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Biodegradation and Decolorization of Reactive Blue MR, Using *Aspergillus* and *Penicillium* Species Isolated from Textile Effluent

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ABSTRACT

The Present investigation focused on isolation and characterization of fungal strains which has an ability to decolorize textile dye, reactive Blue MR. Initially, the effluent sample was collected from the textile industry situated in ichalkaranji, India. The effluent sample was analysed for their physicochemical properties. Various fungal species were isolated, and decolorization capabilities of these fungal species were evaluated for reactive Blue MR dye (50%) in minimal medium, under optimum conditions. It was found that Aspergillus and Penicillium species showed higher decolonization capabilities after 48 hours of incubation. So these fungal isolates are potential candidates for bioremediation.

Keywords: Decolorisation, Textile dye, Reactive blue MR, *Aspergillus*, *Penicillium*.

INTRODUCTION

Dye production in India is estimated to be around 64,000 tonnes, which is about 6.6% of world production. There are around 700 varieties of dyes and dye intermediates produced in India, mainly direct dyes, acid dyes, reactive dyes and pigments [1]. The worldwide annual production of dyes is over 7.105 tons [2,3]. Synthetic dyes mostly used in textile industries. These dyes have not been tested for its toxicity. Textile industries use large quantities of water (1 kg of fabric requires about 150 liters of water) for different processes. Textile effluents color the drains and ultimately water bodies diminish the water quality [4].

An average textile mill produces 60 × 104 m of fabric and discharges approximately 1.5 million liters of effluent per day in India [5,6]. These effluents contain a considerable amount of suspended solids, additives, detergents, surfactants, carcinogenic amines, formaldehyde, heavy metals, and dyes. Fluctuating pH, high temperature, COD and complex coloration are the main characters of textile effluent; it poses serious environmental threats to receiving water bodies [7,8].

Commonly applied treatment methods for color removal from colored effluents consist of integrated processes involving various combinations of biological, physical and chemical decolorization methods [9-11] but these methods have certain drawbacks, they have limited efficiency, requires a large number of chemicals hence costly. Biological treatment methods are more desirable as they are environmentally friendly, do not produce secondary pollutants and have a higher possibility of wider application [12].

However, viable biological treatment using microorganisms requires cheap carbon sources. In some cases, traditional biological procedures were combined with chemical or physical treatment processes to achieve better decolorization. These micro-organisms have the ability to decolorize, degrade, and detoxify textile effluent. Bioremediation offers an effective alternative method for degrading dyes present in wastewater. Fungi, in particular, are known to decolorize and metabolize dyes [13,14], and in some cases, have the potential to decolorize these dyes within a comparatively shorter time [15]. The present study deals with the isolation of fungal species from textile effluents, having the ability to decolorize reactive blue MR 250. Bacteria will be isolated from textile effluent by using a minimal medium.

Decolorisation ability of organisms will be tested by using minimal medium containing an equal amount of reactive blue MR 250.

MATERIALS AND METHODS

Sampling

The samples used for investigation were textile industry effluent collected in bottles. The samples were transported to the laboratory for further analysis. Before use filtration was done by using ordinary filter paper to remove particulate matter present in the effluent.

Dye

In the present investigation, textile dye (Reactive Blue MR) (Figure 1) was used for decolorization and was obtained from ichalkaranji textile mills.

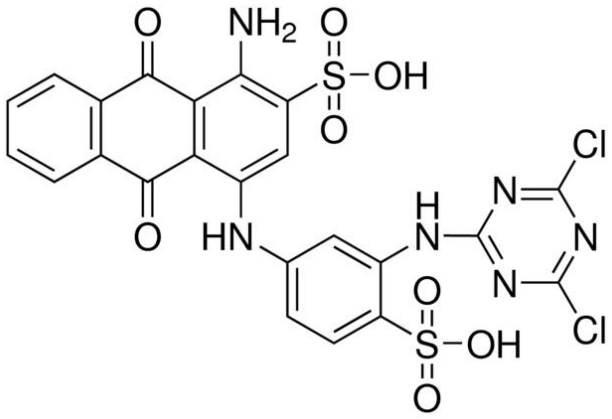

Figure 1. Chemical structure of Reactive Blue MR

Chemicals

All media components and chemicals used in the present investigation were of analytical grade and purchased from Hi-Media Laboratories, INDIA.

The medium used (minimal salt medium)

Minimal salt medium used in process showed in (Table 1).

Table 1. Minimal salt medium

Component	Weight in gm/ml
K_2HPO_4	0.7
KH_2PO_4	0.3
Sodium citrate	0.05
$MgSO_4.7H_2O$	0.01
Di-ammonium sulphate	0.1
Trace salt solution	0.5 ml
Glucose	0.1
D/W	100 ml

Trace salt solution

Trace salt solution used in this process is shown in (Table 2).

Table 2. List of Trace salt solutions involved

Component	Weigh in gm/ml
$FeSO_4.7H_2O$	0.05
$ZnSO_4.7H_2O$	0.05
$MnSO_4.3H_2O$	0.05
D/W	100 ml

Physicochemical analysis of effluent

The effluent of the textile dye industry was filtered through Whatman filter paper no. 1. And it is analyzed for different characters such as color, texture, total dissolved solids, total suspended solids, chemical oxygen demand, and biological oxygen demand.

Determination of absorption maxima of reactive Blue MR

The absorption maximum of Reactive Blue MR was determined by using a spectrophotometer. The optical density of dye solution in water was observed at a different wavelength. The wavelength was dye showed maximum absorbance is taken as absorption maximum of dye, for Reactive Blue MR it is 600 nm.

Enrichment, isolation, and identification of dye decolorizing fungi

Collected effluent was used as inoculum to isolate dye degrading organisms. For enrichment 1 ml of effluent was inoculated aseptically to the sterile minimal broth and it was incubated under optimum conditions for an optimum period of time. For the isolation of fungal species, minimal agar medium has used a loopful of the enriched sample was streaked on a sterile minimal agar plate and incubated under optimum conditions for an optimum period of time. After incubation fungal species with different growth patterns and morphology were isolated and preserved on minimal agar slants at 4°C refrigerator and were served as stock cultures selected isolates were identified on the basis of growth patterns, staining, biochemical reactions by using Bergey´s manual of determinative bacteriology.

Decolorization assay

The ability of fungal strains to decolorize textile dye was determined in minimal medium, added with reactive blue MR. Sterile test tubes containing 5 ml of sterile minimal medium plus 5 ml of reactive blue MR dye were inoculated with fungal species separately. The tubes were incubated at room temperature and after each 24 hours sample were removed aseptically, centrifuged at 10,000 rpm for 10 min and the supernatant was scanned in spectrophotometer at 600 nm one control was also maintained containing 5ml of minimal medium plus 5 ml of dye but no fungal species percentage of decolorization were calculated by applying formula

Decolorization % = Ao- At/ Ao × 100

Where:

Ao is the initial absorbance of the sample

At is the absorbance at a different time interval

RESULTS AND DISCUSSION

Physiochemical characterization of textile effluent

The effluent of the textile industry was blackish-blue in color with a pungent smell, the black color may be due to the various dye and chemicals used in the textile industry. Total dissolved solids 50 mg/L total suspended solids biological oxygen demand 450 mg/L chemical oxygen demand 600 mg/L.

Isolation and identification of fungal species

Various fungal species were isolated from effluent using minimal medium containing an equal amount of dye. Out of which only two fungal species found to be effective in decolorizing effluent, were identified after staining with lactophenol cotton blue and microscopic analysis viz, *Aspergillus* and *Penicillium* species.

These fungal species may be adapted to use effluent as an energy source.

Decolorisation assay

Decolorisation assay was carried out in a liquid medium (Minimal medium containing an equal amount of Reactive blue MR dye). It was found that *Aspergillus* and *Penicillium* species have maximum decolorization ability under optimum conditions than any other isolates (Figure 2).

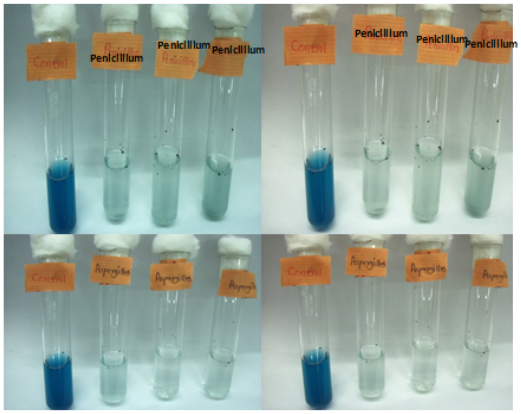

Figure 2. Decolorization assay by *Penicillium* and *Aspergillus* spp

Optical density at 600 nm

Optical density after inoculation

Optical density after inoculation of the sample (Figure 3 and Tables 3 and 4).

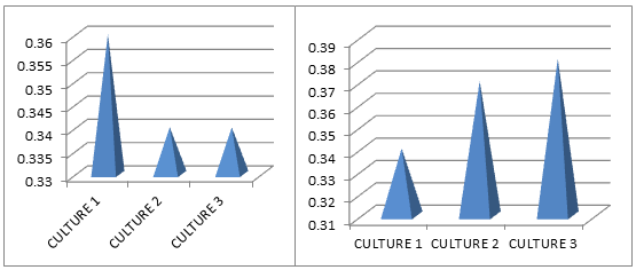

Figure 3. Optical density after the inoculation of samples

By *Aspergillus* spp.

Table 3. Optical density of *Aspergillus* samples

Culture	O.D.
1	0.36
2	0.34
3	0.34

By *Penicillium* spp.

Table 4. Optical density of *Penicillium* samples

Culture	O.D.
1	0.34
2	0.37
3	0.38

Optical density after 12 hours

Optical density of cultured samples after 12 hours (Figure 4 and Tables 5 and 6).

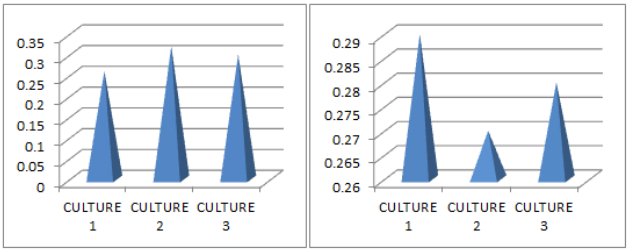

Figure 4. Optical density of sample after 12 hours

By *Aspergillus* spp.

Table 5. Optical density of *Aspergillus* samples

Culture	O.D.
1	0.26
2	0.32
3	0.30

By *Penicillium* spp.

Table 6. Optical density of *Penicillium* samples

Culture	O.D.
1	0.29
2	0.27
3	0.28

Optical density after 36 hours

Optical density of cultured samples after 36 hours (Figure 5 and Tables 7 and 8).

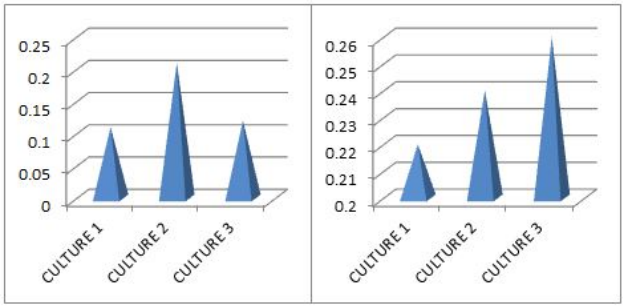

Figure 5. Optical density of sample after 36 hours

By *Aspergillus* spp.

Table 7. Optical density of *Aspergillus* samples

Culture	O.D.
1	0.11
2	0.21
3	0.12

By *Penicillium* spp.

Table 8. Optical density of *Penicillium* samples

Culture	O.D.
1	0.22
2	0.24
3	0.26

CONCLUSION

The wastewater treatment by traditional methods requires the use of chemicals which make it uneconomic and it also causes environmental problems. Hence eco-friendly methods by using bacteria can be applied. The present study reveals that the enriched cultures of *Aspergillus* and *Penicillium* species are effective in biodegradation and decolorization of Reactive Blue MR. these organisms show maximum decolorization at neutral pH and 37℃ temperature. So these organisms can be used for textile wastewater treatment.

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