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Determination of complex dye decolourization level by fungi.

Erandi Rathnayake Weerakoon[1*] and H. H. K. Achala[2]

[1]School of Science, Business Management School (BMS), Sri Lanka

[2]Industrial Technology Institute (ITI), 363 Bauddhaloka Mawatha, Colombo 00700, Sri Lanka

*erandi.weerakoon@bms.ac.lk

Abstract

Textile dyes, when released to the environment, are difficult to decolorize and removed. Chemical and physical methods of textile dye decolourization are considered expensive. On the other hand, the biological methods of textile dye decolorization are environmentally friendly and effective. This research was aimed at studying the textile dye decolourization ability of the fungus *Trametes versicolor* using three textile dyes (Dianix Royal Blue CC (Dye 1), Dianix Blue XF (Dye 2), and Dianix Red CC (Dye 3)). The mushrooms were trimmed to about 1cm^2 pieces and placed on the centre of the PDA plates. After 7 days of incubation they were subcultured on a new plate to obtain pure cultures. Both solid and liquid media were used to study dye decolorization. Inoculation from pure cultures was done under aseptic conditions on solid media. Inoculums were subcultured onto dye incorporated plates and were incubated at room temperature (25 ºC) under exposure to light for three days. The inoculums were incubated at 25°C for 3 days in liquid media. Surface sterilization was successful as no contamination was observed. The fungus showed a fast growth, with a growth rate of 0.145 cm/day. Dye decolorization was observed in both solid and liquid media. The fungus was most effective in decolourizing Dianix Blue XF in both liquid and solid media. The procedure can be further improved by controlling the temperature and the light intensity. The surface disinfestation procedure can be further used. The procedure has the possibility of further use in studying textile dye decolorization by fungi with further improvements and replicates.

Keywords: *Trametes versicolor,* decolorization, textile dyes, fungus

1. Introduction

Dyes are widely used in industries like textile colourization and printing processes[1,2]. Commercial colours and dyes are resistant to degradation, light, and heat. Intense utilization of these synthetic chemicals has caused severe problems, such as contamination, environmental pollution, and toxicity[3]. Several treatment technologies are used nowadays for the removal of dye from industrial wastewater including coagulation, adsorption, biological methods, advanced oxidation processes (AOPs), membrane technology and electrochemical methods[4]. Dye wastewater is treated physically and chemically by flocculation, coagulation, adsorption, membrane filtration, precipitation, irradiation, ozonization and Fenton's oxidation[5-8]. *Trametes Versicolor* (common name: Turkey Tail), a member of the family Polyporaceae, is one of the most popular medicinal macro fungi[5].

Figure 1. *Tremetes Versicolor* mushroom[9]

Wastewater from textile industries can be refined by applying biological, physical, and chemical approaches. However, there are

disadvantages such as high costs, toxic side produces, excessive energy consumption, concentrated mud formation and difficulties in adapting to different wastewater resources[1].

Biological methods of bioremediation are more economical and have no destructive effects on the environment. There have been many studies on the fission and decolourization of dye using microorganisms and, found that there are microorganisms that possess the capacity[1,5]. The enzyme laccase which is secreted by *Trametes Versicolor* has been used in the pretreatment of lignocellulosic biomass, bioremediation, triclosan biodegradation, blue wastewater biodegradation and dye decolourization[5,11].

Due to the use of living organisms capable of natural bioremediation in biological systems, they are more adapted to environmental conditions. Recent studies have shown that use of white-rot fungus in the decolourization process was very effective[3]. In the field of waste and environmental biotechnology, white rot fungi are widely used[3]. This group of fungi can be used in several industrial applications, such as pulp delignification, textile dye bleaching, effluent detoxification, biopolymer modification and bioremediation[2,12]. The successful rescue of wild strains and their ability to produce fruiting bodies under artificial cultivation conditions have been highlighted[5].

2. Methodology

2.1 Fungus isolation and pure culture preparation. The fresh fruiting bodies of *Trametes Versicolor* were collected from the environment with the descriptors for *Trametes Versicolor* identification using the articles and the internet. To prepare the potato dextrose agar (PDA) medium, the potato was cut into small pieces and boiled till the potato pieces could be smashed. Then it was filtered using a cheesecloth to obtain potato infusion without any potato pieces, and after cooling down, 4 g of Dextrose was added, and mixed. The pH measured was 5.5 (Standard range 5.6 ± 0.2). Then the agar was added and closed the mouth of the conical flask using a cotton plug and was covered with aluminium foil. Then autoclaved at 60°C and 121 psi. After autoclaving the media was poured into the sterile petri dishes and was allowed for solidification. Then the mushroom piece was washed using running tap water for about 10 minutes. This step removes any dust particles, and then the mushroom piecewas soaked with liquid soap and rinsed with distilled water three times. Then the mushroom piece was put into a sterile beaker, and transferred to laminar flow.

2.2 Surface sterilization and pure culture isolation. The mushroom piece was washed with sterile distilled water. Then it was washed with 70% ethanol (made using 25 ml by dissolving 17.5 ml of 100% alcohol and 7.5 ml of water) for 1 minute and again washed with sterile distilled water. Next, it was washed with 5.0% Sodium hypochlorite (Clorox) (made using 12.5 ml of 10% Clorox and 12.5 ml deionized water) for 5 minutes and washed with sterile distilled water. Then the mushroom piece was kept drying for about 5 minutes. Then it was cut into (1–2 mm diameter) plugs of the fruiting body using a sterile blade and cultured on a PDA plate using sterile forceps and this was done under aseptic conditions. After inoculating for seven days at 28°C, the mycelial disks (6 mm diameter) from the peripheral region in the PDA were inoculated on a fresh PDA and incubated at 28 °C for five days and a pure culture was obtained.

2.3 Preparation of Potato Dextrose Broth (PDB) media. A volume of 300 ml of PDA media was made using 60 g of potatoes, 6 g of sucrose, and 4.5 g of agar. Before adding agar, the pH was measured (6.4) which should be in between 6.5±0.2. Then the agar was added and poured into three conical flasks (100 ml each) and autoclaved at 121 ºC and 15 psi. Then the flasks were transferred to the lamina flow and the dyes were added. 11 mg of each dye was added to each flask and mixed well before pouring onto the plates. Then poured into the plates and labeled and refrigerated at 40ºC after solidification.

2.4 Preparation of PDB media. A volume of 500 ml of PDB media was prepared using 100 g of potatoes and 10 g of sucrose. Then the pH was measured (6.5) which should be at 6.5±0.2. The mixture was poured into 5 conical flasks, 100 ml each; and 1.5 g of agar was added to each, before autoclaving. 11 mg of each dye was added to conical flask and 100 ml PDB media was added and mixed. The mixture was separated into four falcon tubes. each containing 25 ml of PDB. To this solution 3 mg of each dye was added. The dyes in the falcon tubes were used as controls to check the

colour changes with the conical flasks. PDA was poured into plates and allowed to set. Inoculation was carried out on solidified PDA plates.

2.5 Inoculation of dye plates. Fungal colonies grown in the sub cultured plates were cut into small pieces and were placed in the centre of the dye plates using sterile blades and forceps under aseptic conditions. Three petri plates were used for each dye, and the controls were PDA without dye but with the fungus, PDA with dye but without fungus and PDA without dye but with agar piece for each dye.

2.6 Inoculation on liquid media. Five equal sized pieces of fungus from the subculture plates were added to each dye PDB media and was shaker incubated for three days. Controls used were PDB + dye 1, PDB + dye 2, PDB + dye 3 and PDB + agar piece each containing 25ml of PDB media in falcon tubes.

2.7 Measuring UV absorbance. The radius of the fungus growth on the dye plates were measured and recorded for three days. After 24 hours of incubation 4 ml of solution was transferred into falcon tubes. And centrifuged (sigma 3 – 16 PK) at 3000 g for 30 minutes. Then the supernatant was taken to cuvette and put into the first well of Agilent Technologies Cary series UV – Vis spectrophotometer for each dye containing the fungus and controls. The graphs were observed to check whether there are any shifts in the graphs with the controls. This was done and recorded for three days.

3. Results

3.1 Isolation of Trametes Versicolor from the environment

Figure 2. Mushroom used for the experiment

3.2 Isolation, culturing, and subculturing of the fungus

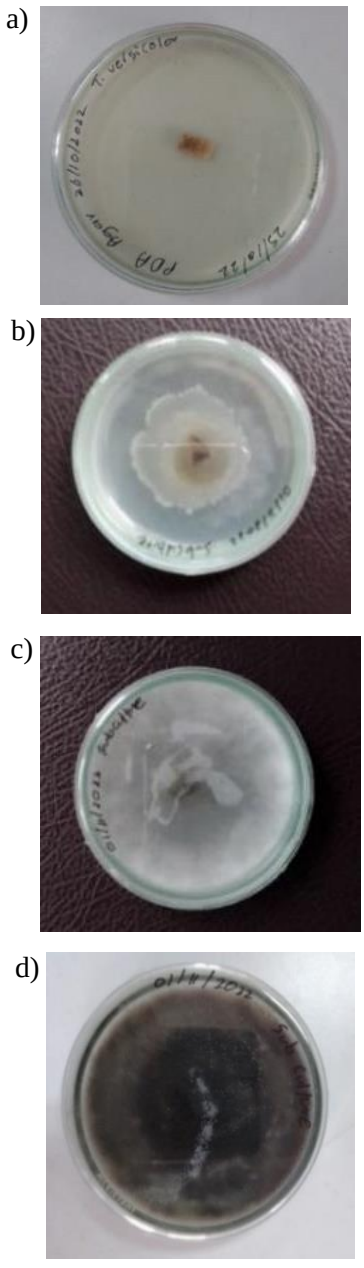


Figure 3. Isolation, culturing, and subculturing of the fungus a) Cultured mushroom piece in the PDA media b) Subcultured mushroom piece c) Fungal growth of subcultured mushroom piece d) After three days of subculturing.

3.3 Subculturing on the dyed plates and liquid media to observe the decolorization of the dye.

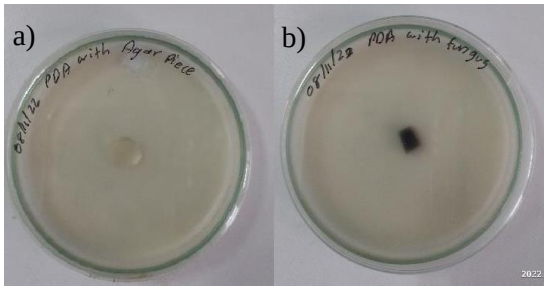


Figure 4. a) PDA with only the agar piece (control) b) PDA with only the fungus (control)

Table 1. Dye incorporated plates with the fungus and the controls from day 0 to day 3

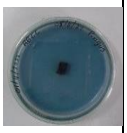
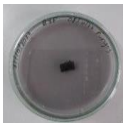
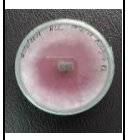
Day	Dyed plate with fungus			
	Control	Dianix Royal Blue CC	Dianix Blue XF	Dianix Red CC
Day 0				
Day 1				
Day 2				
Day 3				

Table 2. Radius and the growth rate of the dye incorporated plates from day 1 to day 3.

Radius of the dye incorporated plates			
	Dianix Royal Blue CC	Dianix Blue XF	Dianix Red CC
Day 1	1.5 cm	1.5 cm	1.8 cm
Day 2	3.3 cm	3.3 cm	3.3 cm
Day 3	3.5 cm	3.5 cm	3.5 cm
Growth rate	0.048 cm	0.048 cm	0.052 cm

Table 3. Dye-incorporated liquid media with the fungus and the controls.

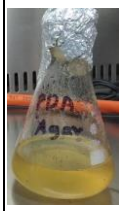
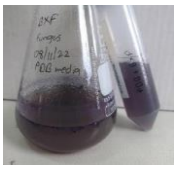
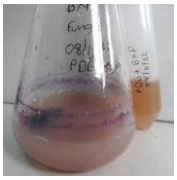
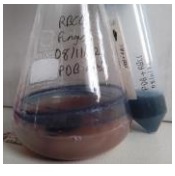
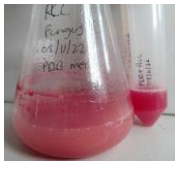
Day 0				
	Dianix Royal Blue CC	Dianix Blue XF	Dianix Red CC	Agar piece
Control				
Potato Dextrose Broth media with fungus				

Table 4. Dye-incorporated liquid media with the fungus and the controls from day 1 to day 3.

Control and the Potato Dextrose Broth media with the fungus			
Day	Dianix Royal Blue CC	Dianix Blue XF	Dianix Red CC
Day 1			
Day 2			
Day 3			

3.4 Absorbance graphs of each dye in the liquid media with the controls.

Table 5. Absorbance graphs of Dianix Royal Blue CC dye in the liquid media from day 1 to day 3.

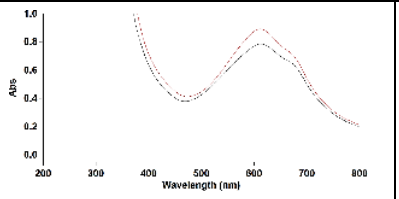
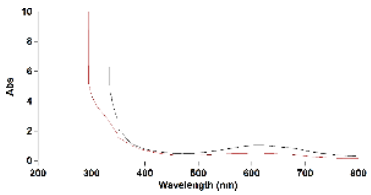
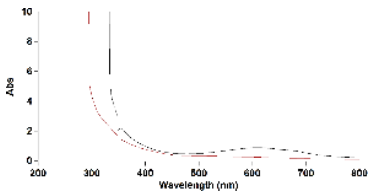
Dianix Royal Blue CC		
Day 1	**Day 2**	**Day 3**
		

Table 6. Absorbance graphs of Dianix Blue XF dye in the liquid media.

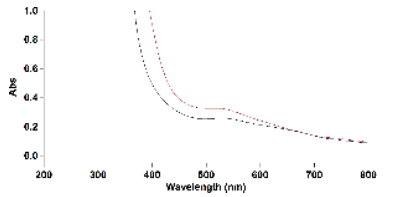
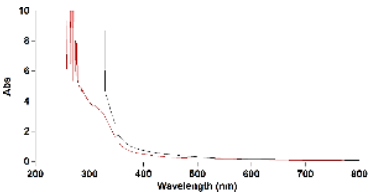
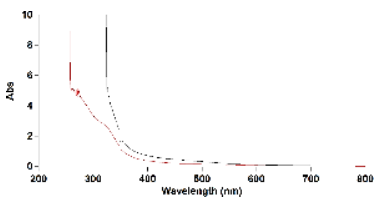
Dianix Blue XF		
Day 1	**Day 2**	**Day 3**
		

Table 7. Absorbance graphs of Dianix Red CC dye in the liquid media.

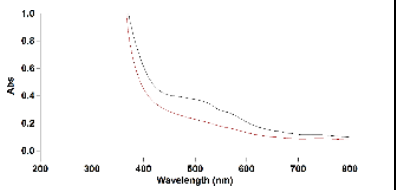
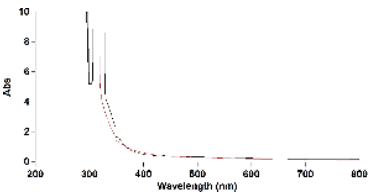
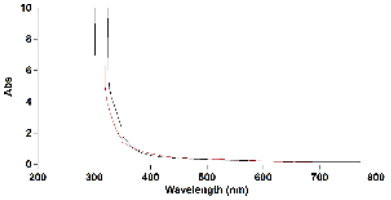
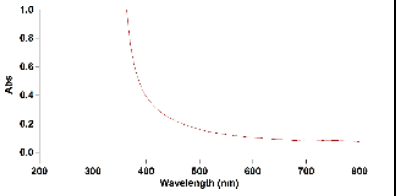
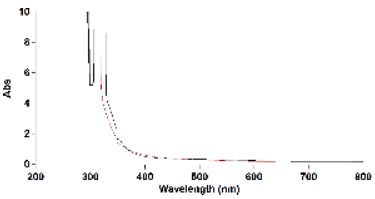
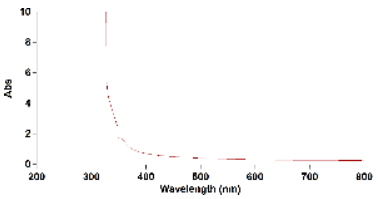
Dianix Red CC		
Day 1	**Day 2**	**Day 3**
		

Table 8. Absorbance graphs of the control in the liquid media.

Control		
Day 1	**Day 2**	**Day 3**
		

4. Discussion

Trametes Versicolor is a wood rotting fungus. It was collected from the environment by observing special features, structure, and color that aligns to the published articles and images. This mushroom has a specific band pattern. Isolated mushroom (Figure 2) piecewas cultured in PDA media plates (Figure 3a). After the growth it was cut with the agar piece and placed onto another petri plate to obtain pure culture (Figure 3b). The fungal growth is denoted in Figure 3c) and sub cultured fungus in figure 3d. The radius of fungal growth was measured to compare the growth rate of the fungus. Dye incorporated plates with the fungus piece Dianix Royal Blue CC, Dianix Blue XF, and Dianix Red and the control from day 0 to day 3 is mentioned in Table 2. The fungal growth can be clearly seen from day 0 to day 3 (Table 2). The growth rate of fungus is higher. Table 4 shows the controls used in the liquid media and they are PDB + Dianix Royal Blue CC, PDB + Dianix Blue XF, PDB + Dianix Red CC, and PDB + Agar piece and the dyed liquid media with the fungus pieces for day 0. Table 5 shows the PDB containing Dianix Red CC with the fungus pieces and the control, PDB containing Dianix Royal Blue CC with the fungus pieces and the control, and PDB containing Dianix Blue XF with the fungus pieces and the control from day 1 to day 3. A clear colour change is observed by naked eye, but absorbance graphs indicate no difference. This may be due to the formation of the pigment from the fungus. The mushroom selected for the study is a pigment producing species. There is a notable difference in the absorbance graphs (Table 5,6,7) compared to the controls (Table 8). But significant difference is not observed among day 2 and day 3 absorbance graphs, when considering the visual difference observed by naked eye. The black color graph shows the control absorbance while the red color graph shows the dye incorporated liquid media with the fungal pieces. These graphs are used to measure and to compare the best dye which can decolorize the most.

Conclusion

In conclusion, fungal growth and decolorization was successful in both methods; however, fungal growth and decolorization is more effective in the liquid media than the solid media. The change of color in the PDB could be clearly detected in the liquid media which can also be observed with the naked eye. This should be done under aseptic technique and the surface sterilization should be done properly to avoid contamination. Further study and optimizations are required regarding controlling the temperature and light intensity. Decolorization can be measured by providing both light and dark conditions to the samples to measure which suits better for the process. Also, screening is required to select *Trametes* strains which do not produce pigments that could interfere with absorption spectra and readings.

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