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RESEARCH ARTICLE

Degradation of Reactive Red HE7B and Yellow FN2R dyes by fungal isolates

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Abstract

The present investigation focused on the isolation and characterization of fungal strains which can efficiently decolorize Red HE7B (C.I. Reactive Red 141) and Yellow FN2R (C.I. Reactive Yellow 206) textile dyes. A total of 6 indigenous fungal strains were isolated from the effluents collected around the discharge site of textile industry situated in Salem District. The fungal isolates were identified as *Aspergillus niger, A. flavus, Fusarium* sp.*, Penicillium* sp.*, Curvularia verruciformis* and *Mucor racemosus*. Decolorization capabilities of these fungal species against the azo dyes were carried out in potato dextrose agar medium under static invitro condition. Highest percentage of degradation was achieved against Red HE7B and Yellow FN2R by *Aspergillus niger* (94%) and *Mucor racemosus* (92%) after 5 d of incubation. This study has confirmed the potential of the test fungi in the decolorization of azo dyes and opened scope for the future analysis of their performance in the treatment of textile effluent.

Keywords: Red HE7B, Yellow FN2R, decolorization, dye degrading fungi, textile dye.

Introduction

Textile industries are major sources of effluents due to the nature of their operations which requires high volumes of water that eventually results in high wastewater generation (Ghorishi and Haghigh, 2003). Wastewater from printing and dyeing units is often rich in color, containing residues of reactive dyes and chemicals and requires proper treatment before being released into the environment (Ramesh Babu *et al*., 2007). Wastewater from the textile industries contains different types of synthetic dyes, which are mostly toxic, mutagenic and carcinogenic. Moreover, their stability to light, temperature and microbial attack, making them recalcitrant compound (Kokol *et al.,* 2007). Textile wastewater includes a larger variety of dyes and chemicals that makes the environment challenge for textile industry not only as liquid waste but also in its chemical composition (Venceslau *et al.,* 1994). Major pollutants in textile wastewater are high-suspended solids, chemical oxygen demand, heat color, acidity and other suitable substrates (Hee *et al.,* 1999).The removal of color from textile industry and dyestuff manufacturing industry's wastewater represents a major environmental concern. In addition, only 47% of dyestuffs are biodegradable (Pagga and Brown, 1986).

Dyes usually have a synthetic origin and complex aromatic molecular structures which possibly come from coal-tar based hydrocarbons such as benzene naphthalene, anthracene, toluene and xylene. Different dyes have different molecular structures. So a microbial capable of decolorizing one dye may have different capacities for the dyes (Knapp *et al*., 1995; Wong and Yu, 1999).

It is estimated that 2, 80,000 tons of textile dyes are discharged every year as such industrial effluents worldwide (Mass and Chaudhaii, 2005). Organic pollutants which originate from organic compounds of dye stuffs, acids, sizing materials, enzymes, tallow etc., are also found in textile effluents. Such impurities are reflected in the analysis of bio-chemical oxygen demand (BOD) and Chemical oxygen demand (COD). In many textile units, particularly engaged in synthetic processing, low BOD/COD ratio of effluent is observed which makes even biological treatment is rich in starch, bicarbonates, chlorides and elements like copper and chromium. Concentration of accumulated metals not only cause mortality but generally results in sub lethal stress and many affect growth rate, reproductive success and their ability to compete with other species in the ecosystem (Ramesh Babu and Parande, 2007).

Currently the major methods of textile waste water treatments involve physical and chemical process. There is also possibility that a secondary pollution problem arise because of excessive chemicals used (Wesenberg *et al*., 2003). Many microorganisms belonging to different taxonomic group of bacteria, fungi (Jadhav *et al.,* 2007), Actinomycetes and Algae (Daneshvar *et al.,* 2007) have been reported for their ability to decolorize azo dyes. Bacterial degradation of these dyes was carried out by their intracellular uptake while the fungi degrade these by extra cellular enzymes (Yoo *et al.,* 2001). The organisms used in most of the study were *Staphylococcus* sp., *E.coli, Bacillus* sp.*, Clostridium* sp., *and Pseudomonas* sp. (McMullan *et al.,* 2001).

Many studies have demonstrated that the white-rot fungi, namely *Phanerochaete chrysosporium, Bjerkandera adusta, Trametes versicolor*, or *Phlebia radiata*, are able to degrade a broad spectrum of structurally diverse dyes. Decolorization of azo, anthraquinonic, heterocyclic, triphenylmethane and polymeric dyes and their partial mineralization by enzymatic and non-enzymatic systems of these fungi have been reported by Ferreira *et al.* (2000). Fungal systems appear to be most appropriate biological agent in the treatment of colored and metallic effluents. In recent years, several adsorbents have been identified as possessing good dye-binding capabilities (Pavan *et al.,* 2008). In particular, biomaterials of microbial origin have been very effective because of their cell wall constituents. Important fungal biosorbents include *Aspergillus* (Fu and Viraraghavan, 2002), *Penicillium* (Iscen *et al.,* 2007) and *Rhizopus* (Kumari and Abraham, 2007*)*. Against these backdrops, this study was aimed to evaluate the efficiency of fungal isolates on the degradation of Red HE7B and Yellow FN2R.

Materials and methods

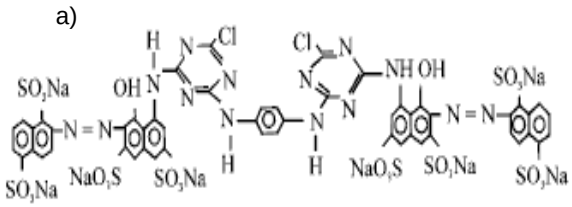
Collection of samples: Twenty different color dye wastes, two soil samples and four dye effluents were collected from Salem town (Tamil Nadu, India) to isolate the dye degrading fungus. They were stored in sterile glass tubes with screw tops at 4±1ºC until use.

Chemicals: Red HE7B (C.I. Reactive Red 141) and yellow FN2R (C.I. Reactive Yellow 206) were purchased from KCS dyes, Salem. The chemical structure of the dyes is shown in Figure 1a and b.

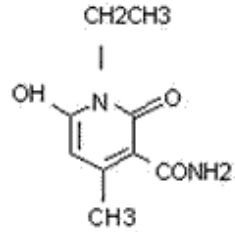
Analysis of physiochemical parameters: The dye effluent was collected in sterile polythene containers and stored at room temperature. Physiochemical properties of the stored effluent like color intensity, pH, Total suspended solids (TSS) and Total dissolved solids (TDS) were analyzed.

Fig. 1a. Red HE7B (C.I. Reactive Red 141);
b. Yellow FN2R (C.I. Reactive Yellow 206).

a)



b)



Measurement of Color intensity: Pure water has no color. The presence of humic acid, fulvic acid, metallic ions suspended matter and industrial waste water may cause color in natural water. Neutralization of anions and cations can remove the color change due to pH. The neutralized sample is read colorimetrically at 620 nm.

Analysis of pH: The instrument being a potentiometer requires to be calibrated before use with buffer solutions when glass electrode is immersed in a solution, it acquires an electrical potential (emf) and gives H^+ concentration or pH of the solution.

Total Suspended Solids (TSS): The suspended matter is determined by filtering or centrifuging the sample, drying the residue and determining the weight difference.

Total Dissolved Solids (TDS): The total dissolved solids can be determined by evaporating and drying a known volume of filtered sample. The maximum permissible limit of TDS for any industrial effluent is 2100 mgL^{-1}.

Isolation and identification of dye decolorizing fungus: The collected dye samples, dye effluents and soil samples spread plated on potato dextrose agar (PDA) plates and incubated for 3-5 days at 25ºC to isolate the fungus. A total of twenty one fungal isolates were made and maintained on potato dextrose agar (PDA) plates. Filamentous fungi were observed under microscope by lacto phenol cotton blue (LCB) staining method and were identified by their characteristics microscopic morphology and with the help of taxonomic guides. The identified fungal strains were preserved on PDA slants at 4ºC in refrigerator and were served as stock cultures.

*Screening of dye decolorizing fungi***:** Twenty one fungal isolates were screened for their dye degrading activity by inoculating on potato dextrose agar (PDA) with respective dye (200 mg/L). Agar plates spread with the fungal isolates were incubated at 30ºC for 7 d. Dye degradation ability of the test t fungi was confirmed by the presence of clear zones around the colonies (Varsha Zope *et al*., 2006).

Dye degradation assay

Inoculum preparation: All the mycelia of the purified fungi were grown on PDA medium for 6 d and suspended in sterile distilled water. After filtering through gauze to remove fungal mycelia, a spore suspension of about 10^7 colony forming unit was prepared and 5 mL of this suspension was added to 150 mL potato dextrose medium (pH, 6) in 500 mL conical flask.

Media preparation for dye degradation assay: After fungal cells were grown for 6 d in PD medium, Red HE7B and Yellow FN2R dyes were added and mixed at the concentration of 200 mg/L and its complete decolorization was noted. Aliquots of the fungal culture after 1, 3 and 5th day incubation following addition of the dyes were centrifuged at 12,000 rpm for 5 min to obtain the supernatant. The dye disappearance of each supernatant was determined spectrophotometrically by monitoring the absorbance at the wavelength maximum for each dye.

Analysis of dye degradation assay: The decolorizing rate of each dye by cell free culture was determined by the difference in the absorbance at the maximum wavelength between the initial and subsequent absorbance values. The percent of dye decolorization was calculated by the formula:

$$\% \text{ Dye decolorization} = \frac{O.D \text{ zero day} - O.D \text{ sample}}{O.D \text{ zero day}} \times 100$$

Results and discussion

Physiochemical characterization of the textile effluent: The problem of environmental pollution is increasing day by day due to removal of dyes from the effluents or their degradation before discharge is a great environmental challenge for the industries. Therefore, economical and environment friendly techniques are required for the removal or degradation of dye waste from the effluent. Fungal treatment of textile dyes and effluents have been found to be influenced by pH, color intensity, TDS and TSS. The physiochemical analysis of the sample textile effluent helped us to measure the pollution level. Thus, the physiochemical parameter's for effluents were conducted and examined. Table1 shows the physico-chemical constituents of dye effluents.

Table 1.Physiochemical characterization of the textile effluent.

Parameters	Values
Color intensity	6935.60
pH	9.8
TSS (mg/L)	0.00878×10^{6}
TDS (mg/L)	15.38×10^{6}

Fungi isolation: Fungi, being aerobic organisms normally showed better dye degradation activities under aerobic condition. Reactive dyes having low degree of fixation with fabrics which lead to less of dyes in effluents. Among dyes, azo dyes specifically known to be color fast and structurally stable on oxygenic condition (Wang *et al*., 2003), due to which they are hard to remove by conventional treatments. Twenty one fungi were isolated from the textile dye effluent by employing spread plate technique.

Screening of dye decolorizing fungi: Decolorization began with the formation of clear zone around the colonies. Among the twenty one isolates 10 fungal strains showed maximum decolorization and hence,.they were selected for decolorization study.

Identification of fungal isolates: All the screened isolates were identified after staining with lacto phenol cotton blue (LCB), microscopic analysis and colony morphology on PDA. They were identified as, *Aspergillus niger, A. flavus, Curvularia verruciformis, Mucor racemosus, Fusarium* sp. and *Penicillium* sp. (Table 2).

Degradation assay: Based on the OD values, the percentage of decolorization was calculated (Table 3 and 4; Fig. 2). Among 21 fungal 10 isolates (50%) showed dye degradation activity.

Table 2. Identification of the fungal isolates.

Colony morphology and microscopic observation	Purified colonies	Isolates
Colonies spreading rapidly, with mycelium white to dark brown to black or purple conidial heads; conidia small, more or less globose, rough, 4-5 µm diameter	I_1 , I_{11}	*Aspergillus niger*
Colonies yellow at first, quickly becoming bright to dark yellow green; conidiophores coarsely roughened up to 1mm long, and 19-20 µm diameter	I_2	*Aspergillus flavus*
Growth moderate, white, peach, to salmon pink or violet. Conidiogenous cells hyaline, enteroblastic, mono or polyphialidic. Micro conidia hyaline, 0-1 or 2 septate, small, macro conidia hyaline, curved (sickle shaped), 3-5 septate, 27-60 × 3-5 µm	I_4, I_6	*Fusarium* sp.
Vegetative hyphae creeping, septate, branched. Conidiophores erect, usually unbranched, septate. Conidia borne in chains which typically form a brush-like head, not enclosed in slime	I_5, I_8, I_{12}	*Penicillium* sp.
Conidia curved, 4- septate, rough walled, 16-26 × 8-12 µm	I_9	*Curvularia verruciformis*
Sporangiophores erect, close, forming, a yellow-brown turf of very variable height, 5-40 mm. high × 8-20 µ wide, branched irregularly in groups	I_{10}	*Mucor racemosus*

Table 3. Degradation of Red HE7B (OD values).

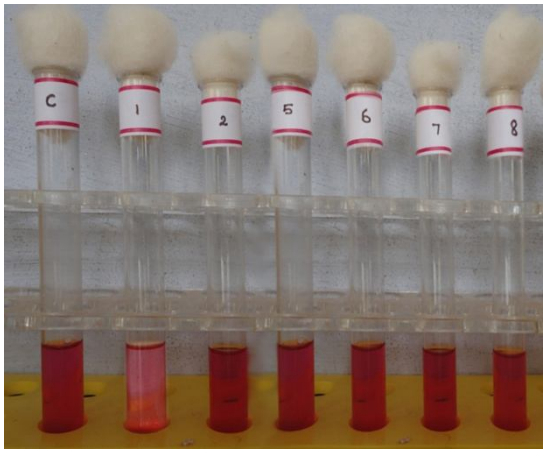
Samples isolated	1st day OD values	3rd day OD values	5th day OD values	Dye degradation on 5th day (%)
C	0.225	0.225	0.225	-
I_1	0.223	0.068	0.012	94
I_2	0.210	0.052	0.016	92
I_5	0.188	0.040	0.019	89
I_6	0.189	0.045	0.016	91
I_9	0.197	0.051	0.015	92
I_{10}	0.218	0.028	0.021	90

Table 4. Degradation of Yellow FN2R (OD values).

Samples isolated	1st day OD values	3rd day OD values	5th day OD values	Dye degradation on 5th day (%)
C	1.512	1.512	1.512	-
I_1	0.928	0.307	0.111	88
I_2	1.049	0.750	0.551	64
I_5	1.037	0.223	0.173	83
I_6	1.276	0.333	0.143	88
I_9	1.436	0.359	0.226	84
I_{10}	1.356	0.263	0.097	92

Fig.3. Degradation of Reactive Red 141 on 5th day.

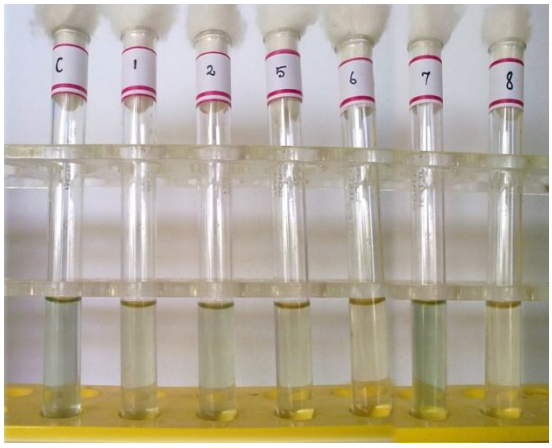
Culture broth with dye



Broth supernatent

Fig.4. Degradation of Reactive Yellow 206 on 5th day.

Culture broth with dye



Broth supernatent

Dye degrading fungal isolates were identified as *Penicillium* sp. (30%), *Aspergillus niger* (20%), *Fusarium* sp. (20%), *A. flavus* (10%), *Curvularia verruciformis* (10%) and *Mucor racemosus* (10%). Among the isolates, *Penicillium* sp. was found to be dominant. This report falls in line with the findings of Naeem *et al.* (2010) who showed 34% prevalence by *Penicillium* sp in his study. Next, to *Penicillium* sp., *Fusarium* and *Aspergillus niger* was found to dominant. Varsha Zope *et al*. (2006) reported about the decolorizing effect of *Aspergillus niger* in his study. After 3 d of incubation, Red HE7B was degraded effectively by *Mucor racemosus* (82 and 87%) and Yellow FN2R by *Penicillium* sp. (78%). Similar finding was done by Erdal and Tuskin (2010) in which decolorizing activity showed 89% by *Penicillium* sp. After 5 d of incubation, Yellow FN2R was degraded by *Aspergillus niger* (94%) and Yellow FN2R by *Mucor racemosus* (92%). The results are in accordance with the report of Andleeb *et al*. (2010) who revealed the formation of decolorization activity around 86% by *Aspergillus niger. Aspergillus niger* and *Mucor racemosus* effectively degraded the reactive dyes under aerobic condition. A study carried out by Varsha Zope *et al*. (2006) showed 93% degradation by *Aspergillus niger.* Similar type of results were reported by Moturi *et al*. (2009) who revealed that, decolorization activity of *Mucor* sp. was around 78%.

This phenomenon might be due to greater removal of dyes by the effective fungal growth with the availability of higher glucose levels. Higher glucose levels also create acidic condition in the culture medium, which thereby supports better removal of dyes. In this study, *Aspergillus flavus* showed lowest degradation (64%). Similarly, Ramalingam *et al*. (2010) reported 74% degradation by *Aspergillus flavus* which is also very low.

Conclusion

Twelve fungal strains were isolated from 21 different dye effluents. Isolated fungal strains were screened for its degradation activity of dyes. Eight fungi showed dye degradation activity namely *Aspergillus niger, A. flavus, Curvularia verruciformis, Mucor racemosus, Fusarium* sp. *and Penicillium* sp. All the 8 fungal isolates were subjected to dye degradation study with Red HE7B and Yellow FN2R. Among the 8 fungal isolates, *Aspergillus niger* (94%) and *Mucor racemosus* (92%) showed high degradation on Red HE7B and Yellow FN2R. To conclude, the selected strains appear to be an attractive option for the treatment of industrial effluents contaminated with dye. *Aspergillus niger and Mucor racemosus* can be used for the treatment of effluents and can be performed in low cost at the industrial site as compared to the anaerobic treatment which requires large input.

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