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Decolorization of Reactive Violet – 2RL Dye by Aspergillus Flavus and Aspergillus Fumigatus from Textile Sludge

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Abstract

Fungi have the ability to degrade a diverse range of pollutants and are attracting wide-spread use in bioremediation. Successful application of decolorization of textile dyes to treat high concentration of industrial effluents will be a mile stone owing to advanced treatment processes. This research paper is aimed to elucidate the decolorization of commercial reactive dye by Aspergillus flavus and Aspergillus fumigatus under static batch experiments. Degradation of Reactive violet – 2RL dye is characterized by HPLC analysis. The enhanced decolorization by Aspergillus flavus was attributed to the highest percentage of decolorization of 89.13% in 20 ppm.

Keywords: Reactive violet – 2RL Dye, Aspergillus flavus, Aspergillus fumigatus, decolorization, HPLC.

Introduction

Dyes are synthetic and aromatic molecular structural compounds. They are used as substrates in food, cosmetics, paper, plastic and textile industries[1]. Among these various industries, textile ranks first in usage of dyes for coloration of fabric. During the dyeing process, approximately 10–15% of the dyes used are released into the aquatic environment like rivers and streams. The presences of these dyes in the aquatic ecosystem are the serious cause of environmental and health concerns[2,3]. Several methods are used to treat textile effluents to achieve decolorization and degradation. The discharged effluents could have a hazardous influence on the environment. The strong color of discharged dyes even at very small concentrations has a huge impact on the aquatic environment caused by its turbidity and high pollution strength[4]. Removal of dyes from textile effluents has been carried out by physical and chemical methods, such as flocculation, membrane filtration, electrochemical techniques, ozonation, coagulation and adsorption[5]. These methods are effective but they are expensive and involve the formation of a concentrated sludge that creates a secondary disposal problem[6]. Considering drawbacks in above mentioned conventional treatment methods, microbial remediation techniques have gained much attention in the last few decades.

Microbial decolorization and degradation is an eco-friendly, cost-competitive and effective method compared to conventional treatment technologies[7,8]. Biological processes represent a good alternative for remediation of environmental pollutants given the ability of some microorganisms to mineralize a wide variety of toxic xenobiotics and to oxidize substrates with low solubility, such as chlorinated phenolics, synthetic dyes, pesticides and polycyclic aromatic hydrocarbons[9-11]. Fungi are able not only to decolorize but also to degrade and mineralize a broad spectrum of different dye structures (azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes), in addition to numerous other toxic organic and recalcitrant compounds[12-14]. This research paper describes the efficiency of decolorization of reactive violet – 2RL dye by *Aspergillus flavus* and *Aspergillus fumigatus*.

Material and Methods

Dyes: Reactive violet - 2RL, commercially available textile dye was used for decolorization studies. The structure of dye is given in Figure-1. All the chemicals were used of analytical grade and used without further purification.

Collection of textile sludge: The textile sludge was collected from dyeing industry located at Perundurai, Erode District, Tamil Nadu, India, in sterile airtight plastic containers and filtered, to remove large suspended particles and stored at 4±1°C until use.

Isolation and maintenance of fungi: The collected textile sludge was serially diluted to the concentration of 10^{-1} – 10^{-12} was inoculated in Czapek Dox Agar at pH-7.0 by the spread plate technique and incubated at room temperature for 3-7 days. The plates were observed for growth of fungi. The fungal isolates were cultured and maintained on Czapek Dox agar at 28±2ºC and sub-cultured consistently every 10 days.

Screening of Fungi, Fungal growth Assay: Fungal mycelial agar disc of ~2 mm diameter were cut from the colony margin (actively growing region) and inoculated on center of petridish containing Czapek Dox agar and un-inoculated plate was maintained as control. These plates were incubated at room

temperature for 7 days. The experiments were performed in triplicate for each culture. The growth of fungi was determined in two perpendicular directions of the plate.

Dye-Agar plate Assay: The isolated fungi were screened for decolorization studies by Dye-agar plate assay. Fungi mycelial agar disc (~2 mm) were cut from the colony margin (actively growing region) and inoculated on center of petridish (in triplicates) containing Czapek Dox Agar supplemented with100ppm of Reactive violet - 2RL dye. All dye agar plates were performed in triplicate and incubated at room temperature (~28^{o}C) in dark for 7 days and un-inoculated dye agar plates were maintained as control. Clearing of the dye indicates decolorization; the size of the decolorization halo was measured in two perpendicular directions of the plate.

Statistical Analysis: Fungal growth and Dye-agar plate assay was conducted in triplicate and results are presented in the mean of triplicates ± standard deviation (SD).

Aqueous Batch Decolorization studies: The batch decolorization studies were conducted in 100 ml Erlenmeyer flasks containing 50 ml of C-limited medium prepared with various concentrations (20,40,60,80,100 ppm) of reactive violet-2RL dye inoculated with 2 mm fungal disc and incubated at 37^{o}C in static condition for 7 days. 5 ml of samples were withdrawn for every 24 h, centrifuged at 10,000 rpm for 10 min and read maximum absorbance at 547 nm by using UV-VIS spectrophotometer (Model :Shimadzu, UV-2400PC series), similarly un-treated medium served as blank. The percentage of decolorization was calculated by using the formula as follows:

$$\text{Percentage of Decolorization} = \frac{\text{ODi} - \text{ODf} \times 100}{\text{ODi}}$$

Where, OD_i is the initial absorbance of dye (mg/l) and OD_f is the final absorbance of dye concentration (mg/l) at different time intervals.

HPLC Analysis: After 7days, 5ml of decolorized samples were taken from 100 ml Erlenmeyer flask containing 20 ppm of dyes with C-limited medium. The decolorized samples were centrifuged and filtered through 0.45 µm of nylon membrane syringe filters of 28 mm diameter. The decolorized samples were extracted thrice with equal volume of dichloromethane (DCM) and evaporated at 50^{o}C in a rotary vaccum evaporator. The extracted residue was dissolved in 2 ml of methanol. The samples were analyzed by using HPLC system Shimadzu (method type: G-ALKA) equipped with detector using C18 reversed phase column with HPLC grade acetonitrile: water in the ratio 60:40 as mobile phase at the flow rate of 0.5 ml / min for 8min, pressure of 46 at room temperature and the UV-VIS detector set at 285 nm.

Results and Discussion

Identification of Fungi: Isolated fungi were identified by using lacto phenol cotton blue staining method and further confirmed at Agharkar Research Institute, Pune. The fungal isolates were identified as *Aspergillus flavus and Aspergillus fumigatus* and the fungal isolates are shown in figures- 2 and 3.

Fungal growth assay: The fungal isolates were grown on Czapek Dox agar and most rapid growth was observed and the statistical data are shown in tables- 1 and 2.

Dye decolorization efficiency by plate assay: The decolorization efficiency of the fungal isolates (*Aspergillus flavus* and *Aspergillus fumigatus*) was screened with reactive violet – 2RL dye on Czapek Dox agar. The distance from the edge of the fungal colony to the growing front of the spreading fungal hyphae was measured. Plates were measured for 7 days and statistical data are shown in tables- 3 and 4. Better decolorization, was achieved by *Aspergillus flavus* for Reactive Violet – 2RL dye.

Decolorization Studies: The fungal strains showed the decolorization of reactive violet – 2RL dye in the concentration of 20, 40, 60, 80 and 100 ppm to more than 65%. The highest percentage of decolorization was showed by *Aspergillus flavus* (89.13%) and *Aspergillus fumigatus* (87.55%) as shown in tables- 5 and 6. Among the fungal isolates, the highest decolorization efficiency of 89.13% was showed by *Aspergillus flavus*.

HPLC analysis of decolorized samples: The HPLC analysis (figure-4) for the samples taken at the 7th day of static incubation showed peak at a retention time of 2.15 min, which represents the retention time of pure reactive violet – 2RL. In the treated sample two peaks were seen with the retention time of 1.66 and 2.24 for *Aspergillus flavus*, 1.68 and 2.22 min for *Aspergillus fumigatus*, which clearly indicate that methanol and dye, decolorized samples were not, degraded. The present study confirms the ability of *Aspergillus flavus and Aspergillus fumigatus* to decolorize the Reactive violet – 2RL dye.

Conclusion

The isolated fungal strains have excellent potential for decolorization of reactive dye, Violet – 2RL. This research paper describes that *Aspergillus flavus* (89.13%) and *Aspergillus fumigatus* (87.55%) were able to completely decolorize Reactive violet – 2RL dye (20 ppm) within 7 days. Analysis of decolorized products by HPLC shows that both the fungi have the ability to decolorize the dyes. It is observed by repeated laboratory studies involving pure cultures of fungi. It also helps in degradation of hydrocarbons in the environment. Fungi have considerable attention due to their extracellular enzymes involved in the diverse applications. Biosorption of dyes by fungi is an effective method, cost-efficient and eco-friendly. The regenerated biomass can be recycled for bioremediation of textile effluents.

References

1. Campos R., Kandelbauer A., Robra K.H., Artur C.P. and Gubitz G.M., Indigo degradation with purified laccases from *Trameteshirsuta* and *sclerotimrolfsii*, *J. Biotechnol*., **8**, 131-139 **(2001)**

2. Fang H., Wenrong H. and Yuezhong L., Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium, *Chemosphere,* **57**, 293–301 **(2004)**

3. Asad S., Amoozegar M.A., Pourbabaee A.A., Sarbolouki M.N. and Dastgheib S. M.M., Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria, *Bioresource Technol*., **98**, 2082–2088 **(2007)**

4. Hao O.J., Kim H. and Chiang P.C., Decolorization of wastewater, *Crit. Rev. Environ. Sci.,* **30**, 449-505 **(2000)**

5. Fu Y. and Viraraghavan Y., Removal of Congo red from an aqueous solution by fungus *Aspergillus niger*, *Adv. Environ. Res*.,**7**, 239-247 **(2004)**

6. Maier J., Kandelbauer A., Erlacher A., Cavaco-Paulo A. and Gubitz M.G., A new alkali-thermostable azoreductase from *Bacillus sp.* strain SF, *Appl. and Environmental Microbiology,* **70**, 837–844 **(2004)**

7. Verma P. and Madamwar D., Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescens*, *World. J. Microbiol. Biotechnol*., **19**, 615–618 **(2003)**

8. Gogate P.R and Pandit A.B., A review of imperative technologies for wastewater treatment II: hybrid methods, *Advan. Environ. Res*., **8**, 553-597 **(2004)**

9. Reddy C.A., The potential for white rot fungi in the treatment of pollutants, *Curr. Opin. Biotechnol*., **6,** 320-328 **(1995)**

10. Rodriguez E., Pickard M.A. and Vazquez-Duhalt R., Industrial dye decolourization by laccases from ligninolytic fungi, *Curr. Microbiol*., **38**, 27-32 **(1999)**

11. Torres E., Bustos-JaimeS I. and Le Borgne S., Potential use of oxidative enzymes for detoxification of organic pollutants, *Appl. Catal. B-Environ*., **46**, 1-15 **(2003)**

12. Machado K.M.G., Matheus D.R., Monteiro R.T.R. and Bononi V.L.R., Biodegradation of pentachlorophenol by tropical basidiomycetes in soils contaminated with industrial residues, *World J. Microbiol. Biotechnol.*, **21**, 297-301 **(2005)**

13. Shah V. and Nerud F., Lignin degrading system of white-rot fungi and its exploitation for dye decolorization. Review, *Canadian J. Microbiol.*, **48**, 857-870 **(2002)**

14. Wesenberg D., Kyriakides I. and Agathos S.N., White rot fungi and their enzymes for the treatment of industrial dye effluents, *Biotechnol. Adv.*, **22**, 161-187 **(2003)**

Table-1
Fungal Growth Assay of *Aspergillus flavus*

Fungal Growth Assay : *Aspergillus flavus*		
Duration/ Incubation Days	**Horizontal (cm)**	**Vertical (cm)**
1.	0.80±0.10	0.86±0.011
2.	1.43±0.25	1.56±0.20
3.	2.33±0.15	2.43±0.20
4.	2.86±0.37	2.90±0.20
5.	3.26±0.55	3.33±0.35
6.	3.50±0.60	3.53±0.45
7.	**3.66±0.65**	**3.73±0.45**

Table-2
Fungal Growth Assay of *Aspergillus fumigatus*

Fungal Growth Assay : *Aspergillus fumigatus*		
Duration/ Incubation Days	**Horizontal (cm)**	**Vertical (cm)**
1.	0.76±0.05	0.76±0.05
2.	1.10±0.10	1.03±0.05
3.	1.20±0.10	1.23±0.05
4.	1.40±0.00	1.36±0.05
5.	1.43±0.05	1.50±0.10
6.	1.60±0.10	1.56±0.15
7.	1.76±0.20	1.73±0.25

Table-3
Dye-Agar Plate Assay of Reactive Violet – 2RL Using *Aspergillus flavus*

Dye Agar plate Assay (cm): *Aspergillus flavus*				
Duration/ Incubation Days	**Reactive Violet – 2RL**			
	Horizontal	**Zone**	**Vertical**	**Zone**
1.	1.66±0.11	1.93±0.05	1.66±0.20	2.03±0.23
2.	1.93±0.40	2.20±0.34	1.90±0.34	2.16±0.28
3.	2.16±0.46	2.40±0.51	2.16±0.46	2.36±0.46
4.	2.33±0.57	2.53±0.57	2.33±0.57	2.53±0.57
5.	2.46±0.64	2.70±0.70	2.46±0.64	2.70±0.70
6.	2.70±0.72	2.90±0.72	2.73±0.77	2.93±0.77
7.	**2.93±0.85**	**3.10±0.79**	**2.93±0.55**	**3.10±0.79**

Table-4
Dye-Agar Plate Assay of Reactive Violet – 2RL Using *Aspergillus fumigatus*

Dye Agar plate Assay (cm): *Aspergillus fumigatus*				
Duration/ Incubation Days	**Reactive Violet – 2RL**			
	Horizontal	**Zone**	**Vertical**	**Zone**
1.	0.83±0.05	1.03±0.05	0.83±0.11	1.03±0.11
2.	1.03±0.05	1.20±0.00	1.10±0.17	1.30±0.17
3.	1.13±0.05	1.33±0.05	1.20±0.17	1.40±0.17
4.	1.23±0.05	1.43±0.05	1.30±0.17	1.50±0.17
5.	1.43±0.23	1.63±0.23	1.40±0.20	1.60±0.20
6.	1.53±0.05	1.66±0.11	1.56±0.15	1.76±0.15
7.	1.80±0.10	2.00±0.10	1.86±0.15	2.06±0.15

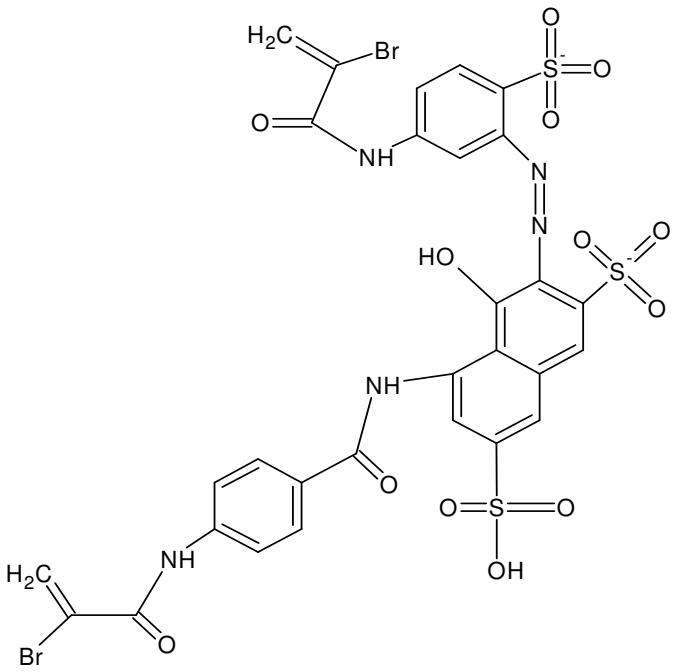
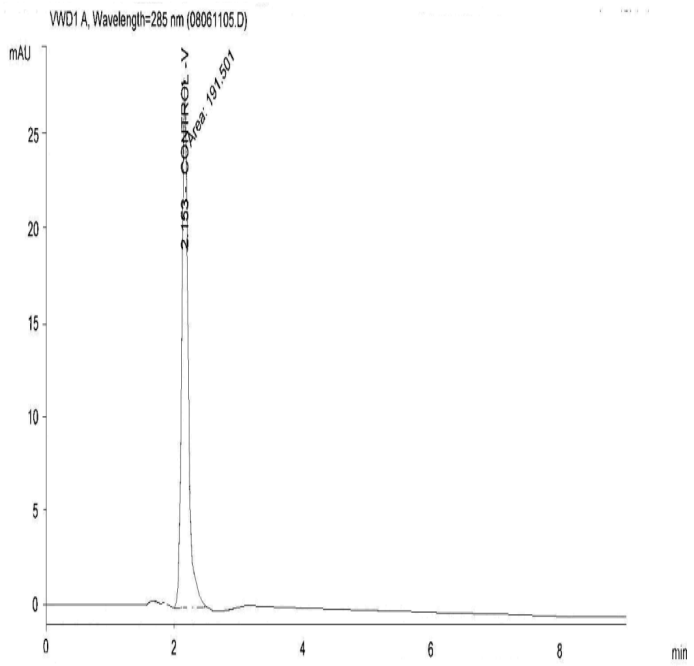
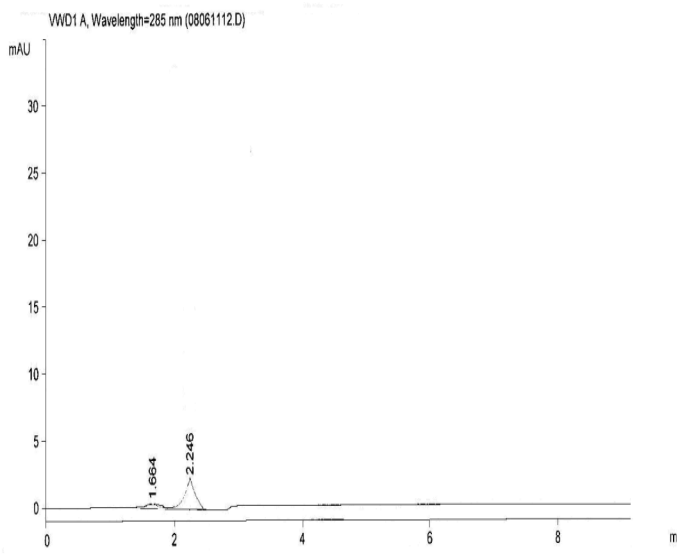


Figure-1
Structure of Reactive Violet – 2RL

Figure- 3
Aspergillus fumigatus

Figure- 2.
Aspergillus flavus





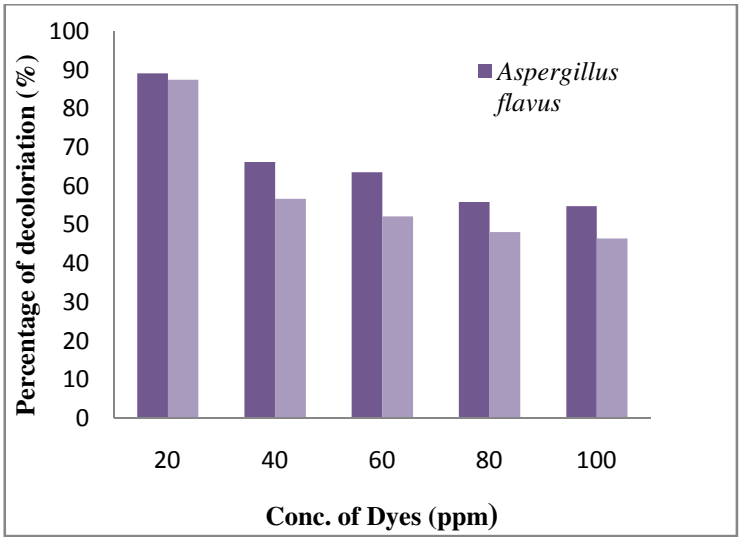


Figure-5
Percentage of Decolorization by *Aspergillus flavus* and *Aspergillus fumigatus*

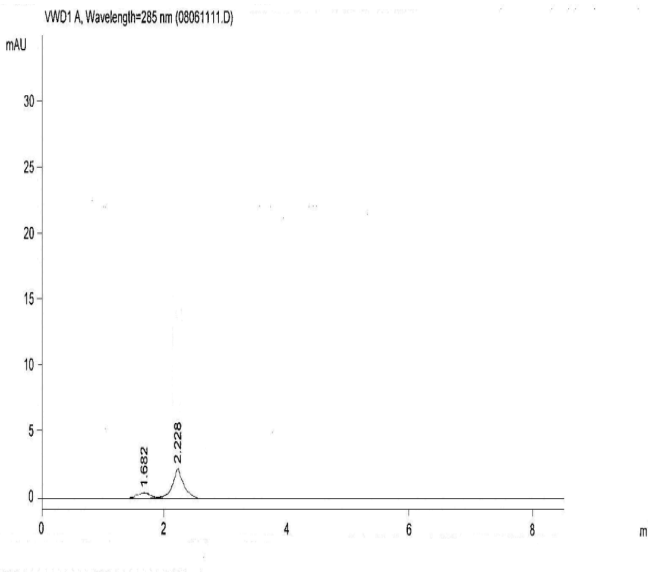


Figure-4
HPLC profile of Reactive Violet – 2RL dye, (a) Control (b) Decolorized sample of *Aspergillus flavus* (c) Decolorized sample of *Aspergillus fumigatus*