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Biodecolorization of Textile Reactive Dyes using *Trametes hirsuta*

***Uma P and S.S. Sudha**
Department of Microbiology, Dr.N.G.P. Arts and Science College, Coimbatore-641048

**Corresponding author: ums_micro@yahoo.co.in*

Abstract

Reactive dyes are important in textile industries. The potential of *Trametes hirsuta* to decolorize reactive textile dyes like Black and Blue FNG was evaluated. Various conditions required for decolorization have been studied and optimized. The fungus laccase activity was measured and decolorization of dyes was studied using crude extract of the enzyme. Decolorization was also studied using immobilized cells. Effective decolorization was achieved in all the cases for Black and Blue FNG dyes from the third day of incubation. Maximum decolorization was observed with Black dye when compared with Blue FNG.

Keywords: *Trametes hirsuta,* Reactive dyes, Laccase, Immobilized cells.

Introduction

Rapid industrialization and urbanization have increased the chances of releasing hazardous compounds at in to the ecosystem. These pollutants are by products in the effort to improve human standards of living but ironically, their unplanned intrusion into the environment can reverse standards of living by impacting negatively on the environment (Asamudo *et al.*, 2005). Among the many contaminants present in water such as acids, bases, toxic organic and inorganic dissolved solids and colours, are considered the most undesirable and they are mainly caused by dyes (Gupta *et al.,* 2005). Dyes are chemical substances, which are used to colour textile fabrics. Dyes are synthetic aromatic water-soluble dispersible organic colorants, having potential application in various industries. Dyes include acidic, basic, azoic, chromic, diazoic, dispersive and reactive sulphur and vat dyes. Approximately 1,00,000 commercial dyes are manufactured. Over 1,00,000 dyes with an annual production of over 7×10^5 metric tons are commercially available (Campos *et al.,* 2001, Mohan *et al.,* 2002).

Colored industrial effluents from the dyeing industries represent major environmental problems. Dye wastewater discharged from textile and dyestuff industries have to be treated. Color can be removed from wastewater by physico-chemical methods. These include adsorption, coagulation, chemical transformation, flocculation, oxidation, filtration, photocatalysis, ozonation and electro-chemical methods. These methods are often laborious and expensive (Sathiyamoorthi *et al.,* 2007). The success of biological processes for color removal from a given effluent depends in part on the utilization of microorganisms that effectively decolorize synthetic dyes of different chemical structures. Many bacteria, actinomycetes, yeast and mitosporic fungi are able to

decolorize dyes. Fungi, mainly Basidiomycetes fungi are able to decolorize, degrade and mineralize a broad spectrum of different dye structures (Machado *et al.*, 2006). White rot fungi from the Basidiomycetes group are a heterogenous group of microorganisms having the capacity to degrade lignin as well as other wood components. The dye degrading ability of the white rot fungi is due to its ligninolytic enzyme system consisting of lignin peroxidase, manganese dependent peroxidase and laccase. Among these enzymes, laccase claims our attention, due to its merits such as wide range of substrate utilization (Kirk and Farell, 1987, Sathiyamoorthi *et al.*, 2007). The present study, aims to determined the ability of *Trametes hirsuta* in the decolorization of Black and Blue FNG, using their enzymatic system.

Materials and Methods

Organism used

The white rot fungi *Trametes hirsuta* (MTCC 1171) was procured from the Institute of Microbial Type Culture Collection, Chandigarh, India.

Growth and Maintanence

The obtained culture was grown in different fungal media like Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Rose Bengal Agar, Corn Meal Agar, Czapek Dox Agar and Sabouraud Dextrose Agar (SDA). Media were prepared, sterilized and inoculated with *Trametes hirsuta*. The plates were then incubated at room temperature for 7 days. The culture was then maintained in Malt Extract Agar (MEA) media and stored at 4°C.

Dyes

Reactive dyes are the dyes, mostly used in the textile industries. Dyes like Black and Blue FNG were purchased from Tirupur. Dyes were selected based on their structural diversity and frequency of use in the local textile industries.

Preparation of Dye Solution

Stock solutions of the dyes were prepared with 100mg of dye in 100ml of sterile distilled water. The pH of the dye solutions was in the range of 6.5- 7.5. These dyes were filter sterilized and used for decolorization studies.

Dye-Agar Plate Assay

Malt Extract Agar (MEA) medium was prepared and sterilized. To each 15ml of the medium 5ml of sterile dye (Black and Blue FNG) solutions were added and poured into petridishes separately. For each plate, a disc (5mm) of fungal mycelium was placed at the center. Uninoculated plates served as a control. The plates were then incubated at 30°C and observed for visual disappearance of color.

Liquid State Decolorization

Decolorization Assay

Decolorization studies were carried out in Malt Extract broth medium. The Medium was prepared, sterilized and supplemented with dye (Black and Blue FNG) solutions. To this medium disc (5mm) of fungal mycelium were inoculated. In the third day of inoculation,

2ml of culture medium was taken from the flask. Then it was centrifuged and read at 594nm for Black and 580nm for Blue FNG using UV-Visible spectrophotometer. Medium without dye and inoculum was used as blank. Medium with dye but without inoculum was used as control. The rate of decolorization was estimated by the following formula

$$\text{Rate of decolorization (\%)} = \left\{ 100 - \frac{\text{Absorbance of treated dye solution}}{\text{Absorbance of control dye solution}} \right\} \times 100$$

Effect of Physico Chemical Parameters on Decolorization

The decolorization ability of *Trametes hirsuta* at various temperatures (25°C, 30°C, 35°C and 40°C), and pH between 3 to 8 were studied. Based on temperature and pH other physico-chemical factors such as different carbon sources (glucose, maltose, mannose and sucrose) and nitrogen sources (yeast extract, peptone, tryptone and beef extract) were studied and the decolorization rate was observed using UV-Visible Spectrophotometer as described above.

Solid Substrate Enzyme Production

Laccases, which are extracellular secretion of white rot fungus, were able to oxidize different substrates like guaiacol, syringoldazine and non-phenolic compounds. The enzyme system of *Trametes hirsuta* was checked based on Trejo Hernandez *et al.*, (2001). 100 ml of enzyme production (Nutrient salt) medium was prepared, sterilized and guaiacol (10µl/100ml) was added as a substrate for screening the production of enzyme and poured into a petridish. The plates were allowed for solidification. A disc measuring 5mm was cut from the actively growing edge of the mycelium and placed at the center of the plate. The plates were then incubated at 30°C in a dark place for 5 days and observed for the presence of a reddish brown zone surrounding the colony.

Liquid Substrate Enzyme Production

500 ml of the production medium was prepared and sterilized. Three discs measuring 5mm each were cut from the actively growing edge of the fungus (*Trametes hirsuta*) and were inoculated into 3 different portions of the medium. After 3 days of inoculation, substrate guaiacol (10µl/100ml) was added. From the following day enzyme assay was checked spectrophotometrically using potassium phosphate buffer and guaiacol as a substrate at 520nm.

Decolorization of Dyes Using Crude Enzyme Extract

Decolorization of dyes was also checked using the crude enzyme extract. 350ml Malt extract broth medium was prepared and sterilized. 25 ml of the sterilized broth was dispensed into 100 ml Erlenmeyer flasks. To this, 25 ml of stock dye solutions (Black and Blue FNG) were added. Then to this medium different concentrations (5%, 10%, 15% and 20%) of crude enzyme extract were added. The flasks were then incubated at 30°C and the rate of decolorization was studied at 24h intervals using UV-Visible Spectrophotometer as said above.

Preparation of Immobilized Cells

Immobilized cells were prepared using sodium alginate. The fungus was scraped using sterile blade into 100ml of sodium alginate solution. These were mixed well and this mixture was aseptically added to 4% calcium chloride solution using a sterile pipette/syringe. The beads were formed and it was allowed to stand for 30 minutes in the solution. Then it was washed with distilled water and used. A similar method was followed for preparing beads using crude enzyme extract. 10ml of the extract was mixed with sodium alginate solution and the above procedure was followed.

Efficacy of Immobilized Cells on Decolorization of Dyes

The prepared immobilized cells were used for decolorization of dyes. 150 ml of Malt extract broth medium was prepared and sterilized. 25 ml of sterilized medium was dispensed into 100ml Erlenmeyer flasks. To this 25 ml stock dye (Black and Blue FNG) solutions were added separately. Then using sterile spatula 10 beads (fungal beads and crude extract beads) were added to the media and incubated at 30°C and the rate of decolorization was studied using UV-Visible Spectrophotometer as described above. Medium with dye but without beads served as control.

Results and Discussion

The fungus *Trametes hirsuta* growth was examined in different media. This fungus is white to yellow in color and when examined microscopically using LCB mount, polypores are seen.

Dye-Agar Plate Assay

The primary screening studies were performed on dye-agar plate to check the ability of the fungus to decolorize dyes. In the solid-state decolorization, the mycelial growth of the fungus started from the first day but decolorization started from the third day. The decolorization rate differed with the dyes. The medium incorporated with Black dyes showed greater decolorization than medium with Blue FNG.

Liquid state Decolorization

Effect of Physico-Chemical Parameters on Decolorization

Black

Decolorization of dyes by *Trametes hirsuta* was observed at different temperatures, pH levels, carbon sources and nitrogen sources. Maximum decolorization was observed as 91.2% at 35°C, 89.8% at pH4, 94.1% with glucose and 89.4% with yeast extract respectively (Table: 1,2,3&4).

Table 1. Effect of Temperature on Decolorization of Black and Blue FNG by *Trametes hirsuta*

S.No	Temperature	Rate of Decolorization (in %)									
		Black FNG					Blue FNG				
		3rd day	4th day	5th day	6th day	7th day	3rd day	4th day	5th day	6th day	7th day
1	25°C	13.14	16.5	24.5	34.2	37.13	72.7	75.8	78	80	80
2	30°C	57	60.3	65.8	71.3	74.3	81.3	84.6	88	89.3	90
3	35°C	72	77.2	82.3	86.9	91.2	40.6	43.3	46.5	49	51
4	40°C	40	47.3	52.3	57.4	60.8	39.3	42.6	44	45.3	45.9

Table 2. Effect of pH on Decolorization of Black and Blue FNG by *Trametes hirsuta*

S.No	pH	Rate of Decolorization (in %)									
		Black FNG					Blue FNG				
		3rd day	4th day	5th day	6th day	7th day	3rd day	4th day	5th day	6th day	7th day
1	pH3	75.2	79.4	81.3	84.6	88.5	76.9	81.2	83.8	86.3	87.2
2	pH4	77.6	80.2	82.3	86.1	89.8	75	78.4	81.03	82.8	84.5
3	pH5	75.1	77.2	78.01	79.5	79.8	55.7	60.3	63.5	68	70.5
4	pH6	65.8	69.4	74.1	77.5	79	60.5	64.9	70.1	72.8	75.4
5	pH7	24.5	28.3	34.2	39.5	44.1	21	25.2	29.4	33.6	35.2
6	pH8	20.1	24.6	28.1	30.9	34.6	18.3	22.9	27.5	32.1	33.9

Table 3. Effect of Carbon sources on Decolorization of Black and Blue FNG by *Trametes hirsuta*

S.No	Carbon source	Rate of Decolorization (in %)									
		Black FNG					Blue FNG				
		3rd day	4th day	5th day	6th day	7th day	3rd day	4th day	5th day	6thday	7th day
1	Glucose	78.5	87.7	90.7	92.8	94.1	71.3	74	76.6	78	78
2	Maltose	27.4	33.3	40.1	44.7	48.1	79.3	82	84	86	87.3
3	Mannose	21	14.3	27.4	40.9	49.8	63.3	65.3	66.6	68	70
4	Sucrose	35	38.8	56.5	64.5	74.3	56	58.7	60.6	62	62

Table 4. Effect of Nitrogen Sources on Decolorization of Black and Blue FNG by *Trametes hirsuta*

S.No	Nitrogen sources	Rate of Decolorization (in %)									
		Black FNG					Blue FNG				
		3rd day	4th day	5th day	6th day	7th day	3rd day	4th day	5th day	6th day	7th day
1	Yeast extract	67.8	75.1	80.8	87	89.4	79.3	82.6	84	86.6	87.3
2	Peptone	9.8	22	30.6	40	43.3	59.3	62	64	65.3	66.6
3	Tryptone	37.14	46.5	53.5	64.08	69.4	72	73.3	75.3	76.6	76.6
4	Beef extract	6.9	17.9	22	29.4	34.3	51.3	53.3	54.6	56.6	57.3

Blue FNG

Decolorization of dyes by *Trametes hirsuta* was observed at different temperatures, pH levels, carbon sources and nitrogen sources. Maximum decolorization was observed as 90% at 35°C, 87.2% at pH3, 87.3% with maltose and 87.3% with yeast extract respectively (Table: 1,2,3&4).

Solid Substrate Enzyme Production

White rot fungi are capable of producing extracellular enzymes like lignin peroxidase, manganese peroxidase and laccase. A dark reddish brown color appeared around the fungus within 24 hours as a result of laccase oxidative polymerization with guaiacol.

Liquid Substrate Enzyme Production

In nutrient salt liquid medium, the mycelial growth of fungi *Trametes hirsuta* started from the first day. A reddish brown color was found within 24 hours when inducer guaiacol was added to the third day of the old culture, which confirmed the presence of laccase in the medium.

Decolorization of Dyes using Crude Enzyme Extract

The effect of crude enzyme on decolorization of dyes was observed at different concentrations like 5%, 10%, 15% and 20% for all the three dyes. Decolorization was observed from second day of incubation. Maximum decolorization was observed at 94.8% for Black and 78% for Blue FNG respectively.

Efficacy of Immobilized Cells on Decolorization of Dyes

In order to test the ability of *Trametes hirsuta* for dye decolorization, the mycelium is immobilized on sodium alginate to from beads, which is used for decolorization. Maximum decolorization was 78.9% for Black dyes and 50.7% for Blue FNG (Table: 5).

Table 5. Effect of Immobilized cells on Decolorization of Black and Blue FNG by *Trametes hirsuta*

S.No	No.of days	Rate of decolorization (in %)	
		Black FNG	Blue FNG
1	3rd	62.8	28.2
2	4th	67.4	37
3	5th	70.8	43
4	6th	74.9	47.2
5	7th	78.9	50.7

Decolorization of dyes with immobilized beads of crude enzyme extract was also observed. Maximum decolorization was observed in Black followed by Blue FNG as 87.8% and 78% respectively (Table: 6).

Table 6. Effect of Immobilized cells (using extract) on Decolorization of Black and Blue FNG by *Trametes hirsute*

S.No	No.of days	Rate of decolorization (in %)	
		Black FNG	Blue FNG
1	3rd	73.3	54
2	4th	78.5	62
3	5th	81.8	71.3
4	6th	83.4	75.3
5	7th	87.8	78

Industrial dyes are usually discarded into water, after r without processing. When the dye concentration :aches higher levels, they exert pressure on ecosystems, nd are said to be pollutants. Physical and chemical nethods available to treat those waters are expensive nd do not produce satisfactory results. Biologically, lecolorization can be achieved by the use of naturally)ccurring microorganisms such as bacteria and fungi. In ·ecent years attention has been directed towards fungal lye-decolorization systems (Murugalakshmi *et al.,* 2007).

In the present study the dye decolorization ability of *Trametes hirsuta* was studied. The fungus decolorized Black and Blue FNG dyes. Compared to Blue FNG, Black dyes were decolorized more effectively. Differences have been observed in the extent of decolorization among the dyes. This could be due to structural variations among the dyes, or various physico-chemical factors like temperature, pH, carbon sources, nitrogen sources employed and laccase production.

In the present study higher percentage of Black dye decolorization was observed in the dye-agar plate method. This result was similar to those reported by Sathiyamoorthi *et al.,* (2007) who observed solid state decolorization of Blue CA, Black B133 and Corazol violet SR dyes.

The effect of temperature on decolorization of dyes was observed as greater for Black (91.2%) at 35°C than Blue FNG. Daneshvar *et al.,* (2006) reported that the range of decolorization rate increased as the temperature rose. The effect of temperature on adsorption by *Rhizopus nigricans* and *Saccharomyces cerevisiae* was detected by Kumari *et al.,* (2006). The results show that acidic pH is mostly required for fungal growth and decolorization. The rate of decolorization was maximum at pH4 (89.8%) for Black dye. This is similar to the results of Mohandass *et al.,* (2007) who observed maximum decolorization in acidic pH. Various carbon sources were used as substrate to investigate their effects on decolorization. Among the four carbon sources used Glucose showed medium decolorization as 94.1% for Black dye. This is similar to results recorded by Mohandass *et al.,* (2007) who used sucrose, mannitol and glucose as carbon sources and found maximum decolorization (more than 95%) in the glucose. According to Xian-chun jin *et al.,* (2006), the decolorization rate was maximum when CMC, glucose, mannitol and sucrose were used as substrates for decolorization. Additional nitrogen sources have a significant effect on dye decolorization. Of the nitrogen sources used yeast extract showed maximum decolorization for Black dye as 89.4%. Xian-chun jin *et al.,* (2006) reported that when 0.2% di-ammonium sulphate or Ammonium chloride is used as nitrogen source, 100% decolorization is achieved.

In *Trametes spp.,* laccases are the major enzymes but peroxidases are also secreted. Decolorization of dyes was also achieved by using crude enzymes at different concentrations like 5%, 10%, 15% and 20%. The greatest decolourization was in 94.1%. The results observed in this study were similar to those of Zouari Mechichi *et al.,* (2005) who worked on decolorization of textile dyes by crude and purified enzymes of *Trametes trogii.* Laccase-based decolorization was also observed by Arulmani *et al.,* (2005) on decolorization of dyes like

Methyl violet and Emerald green using *Pleurotus sajorcaju*.

Many white rot fungi have been characterized for color removal with various immobilization methods. In the present study, *Trametes hirsuta* was immobilized using Sodium alginate crystals used for decolorization of dyes. The rate of decolorization was 78.9% for Black dye. Arulmani *et al.,* (2005) has also reported a simple immobilization method using lignocellulosic fiber. Immobilization of fungal laccases on various carrier materials such as activated carbon, agarose, sepharose and porosity glass has been shown to increase stabilities of the enzymes at high pH and tolerance to elevated temperatures has been studied by Abadulla *et al.,* (2000).

The present study confirms that the white rot fungus *Trametes hirsuta* is able to decolorize Black and Blue FNG dyes effectively. When compared to Blue FNG, Black dyes are decolorized more effectively. The results of the present study show that the test fungus is efficient in dye decolorization and can be used for treatment of dyes to avoid environmental problems.

References

Arulmani M, Murugesan K, Arumugam P, Dhandapani P, Thangavelu Kalaichelvan P (2005). Efficacy of *Pleurotus sajor-caju* on the decolorization of methyl violet, Emerald Green and dyeing effluent, *Indian J App Microbiol*, 47-52.

Asamudo N U, Daba A S and Ezeronye O U (2005). Bioremediation of textile effluent using *Phaenerochaete chrysosporium*, *African J Biotechnol*, 4(13): 1548-1553.

Campos R, Kandelbauer A, Robra K H, Artur Cavalo Paulo and Gubitz G M (2001). Indigo degrtadation with purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii.*, *J Biotechnol* 8: 131-139.

Daneshvar N, Ayazloo M, Khataee A R, Pourhassan M (2006). Biological decolorization of dye solution containing Malachite Green by microalgae Cosmarium sp, *Bioresource Technol,* 4 (2): 53-59.

Gupta V K, Mittal A, Gajbe V (2005). Adsorption and desorption studies of a water soluble dye, Quinoline Yellow using waste materials, *J Colloid Interf* Sci, 284: 89-98.

Kirk T K and R L Farell (1987). Enzymatic Combustion: The microbial degradation of lignin, *Ann, Rev Microbiol*, 41: 465-509.

Kumar K, Saravana devi S, Krishnamurthi K, Gampawar S, Nilesh mishra, Pandya G H, Chakrabarti T (2005). Decolorisation, biodegradation and detoxification of benzidine based azo dye, *Bioresource technol* 3: 312-316.

Kumari K and Emilia Abraham T (2006). Bisorption of anionic textile dyes by nonviable biomass of fungi and yeast, *Bioresource Technol* 5(2): 149-154.

Machado K M G, Compari L C A, Morais R O, Luiz H Rosa, Mercia H Santos (2006). Biodegradation of reactive textile dyes by Basidiomycetous fungi from brazilian ecosystems, *Brazilian J Microbiol*, 37: 481-487.

Mohan S V, Roa C N, Prasad K K, Karthikeyan J (2002). Treatment of simulated reactive yellow 22 (azo) dye effluents of Spirogyra sp, *Waste Manage,* 22: 575-582.

Mohandass Ramya, Anusha B, Kalavathy S and Devilakshmi S (2007). Biodecolorization and biodegradation of Reactive Blue by Aspergillus sp, *African J of Biotechnol*, 6(12): 1441-1445.

Murugalakshmi C N, Anand R and Sharanya V (2007). Studies on textile dye decolorization by different fungal species, *Nature Environ and Pollution Technol*, 6(3): 377-380.

Sathiya Moorthi P, Periyar Selvam S, Sasikalaveni A, Murugesan K and Kalaichelvan (2007). Decolorization of textile dyes and their effluents using white rot fungi, *African J Biotechnol*, 6(4): 424-429.

Trejo Hernandez M R, Lopez mungula A, Quintero Ramirez R (2001). Residual compost of Agaricus bisporus as a source of crude laccase for enzymatic oxidation of phenolic compounds, *Process Biochem*, 36: 635-639.

Xian-Chun Jin, Gao-Qiang Liu, Zheng-Hong Xu-Wen-Yi Tao (2006). Decolorization of a dye industry effluent by Aspergillus fumigatus XC6, *Appl, Microbiol, Biotechnol*, 6: 321-329.

Zouari-Mechichi H, Tahar Mechichi, Abdelhafidh Dhouib, Sami Sayadi, Angel T Martinez, Maria Jesus Martinez (2005). Laccase Purification and characterization from Trametes trogii isolated in Tunisia: decolorization of textile dyes by the purified enzyme. *Enzyme Microbiol Technol* 4(2): 532-541.

Norman Borlaug a great scientist and humanist passed away on Saturday, September 12th 2009. Let us remember him as a path finder in the basic science of agriculture. He pioneered the concept of Green Revolution.

Dr. Borlaug started his research career in agriculture in Mexico at a time when the world was passing through a serious food crisis. During 1942-1943, nearly two million people died of hunger during the Great Bengal Famine. China also experienced wide-spread and severe famine during the 1950's. Famines were frequent in Ethiopia, the Sahelian region of Africa, and many other parts of the developing world. It was in this background that Dr. Borlaug decided to look for a permanent solution to recurrent famines by harnessing science to increase the productivity, profitability, and sustainability of small farms.

He was widely described as the father of the broad agricultural movement called the Green Revolution.

"More than any other single person of this age, he has helped provide bread for a hungry world," said the Nobel committee in presenting him with the Peace Prize. "We have made this choice in the hope that providing bread will also give the world peace."

The Indian Counterpart of Borlaug Dr. M.S. Swaminathan

(Courtesy, The Hindu dated 6th October 200