

Development of Industrial Effluent Treatment System Using White-Rot Fungi, Isolated from Nature and their Enzymes

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Abstract

Industrial effluents containing wastewater from textile and dyestuff industries is one of the most difficult to be treated. Since it contains various kinds of synthetic dyes with complex aromatic molecular structures, illustrating the need for a non-specific method of decolorization. Due to the low biodegradability of synthetic dyes, they can cause serious environmental pollution. Hence there's an urgent need to develop treatment system for bioremediation of dye containing industrial effluents to reduce pollution in a non-toxic manner and provide environmentally friendly treatment technology for sustainable development. In recent years white-rot fungi have attracted increasing attention as their lignolytic enzymes have the ability to degrade recalcitrant compounds and synthetic dyes. Owing to extracellular, non-specific, free radical based lignolytic systems of WRF; they can completely eliminate a variety of xenobiotics, including synthetic dyes and industrial dyes giving rise to non-toxic compounds. Hence five white-rot fungi (isolated from nature) were evaluated for their potential to declorize two dyes Rhodamine-B and Methyl Orange in order to develop them in future as target organisms for treatment of Industrial effluents. All the fungal isolates showed high rates of decolorization, with KV10 and KV12 removing 76% color within 96 hrs. All the fungi not only decolorized the dyes but also degrade them as is evident from the shift in their absorption spectra. Linear increase in laccase production by all five fungi, was observed with a simultaneous increase in extent of decolorization, irrespective of the type of dye. The presence of the dyes in liquid medium had little or no effect on the mycelial growth, at the concentration tested.

Keywords: Rhodamine-B; Xenobiotics; Anthraquinone; Chromophore; Synthetic dyes; White-rot fungi

Introduction

Synthetic dyes represent a large group of chemically different compounds, which are classified by their chromophore into azo, anthraquinone, triphenylmethane, heterocyclic or pthalocyanine dyes [1]. Over 10,000 dyes with an annual production of over 7×10⁵ metric tons are commercially available. Approximately 50% of these dyes are released in the industrial effluent [2]. The majority of synthetic dyestuffs are hardly removed from textile waste water by conventional waste water treatment, such as activated sludge [3] and physiological processes. Due to the low biodegradability of synthetic dyes, they can cause serious environmental pollution [4]. The strong color of discharged dyes even at very small concentrations has a huge impact on the aquatic environment caused by its turbidity and high pollution strength: in addition toxic-degradation products can be formed [5]. Most dyes are very stable to light, temperature and microbial attack, making them recalcitrant compounds. Many of them are toxic, carcinogenic and mutagenic [6,7]. In recent years white-rot fungi have attracted increasing attention as their lignolytic enzymes have the ability to degrade recalcitrant compounds and synthetic dyes [8]. Owing to extracellular, non-specific, free radical based ligninolytic systems of WRF; they can completely eliminate a variety of xenobiotics, including synthetic dyes and industrial dyes giving rise to non-toxic compounds [9]. Dye decolorization by white-rot fungus consists of two simultaneous steps, namely, adsorption between the mycelia and the macromolecular dyes and the enzymatic degradation [10]. Industrial effluents generally contain various kinds of dyes, illustrating the need for anon-specific method of decolorization [11]. The objective of this paper was to evaluate decolorization potential of five laccase producing white- rot fungi, isolated from nature to decolorize, two Synthetic dyes-Methyl orange and Rhodamine-B. The fungi used in this study have earlier shown their potential to decolorize and degrade Triphenyl methane dyes, in a non-toxic eco friendly manner [12], therefore an attempt was made to test their potential to decolorize different kinds of dyes, in order to develop them in future, as target organisms for treating industrial effluents in a non-toxic eco friendly manner and provide safe technology for bioremediation of wastewater polluted due to discharge of industrial effluents from dyeing industries.

Materials and Methods

Organisms

Fungi used in the study, were isolated from nature. These newly isolated fungi from natural sources were- KV10, KV11, KV12, KVA5, AKCH. All fungal strains were grown on 2% Malt Extract medium [13] containing Malt extract 20 g/l, $Ca(NO_{3)2}.4H_2O$ 0.5 g/l, $MgSO_4.7H_2O$ 0.5 g/l, $KH_2PO_40.5$ g/l, Agar 20 g/l(MEA) and incubated at 28°C. Pure cultures of these strains were maintained by repeated sub culturing on Malt Extract Agar (MEA). The strains were kept at 4°C till use. All strains were examined microscopically for, mycelial characters, presence or absence of spores, septa and clamp connections and named alphabetically.

Chemicals

Malt extract (Himedia), Calcium nitrate tetrahydrate (Qualigens), magnesium sulphate (Qualigens), potassium dihydrogen phosphate (Qualigens). 10 mM Guiacol, prepared in 0.1M Citrate-phosphate

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buffer (SRL), Methyl orange (Qualigens), Rhodamine-B (SD).

Culture conditions

All WRF were grown in 250 ml Erlenmeyer flasks containing 50 ml of sterile 2% Malt extract broth (MEB) containing Ca (NO₃)2 .4 H_2O 0.5 g/L, MgSO₄.7 H_2O 0.5 g/L, KH₂PO₄ 0.5 g/L, pH 5.4 and incubated at 28°C. Each flask was inoculated with two 8mm diameter fungal discs, obtained from the periphery of the actively growing fungal cultures on MEA plates and incubated at 28°C. After 8 days of growth on liquid media, 1 ml of Methyl orange and Rhodamine B (1 mg/ml) was added to each flask, containing respective grown fungal culture. The time of addition of dyes in the grown cultures was recorded as 0 hrs and 1 ml of extracellular culture filtrate was removed aseptically, at selected intervals. The un-inoculated Malt extract broth (MEB) with respective dye served as abiotic control.

Decolorization assays

The extent of decolorization of the two dyes in the culture filtrates was analyzed, spectrophotometrically at the wavelength maxima of the respective dyes, i.e. 422 nm for Methyl orange and 510 nm for Rhodamine B.

The decolorization efficiency was determined as described:

% decolorization = [(A λ initial – A λ final)/ A λ initial x 100].

Where A λ initial is the absorbance of dye in un inoculated control and A λ final is the absorbance of dye in inoculated cultures.

UV-VIS characterization

The dye degradation products produced duringbiodegradation under static cultivation conditions, in the culture filtrates of all five fungi, with each of the dyes, were monitored by following the change in the absorption spectra in range of 200-800 nm using a UV-VIS spectrophotometer.

Enzyme assays

The 250 ml Erlenmeyer flasks with 30 ml of MEB were inoculated in same way as for decolorization assay. Enzyme activity was measured in culture filtrates from replicate flasks, obtained after mycelia removal by filtration filter paper. Laccase activity was was determined spectrophotometrically, by monitoring increase in absorbance at 470 nm due to oxidation of guaiacol according to [14]. Heat killed enzyme was used as control. The change in absorbance of 0.01/ml/min, was defined as 1 U of laccase activity.

Determination of biomass production

Biomass production in liquid media was evaluated by determining the dry mass of mycelia. Mycelia were harvested selected time intervals from liquid cultures by filtration through what man filter paper no.1, dried at 80°C for 48 hours and weighed.

Results

The fungal isolates, when observed microscopically, showed the presence of clamp connections, the characteristic feature of basidiomycetous fungi. All newly isolated white rot fungi from natural sources, were basidiomycetes (namely, KV10, KV11, KV12, KVA5, and AKCH). All the strains grew well on 2% Malt extract agar medium.

Dye decolorization by fungi in liquid culture

Both the synthetic dyes Methyl orange and Rhodamine-B were

found to be decolorized by all the five fungi isolated from nature (Figures 1a and 1b), though to different extents (Figures 2a and 2b). Among the five fungi tested all of them showed considerable high rate of decolorization (Figure 3), with KV10 and KV12, being the most efficient, removing 76 and 79% of methyl orange within 96 hours of incubation. Among the two dyes, Methyl orange was decolorized to a much higher extent (nearly 80%) compared to Rhodamine-B (just more than 60%), Irrespective of fungal strain used for decolorization.

All the five fungal strains not only decolorized these dyes, but also degraded them which is evident from the visible absorption spectra 200-800 nm of each of the dyes being degraded by fungal strains, taken at selected intervals (Figures 4a-4h).

There was correlation between extent of decolorization and lac case production, by all five fungal cultures showed linear increase in laccase production with a simultaneous increase in extent of decolorization, irrespective of the type of dye, indicating the involvement of laccase in decolorization of synthetic dyes. There was little or no effect of



Figure 1: (A) methyl orange uninoculated control (left) and decolorized by fungus KV 12. (B) rhodamine B uninoculated control (left).





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Discussion

Five fungi tested, have shown faster decolorization rates, showing more than 50% decolorization within first 48 hours, which is much faster than reported elsewhere Vasdev [15], reported decolorization time of 96 hours for various species of *Cyathus*, while Yesilada [16],

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Figure 4g: Degradation of methyl orange by KVA5 at the 0 hours.

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Figure 4h: Degradation of methyl orange by KVA5 after 48 hours.

Fungal Cultures	Malt extract (ME Broth)	ME + Methyl orange	ME + Rhodamine B
AKCH	0.184	0.187	0.179
KVA5	0.245	0.249	0.241
KV10	0.253	0.257	0.251
KV11	0.137	0.134	0.141
KV12	0.372	0.369	0.374

Table 1: Biomass of the five fungal species in grams.



Table 2: Structures of the dyes.

reported decolorization time of 72 hours for 62% decolorization of crystal violet by *P. chrysosporium* ME446, while Knapp et al. 1995, reported 216 hours of decolorization time for *P. chrysosporium* NCIM1197.

Among the five fungi capable of decolorizing the two dyes, fungal isolate KV10 and KV12 were found to be the most efficient in decolorization of dyes. Which is in agreement with suggestion of Goszczynski [17] that among the various factors affecting the dye decolorization rate, besides differences in the structure of the dyes, is fungal physiological characteristics.

In the present study, Methyl orange was decolorized to a much higher extent, as compared to Rhodamine - B which is explained on the basis of differences in their chemical structure (Table 2). This shows that dyes belonging to chemically different groups are not decolorized to same extent and structural differences in the dye molecule strongly affect the decolorization process [18]. The decolorization process requires the destruction of chromophores. Small structural differences also affect the decolorization process. Rhodamine B is a diazo dye, while Methyl orange has no azo bond (Table 2). The degradation of the azo dye involves aromatic cleavage, which depends on the identity, number and position of functional groups in the aromatic region, and the resulting interaction with the azo bonds [7]. Similar results have also been reported by Si Jing [11]. High level of decolorization of dyes by all the five fungi used in this study, could actually be due to biodegradation of these dyes, as was evident from change in the absorption spectra of the dyes, before and after decolorization. This is in agreement with study of Franciscon [19] on biodegradation of dyes by S. arlette, our earlier study [12] and Huang [20], have also suggested that if dye removal is attributed to biodegradation, either the major UV-VIS light absorbance peak would completely disappear or a new peak would appear. Similar results were observed in our present study, there was complete shift in the absorbance peak at their wavelength maxima, (Methyl orange at 590 nm, and Rhodamine B at 620 nm), in the spectrum, along with exponential fall, in the U.V.-Visible spectra, indicating degradation of dyes by all five fungal cultures.

This significant positive correlation observed between increase in laccase production and extent of decolorization of dyes, by all five fungi, indicates the involvement of laccase in degradation of synthetic dyes. It has also been reported by Diwaniyan [21], Vasdev [15], Vasdev and Kuhad [14].

Despite structural variations in the dyes tested, none of the dyes, showed, any significant effect, on the growth of these fungi, as is evident from their biomass at the end of decolorization period (Table 2). This indicates that the fungi are able to neutralize or degrade these dyes without forming any toxic intermediates, which could hinder the growth of these fungi.

Similar results were obtained in our earlier study with triphenylmethane dyes [12]. This is a novel and very unusual characteristic of these fungal isolates, as in most studies there is reduction in the biomass of fungi on media in presence of dyes as compared to control media in the absence of dyes. Eichlerova [18] reported it in case of *Ischnoderma resinosum*, Ngieng [22] reported itin endophytic fungi isolated from *Melastoma malabathricum*.

Conclusion

The results from the present study demonstrate the ability of five fungal isolates to decolorize and degrade both dyes- Methyl orange and Rhodamine-B, irrespective of the complexity in their structures. With their high decolorization efficiency, faster rate of decolorization, tolerance to high concentration of dyes and their ability to degrade them completely in a non-toxic, eco-friendly manner, as is evident from no adverse effect on growth of these white-rot fungi, in presence of dyes, also ability to produce high level of lac case during decolorization. As these fungi have earlier shown their potential to decolorize and degrade triphenyl methane dyes also, they can be developed and exploited in future for bioremediation of industrial effluents from dyeing industries [23-26].

Since, they can decolorize chemically diverse group of dyes-synthetic dyes as well as triphenyl methane dyes. Their ability to produce laccase during decolorization, shows that this enzyme can be overproduced, purified, isolated and immobilized, or can be bioengineered into nano particles and developed as potential source for bioremediation of toxic industrial effluents, pesticide and insecticides. These fungi as well as enzyme produced by them can therefore be most suitable eco friendly

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agents for reducing pollution and developing technology for clean and safe environment for sustainable development [27].

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