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Research Article

SCREENING OF FUNGI FOR THE DEGRADATION OF TEXTILE DYES FROM INDUSTRIAL EFFLUENTS

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Abstract

The objective of the study was to investigate the potential dye degrading fungal species from textile mill effluents in a cost effective and eco friendly manner. Decolorizing isolates of Zygomycotina, Deuteromycotina and Ascomycetes were isolated from dye industry effluents. Decolorization assay was carried out on both solid and liquid assay medium along with laccase activity. Among all the fungi, *Aspergillus* was a dominant species recorded among the effluents. All the fungal isolates were tested for decolorizing activity against methylene blue, crystal violet, sudan black, malachite green and methyl red. On solid and liquid medium, *Aspergillus terreus, A. niger,* and *Fusarium moniliforme* were found to decolorize maximum number of dyes and registered maximum percentage of color reduction. Laccase assay was done using Lignin modifying enzyme basal medium with Hydroxybenzotriazole, and among the isolates, *F. moniliforme* and *A. terreus* were found to produce the laccase enzyme. Among the 13 species of fungal isolates, the maximum degradation activity was shown by *A. terreus* (86.33 %), followed by *A. niger* (84.20) and *F. moniliforme* (80.00 %) seems to be potential candidates to degrade commonly used dyes. So these strains can be used for treating the dye containing effluents as well as in bio remediating degraded aquatic and land polluted by these effluents. So, commercial development and application of this fungus for textile wastewater treatment will be an advantage to dye removal process.

Keywords: *Aspergillus*, Biodegradation, Decolorization, Dye, Effluent, Laccase,

INTRODUCTION

Now a day the population explosion coupled with industrial expansion has resulted in pollution of water, air and soil. The discharge of pollutants from various industries poses threat to the biodiversity of the earth. Off late the human race has understood the importance of environmental health, and suddenly it is the global move to bio remediates the damaged habitats. The textile industry covers two-third of the gross dye stuff market. During manufacturing and usage, approximately 10- 15 % of the dye is lost directly to wastewater that finds its way into the environment[1,2]. Color present in the industrial effluent gives a direct indication that the water is polluted. Hence color is the first contaminant recognized in the textile effluent and it has to be removed before discharging into rivers[3]. Industrial effluents are of various types, which depend on the process involved. Textile industry goes hand in hand with modernization and dyeing is the major process involved. Dyeing house effluents are being continuously discharged into the sewage and adjoining water courses including the ground water tables rendering a catastrophic impact on the aquatic environment. The textile dye effluents have created significant concern because it imparts toxicity and these dye effluents are objectionable because of the fact that they reduce penetration of light through water bodies. With regard to their color removal by conventional treatment methods leads to severe water pollution thus creating a need for developing cost effective clean-up operations. Furthermore, some synthetic dyes, such as azo dyes, may be carcinogens or mutagens. Under anaerobic conditions, they are transformed into aryl amines that are potentially more toxic than the parent compounds. Microbial degradation seems to be promising compared to other methods available. Several combined anaerobic and aerobic microbial treatments had been suggested to enhance the degradation of textile dyes. Furthermore no single conventional treatment method had been found to be effective for all dye classes. Thus, there is a need for the development of new technologies for color removal. Microbial treatment which involves bacteria and fungi is an attractive option as it could be cost effective and environmental friendly[4]. Fungi are a heterogeneous group and have a range of features that separate them from other organisms. Certain bacterial anaerobic reduction of dyes generates colorless, dead-end aromatic amines which are generally more toxic than parent compounds. Aerobic bacterial dye degradation seems to be promising but confined to a single dye usually. Although bacteria are fast growing and can respond to a changing environment by populations utilizing the energy source present, there are added advantages of using fungi for biodegradation. Many of the pollutants are toxic to the organisms that are supposed to degrade them. Over the past decade, the white rot fungi have been studied for their ability to degrade maximum dyes which also used to indicate ligninolytic activity. The low specificity of lignin degrading enzymes suggests that they may be suitable for treating the textile effluents and enzymes such as lignin peroxidase, manganese peroxidase and laccase are involved. The present study was an attempt to investigate the potential dye degrading capabilities of commonly

available fungal species isolated from textile mill effluents in a cost effective and eco friendly manner.

MATERIALS AND METHODS

Collection of Samples

The samples were collected from effluents of dying industries. Decolorization activities were tested against various industrially important dyes like malachite green, methyl red, malachite green, crystal violet and sudan black.

Isolation and Identification of fungi

The fungal strains from effluent samples were isolated using dilution-plating technique. The fungal isolates were then transferred to fresh plates for purification. Using standard reference manuals fungi were identified microscopically and the isolates were preserved on potato dextrose agar slants for further study. The dyes used in the study included Methylene blue, Crystal violet, Sudan black, Malachite green, and Methyl red which are of commercial importance.

Dye degradation in solid medium

Mineral salt medium was prepared and dye concentration of 500 µg/l was added to the medium. Along with control plates they were incubated at 30^0C for 5-8 days. The extent of zone formation around the colonies was observed and recorded[5].

Dye degradation in broth culture

Dye decolorization experiments were carried out in 250 ml flasks containing 100 ml of Potato dextrose broth and different dyes (500 µg/l) were inoculated with spore suspension of all the fungal isolates. The flasks were kept in mechanical shaker and incubated at 25^0C for 5 days, 10 ml of the sample was filtered and centrifuged at 6000 rpm for 10 minutes. Decolorization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wavelength maxima (λ max) of respective dye. Uninoculated flasks served as controls for a biotic decolorization. The optical density values were measured using a spectrophotometer at different wavelength for different dyes.

Decolourization Assay

Decolorization activity was expressed in terms of percentage decolorization and was determined by monitoring the decrease in absorbance at absorption maxima (λ max) of respective dyes. This was calculated using the following formula as described by[6]

$$\text{Decolourization (\%)} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

Laccase assay

Solid medium in Petri plates were prepared using Lignin modifying enzyme basal medium (LBM) and inoculated with a purified fungal culture. Plates were incubated at 25°C for 7-14 days. After incubation, the mycelia were scraped gently without disturbing the medium and the laccase substrate i.e., 0.1 % hydroxybenzotriazole (in 95 % ethanol) was added over the medium and kept for 30 minutes. The area of decolorized zone was recorded which indicated the laccase activity[7].

RESULT

Isolation and Identification

A total 10 fungal species under 7 genera were isolated from the textile effluents and identified. The total number of species included 2 members of Zygomycotina i.e., *Mucor racemosus* and *Rhizopus stolonifer* and 8 species in 5 genera of Deuteromycotina and Ascomycetes such as *Aspergillus niger, A. fumigatus*, *A. flavus, A. terreus, Penicillium oxalicum, Fusarium moniliforme, Cladosporium cladosporioides* and *Trichoderma viride*. Among the fungal members the species of *Aspergillus* occurred in large numbers.

Decolourization experiments by fungal systems

Solid plate assay

Initial evaluation of dye decolorization was done using solid medium. By day 4, the extent of mycelia growth on the agar plates was indifferently similar for all cultures whether or not any dye was present. Decolourization began with the formation of clear zones around the colonies. Complete decolourization was assessed as the total disappearance of color without any visible sorption to the biomass. *M. racemosus, C. cladosporioides, P. oxalicum* and *T. viride* did not decolorize any of the dyes tested. In the 8-day incubation period, *A. terreus, A. niger* and *F. moniliforme* decolorized maximum number of dyes to the greatest extent. Methylene blue (MB), Sudan black (SB) and Malachite green (MG) were completely decolorized while there were faint traces of color on the Crystal violet (CV) and Methyl red (MR). The MB and MG were almost completely decolorized by *A. terreus,* while regions of strong color remained in plates with *A. niger* and *F. moniliforme*. MB, SB and MG were faintly decolorized by *M. racemosus*, *A. flavus* and *A. fumigatus* which could be visualized on the agar surface around the colony.

Assay of decolorization of liquid culture

When fungal cultures were incubated on rotary agitation at 200 rpm, exhibited uniform mycelial growth of 2-3 mm in diameter were formed on the surface of the growth medium by day 4 and a simultaneous decrease in dye absorbance was accompanied with visible sorption of the dyes to the fungal mat. The percentage of color removal increased with further incubation period. Among the fungal cultures, *A. terreus, A. niger* and *F. moniliforme* registered the maximum percent of color reduction (above 80 %), while the *M. racemosus, C. cladosporioides, P. oxalicum* and *T. viride* demonstrated low amount of color reduction (below 25 %). In dyes, Methylene Blue (MB), Sudan Black (SB) and Malachite Green (MG) were completely decolorized, while 40 % - 60 % decolorization was registered for Crystal Violet (CV) and Methyl Red (MR). These fungi were able to decolorize the dye with maximum of 500 µg/l dye added and the percentage of decolorization after 5 days. The maximum degradation activity was shown by *A. terreus* (86.33 %), followed by *A. niger* (84.20 %) and the lower level degradation was observed in *C. cladosporioides* (3.14 %), *P. oxalicum* (4.25 %) *M. racemosus* (4.54) and *T. viride* (5.09 %) and thus the degradation activity were found in the range of 3.14 % and 86.33 %.

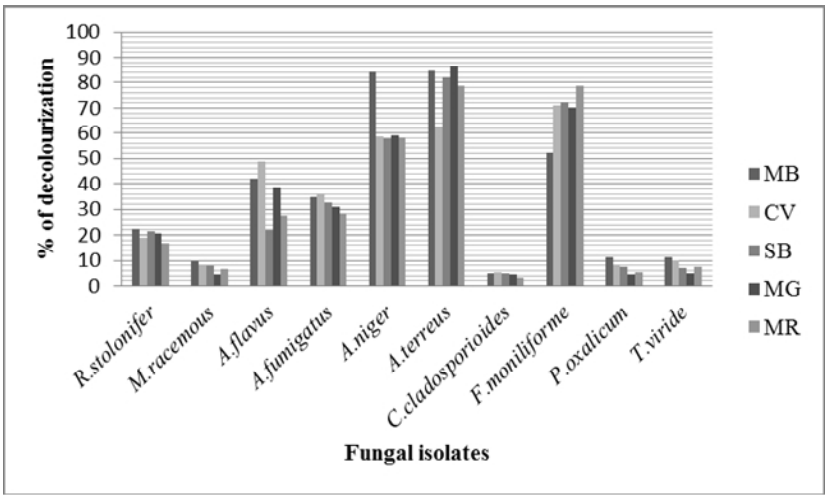

Figure 1: Assay of decolorization in liquid culture

Laccase assay

Among 13 species, *A. terreus* and *F. moniliforme* showed clear zone around the colonies on Lignin modifying enzyme basal medium (LBM) plates. It indicated the formation of a strong oxidation zone in the zone of dye degradation when incubated with hydroxybenzotriazole for 30 minutes which is due to laccase secretion.

DISCUSSION

The present study was aimed to isolate the Ascomycetes and Zygomycetes from dye effluents and find out their dye degrading capacity. The results obtained from the present investigation revealed the ability of fungal species in degrading various commercial dyes in effluents. Dye colour removal by fungal species was monitored for different concentrations in the stimulated basic dye solution at different time intervals based on the dye decolorization. The dye removal profiles showed four distinct phases. Initially the dye uptake was rapid which may be due to bio sorption. In the second phase, the dye color uptake retarded which may be due to acclimatization phase and during the third phase, the dye color removal attained a rapid uptake which may be due to dye uptake by fungal cells and finally leveled and attained saturation after a contact time of 5 days and remained more or less constant thereafter up to the end of 8 days after which the study was terminated. From the results, it was noted that at least 48 hours of contact time was required for the initial acclimatization of the fungal species to the existing stimulated dye environment. Based on the biomass study, the dye removal pattern showed that increase in the biomass concentration indicated increased dye removal capacity, which may be attributed to the fact that the increase of biomass of fungal species gives more surface area for sorption of the dye molecules on the surface. Dye degradation has been proposed as a quick, reproducible and inexpensive screening method to determine ability to degrade many aromatic compounds. In the present study different species of Ascomycetes and Zygomycetes were screened for decolorization of commercial textile dyes. *A. terreus, A. niger, A. flavus, A. fumigatus, R. stolonifer, P. oxalicum, F. moniliforme. M. racemosus, C. cladosporioides* and *T. viride* showed decolorizing activity in all the dyes which includes MB, CV, SB, MG and MR thus exhibiting their potential biodegrading capability. However, *A. terreus* seemed to be more potential on the dyes as their zone of inhibition in the dye containing medium was larger. *A. terreus, A. niger* and *F. monilforme* decolorize dyes namely MB, SB and MG; however with synthetic dyes like methyl red and crystal violet complete decolourization could not be achieved comparatively to the other dyes. This may be due to the chemical functionalities of dyes and a clear relationship between dye structures and fungal dye biodegradability has not been established so far[8]. Though the dyes MB and MG were completely decolorized by the mycelium in liquid medium, it was very slow in solid medium. The efficiency of decolorization by the fungus varies among the dyes. All the other dyes were decolorized up to 50 to 86 % within 8 days except CV and MR. In the case fungal systems, the rate of color removal increased with the incubation period. Among the 10 strains used in the study *A. terreus* and *F. moniliforme* recorded almost similar rate of decolourization on an 8 days period[9]. In liquid culture, rapid dye decolorization by the fungus was observed within 24 h. It was mainly due to the high adsorption of the dye by mycelium. In subsequent days, dye decolorization may be due to production of extracellular enzyme[10]. The azo dyes are degraded by bacteria using their extracellular hydrolytic and oxidative enzymes[11,12]. In the present study, *F. moniliforme* and *A. terreus* were able to oxidize non phenolic compounds such as hydroxybenzotriazole (HBT). As HBT is considered as a unique laccase substrate, it is confirmed that the enzyme liberated is a true laccase. Among the different lignolytic enzymes, laccase has been received more attention in recent years because of its ability to catalyze the oxidation of a wide spectrum of a molecules containing aromatic rings substituted with electron withdrawing groups. Its low specificity makes laccase a promising tool in transforming many toxic substituted phenols or aromatic compounds[13,14]. The results suggested that the fungal strains of *A. terreus, A. niger* and *F. moniliforme* have potential for dye degradation and the laccase production indicated their enormous potential to degrade other pollutants also.

CONCLUSIONS

Dyes are designed to resist fading even against chemicals such as oxidizing agents. It is really amazing that these dyes stand no chance against microbial degradation. The fungal strains isolated from textile effluents, were screened for their

ability to decolorize MB, CV, SB, MG and MR. Among the fungi isolated, *A. terreus, A. niger* and *F. moniliforme* found to be more efficient in decolourizing textile dyes. Bioremediation has proved to be very effective method in countering the textile dye pollution in an eco-friendly way. This approach creates a promising hope to remediate the environments polluted by textile dyes. Moreover, a lot of future work is needed to isolate new microorganisms capable of effectively degrading wide range of textile dyes and to create an environment free from textile dye pollution.

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