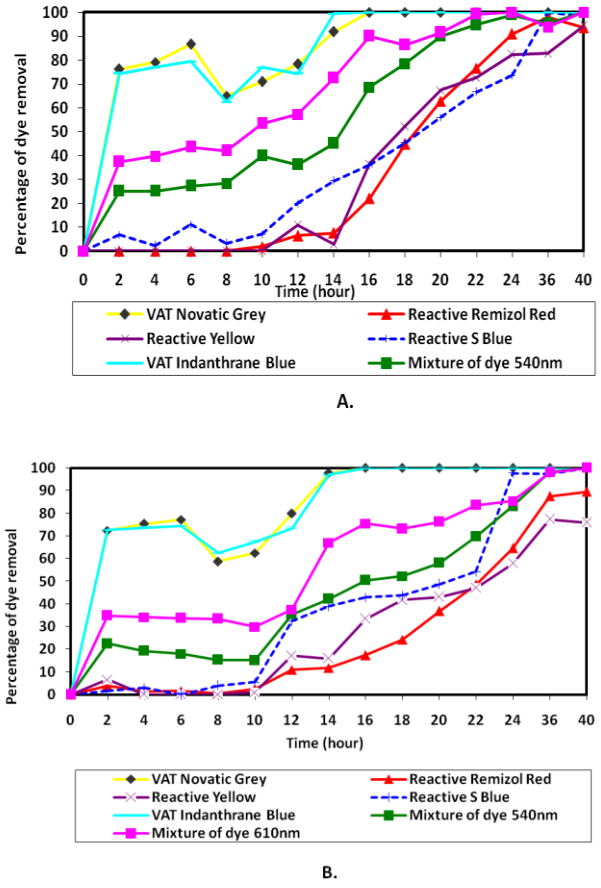

Fig. 2: Percentage reduction of various dyes vs time when biomass is in growing mode i.e. bioaccumulation at initial dye concentration A.) 50 mg/L; B.) 100 mg/L

Table 2: Specific dye removal per unit fungal biomass after 40 hours of growth

S. No.	Dyes	Initial concentration of dye 50 mg/L			Initial concentration of dye 100 mg/L		
		Biomass (g/L)	Dye removal (mg/L)	Specific dye removal (mg/g)	Biomass (g/L)	Dye removal (mg/L)	Specific dye removal (mg/g)
1.	Vat Novatic Grey	6.97	48.97	7.03	4.77	93.79	19.66
2.	Reactive Remazol Red	6.29	42.94	6.83	6.41	85.41	13.32
3.	Reactive Yellow	5.2	47.25	9.09	7.27	79.13	10.88
4.	Reactive S Blue	6.58	~50	8.05	5.9	~100	19.94
5.	Vat Indanthrane Blue	5.22	~50	10.82	4.9	~100	22.01
6.	Mixture of dye 540nm	6.27	~50	9.23	5.25	~100	21.87
7.	Mixture of dye 610nm	6.27	48.33	7.71	5.25	96.48	18.37

3.3 Microscopic Examination

The *A. lentulus* pellets were microscopically examined under 10X, which revealed the accumulation of dyes within the fungal hyphae in the colored pellet (Fig. 6).

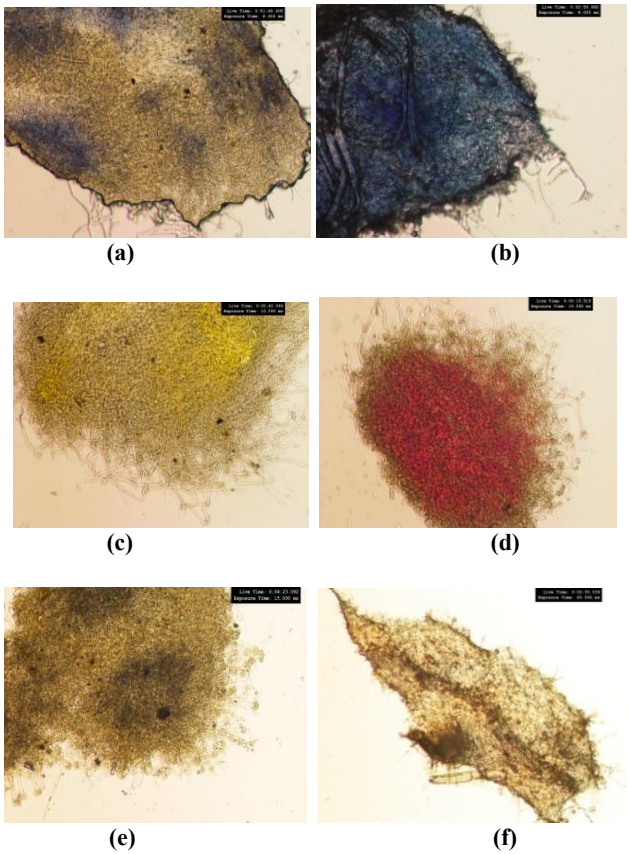

Fig. 6: Microscopic images of the pellets *A. lentulus* and accumulated dye in the hyphae, a.) VAT Indanthrane Blue, b.) Reactive S Blue, c.) Reactive Yellow, d.) Reactive Remizol Red, e.) VAT Novatic Grey, f.) Dye Mixture

4. CONCLUSION

In the present study, the fungal strain *Aspergillus lentulus* was capable of removing Vat as well as Reactive dyes by biosorption as well as bioaccumulation. Moreover, the dye mixture was also efficiently removed with a very high dye uptake capacity of 21.87 mg/g of fungal biomass. Such high efficiency of dye removal in less time by *A.lentulus* could be harnessed in decolourization of colored textile effluents, hence, decreasing the toxic effects of textile dyes.

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REFERENCES

[1] R. Babu and B. Parande, "S and Prem Kumar, T. 2007. Textile technology cotton textile processing waste generation and effluent treatment," *J. Cott. Sci*.

[2] R. G. Saratale, S. S. Gandhi, M. V Purankar, M. B. Kurade, S. P. Govindwar, S. E. Oh, and G. D. Saratale, "Decolourization and detoxification of sulfonated azo dye C.I. Remazol Red and textile effluent by isolated Lysinibacillus sp. RGS.," *J. Biosci. Bioeng.*, vol. 115, no. 6, pp. 658–67, Jun. 2013.

[3] K. Schneider, C. Hafner, and I. Jäger, "Mutagenicity of textile dye products.," *J. Appl. Toxicol.*, vol. 24, no. 2, pp. 83–91, April 2004.

[4] D. Wesenberg, I. Kyriakides, and S. Agathos, "White-rot fungi and their enzymes for the treatment of industrial dye effluents," *Biotechnol. Adv.*, 2003.

[5] H. Lata, V. K. Garg, and R. K. Gupta, "Removal of a basic dye from aqueous solution by adsorption using Parthenium hysterophorus: An agricultural waste," *Dye. Pigment.*, vol. 74, no. 3, pp. 653–658, Jan. 2007.

[6] S. D. Hosseini, F. S. Asghari, and H. Yoshida, "Decomposition and decoloration of synthetic dyes using hot/liquid (subcritical) water.," *Water Res.*, vol. 44, no. 6, pp. 1900–8, Mar. 2010.

[7] I. M. Banat, P. Nigam, D. Singh, and R. Marchant, "Microbial decolourization of textile-dyecontaining effluents: A review," *Bioresour. Technol.*, vol. 58, no. 3, pp. 217–227, Dec. 1996.

[8] C. Doğar, A. Gürses, M. Açikyildiz, and E. Ozkan, "Thermodynamics and kinetic studies of biosorption of a basic dye from aqueous solution using green algae Ulothrix sp.," *Colloids Surf. B. Biointerfaces*, vol. 76, no. 1, pp. 279–85, Mar. 2010.

[9] L. Coulibaly, G. Gourene, and N. S. Agathos, "Utilization of fungi for biotreatment of raw wastewaters," *African Journal of Biotechnology*, vol. 2, no. 12. Academic Journals (Kenya), pp. 620–630, 27-Feb-2004.

[10] P. Kaushik and A. Malik, "Alkali, thermo and halo tolerant fungal isolate for the removal of textile dyes.," *Colloids Surf. B. Biointerfaces*, vol. 81, no. 1, pp. 321–8, Nov. 2010.

[11] P. Kaushik and A. Malik, "Comparative performance evaluation of Aspergillus lentulus for dye removal through bioaccumulation and biosorption," *Environ. Sci. Pollut. Res.*, vol. 20, no. 5, pp. 2882–2892, 2013.

[12] K. Kumari and T. Abraham, "Biosorption of anionic textile dyes by nonviable biomass of fungi and yeast," *Bioresour. Technol.*, vol. 98, no. 9, pp. 1704-1710, Jul. 2007.

[13] P. Kaushik and A. Malik, "Removal of Dyes by Aspergillus sp. in Growth Supportive and Growth Restricted Modes", *Proceedings Of International Conference On Energy And Environment*, pp. 239-241, Marsh, 2009.

[14] M. Ramya and B. Anusha, "Biodecolourization and biodegradation of Reactive Blue by *Aspergillus sp*.," *African J. of Microbiology.*, vol. 6, no. 12, pp. 1441-1445, Jun. 2007.