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Evaluation of the bioremediation ability of the isolated white rot fungus on the textile effluents

R Indira Priyadarsini*, V Bhuvaneswari, K Suresh Kumar

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Abstract

Every one contributes to the environmental pollution by their activities either directly or indirectly and it is our moral responsibility to uncover a solution in order to come out of it also. Hence an attempt was made to isolate a white rot fungus with better remediation ability. The white rot fungus was isolated from decay wood sample and identified as *Trametes hirsuta* by molecular identification methods. The assessment of the bioremediation ability of the isolated organism was done with four textile effluent samples and the physico-chemical parameters namely colour, pH, TSS, TDS, COD, BOD, Total Hardness, level of Calcium, Magnesium, Chloride and Sulphate were evaluated. The change in the initial pH values of the four textile effluents after treatment with the isolated organism was not significant. All the other parameters were found to be reduced from their respective initial level after treatment with the isolated white rot fungus.

Key words: Azo dyes, BOD, COD, Decay wood, Textile effluent, *Trametes hirsuta*

Introduction

In developing countries including India textile, leather and paper industries represent an important economic sector. Huge amount of capital and Human resource is engaged in these industries. These industries are one of the most important sources of environmental pollution (Meshram et al. 2010). Small scale industries in India contribute 3900 million liters waste water per day. Presence of very low concentrations of dyes is visible and undesirable. Some of these dyes are mutagenic, carcinogenic and toxic (Kaushik and Malik 2009). Dyes are widely used in the textile, rubber product, paper, printing, color photography, pharmaceuticals, cosmetics and many other industries. Amongst these, azo dyes represent the largest and most versatile class of synthetic dyes. Approximately 10 - 15% of the dyes are released into the environment during manufacturing and usage. Since some of the dyes are harmful, dye-containing wastes pose an important environmental problem (Gurulakshm et al. 2008). Furthermore, their discharge into surface water leads to aesthetic problems and obstructs light penetration and oxygen transfer into bodies of water, hence affecting aquatic life (Pinheiro et al. 2004). Moreover, it is very difficult to treat textile industry effluents because of their high BOD, COD, colour, pH and the presence of metal ions (Gogate and Pandit 2004).

The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages. The process is relatively inexpensive, it is a simple method and the running costs are low and the end products of complete mineralization are not toxic. Their enzyme producing activity makes them effective decolourizers; they remove toxic metals by biosorption ultimately rendering the effluents more ecofriendly (Bhargavi and Charya 2010).

In order to handle the present pollution load, the present investigation was designed to identify a new white rot fungus and realize its efficiency on textile industry azo dyes. The study was an attempt to isolate dye degrading white rot fungus from natural source such as decay wood. The efficiency of the isolated fungus was assessed by evaluation the physico-chemical characteristics of the textile industry effluent before and after treatment with the isolated white rot fungus.

Materials and Methods

Sample collection

Natural sample such as decay wood was collected from Hosur, Tamil Nadu, India, in sterile plastic covers and were brought to the laboratory without exposing to the external environment. The

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R Indira Priyadarsini

Department of Biotechnology, The Oxford College of Engineering,
Hosur Road, Bangalore-560 068, India

Ph: 0091 9790022658
*Email: indira8791@yahoo.com

V Bhuvaneswari

Department of Biochemistry, Avinashilingam University for
Women, Mettupalayam Road, Coimbatore-641 043. Tamil Nadu

K. Suresh Kumar

Department of Biotechnology, SASTRA University, Thanjavur-
613402, Tamil Nadu, India

sample was surface sterilized to eliminate surface contaminants and then the material was used for fungi isolation on the potato dextrose agar followed by malt extract agar (MEA) medium. The fungi culture was maintained through periodic culturing on MEA of pH 6.5 to 7.0 at 30°C.

Effluent collection

Textile industry effluent samples were collected from textile industry located at Tirupur, Tamil Nadu, India. The four different effluent samples E1, E2, E3 and E4 contained the azo dyes namely Reactive Black 5H, Direct blue 71, Direct red 80 and Acid orange 5 respectively were collected in sterile containers, brought to the laboratory without exposure to the environment. The samples were stored at -4ºC until use. The effluent was used for media preparation instead of distilled water. Five loops from seven days old culture of white rot fungus were inoculated in to the tubes and the study was done after seven days. Blank was performed throughout the phase in which no organism was inoculated. All the procedures were performed in triplicates.

Physicochemical properties of textile effluents

The physicochemical properties of the effluent such as colour, pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biochemiical Oxygen Demand (BOD), Total Hardness, levels of Calcium, Magnesium, Chloride and Sulphate were evaluated.

pH and colour

pH was determined using digital pH meter. The intensity of the color present was studied by decolorisation assay. The study was done for the effluent samples at the respective ʎ$_{max}$ values. Dye decolourisation was expressed in terms of percentage calculated according to the following equation (Sarnthima and Khammuang, 2008):

$$Decolourisation\ (\%) = \frac{A_0 - A_t}{A_0} * 100$$

Where A_0 is an absorbance at ʎ$_{max}$ at time 0 and A_t is an absorbance at ʎ$_{max}$ of each dye after each time intervals.

Other parameters

Total Suspended and Total Dissolved Solids were determined by Filtration Method (APHA, 1998). Chemical Oxygen Demand was determined by Open Reflux Titrimetric Method (APHA, 1998) while Biochemical Oxygen Demand was determined by Winkler's Iodometric Mehtod (APHA, 1998). Total Hardness, Calcium levels were estimated by EDTA Titrimetric Method (APHA, 1998). Chloride level was examined by Silver Nitrate Tirimetric Mehtod (Vogel, 1964) while sulphate level was determined by Turbidometric method (APHA, 1998).

Assessment of the decolourisation efficiency of isolated white rot fungus on azo dye (Reactive Black 5H) - HPLC analysis

HPLC analysis of Reactive Black 5H before and after treatment with the isolated fungus was done on the phenylhexyl column with the following elution program: 0–10 min, isocratic at water/acetonitrile/formic acid 90:10:0.1 for 10–11 min, linear gradient to water/acetonitrile/formic acid 85:15:0.l; 11–40 min, isocratic at water / acetonitrile / formic acid 85:15:0.1: 40–50 min, linear gradient to acetonitrile/formic acid 100:0.1. Products were identified by comparing their retention times with those of standards (Srebotnik and Hammel, 2000). The dye molecule Reactive Black 5H was used as standard for HPLC analysis

Results and Discussion

The present investigation gave an attempt to isolate white rot fungus with better capacity of dye degradation. In this view there were seven fungal strains isolated from the collected decay wood sample. After subjecting to various preliminary procedures two of the white rot fungal strains could decolorize the azo dye molecule out of which one had edged over the other one. Hence the white rot fungus with better performance in dye decolorization studies was selected and subjected to molecular identification using basidiomycetes-specific internal transcribed sequence primer followed by phylogenetic analysis. The isolated white rot fungus was identified as *Trametes hirsuta* (results not shown here). The study was designed in order to understand the efficiency of the isolated white rot fungus to remediate textile effluents. Since the white rot fungus was isolated and identified as *T. hirsuta*, control procured from MTCC, Chandigarh, India (*Trametes hirsuta* with MTCC accession number 1171) was used for comparison. The parameters were assessed before and after treatment with the control and isolated white rot fungus. All the procedures were performed in triplicates.

Colour

Presence of colour in the waste water is one of the major problems in textile industry. Colour is easily visible to human eyes even at very low concentrations. Hence, colour from textile wastes carries significant esthetic importance. Most of the dyes are stable and have no effect of light or oxidising agents. They are also not easily degradable by the conventional treatment methods. Removal of dyes from the effluent is a major problem in most of textile industries (Sengupta, 2007).

The colour of the effluents under study was contributed by the molecules dissolved in it. The samples which were turbid initially changed to clear solution after the incubation period. Hence it could be concluded that the white rot fungus inoculated in the solution could have either used the molecules responsible for turbidity or could have just degraded them in to smaller products resulting in reduction of the turbidity.

The ability of the white rot fungi to decolourise the textile effluent samples was assessed. The colour removal would be proportional to the amount of chromogens removed from the sample. Chromogens could be removed as a result of degradative ability of the white rot fungi inoculated in the sample. The per cent decolourisation of the effluent samples E1, E2, E3 and E4 by control were 69, 80, 72 and 75 respectively. The percent decolourisation by the isolated *T.hirsuta* were 73, 84, 74 and 78 for E1, E2, E3 and E4 respectively (Figure 1). The decolourisation efficiency of the isolated white rot fungus was more than the control organism. This could be substantiated as the test *T.hirsuta* was isolated from decay wood sample wherein the degradative ability of the organism would be maximally active while the control *T.hirsuta* is maintained under normal conditions and hence needs to be induced better for the degradative ability.

The ability of white rot fungi to decolourise various synthetic textile dyes has been extensively studied (Ramsay et al. 2005). There was an earlier study on the decolourisation of RB5 by Ramsay and Nguyen (2002) where they had reported the decolourisation of eight textile dyes including RB5 by *T.versicolor*. Fu and Viraraghavan (2001) suggested that a biosorption mechanism might also play an important role in the decolourisation of dyes by living fungi in addition to biodegradation. Yang et al. (2009) reported the decolourisation of the Acid Red, Malachite Green and Crystal Violet dyes by the extracellular laccase activity from *Trametes sp.* SQ01. Selvam et al. (2003) showed that laccase from *Thelephora sp.* degraded three azo dyes without a redox mediator. Sathiyamoorthy et al. *(*2007) reported the decolourisation of Blue CA, Black 133 and Corazol violet SR and the decolourisation of effluent collected from dye house by *Pleurotus florida* and *T.hirsuta*. Effective decolourisation of the effluent was obtained by *P.florida* in 2% glucose medium.

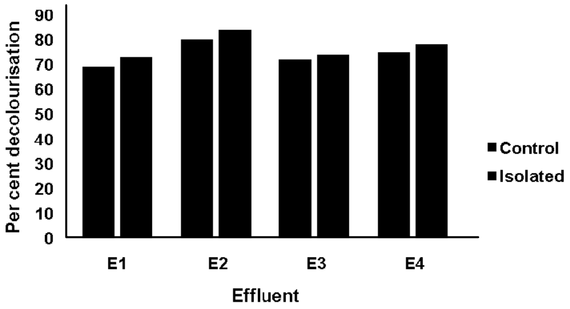

Figure 1. Per cent decolourisation of the textile effluents by the control and isolated *T.hirsuta* (E1 – Effluent containing Reactive Black 5 H; E2 – Effluent containing Direct Blue 71; E3 – Effluent containing Direct Red 80; E4 – Effluent containing Acid Orange 5)

Synthetic textile wastewater and real wastewater from Batik dyeing process obtained from the factory in Lamphun province, Thailand were decolourised by immobilised white-rot fungus *Coriolus versicolor* RC3 in repeated-batch system (Srikanlayanukul et al. 2006). Dayaram and Dasgupta (2008) had reported for the first time that *Polyporus rubidus* isolated from outskirts of Mumbai to have laccase activity. They demonstrated its dye degrading ability by testing on textile wastewater from four textile units in the State of Maharashtra, India. Earlier studies with white rot fungi, *Trametes hirsuta, Pleurotus ostreatus* and *Ischnoderma resinosum* have already shown the use of redox mediator such as violuric acid and hydroxybenzotriazole (HBT) to decolourise recalcitrant dyes such as Acid red 97, Acid green 26, Reactive black 5, Reactive red 22 and Reactive yellow 15 (Couto and Sanroman, 2007). Mazmanci et al. (2009) studied the decolourisations of different textile dyestuffs with *Coriolus versicolor, Laetiporus sulphurous, Pleurotus ostreatus, P. chrysosporium, Pleurotus eryngii* and *Funalia trogii*. The decolourisation efficiency was found to be different for different organisms.

Addition of urea as nitrogen source and glucose as carbon source significantly enhanced decolourising capacity (up to 87%) of *P. chrysosporium* on congo red, malachite green and crystal violet. A significant reduction in COD content of dye solutions (79-84%) were recorded by fungus supplied with additional carbon and nitrogen. A significant correlation between colour and COD of dye solutions was recorded (Pant et al. 2008).

pH

pH of the textile industry effluent reflects the nature of the molecules present in the effluent and also would reflect the impact of the processes involved in dyeing. The pH values of the four textile effluent samples are given in Table 1. The initial pH of the effluent samples was found to be 9.45, 10.35, 9.57 and 11.02 for E1, E2, E3 and E4 respectively. The high pH values might be attributed to the basic salts used in the textile dyeing processes which were discharged with the effluent. The pH values were increased for E1 and E2 by both the control and the isolated *T.hirsuta* whereas the pH values were reduced for E3 and E4 by both the organisms.

pH is one of the important parameters because microbes used in biological treatments can only survive in a narrow pH range that is between 6.0 and 8.0 (Meenakshipriya et al. 2008). Hence the pH values of the effluent samples were brought down to neutral pH after which all the other parameters were assessed.

Table 1. pH of the textile effluents before and after treatment with the control and isolated *T.hirsuta*

Sample	Initial	Control treated	Isolate treated	# IS tolerance limit IS – 2490 - 1981
E1	9.45±1.10	9.88±0.65	9.57±0.51	5.5 - 9.0
E2	10.35±0.58	10.86±0.48	10.92±0.5	
E3	9.57±0.40	9.27±0.73	9.47±0.29	
E4	11.02±0.38	6.68±0.30	8.56±0.23	

- Tolerance limit for industrial effluents discharged into inland surface water prescribed by the Bureau of Indian Standard Values are expressed as Mean ± Standard deviation of triplicates (E1 – Effluent containing Reactive Black 5 H; E2 – Effluent containing Direct Blue 71;E3 – Effluent containing Direct Red 80; E4 – Effluent containing Acid Orange 5)

Total suspended solids (TSS) and Total dissolved solids (TDS)

Total suspended solids include all particles suspended in water which will not pass through a filter. Suspended solids are present in sanitary wastewater and many types of industrial wastewater and the components of TSS include chlorides, sulfates, magnesium, calcium, and carbonates. Different processes in textile industry contribute to TSS in textile effluents.

Presence of TSS leads to turbidity resulting in poor penetration of light in aquatic system, thereby curtailing the light for photosynthetic activity. Further the settling of suspended particles on soil, soil fauna, might lead to various damages like change in soil porosity, soil texture, water holding capacity on one hand and clogging of gills and respiratory surface of fishes on the other hand (Mohammed et al. 2004).

The levels of TSS and TDS are given in Table 2. The initial TSS values of the effluent samples E1, E2, E3 and E4 were reduced by both the control and the isolated *T.hirsuta*. The per cent reduction (Figure 2) by the white rot fungus was found to be maximum in E3 (Control-52% and Isolate – 41%). The control organism could reduce TSS more than the isolated organism.

TDS is the measure of total inorganic salts and other substances that are dissolved in water (Nasrullah et al. 2006). High levels of TDS are aesthetically unsatisfactory and may also produce distress in human and livestock (Patil et al. 2009). As registered in Table 2, the initial TDS values of the effluent samples were 4500, 3800, 5200 and 6100 mg/L for E1, E2, E3 and E4 respectively. The amount of TDS was reduced in all the four samples by the control and the isolated organism. The per cent reduction of TDS in E2 was 45% and 55% by the control and isolated *T.hirsuta* respectively (Figure 3) which was more than the other three samples. The efficiency in reducing TDS in all the four samples was found to be more with the isolated *T.hirsuta* than the control organism.

Chemical Oxygen Demand (COD) and Biochemical oxygen demand (BOD)

Organic pollutants, which originate from organic compounds of dye stuffs, acids, sizing materials, enzymes and tallow are also found in textile effluent. Such impurities are reflected in the analysis of COD and BOD. These pollutants are controlled by use of biological treatment processes. In many textile units, particularly engaged in synthetic processing, low BOD/COD ratio of effluent is observed which makes even biological treatment not a ready proposition (Sengupta, 2007).

Table 2. Levels of Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) in the textile effluents before and after treatment with the control and isolated *T.hirsuta*

Parameters (mg / L)	Sam -ple	Initial	Control treated	Isolate treated	# IS
TSS	E1	2100±130	1200±100	1300±150	100
	E2	3400±250	2500±160	2700±240	
	E3	2900±140	1400±130	1700±210	
	E4	3600±300	2900±2500	3200±350	
TDS	E1	4500±370	3800±490	3300±220	2100
	E2	3800±650	2100±370	1700±220	
	E3	5200±160	4000±410	3500±770	
	E4	6100±290	4700±360	4000±240	

#IS - Tolerance limit for industrial effluents discharged into inland surface water prescribed by the Bureau of Indian Standard IS – 2490 - 1981. Values are expressed as Mean ± Standard deviation of triplicates (E1 – Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3 – Effluent containing Direct Red 80 ; E4-Effluent containing Acid Orange 5)

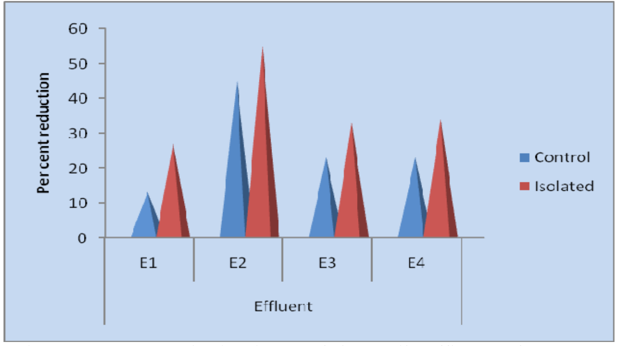

Figure 2: Percent reduction in tss of the textile effluents after treatment with the control and isolated *T.hirsuta* (E1 – Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3 – Effluent containing Direct Red 80 ; E4 – Effluent containing Acid Orange 5)

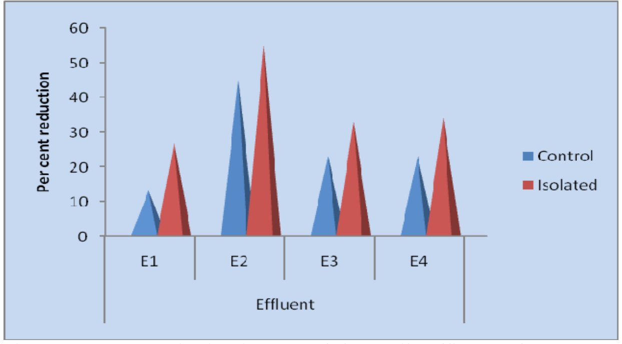

Figure 3: Percent reduction in TDS of the textile effluents after treatment with the control and isolated *T.hirsuta* (E1 – Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3 – Effluent containing Direct Red 80 ; E4 – Effluent containing Acid Orange 5)

COD and BOD levels of all the effluent samples (Table 3) were reduced by the control and the isolated organisms. The maximum per cent reduction of COD by the control was 42% in E4 while it was 67% for the isolated organism in E3. The ability of the fungi in terms of per cent reduction was observed to be higher (Figure 4) for the isolated organism than the control organism.

Figure 5 gives a clear picture of the per cent reduction in BOD levels of the four effluent samples The efficiency of the control organism in reducing the level of BOD was found to be maximum (61%) in E3 while the maximum reduction efficiency of the isolated organism was 29% in E2.The control organism was found to be better in reducing BOD while the isolated *T.hirsuta* was more efficient in reducing COD values of the samples.

COD:BOD is a good indicator of assessing the biodegradabale nature of a compound. For BOD_5, an indication is that a COD:BOD ratio of greater than 100 means that the compound is relatively non-biodegradable and a ratio less than 10 means it is relatively degradable. However, low BOD_5 may merely indicate that the test microbes need longer than the test period to begin breaking the compound down and, therefore, ultimate BOD or other biodegradation testing is generally much more reliable (http://www.cefic.com). The ratio between initial level of COD and BOD for the samples E1, E2, E3 and E4 were 18, 8, 43 and 14 indicating that all the samples were easily biodegradable.

Table 3. Levels of COD and BOD in the textile effluents before and after treatment with the control and isolated *T.hirsuta*

Parameters (mg / L)	Sample	Initial	Control treated	Isolate treated	# IS
COD	E1	1600±140	1100±110	700±06	250
	E2	1300± 80	1000±30	900±40	
	E3	2100±40	1400±150	700±50	
	E4	2400±160	1400±240	1000±120	
BOD	E1	89±4.90	54±2.16	64±2.94	30
	E2	154±2.94	79±4.55	109±1.63	
	E3	49±2.16	19±0.82	39±0.82	
	E4	169±3.74	124±3.27	129±2.94	

#IS - Tolerance limit for industrial effluents discharged into inland surface water prescribed by the Bureau of Indian Standard IS - 2490 - 1981. Values are expressed as Mean ± Standard deviation of triplicates (E1 – Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3-Effluent containing Direct Red 80; E4-Effluent containing acid orange 5)

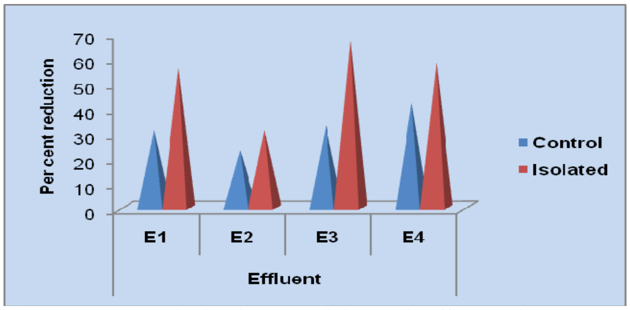

Figure 4. Percent reduction in COD of the textile effluents after treatment with the control and isolated *T.hirsuta*

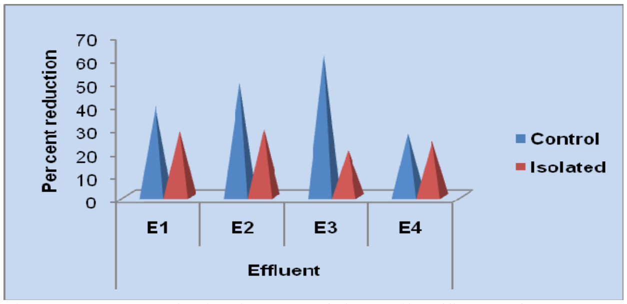

Figure 5: Percent reduction in BOD of the textile effluents after treatment with the control and isolated *T.hirsuta* (E1 – Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3 – Effluent containing Direct Red 80 ; E4 – Effluent containing Acid Orange 5)

A high BOD and COD value show that the effluents have highly oxygen demanding wastes which cause the depletion of DO which is a fundamental requirement for aquatic life. Moreover, high BOD and COD produce unaesthetic colour, endanger water supplies and decrease recreational value of water ways (Nosheen et al. 2000). Singh and Thakur (2004) reported the efficiency of *Paecilomyces sp.,* amongst the eight fungal isolates of four strains isolated from pulp and paper mill effluent on the decolourisation and COD reduction as 81% and 75% respectively.

Table 4. Levels of Total Hardness, Calcium and Magnesium in the textile effluents before and after treatment with the control and isolated *T.hirsuta*

Parameters (mg / L)	**Sample**	**Initial**	**Control treated**	**Isolate treated**	**# IS**
Total Hardness	E1	4400±500	2800±400	1700±100	NM
	E2	7000±540	3500±150	3600±50	
	E3	4600±240	2500±80	1600±190	
	E4	8000±740	3700±100	2900±250	
Calcium	E1	2900±100	2000±340	1000±310	NM
	E2	5200±590	3000±980	3000±410	
	E3	3000±220	1600±530	120±160	
	E4	6200±540	3100±760	2400±240	
Magnesium	E1	1500±220	800±190	700±80	NM
	E2	1800±230	500±150	600±90	
	E3	1600±380	900±100	400±16	
	E4	1.8±0.04	600±20	500±12	

#IS - Tolerance limit for industrial effluents discharged into inland surface water prescribed by the Bureau of Indian Standard tolerance limit IS – 2490 - 1981. Values are expressed as Mean ± Standard deviation of triplicates NM- Not mentioned (E1– Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3 – Effluent containing Direct Red 80 ; E4 – Effluent containing Acid Orange 5)

An investigation was conducted on lignin degradation and COD reduction in black liquor from a pulp and paper mill using five white-rot fungi, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Lentinus edodes*, *Trametes versicolor* and S22 (Wu et al. 2005). Prasongsuk et al. (2009) performed the decolourisation of pulp mill wastewater using thermotolerant white rot fungi. Both *P. chrysosporium* and *Daedaleopsis sp.* were able to decolourise wastewater on wastewater agar plates. They also reported that *Daedaleopsis sp.* and *P. chrysosporium* exhibited the highest ability to decolourise wastewater from the pulping process (52%) and wastewater from the pulping process combined with that from the paper recycling process (86%). All fungi tested reduced the COD by 59–71% and 66–83% respectively in the above mentioned wastewater samples.

Adebayo et al. (2010) performed the biological treatment of effluent generated from international textile industry (Nig) Ltd., Nigeria with mixed culture of *Aspergillus niger* nd *Aspergillus wentil*. The results indicated remarkable reduction in the levels of COD (75%), BOD (97.3%), TSS (99.5%), DS (99.6%) and SS (99.3%).

Total hardness, calcium and magnesium

Hardness in water arises due to the presence of calcium and magnesium salts and other metal ions such as iron and strontium. Calcium, magnesium and total hardness in the water are inter-related. An important source of calcium is the dissolution of small quantities of carbonate compounds from industries. Magnesium usually occurs in lesser concentration than calcium due to the fact that the dissolution of magnesium rich minerals is a slow process and that of calcium is more abundant in the earth's crust (Geetha et al. 2008).

The levels of total hardness, calcium and magnesium of the four effluent samples are shown in Table 4. As indicated in Figure 6, the total hardness of all the effluent samples was reduced by both the control and isolated *T.hirsuta*. The maximum per cent reduction by the control organism was 54% in E4 while it was 65% by the isolated organism in E3. It could be stated that the extent of reduction of total hardness in all the samples was better by the isolated *T.hirsuta* than the control organism.

Both control and isolated fungi showed reduction in the levels of calcium and magnesium from the respective initial values in all the four effluent samples. The percentage reduction of calcium was found to be maximum in E4 and E1 for control and isolated *T.hirsuta* respectively (Figure 7).

Figure 8 gives the per cent reduction of magnesium level of the effluent samples after treatment with the fungal species. Among the two fungal species used in the study, control *T.hirsuta* recorded the maximum per cent reduction of magnesium in E2 from the initial level whereas the isolated *T.hirsuta* registered the maximum per cent reduction of magnesium in E3 from the initial value. The isolated *T.hirsuta* was found to be more efficient than the control organism in reducing the levels of magnesium and calcium.

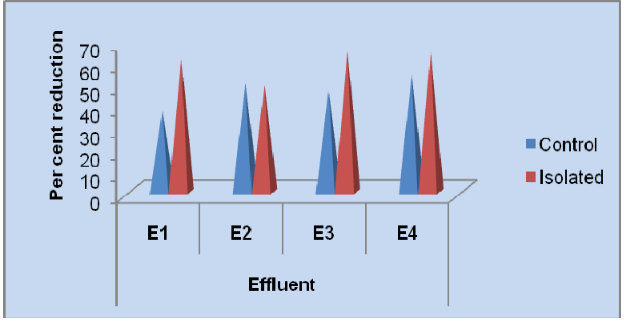

Figure 6: Percent reduction in Total Hardness of the textile effluents after treatment with the control and isolated *T.hirsuta*

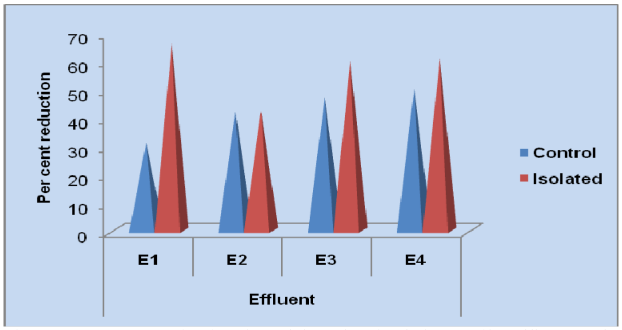

Figure 7: Percent reduction in calcium level of the textile effluents after treatment with the control and isolated *T.hirsuta*

Chloride and Sulphate

Table 5 records the levels of chloride and sulphate in the effluent samples after treatment with the control and isolated organism. Both chloride and sulphate levels were reduced from their respective initial values in all the samples by both the organisms. It is apparent from Figure 9 that the efficiency of the isolated organism in reducing the chloride level was higher than the control organism. The per cent reduction obtained for control was 50% in sample E4 while the value was 66% in E1 for the isolated *T.hirsuta*.

Figure 10 depicts the per cent sulphate reduction in all the four effluent samples. The sulphate levels of the four textile effluent samples were found to be reduced after treatment. The maximal per cent reduction in sulphate level was observed in E1 (54% by control and 57% by isolated *T.hirsuta*).

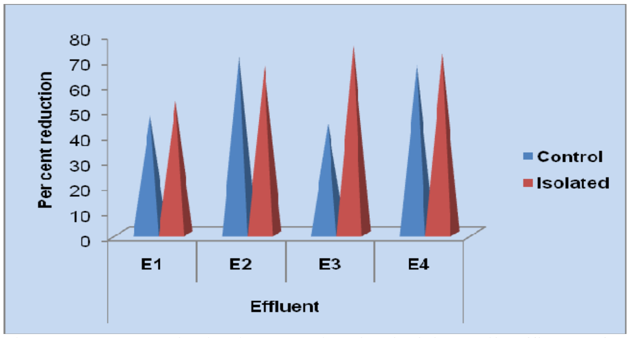

Figure 8. Per cent reduction in magnesium level of the textile effluents after treatment with the control and isolated *T.hirsuta* (E1 – Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3 – Effluent containing Direct Red 80 ; E4 – Effluent containing Acid Orange 5)

Table 5. Levels of chloride and sulphate in the textile effluents before and after treatment with the control and isolated *T.hirsuta*

Parameters (mg / L)	Sample	Initial	Control treated	Isolate treated	# IS
Chloride	**E1**	650±22.91	510±8.29	480±7.35	1000
	E2	550±11.86	480±9.39	400±8.60	
	E3	600±11.84	480±9.39	430±5.72	
	E4	765±17.96	582±13.12	500±13.20	
Sulphate	**E1**	441±4.08	205±6.98	191±6.16	1000
	E2	265±9..09	170±7.48	150±6.53	
	E3	415±3.74	240±8.29	228±7.48	
	E4	752±13.06	555±6.98	415±13.49	

#IS - Tolerance limit for industrial effluents discharged into inland surface water prescribed by the Bureau of Indian Standard IS – 2490 - 1981 Values are expressed as Mean ± Standard deviation of triplicates (E1 – Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3 -Effluent containing Direct Red 80; E4-Effluent containing acid orange 5)

Ramadevi et al. (2006) analysed chloride and sulphate level along with the other physico-chemical parameters namely pH, electrical conductivity, TSS, TDS, COD, BOD, DO, total hardness, calcium, magnesium, sodium, potassium, chromium, zinc and cadmium in a dyeing industry effluent and the values were found to be higher when compared to those of water sample used in the dyeing process and were above the permissible limits prescribed by the Bureau of Indian Standards (BIS). In contrast, the initial chloride and sulphate levels were found to be within the permissible limits in the present investigation.

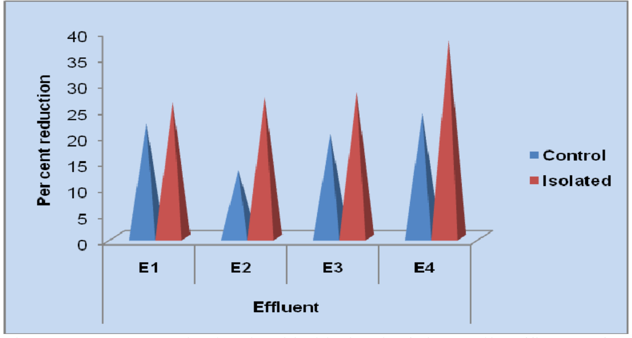

Figure 9. Per cent reduction in chloride level of the textile effluents after treatment with the control and isolated *T.hirsuta*

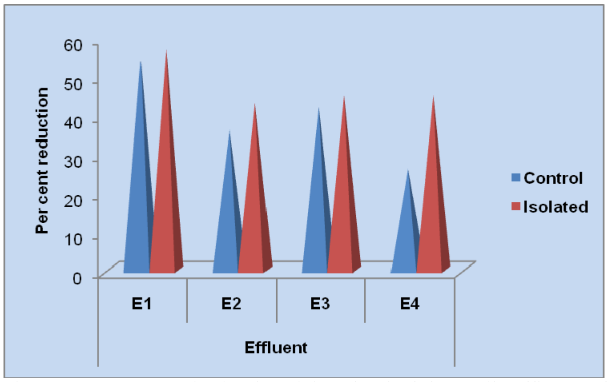

Figure 10. Per cent reduction in sulphate level of the textile effluents after treatment with the control and isolated *T.hirsuta* (E1 – Effluent containing Reactive Black 5 H ; E2 – Effluent containing Direct Blue 71; E3 – Effluent containing Direct Red 80 ; E4 – Effluent containing Acid Orange 5)

Assessment of the decolourisation efficiency of isolated white rot fungus on azo dye (Reactive Black 5H) - HPLC analysis

The standard Reactive Black 5H before treatment had given a single peak with the retention time of 7.6 min in the chromatogram (Figure 11). The HPLC analysis after treatment with the isolated white rot fungus indicated the presence of multiple peaks and also the absence of a peak at 7.6 min in the chromatogram. This could confirm that the azo dye molecule present in the sample before treatment was degraded in to a number of other metabolites by the inoculated white rot fungus which might indicate the decolourisation efficiency of the isolated *T.hirsuta.*

The study on dye degradations by different fungal strains were performed by a number of scientists on various azo dye molecules. Zhao and Hardin (2007) performed HPLC of two commercially used disperse azo dyes, Disperse Orange 3 and Disperse Yellow 3 after treating with *Pleurotus ostreatus* and determined several degradation products in the chromatogram.

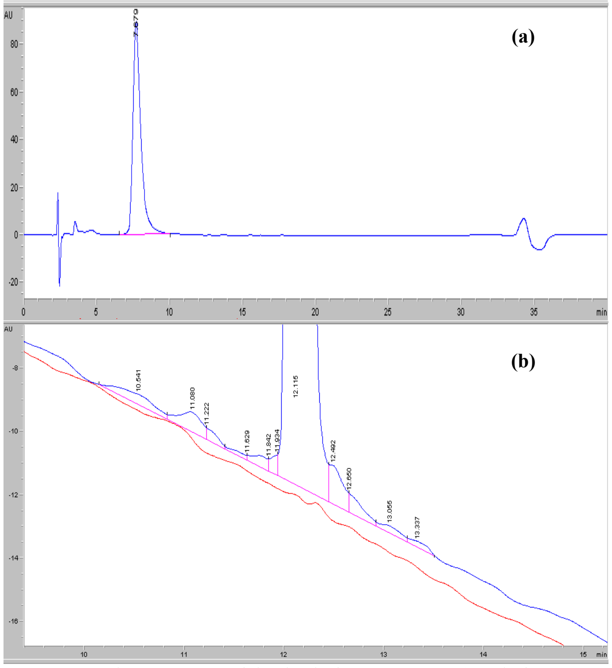

Figure 11: Chromatogram of the dye before treatment (a) and after treatment (b) with the isolated *T.hirsuta*

Ramya et al. (2007) carried out HPLC analysis of Reactive Blue after treatment with *Aspergilus sp.* They reported the peaks with the retention time of 4.32 min and 4.61min and not with 3.69 min (retention time for Reactive Blue) This indicated the degradation of the dye by the organism. The products of phenolic and nonphenolic synthetic lignins after depolymerisation by *Trametes villosa* laccase were analysed by HPLC (Srebotnik and Hammel, 2000). The chromatogram exhibited various peaks for the degraded products. Patel and Suresh (2006) analysed the products of RB5 decolourisation by magnesium-palladium system using UV and HPLC studies. Reversed-phase preparative HPLC has been successfully used to isolate several sulphonated azo dyes (Acid Red 1, Acid Red 8, Acid Red 106, Acid Violet 5, Chromotrope 2R, Reactive Orange 16 and Cibacron Brilliant Red 3B-A) from their impurities. HPLC analysis peformed by Tauber et al. (2005) indicated that Acid Orange 5, Acid Orange 52, and Direct Blue 71 were decomposed to an extent of 65 to 90% and 90 to 100% after 1 hr and 2 hrs of treatment respectively by laccase.

Conclusion

The present study evaluated the remediation ability of the isolated *T.hirsuta* in comparison with the wild *T.hirsuta*. It could be concluded that the isolated organism was better than the wild strain in reducing the level of TDS, COD, Total hardness, level of calcium, chloride and sulphate in all the four textile industry effluent samples. The decolorisation ability of the isolated organism was also found to be better than the wild strain and the study could be extended to know the molecules formed as a result of degradation function.

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