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Evaluation of different *Aspergillus* species for degradation of a reactive dye, Orange M2R

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ABSTRACT

Decolorization study of reactive dye Orange M2R by four fungal strains, Aspergillus flavus, A. niger, A. oryzae, A. terrus and their consortia were carried out using Czapek Dox broth containing 200mg/l of Orange M2R. The effects of physiochemical parameters (carbon source, temperature, pH, shaking vs static) on the decolorization potential of Aspergilli and their consortia was investigated. The fungal consortia have maximum decolorization (89.35±0.63%) with sucrose as carbon source followed by glucose (83.08±1.02%), A. niger decolorized maximally (81.37±0.35%) in sucrose containing medium. It was found that shaking favored the dye decolorization effectiveness of all tested aspergilli as well as their consortia. The effect of initial pH was also tested and found that highest decolorization showed by fungal consortia (91.84±0.91%) at an alkaline pH 8, but A. flavus showed maximum decolorization (83.36±0.63%) at acidic pH 5 and A. niger (80.10±0. 59%) at pH 6. Effect of incubation temperature had a significant role in the degradation of dye; fungal consortia (93.61±0.79%) showed maximum decolorization at 35°C; A. niger and A. oryzae showed optimum decolorization at 30°C. The study confirmed the potential of the above fungi and their consortia in the decolorization of Orange M2R and opened up scope for future analysis of their performance in the treatment of textile effluent.

Key words: Aspergilli, fungal consortia, Orange M2R, decolorization, physiochemical

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INTRODUCTION

Environmental pollution from human activities is a major challenge for the present world [1]. Textile, cosmetics, pharmaceuticals and dying industry effluents constitute a major source of water pollution. Dyes or their breakdown products are known to be highly toxic and carcinogenic for living organisms [2]. The wastewater generated from textile industries vary in their characteristics depends on the process employed such as desiring, scouring, bleaching, mercerizing, dyeing, printing and finishing [3, 4]. The concentration of dye contained in the effluent varies between 10-200 mg/ml depending on the dyeing process and type of treatment method employed. Many dyes and pigments are hazardous and toxic for human as well as for aquatic life in the concentration at which they are being discharged to receiving water bodies [5]. The high concentration of dyes leads to ulceration of the skin and mucous membrane, dermatitis, perforation of nasal septum, severe irritation of respiratory tract and on ingestion may cause vomiting, hemorrhage and diarrhea [6]. Dyes used in the textile industry are difficult to remove by conventional methods that are recalcitrant against light, oxidizing agents and biodegradation processes [7, 8]. The slow rate of decomposition of dyes present in wastewater desperately needed novel treatment methods to accelerate the process [9, 10]. The methods employed for alleviating the environmental problems caused by the textile dye effluent include physical,

chemical and biological treatment processes. The physico-chemical methods include adsorption, chemical precipitation, flocculation, oxidation via chlorine, peroxide, electrolysis and ozone treatment, reduction and electrochemical destruction, have been used to treat textile effluents, but these methods have many disadvantages and limitations [11]. Biological methods of removal involve the use of microorganisms such as fungi, bacteria, algae and actinomycetes [12] to convert the pollutants into non-toxic harmless substances. Biological processes convert organic compounds to water and carbon dioxide [4], have a low cost, sustainable and are easy to use. Microbial metabolism and degradation of dyes depend upon the climatic condition, presence or absence of oxygen, presence of alternate carbon or energy source, optimum pH and temperature etc. Therefore, the present study aims to investigate the effect of physiochemical parameters on decolorization potential of four *Aspergillus* species for a reactive dye, Orange M2R.

MATERIALS AND METHODS

Four fungal strains used in this study *Aspergillus flavus*, *A. niger*, *A. terrus* and *A. oryzae* were recovered from dye disposal soil reported in earlier papers [13, 14]. Here, the decolorization potential was evaluated against a dye Orange M2R. The textile dye used for this study was obtained from M/s Sheena Export and Textiles, Panipat (Haryana, INDIA). The chemical structure of the dye is shown in Fig. 1. Czapek Dox media constituents and other chemicals used were of analytical grade and purchased from Hi-media (Mumbai). The following chemical composition of the Czapek Dox broth was used (all in g/l) [K_2HPO_4, 1.0; $NaNO_3$, 3.0; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01; Yeast extract 5.0; Sucrose 5.0].

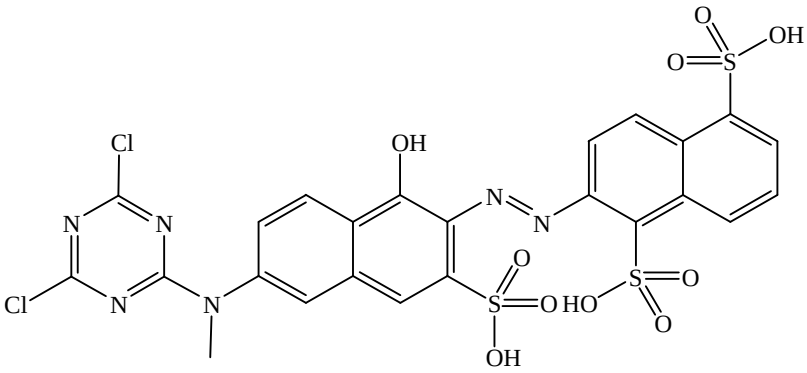

Figure 1: Chemical structure of Orange M2R [15]

The decolorization study was carried out in the aqueous medium (100ml) with dye (200mg/l); subsequently inoculated with each *Aspergillus* strain biomass (8mm disc) and in one set a consortium (8mm disc of testing aspergilli biomass), separately. The cultures were grown for 10 days in an incubator shaker at 25±2ºC and 120 rpm. Samples were withdrawn aseptically on alternate days, centrifuged at 5000 rpm for 10 min and the supernatant was scanned in spectrophotometer at λ_{max} (494nm) of the dye. Two control flasks were also maintained *viz.*, one flask contained medium without dye and second flask contained medium with dye and no fungal biomass. Dye degradation was reported as percent decolorization and expressed as [16]:

$$\text{Decolorization (\%)} = \frac{\text{Initial O. D.} - \text{Final O. D.}}{\text{Initial O. D.}} \times 100$$

To investigate the effect of various physiochemical parameters such as different carbon sources (sucrose, glucose and fructose), pH (5-9), temperature (20-40°C) and shaking/static condition same protocol was used. Effect of shaking was studied by incubating the culture flasks at 25±2°C in an incubator shaker with an agitation speed of 120 rpm. Experiments were performed in duplicates and the results were expressed as the mean±SD (standard deviation).

RESULTS AND DISCUSSION

Dye decolorization by fungi in aqueous medium has been widely employed to identify the ligninolytic potential and potential degradation of xenobiotic compounds by basidiomycetes [17]. The time required by fungal consortia and pure culture to decolorize the synthetic dye was similar to that reported for other basidiomycetes (6 to 10 days) as shown in Fig. 2. The decolorization efficiency of fungi can be due to the presence of chitin with hydroxyl and amino groups in their cell wall, which make them an efficient adsorbent of dye effluent [8]. Differences in the capacity of dye decolorization between fungi have been related to inter and intraspecific variations, the molecular complexity of the dye and culture conditions [19, 20, 21]. Therefore, some parameters that influence the decolorization of dyes were studied using this dye with pure and a mixed culture.

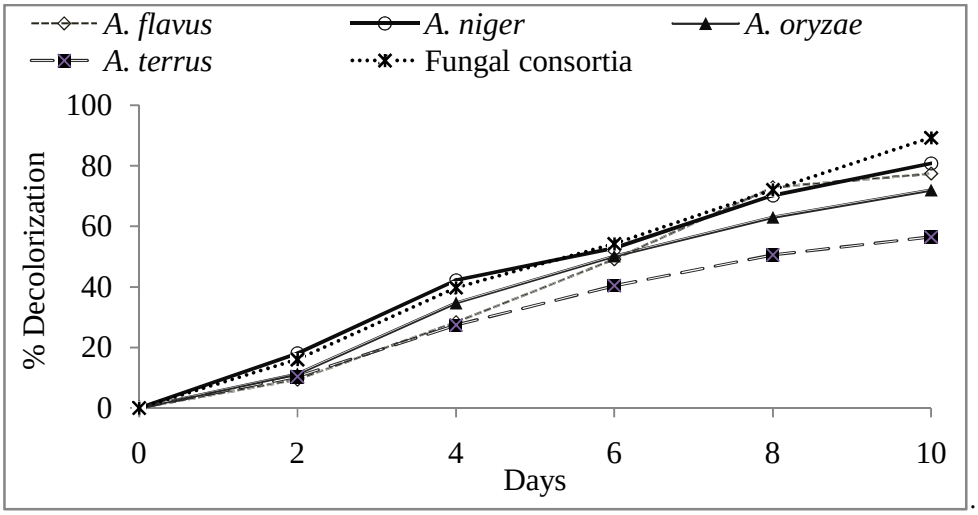

Figure 2: Decolorization kinetics of Orange M2R by fungi

Effect of shaking/static on dye degradation

Shaking favored the decolorization of Orange M2R (Fig. 3), with (89.35±0.63%) decolorization being shown by fungal consortium, whereas *A. flavus* and *A. niger* degraded the dye about 60-70% in static culture after ten days. Although shaking has been shown to suppress the expression of the ligninolytic system in *Phanerochaete chrysosporium* [22], this condition generally results in higher dye decolorization than obtained with static cultures due to an increase in mass and oxygen transfer rate between cells and the medium, factors that optimize the action of oxidative enzymes. Thus, shaking has been found to significantly increase dye decolorization by *P. chrysosporium*, *Trametes versicolor* and *Bjerkandera* Sp. BOS55 [23]. Shaking cultures of *Bjerkandera fumosa*, *Kuehneromyces mutabilis* and *Strofariarugoso annulata* promoted a higher dye decolorization than static cultures [24]. However, *Phlebia tremellosa* shows better dye decolorization under static conditions [25], but decolorization was only determined after 14 days of incubation and therefore, monitoring of the decolorization kinetics was not possible.

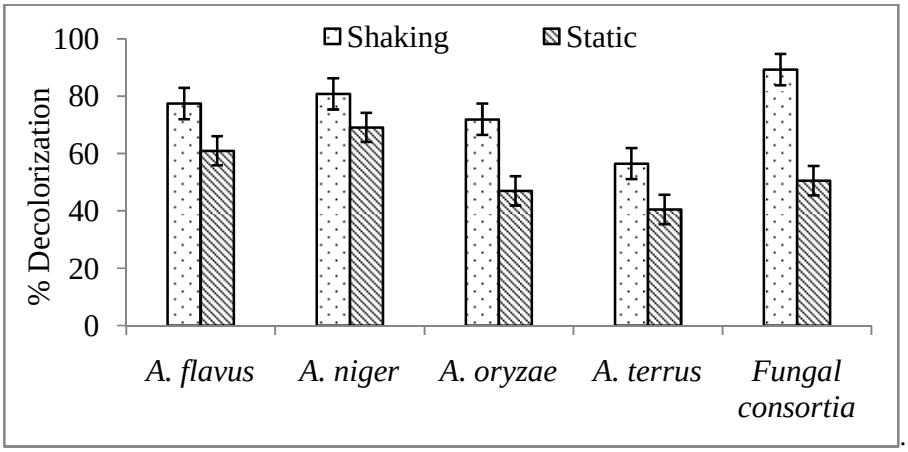

Figure 3: Effect of shaking/static on degradation of Orange M2R

Effect of carbon source on dye degradation

Various carbon sources *viz*., glucose, fructose and sucrose, at 5g/l were used, as co-substrates to investigate their effects on dye decolorization after 10 days of incubation by pure fungi and their mixed culture. The addition of glucose as co-substrate reduced the color (83.08±1.02%) by mixed culture where as with fructose the dye degraded (74.90±1.50%) by *A. oryzae*; while the addition of sucrose as an additional carbon source enhanced the rate of dye decolorization and degraded (89.35±0.63%) by fungal consortia followed by *A. niger* (81.37±0.35%), *A. flavus* (77.47±1.90%) and *A. oryzae* (72.01±0.99%) as shown in Fig. 4. Even without any additional carbon source, a moderate reduction (43.94±1.06% – 49.66±0.27%) in the color was shown by *A. niger* and fungal consortium. It is notable that when sucrose was used as co-substrates the decolorization rates of mixed culture reached approximately 90% under similar culture conditions. While comparing our previous study results on Reactive Black HFGR by a pure culture of *Aspergillus allhabadii* MTCC 9988 degrade maximum in glucose-supplemented medium in shaking condition [26]. The culture media improve the growth and the adsorption/degradation ability of fungi and in the presence of additional carbon source, the decolorization increased up to 90- 95% in 8-10 days [22, 26]. Further, the rate of color removal could be linked with the available co-substrates and with the exponential growth phase [27, 28].

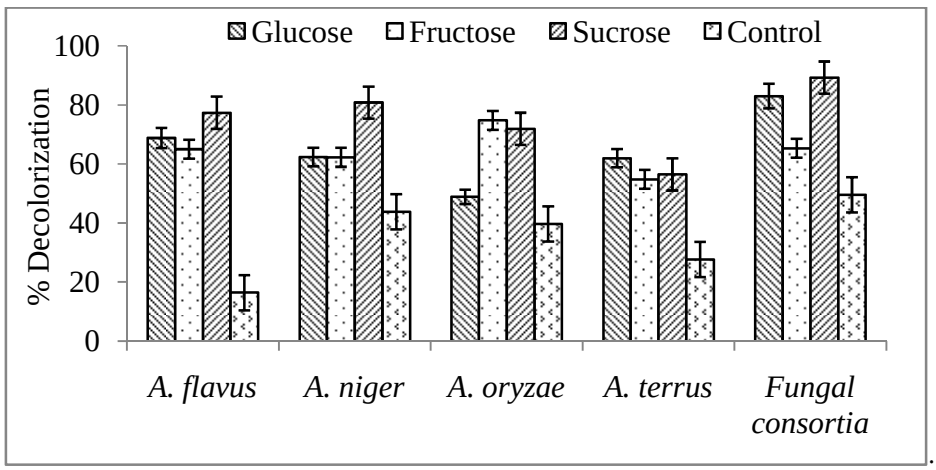

Figure 4: Effect of carbon source on degradation of Orange M2R

Effect of initial pH on dye degradation

The effect of initial pH on the decolorization of dye by pure fungal strains and their consortium is shown in Fig. 5. The results revealed that *Aspergillus* species were capable of decolorizing this dye over a pH range of 5.0–9.0 efficiently. The fungal consortia degraded the dye (91.84±0.91%) at an alkaline pH 8, whereas, *A. flavus*

(83.36±0.63%) and *A. niger* (82.10±0.59%) reduced the color at acidic pH 5 and 6 respectively. The optimum pH for color removal is often 4.5 to 11.5 for most of the dyes and observed decrease in decolorization towards both ends of the optimum pH values [11]. Higher uptake obtained at lower pH value may be due to the electrostatic attraction between positively charged cell surface and dye anions.

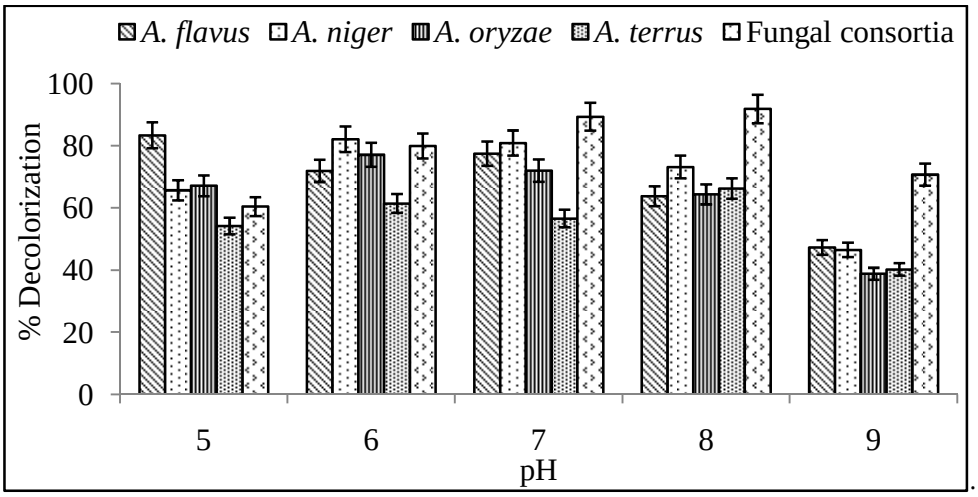

Figure 5: Effect of initial pH on degradation of Orange M2R

Effect of incubation temperature on dye degradation

The incubation temperature had a significant effect on the dye decolorization ability and maximum decolorization (93.61±0.79%) by mixed culture at 35°C; whereas *A. niger* reduced the color (83.94±0.59%) at 30°C as shown in Fig. 6. *A. flavus* and *A. oryzae* showed higher degradation at 25°C whereas *A. terrus* moderately decolorized the dye at 30°C. Remazole Black B decolorizes efficiently by mix culture at 30°C in an earlier report [29]. The alleviating temperature may enhance the production of enzyme that increases the respiration rate and substrate metabolism. The degradation of pollutant by microorganisms relies on optimum temperature that favorably supports the microbial activity [30].

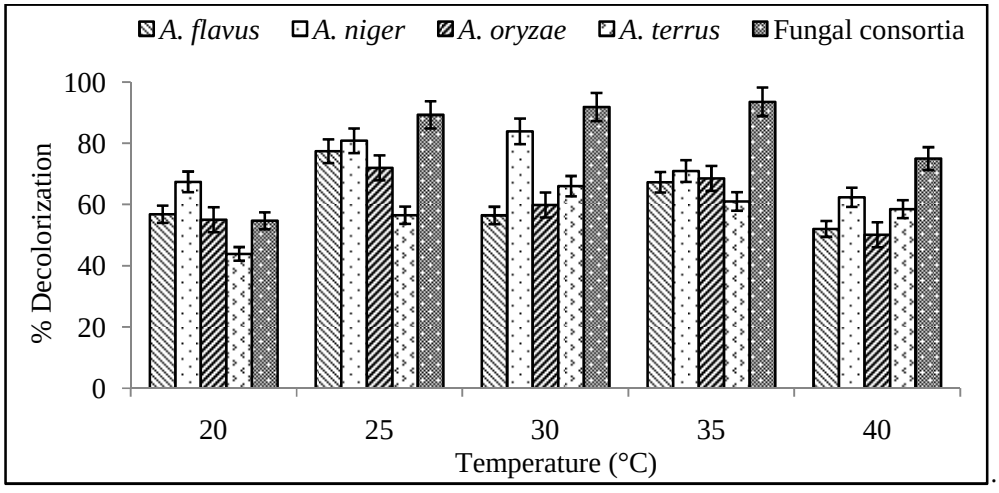

Figure 6: Effect of incubation temperature on degradation of Orange M2R

In the mixed culture of *Aspergillus flavus, A. niger, A. terrus* and *A. oryzae*, a greater reduction in dye was observed in the presence of additional carbon source. Dye decolorization was faster in the mixed culture compared to the pure culture with approximately 94% color reduction within 10 days. These results support the importance of studies involving mixed cultures in order to better understand the possible relationship between different enzymatic systems

in the degradation of pollutant molecules. The present results demonstrated differences between four aspergilli grown in pure or mixed cultures in terms of their ability to degrade reactive textile dye. The excellent performance of fungal consortia and *A. niger* in the decolorization of dye Orange M2R reinforces the potential of these fungi for environmental decontamination to attain greener environment.

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