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Studies on Dyes Decolorization of White Rot Fungi Isolated From Soil

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Abstract:

The present study was carried out for biodegradation of dye by using test organisms *T. hirsuta and WRF-Isolates.* Decolorization is an important step in the degradation of the Dye and complete decolorization was achieved by the organisms when the pH of the medium was in a range 4.0-4.5.During the incubation period in the decolorization process by the microorganisms, namely *T. hirsuta and WRF-Isolates* always showed a decrease in the pH from the initial condition indicating the release of the degradation products. Solid state decolorization was observed to be faster (Maximum 95% with WRF Isolates) compare to the aqueous state decolorization process. In aqueous state decolorization process, WRF isolate were showed faster decolorization of congo red (77.28 % Decolorization) and Methylene Blue (70.67%) while T. hirsuta one of the well known culture for dye decolorization showed less decolorization within five days (CR-69.10% and MB- 65.34%). The finding in the present study can serve as an important base for the development of a economical as well as simplified biological treatment system using microorganisms for providing reusable clean water for industrial purpose.

Keywords:
White Rot Fungi, Decolorization, T. Hirsuta and Dy

Introduction:

Due to rapid industrialization and urbanization, a lot of chemicals including dyes are manufactured and used in day-to-day life. Dyes are synthetic and aromatic molecular structural compounds. They are used on several substrates in food, cosmetics, paper, plastic and textile industries (*Sathiya et al.,2007*).Wastewater from textile industries poses a threat to the environment and are often carcinogenic (*Kim et al., 2003*). Further, the adsorption of light by these textile dyes creates problems for photosynthetic aquatic plants and algae (*Singh and Singh, 2006*). Earlier studies have shown that many reactive dyes are not degraded in ordinary aerobic sewage treatment processes and that they can be discharged unaffected from the treatment plant (*Carliel et al., 1996).*

The strong color of discharged dyes even at very small concentrations has a huge impact on the aquatic environment. Currently, textile effluents are treated by physico-chemical methods that are often quite expensive (*Robinson et al., 2001*), Some anaerobic textile wastewater treatment methods have been

developed at a laboratory scale and have shown to remove color efficiently (*Kapdan et al., 2000).* Furthermore, there may be a risk of reverse colorization when anaerobic degradation products are exposed to oxygen (*Knapp and Newby, 1995).*

Decolorization of dye wastewater by fungal metabolic activities is the subject of many studies (*Hu, 2001; Wong and Yuen, 1996).* Several combined anaerobic and aerobic microbial treatments have been suggested to enhance the degradation of textile dyes (*Bortone et al., 1995; Haug et al., 1991; O' Neill etal.,2000*). Many of which are mutagenic and/or carcinogenic (*Chivukula et al., 1995, Chung et al., 1993, O'Neill et al., 2000*).

A great number of white rot fungi have been shown to excrete extra cellular enzymes like lignin peroxidase, mangnese-peroxidase and laccase *(Hatakka, 1994*). Fungi from the Basidiomycetes group, known as white rot fungi are a hetereogenous group of microorganisms but have in common the capacity to degrade lignin as well as other wood components *(Kirk and Farrell, 1987). Trametes hirsuta* and a purified laccase from this organism were able to degrade triarylmethane, indigoid, azo, and anthraquinonic dyes *(Abadulla et al., 2000).*It is found all year round and persists due to its leathery nature *(Phillips, Roger., 2006).* Laccase based decolorization treatments are potentially advantageous to bioremediation technologies (*Gian Freda et al., 1999; Rodriguez et al., 1999).*

The white rot fungi are by far the most efficient ligninolytic microorganisms. They are able to degrade a wide variety of recalcitrant pollutants including various types of dyes. *(Machado et al., 2006).*

Most information on the biodegradation of synthetic dyes by ligninolytic fungi has been obtained with *Phanerochaet echyrsosporium* (*Paszczynski and Crawford.,1995).* Many white rot fungi studiedin connection with their ligninolytic enzyme production and their decolorisation ability *(Revankar and Lele, 2007; Mechichi et al., 2006; Maxima and Costa-Ferreira, 2004; Susla et al., 2007; Singh et al., 2007; Demir et al., 2007).*In the present study the main thrust is to determine the ability of *WRF-Isolate* and *Trametes hirsute* in the decolorization of the dyes. The study also aimed to assay the solid and aqueous state dye decolorization.

Material and methods:

The white rot fungi *Trametes hirsute (NCIM-1201*) was procured from National Chemical Laboratory, Pune, India. One White Rot fungal culture was isolated and identified from soil sample of Katol Dist. Nagpur (MS), area based on the *Nyakundi et al., (2011). T. hirsute* and *WRF-Isolate* were maintained in Potato Dextrose Agar *(PDA*) media and stored at 4°C.Methylene blue and congo

red dyes were selected for decolorization study. Sampling was done using stratified random sampling method from Katol region. Isolation and Identification of White Rot Fungi was done on the basis of Nyakundi method *(Nyakundi et al., 2011).* Laccase was confirmed by using inducers, guiacol. Solid Substrate Enzyme Production, Solid state Decolorization and Aqueous batch Decolorization studies were done on the basis of (*Sathiya et al.,2007).*

Rate of dye decolorization was estimated by following formula

$$\textbf{Rate of Decolorization (Solid State)}(\%) = \frac{diamaterofzonedicolorization}{diamaterofplatetaken} * 100$$

$$Rateofdecolorization\ (Auueous\ State\ )(\%) = 100 - \left(\frac{Absorbanceoftreateddyesolution}{Absorbanceofcontroldyesolution}\right) * 100$$

Screening of fungi for extracellular enzyme production

White rot fungi are capable of producing extracelluar enzymes such as lignin peroxidase, manganese peroxidase and laccase. Laccase is mainly responsible for the decolorization of aromatic compounds. It is able to oxidize substrates such as ABTS, guiacol *(Sathiya et al., 2007).* In this test dark reddish brown zones appeared on both the culture plates.

In *WRF-Isolate* inoculated plate, the appearance of dark reddish brown color takes place within 24 h and the complete color change was observed in fourth day. In *T. hirsute* inoculated plate, the color change starts at the first day but active change starts only from the second day. These findings of present studies agree with observations made earlier by (*Sathiya et al 2007).*

Result and discussion:

White rot fungus *T. hirsute* and *WRF-Isolate* were tested for their dye decolorizing ability. Their activity was tested against most common dyes such as Methylene Blue and Congo Red. The various parameters such as solid-state decolorization, aqueous state decolorization were analyzed.

Solid State Decolorization Assay

In solid state decolorization the mycelial growth of the microorganisms and visible decolorization starts at the first day. *WRF-Isolate* was found to be fast growing fungi as compared to the *T. hirsuta*, and it also starts production of basidiospores from third day onwards.

The deolorization of dye by *WRF-I* was observed to be 2 fold faster than the decolorization by *T. hirsuta.* About 93.75% decolorization by *WRF-Isolate* was observed on the 4th day of incubation and in *T. hirsuta* 61.87% of decolorization was observed. (Figure3).

Methylene Blue

At the concentration of 100 mg/l *WRF-Isolate* decolorized the dye by 95% on 4th day of incubation and 63.1% of decolorization by *T. hirsuta*was observed.

Aqueous Batch Decolorization Assay

Decolorization of Congo red and Methylene Blue in 0.5% glucose medium was observed at 340nm and 664nm respectively in Shimadzu UV-Spectrophotometer daily for 5 days.

Congo red

At the concentration of 100 mg/l visual decolorization by *WRF-Isolate* and *T. hirsuta* was observed within 24 h. Maximum decolorization was found to be 77.28% *WRF-Isolate* on the 5th day of incubation and 69.10% was found to be in *T. hirsuta* (Fig.7)

Methylene Blue

At the Concentration of 100 mg/liter visual Decolorization by WRF isolates and *T. hirsuta was observed within 24 Hrs. Maximum Decolorization was found to be 70.67 % WRF* fungi isolates on the 5 day of incubation and 65.34% were found to be in *T. hirsuta*

Batch studies were conducted in a defined medium with pH 7.0, for both the test organisms, namely, *T. hirsuta*and *WRF-Isolate.* As the decolorization progressed there was a substantial reduction of pH to a range between 4.0 - 4.5 exhibited by both the test organisms. Reduction in pH of the medium might be due to the presence of conversion products, Livernoche *et al.*,(1983) and Latha*et al.*,(*1997*). According to them, there was a correlation between the variation of pH and color removal. When a particular concentration of sucrose or glucose provided the decolorization or color removal process by white rot fungus was enhanced (*Srinivasan and Murthy, 2000).*

The laccase from *Polyporus rubidus* could decolorize synthetic dyes in a broad range of concentration without the need for redox mediators. Earlier studies with white rot fungi, Trametes hirsuta, *Pleurotusostr eatus*, and *Ischnoder maresinosum*, have already shown the use of redox mediatorto decolorise recalcitrant dyes. *(Couto and Sanroman, 2007; Eichlerova et al., 2005).*

In this study, the biodegradation of dye by the test organisms namely, *T. hirsuta* and *WRF-Isolate* have been described. Guaiacol is one of the well known substrate for Laccase extracellular enzyme, secreated by White Rot fungi. In the present study it was confirmed that PDA plates supplemented with Guaiacol, was found to be an excellent screening medium for White Rot fungi from soil sample. Also Guaiacoland Gallic Acid act as good inducers for Laccase Production from White Rot fungi.

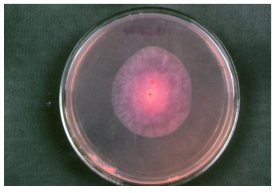

Figure-1 Solid State Decolorization of Congo Red Dye by *WRF-Isolate*

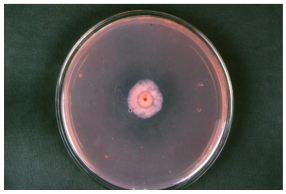

Figure-2 Solid State Decolorization of Congo Red Dye *T.hirsuta*

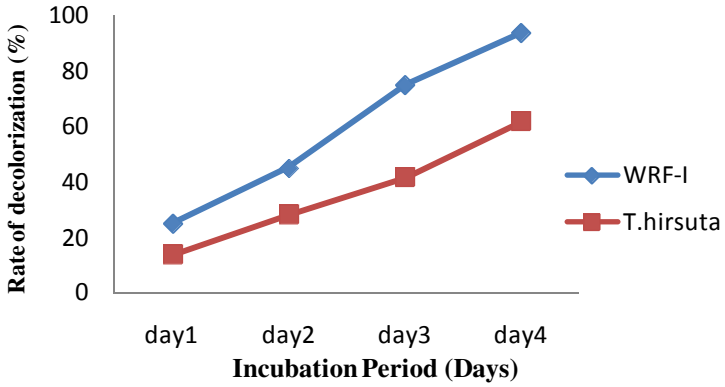

Figure- 3 Solid State Decolorization of Congo red by white rot fungi. And *T. hirsuta*

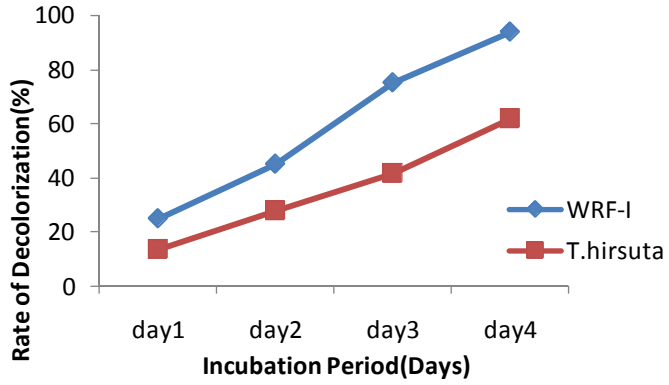

Figure- 4. Solid State Decolorization of Methylene Blue by white rot fungi and T. hirsuta

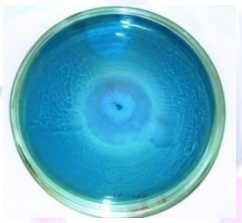

Figure-5 Solid State Decolorization of Methylene Blue Dye *WRF-Isolate*

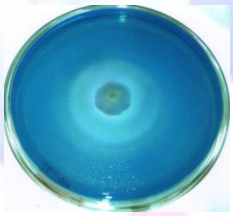

Figure- 6 Solid State Decolorization of Methylene Blue Dye by *T.hirsuta*

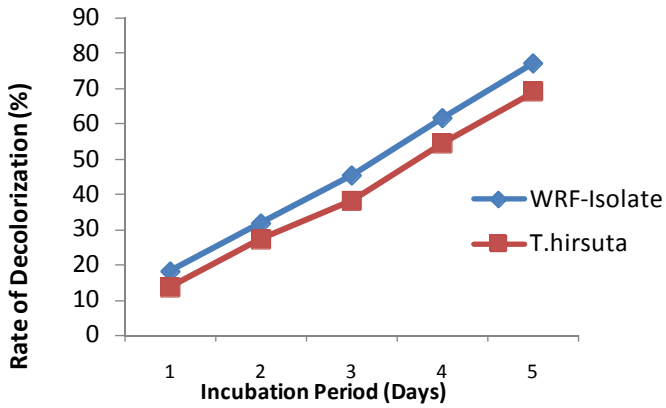

Figure-7. Aqueous State Decolorization of Congo Red by white rot fungi and T. hirsuta

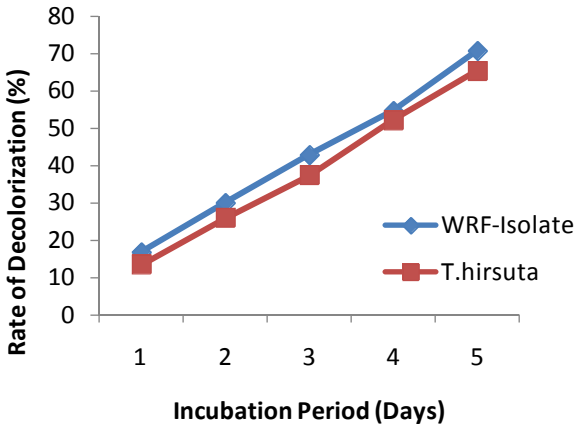

Figure-17 Aqueous Batch Decolorization of Methylene Blue by white rot fungi.

Figure. 8. Aqueous State Decolorization of Methylene Blue Dye by *White Rot fungi.*

Figure.9. Aqueous State Decolorizationof Congo Red Dye by White Rot Fungi.

Conclusion:

The findings in the present study showed the potential of *WRF-Isolate* and *T. hirsuta* for decolorization of dyes and on the basis of results the aqueous decolorization is recommended for dye decolorization. The findings in the present study can serve as an important base for the development of an economical/eco-ethical as well as a simplified biological treatment system using micro-organisms. The alternative is clear: man, a material-using organism, must also make moral choices and try to aim for a preferred limiting. In Aqueous state decolorization process, *WRF-Isolate* were showed faster decolorization of Congo Red and Methylene Blue while *T. hirsuta* one of the well known culture for dye degradation showed less decolorization within five days

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