

Bioremediation potential of textile *Aspergillus flavus* TEAK 07 Against textile dye and their toxicity assessment

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ABSTRACT

The study focused on the isolation, decolorization efficiency of the indicator organism and their toxicity assessment. The predominant isolate was morphologically identified, characterized by 18SrRNA and named as *Aspergillus flavus* TEAK 07. The indicator and the reference fungi *Aspergillus flavus* MTCC 1883 strain were assessed for their efficacy to decolorize the reactive Red 120. The indicator organism *Aspergillus flavus* TEAK 07 expressed the best decolorization efficiency than the reference isolate. The various factors affecting Reactive red decolonization were optimized, include temperature, pH, different carbon and nitrogen sources, and different agro substrate. Results showed that pH 5.5 and temperature of 28°C was optimal. In addition to this, glucose and ammonium were found to be a better carbon source, and nitrogen source, the initial inoculum concentration of 1% and 0.1 mg/ml of initial dye concentration was found to give maximum decolorization. Vigna mungo seed germination tests proved, decolorized dye was less toxic than the original dye.

Keywords: Reactive dye 120, *Aspergillus flavus* TEAK 07, Decolorization, Seed Germination.

INTRODUCTION

Reactive dyes, including many structurally different dyes, are extensively used in the textile industry because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimal energy consumption. The three most common groups are azo, Anthraquinone and Phthalocyanine dyes, most of which are toxic and carcinogenic. Disposal of these dyes into the environment causes serious damage, since they may significantly affect the photosynthetic activity of aquatic plants by reducing light penetration and also they may be toxic due to their breakdown products. Studies indicated that approximately 15 % of produced synthetic dyes per year have been lost during manufacturing and processing operations. Color is one of the pollutants,

because of several reasons: (i) it is visible and even small quantities of dyes (≥ 0.005 mg/L) is not allowed; (ii) color can interfere with the transmission of sunlight into natural streams; (iii) many of the azo dyes and their intermediate products, such as aromatic amines, are toxic to aquatic life, carcinogenic and mutagenic to humans. Consequently, dyes have to be removed from textile wastewater before discharge (Flores et al., 2008). Furthermore, commercial reactive azo dyes are intentionally designed to resist degradation, many of these substances are considered toxic, even carcinogenic. Over the past couple of decades, manufacturers and users of dyes have faced increasingly stringent legal regulations promulgated to safeguard human health and the environment. So, there is a clear need to treat dye wastewater prior to discharge into the

primary effluent. Their discharge is undesirable, not only for aesthetic reasons, but also because many azo dyes and their breakdown products such as aromatic amines have been proven toxic to aquatic life and mutagenic to humans (Chung and Cerniglia, 1992). Though several physical methods are available for decolorization, Bioprocessing can overcome the defects like high-energy costs, high-sludge production, and formation of by-products and it is cost saving, effective and more environmentally friendly. There is a concern azo dye reduces to certain aromatic amines (arylamines) because they are found to be a carcinogen and mutagenic. To overcome this problem, dye selected for study was Reactive Red and investigated to reduce its toxicity, the way to achieve cheap and economical bioremediation process. .

MATERIALS AND METHODS

Dyes:

The textile dye, Reactive red 120, obtained from the textile industry in Tiruppur, Tamilnadu. **Stock solution** (0.1g in 10ml) of Reactive red was prepared by dissolving the dye in double distilled water. *Aspergillus flavus* MTCC 1883 obtained from the Microbial Type Culture Collection (MTCC), Chandigarh was used as a reference organism in the present study. The cultures were maintained on PDA slants at 4 °C. The 3 days old culture of test and reference isolate was used as an inoculum for the entire study.

Screening for Reactive Red Decolourization and Acclimatization (Kalme et al., 2006)

A loopful suspension of the selected culture from 72 hours SDA broth was inoculated on Potato dextrose agar plate amended with Reactive red 120 at a concentration of 0.1mg/ml and incubated for 72 hours based on the decolorization zone around the colony, positive and better zone formed strain was taken. Colony morphology, growth characteristics of effluent isolates were observed on SDA plates as described by Harley and Prescott (1993).

The characterized fungal isolates were sub cultured in fresh medium and submitted to National Centre for Cell Science (NCCS), India

for molecular identification based on their 18S rRNA gene sequences. The data subjected to BLAST analysis. The sequence data of novel isolates obtained were submitted at NCBI, GenBank to acquire unique accession numbers. The acclimatization was done by gradually exposing the isolates to the increasing concentration of dye. The successive transfer of culture into fresh medium containing 0.1, 0.5 and 1% of reactive red dye was done at 28°C in static condition. The acclimatized organisms were used for further studies.

Decolourization Assay: (Kalyani et al., 2007).

The dye decolorizing fungal isolate was inoculated into 100 ml of reactive red (10mg/100ml) incorporated potato dextrose agar medium, sterilized and 1 % inoculum was transferred aseptically into the medium and incubated at 28°C for 72 hours at 100rpm. After incubation, the culture broth was centrifuged. Percentage of decolourization was determined by monitoring the decrease in absorbance at the maximum wavelength of the dye (i.e., 518 for reactive red). UV-Vis spectrophotometer was used for absorbance measurement. Decolourization activity was calculated as follows:

% of decolorization = (Initial absorbance – final absorbance) / final absorbance x 100

Effect of Various Parameters on Reactive Red Decolorization: (Bras et al, 1997)

500ml of sterile reactive red incorporated potato dextrose agar medium was prepared, 1% inoculum was added aseptically into respective flasks and incubated at 28°C in a shaker at 100 rpm. Around 10 ml of culture was aseptically drawn periodically at 24 hour intervals up to 72 hours from the flasks. Percentage of decolourization was calculated. Same way the effect of temperature (28°C, 30°C, 32 °C, 35°C, and 37°C), pH (4, 4.5, 5, 5.5, 6, 6.5, 7, 8), dye concentration (0.1 to 1%), an influence of 1% different carbon sources (Fructose, Glucose, Lactose, Maltose, Mannitol, Xylose and Sucrose) and 0.2% nitrogen sources (Peptone, Ammonia chloride, Potassium nitrate, Ammonium sulfate, Ammonium nitrate) were investigated using potato dextrose agar medium.

Dye Decolourization using Cheap Sources:

Various natural cheap sources like Rice bran, Wheat bran, and Pomegranate peel powder were used (1% concentration) as substrates (by replacing sugar components in production medium) for effective decolourization. Cheap sources were collected, dried, powdered, sterilized and seeded with 1% of inoculum and incubated at 28°C for 72 hours. Percentage of decolorization was estimated at 518nm.

Immobilization (Jo-Shu Chang *et.al.*, 2000).

A 2% (w/v) slurry of sodium alginate was prepared in hot (60°C) distilled water, cooled, 0.1% of the biomass of isolate was added and stirred, introduced into a syringe, and was then pressured to drop in 0.1M CaCl₂ solution to form particles of 2.5-3.0 mm in diameter. The particles were suspended in the CaCl₂ solution for 12 h to enhance their mechanical stability. Immobilized cells were suspended into 50ml of reactive red incorporated media; decolorization with an identical amount of cell-free immobilization matrices were also conducted. After complete decolorization, the immobilized cell particles were collected, rinsed twice with sterile deionized water and transferred into a fresh decolorization medium for cost effectiveness.

Toxicity Assessment: (Prashanth and Mathivanan, 2010)

Seeds of *Vigna mungo* were surface sterilized with 70% ethanol and then treated with 0.2% Mercuric chloride and washed three times with sterile distilled water and blot dried under aseptic conditions. Surface sterilized seeds were soaked for 30 minutes in 40ml of the decolorized supernatant for test, the same volume of water and reactive dye was used as positive and negative control. Seeds were then dried under aseptic conditions for 4 hrs. A total of six seeds was placed in each Petri plate containing a moist filter paper and the plates were maintained in a growth chamber for 7 days. The germination percentage was calculated.

RESULTS AND DISCUSSION

Decolorization study revealed *Aspergillus flavus* TEAK 07 (Fig 1.a) showed positive and better decolorizing nature than the other isolates and reference strain, *Aspergillus flavus* TEAK 07 was subcultured and used for further studies. Incubation time plays a substantial role in organism growth and decolorization effect on reactive red. Decolorization observed under static conditions, whereas up to 65% decolorization was observed under shaking conditions (Fig 1.b). The lower level of decolorization is likely due to lower azoreductase activity under aerobic conditions (Bor-Yann, 2002; Abubacker *et.al.*, 2013). The rate of color removal increases with increasing temperature, within a defined range.



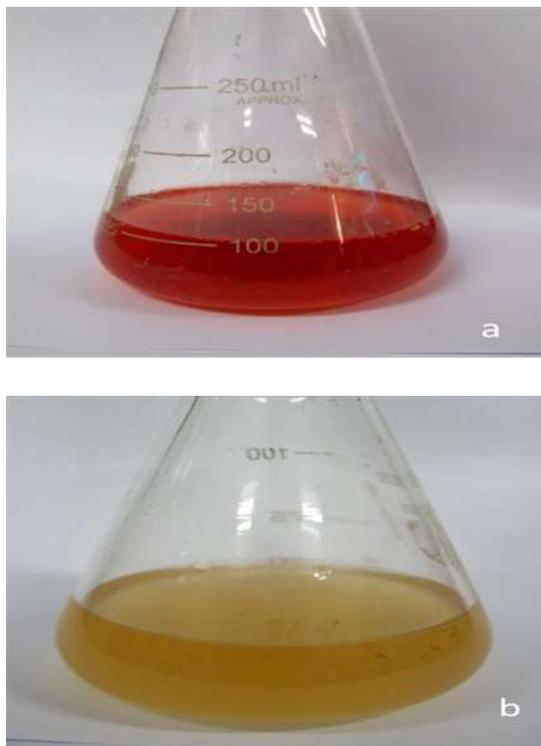
Figure-1a. *Aspergillus flavus* TEAK07 on PDA plates



Figure-1b. Decolourization assay

The growth study of the organism is essential for knowing decolorization process. Maximum decolorization was observed for 48 hours to 96 hours. Based on the results analyzed at different time intervals, it was observed that maximum decolorization was at 72 hours. But considering the reaction process and incubation time, the

decolorization was higher comparatively from 48 hours of incubation and considerably increased till 72 hours. So the incubation time of 72 hours was chosen for further study of the decolorization process using other parameters (Fig 2 a, b).



Figure–2. PDA medium (Test) amended with Reactive red. a. before decolorization & b. after decolorization

The temperature required to produce the maximum rate of color removal tends to correspond with the optimum cell culture growth temperature of 28–30°C (75.80%) for the selected strain. Some studies dealing with microbial decolorization of azo dyes has been undertaken (Dos Santos et al., 2004), in which narrow temperature ranges were determined as being necessary for the decolorization of azo dyes by extremely complex consortia of microorganisms inhabiting active sludge.

The medium pH is also an important factor with regard to decolorization. The pH has a major effect on the efficiency of dye decolorization, and the optimal pH for color removal is often between 4.0 and 8.0 (Chen et al., 2003; Kilic et al., 2007) which correlates the present study,

isolate was able to decolorize Reactive red over the pH range 4-6, with maximum decolorization (78%) at pH 5.5. The rate of color removal is higher at the optimum pH, and tends to decrease rapidly at neutral or strongly alkaline pH. It is believed that the effects of pH may be related to the transport of dye molecules across the cell membrane, which is considered as the rate limiting step for the decolorization (Kodam et al., 2005).

Among the different carbon and nitrogen sources glucose (82.32%) were efficient for decolorization of reactive red 120. Azo dyes are deficient in carbon sources, and the degradation of dyes without any supplement of carbon or nitrogen sources is very difficult (Sani and Banerjee, 1999) and Mamidala (2013). Two opinions have been argued for many years: one deems that dyes are not a carbon source since the microorganism obtain energy from the glucose instead of the dyes and glucose can enhance the decolorizing performance of biological systems (Sarioglu and Bisgin, 2007); while another deems that glucose can inhibit the decolorizing activity (Chen et al., 2003). The variability may be due to the different microbial characteristics involved. Our results showed that a certain concentration of carbon source (such as glucose) was necessary for the decolorizing process, 82.32% of decolorization was achieved with glucose as carbon source.

Among all nitrogen sources, Ammonium nitrate was the best nitrogen source for efficient decolorization (85.60%) of Reactive Red by the selected isolate. The nitrate was obviously a better electron acceptor than the azo bond. This may lead into the competition in a reduction reaction between the nitrate in the liquid and the also bonds in the azo dye (Panswad and Luangdilok, 2000). This agrees with the statement by Carliell et al., (1995). As the dye concentration increased in the culture medium, a decline in color removal was obtained. This may be attributed to the toxicity of dye to fungal cells through the inhibition of metabolic activity, saturation of the cells with dye products, inactivation of the transport system of the dye or the blockage of active sites of azoreductase

enzymes by the dye molecules (Vijaykumar et.al, 2007; Isik and Sponza, 2004) and Krishna Kumar (2013). Furthermore, the higher the dye concentration, the longer the time required to remove the color (Wuhrmann et al., 1980).

The major restriction in the commercialization of industrial bioremediation is their high processing cost. The use of readily available cheap agro-industrial residues as the carbon sources may reduce the high cost. In our study different cheap substrates like rice bran, wheat bran, and pomegranate peel powder were utilized for reactive red decolorization. Among the cheap sources rice bran shows the best cheap substrate for reactive red decolorization.



Figure-3a. Immobilized Beads.
b. Decolorization of Reactive red using Immobilized cells control and immobilized cells

By offering many advantages over other methods, immobilized microbial cell technology has been applied widely in the field of wastewater treatment. For decolorization of azo dyes in wastewater, natural gels such as alginate (Chang et al., 2001); carrageenan; synthetic gels such as polyvinyl alcohol. Alginate is one of the most widely studied gel matrices for cell

entrapment with alginate gel beads offering high biomass loading and good substrate diffusion within the matrix. In the present study fungal cells immobilized with alginate beads were utilized for reactive red decolorization and the maximum of 75% of decolorization achieved followed by 68% during the second time using the same immobilized beads (Fig 3 a, b).



Figure-4. Phytotoxicity (*Vigna mungo*) seed germination test.
a. Reactive red,
b. Distilled water,
c. Decolorized supernatant

Besides, the use of dye contaminated water is harmful to agriculture. Thus, it was of prime interest to assess the phytotoxicity of the dye and

its extracted metabolites after degradation. Phytotoxicity tests were conducted to assess the impact of the treated colored water on vegetation once it is thrown to the ecosystem as well as to explore the possible reuse of the treated solution in irrigation fields such as parks and golf-courses, etc. (Fig 4 a, b, c). Results suggest that biodegradation is able to illuminate the phytotoxicity of azo-dye aqueous solutions. Anyhow, the germination of seeds and the growth of the plant treated with the decolorized and degraded water could help in promoting the reuse of the treated water in the irrigating field under more circumstances (Pollock et al., 2002).

CONCLUSION

Though dyes can be removed from waste water by chemical and physical methods, both the physical and chemical methods have many disadvantages in application, such as high-energy costs, high-sludge production, and formation of by-products. Conversely, bioprocessing can overcome these defects because it is cost saving and environmentally benign. Bacteria, Fungi and algae have been used in dye decolorization. The above said work concludes that the new fungal strain *Aspergillus flavus* TEAK 07 capable of degrading a wide range of structurally different dyes under optimized conditions

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