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DECOLORIZATION AND DETOXIFICATION OF TEXTILE DYES AND EFFLUENT WITH A LACCASE ENZYME FROM *TRAMETES HIRSUTA*

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Abstract:
A large group of chemical dyes are manufactured on an industrial scale with an annual production of over 7×10^5 metric tons. Reactive dyes are important chemical pollutant from textile industries. These dyes are discharged from textile and dye industries directly into water bodies and responsible for toxicity and carcinogenicity. The species of white rot fungi, *T. hirsuta* was evaluated for its ability to decolorize dyes, reactive pink and reactive and direct effluent. Laccase is lignolytic enzyme extracellularly secreted by white rot fungi. The laccase activity was measured using both solid and aqueous state assays. The effective decolorization was started after 72 hrs of incubation, with different concentrations 5 mg/l, 10mg/l, 15mg/l, 20mg/l & 50mg/l & complete decolorization was observed in higher concentration within 9 days of incubation. A toxicity study shows that effluent after treatment was less toxic as compound to untreated effluent. The absence of zone of inhibition on agar plates indicated that the fungal degraded dye metabolites are nontoxic to beneficial micro-flora. Therefore, *T.hirsuta* has promising potential in color removal from textile wastewater containing reactive dyes.

Keywords: Reactive dyes; decolorization; *Trametes hirsuta;* detoxification

INTRODUCTION:

Various chemical substances discharged from the industries become a persistant environmental contaminant. In our day-to-day life a lot of chemicals including dyes are manufactured due to industrialization & urbanization. Textile processing industries were found in most of the countries & their numbers have been increased. In textile industries, color is applied to finished product through dying, resulting in the generation of different wastewaters. Textile industries release large quantity of intensely colored & toxic effluent, which cause serious environmental pollution. The dyes includes such as acidic, reactive basic, disperse, azo, diazo, anthraquinone. Reactive dyes are most common dyes because of many advantages such as operating at mild conditions, give bright colors & stable structures. Reactive dyes are characterized by azo bond's (N=N) & used to dye cellulose fibre. The color of azo dyes is due to the presence of azo bond associated with chromophores. Effluent from industries containing reactive dyes causes serious environment pollution because presence of such dyes in water is highly visible & affects their transparency & aesthetic even if the concentration of the dyes is low.

White rot fungi are basidiomycetes that are capable of degrading a lingo cellulose substrate. They are called white rot because the degradation process results in a bleaching of wood substrate. Fungi are important organisms that have high tolerance to toxic environment making them ideal to use for bioremediation process. Three types of enzymes are produced by white rot fungi, Lignin peroxidase (Lip), Manganese-dependent peroxidase (Mnp) & Laccase (Lac).

Laccase, EC 1.10.3.2, p-diphenol: oxygen oxido-reductase, is part of larger group of enzymes termed the Multi Copper Oxidases (MOC) (Komori et al., 2009) belonging to the group of blue-copper proteins. This enzyme is found in many organisms, including plants, bacteria fungi & human. This enzyme is generally extracellular & catalyzes the oxidation of several phenolic compounds, aromatic, amines, thiols & some inorganic compounds using molecular oxygen as electron acceptor .Fungal laccases have been confirmed for their ability to degrade several azo dyes.

The aim of the present work was to exploit biodecolourization of reactive black & reactive pink by *Trametes hirsuta* with following objectives: to assess the ability of fungal cultures to decolorize & degrade the dye, the actual dye industry waste, confirmation of degradation of dye & to assess the toxicity of degraded products.

MATERIAL AND METHODS:

The fungus, *T. hirsuta* was isolated from soil. The solid medium used for fungal growth contained per liter: 10 g of malt extract, 4 g of yeast extract, 4 g of glucose and 20 g of agar (pH 5.5).

Dyes:

Reactive dyes are the dyes, which are mostly used in the textile Industries. The following dyes

were selected for use for the study Reactive Pink and reactive Black

Solid states enzyme production:

The laccase production medium containing wheat bran flakes 4.5%, yeast extract 1.5%, glucose 1%, NH4Cl 0.25% and pH 5.0 was prepared and gallic acid (50 µg/100ml) was added as a substrate for laccase screening. For laccase production, a disc of 6 day-old culture was placed in the center of the plate. The plates were incubated in dark and observed for reddish brown zone in the medium which will be formed as a result of laccase oxidative polymerization with gallic acid.

Laccase confirmation using inducers:

Nutrient salt medium containing (g/l) glucose 10 g, $(NH_4)_2PO_4$ 0.2 g, KH_2PO_4 1.0 g, $MgSO_4.7H_2O$ 0.5 g, KCL 0.5 g, $FeSO_4.7H_2O$ 0.005 g, thiamine hydrochloride 0.001 g and pH adjusted to 6.0 was prepared and gallic acid 0.05 g/100 µl was prepared and added in 50ml of media. The medium was inoculated with crude enzyme but without inducer was maintained as control and observed for color change in the medium.

Estimation of biomass to be inoculated:

The biomass to be inoculated in the flask as determined by taking the initial weight of plate of silver foil with 5 sterile discs of filter paper (2mm size) and then weighing plates with inoculated 6 days old culture discs 5 in number of respective culture. The biomass was then obtained by subtracting initial weight from final weights.

A study of Solid state decolonization:

Dye solutions of 50mg/L concentration were prepared. After sterilization media, two ml of respective dye solution was added aseptically to 50ml of medium and a disc of 6 day-old of respective culture was inoculated. Plates were incubated at room temperature for decolorization of dyes.

Calibration of UV Spectrophotometer:

To find out the maximum absorption of dyes and effluent, wavelengths ranging from 400nm to 750nm were selected and wavelength with maximum absorption was selected for further study.

A study of dye decolorization using submerged fermentation:

Dye solution of concentration 5mg/l, 10mg/l, 20mg/l were prepared and to 20% of Nutrient salt medium (glucose 10g, $(NH_4)_2PO_4$ 0.2g, KH_2PO_4 1.0g $MgSO_4.7H_2O$.KCl 0.5g, $FeSo_4.7H_2O$ 0.005 g, thiamine hydrochloride 0.001 g ,distilled water 1000ml have pH 6.0 was added. 5 discs of respective culture per tube were kept at room temperature. Decolorization rate was observed by UV – Visible Spectrophotometer.

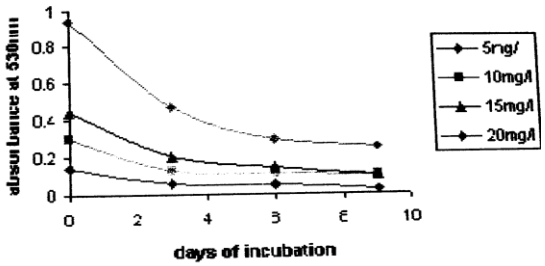
Effluent decolorization using submerged state fermentation:

The effluent containing reactive dyes was collected from Vadodara, Rangreg textile, Gujarat. The initial pH of the effluent was pH 8.7 and the pH was adjusted to 6. Various dilutions of effluent (40%, 60% and 80%) were prepared with Nutrient salt medium. 5 discs of respective inoculums per tube were added and kept to room temperature for decolorization , Decolorization rate was observed by UV - Visible spectrophotometer.

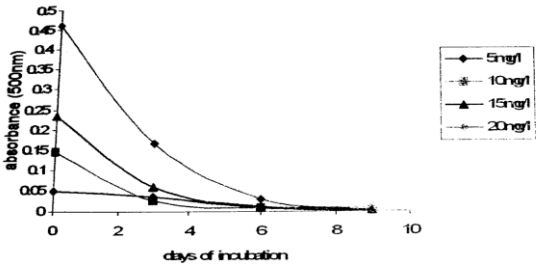
RESULT AND DISCUSSION:

In present study the dye decolorization ability of *T. hirsuta* was studied using their extracellular laccase enzyme system. Decolorisation depends upon laccase production, media and dyes. The similar observation regarding dye degradation by white rot fungus *P. chrysosporium* has been observed. Decolorization of various concentrations of dyes was determined after every 36 hrs of incubation at room temperature. Decrease in concentration of dye was then compared with respective uninoculated control media.

Decolorization of reactive pink and reactive black in 20% Nutrient salt medium was observed at 530nm, on spectrophotometer for 10 days with 3 day interval. At lower concentration of 5mg/l visual decolorization *T. hirsuta* was observed and complete decolorization was found within 9 days of incubation. The rate of decolorization was observed at 560nm for incubation period of 5 days at everyday interval. Effluent of unadjusted pH (11) was decolorized by T. hirsuta, 37.55%. Detoxification study showed that after 24hrs of incubation at 37^0 C, the untreated effluent showed zone of inhibition on both plates containing cultures of *B. substilis* and E. coli. On plate containing *B. substilis* zone diameter of 1.5mm was observed which was greater as compared to zone of diameter of 1.2 mm on *E. coli.* While no zone of inhibition was observed on plate's containing the treated effluent for both the cultures. This indicated that the effluent was detoxified.



Graph. 1: Decolorisation study of Reactive Pink by *T.hirsuta.*



Graph. 2: Decolorisation study of Reactive black by *T.hirsuta.*

Fig. 1: Laccase production

Fig. 2: Decolorization of reactive pink

Fig. 3: Decolorization of reactive black

Fig. 4: Decolorization on plates containing reactive pink

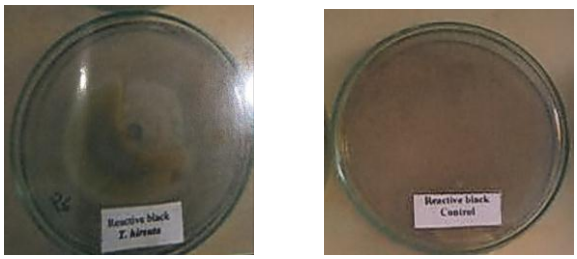


Fig. 5: Decolorization on plates containing reactive black

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