Analysing The Evolution of Fungal Strains in Textile Dyes

Sakshi Yadav[1], Dr. Nandlal Vyas[2]

[1]Research Scholar in Jodhpur National University, Jodhpur, Rajasthan

[2]Dean, Professor, Department of Microbiology, Jodhpur National, University, Jodhpur, Rajasthan

Abstract

The potential of 10 indigenous fungi isolated from soil samples of dye disposal sites was evaluated to decolorize textile azo dyes *viz*., Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B. In pure culture, it was observed that *Humicola insolens*, *H. brevis*, *Aspergillus terrus*, *A. flavus*, *A. niger* and *Rhizopus* sp. were efficient in decolorizing textile dyes. The study also depicted that *Rhizopus* sp. was highly efficient in decolorizing (81.01%) a mixture of 5 dyes used in the present investigation followed by a fungal con- sortium consisting of all the ten fungal strains (78.73%). Recalcitrant dye yellow M4G was also efficiently degraded by fungal consortium (55.76%) compared to pure cultures. This study reinforces the potential of indigenous adapted fungal consortia for the decolorization of textile effluents.

Keywords: Decolorization, Biodegradation, Fungal Strains, Indigenous Fungi, Textile Dyes.

1. INTRODUCTION

Many scientists in the field of biology and chemistry have given significant contribution to science and technology by utilizing the natural resources. Industrialization processes are considered as one of the main aspects for enhancing economic growth worldwide; however, the effluents released from these processes are major contributors to pollution and eco-toxicity [1]. Colored effluents are produced because all industrial processes, such as textile, dye manufacturers, pharmaceuticals, foods, plastics, cosmetics, leathers, rubbers, papers, and pulp, contain dyes [2].

Microorganisms are able to degrade synthetic dyes to non-coloured compounds or even mineralize them completely under certain environmental conditions Bioremediation is one of the most effective and successful cleaning techniques for the removal of toxicants from polluted environments.[3] Fungi and bacteria, both are the principal degraders of organic matters, but fungi are better known for the purpose due to their superiority in the enzyme production.[4] Traditionally, fungi have been classified as white-, brown-, or soft-rot fungi on the basis of technical decay and descriptions, regardless of their taxonomic position. Because the enzyme systems and metabolic pathways involved in the breakdown of carbohydrates and lignins probably are truly distinct in these fungi, rather than just modified in one or a few specific enzyme activities, decay type is of significant taxonomic importance. One important physiological characteristic of decay fungi in culture is the production of extracellular enzymes phenoloxidases and peroxidases.[5]

2. LITERATURE REVIEW

Samta Saroj (2015) Azo dyes are extensively common environmental pollutants that are resistant and have negative biological impacts on biodegradation processes. Therefore it is important for the treatment of textile wastewater to explore new microbiological agents and to create an environmentally friendly and cost-effective procedure. A fungal consortium of three strains has been developed: Penicillium oxalicum SAR-3, Aspergillus niger SAR-6 and Aspergillus flavus SAB-3. At different starting concentrations, the consortium has a remarkable capability for azo dyes degradation (Acid Red 183, Direct Blue 15, Direct Red 75). All three teeth decolorized nearly entirely at modest starting levels (200–400 mg L−1) was seen in the consortiums utilised. Simulate textile wastewater was successfully discoloured by the collaboration. The dyes degradation was reported by UV–Visible and FTIR spectroscopic analyses. Moreover, metabolite toxicity study produced after degradation showed a substantial decrease in dyes' toxicity[6].

Babita Rani (2014) Applied to two fungal isolates Aspergillus niger and Phanerochaete Chrysosporium, isolated from dye effluent soil, biodegradation and detoxification of dyes, Malachite gren, Nigrosine and Basic fuchsine have been conducted. The biodegradation technique, namely agar surplus, and liquid media; stationary and shaking conditions at 25 °C were chosen for three approaches. The de-colorization of the Basic fuchsin (81.85%) was reported

by aspergillus niger, followed by Nigrosin (77.47%), Malachite green (72.77%) and Fishing Mixture (33.00%). In contrast, P. chrysosporium has registered greatest decoloration with Nigrosin (90%), followed by Basic Fuchsin (89%), Malachite (83%) and Mixture (85%). (78.4 percent). In comparison with the stationary technique the chosen fungal strains were better performed under shaking conditions; in addition, pH of the dye solutions was neutral from acidic inoculated. The seed germination bioassay research shows that seed germination is present, but uninoculated dyes hinder germination, even after four days of observation, when inoculated colouration solutions are employed. Likewise, uninoculated dyes have reduced microbial growth. A. niger and P. chrysporium performance in the biodegradation of textile teeth of various chemical structures shows and strengthenes the environmental decontamination capability of these funguses[7].

Babita Rani, Vivek Kumar, Jagvijay Singh, Sandeep Bisht, Priyanku Teotia, Shivesh Sharma, Ritu Kela (2014) Biodegradation and detoxification of dyes, Malachite green, Nigrosin and Basic fuchsin have been carried out using two fungal isolates Aspergillus niger, and Phanerochaete chrysosporium, isolated from dye effluent soil. Aspergillus niger recorded maximum decolorization of the dye Basic fuchsin (81.85%) followed by Nigrosin (77.47%), Malachite green (72.77%) and dye mixture (33.08%) under shaking condition. Whereas, P. chrysosporium recorded decolorization to the maximum with the Nigrosin (90.15%) followed by Basic fuchsin (89.8%), Malachite green (83.25%) and mixture (78.4%).[9]

Hinsch et al. (2015) reported that the fungal pigments isolated from rotting hardwood logs in Canada produced xylindein (green pigment from C. aeruginosa), draconin red (red pigment from S. cuboideum), and yellow pigment (S. ganodermophthorum) and were able to dye multi fabric test strips. Their results indicated that all these pigments could be used to dye fabrics without the need for additional chemicals. Xylindein exhibited good potential to dye garment fabrics and draconin red for second layer garment fabrics. Awkwardly, dichloromethane (DCM) is used to extract colorants from spalting fungi and causes environmental problems and health issues, which is one of the major hurdles holding back spalting fungi from commercialization. Researchers have found that natural oils could be used to transfer pigments from Chlorociboria sp., Scytalidum cuboideum, and Scytalidium ganodermophthorum onto host substrates.[10]

3. MATERIALS AND METHODS

Media and chemicals

The textile dyes (Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B) used for the decolorization in the present investigation was a gift from M/s Maddy Fashion Kolkata, Kolkata (West Bengal). All media components and chemicals used in the present study were of analytical grade and purchased from Hi- Media Laboratories (Mumbai, India).

Sampling

Soil samples and waste water samples from Maddy Fashion Kolkata (MFK), S M Textile (SMT) and Maya Textile Processing (MTP), Kolkata, West Bengal state were collected in sterile polypropy- lene bags and glass bottles respectively. The soil samples were kept in a refrigerator at 4°C until the fungi were isolated. Waste water samples were immediately processed for physicochemical analysis.

Isolation of fungal strains

For isolation of potential strains, eight different types of samples from various environments (sludge samples from industrial effluent, waste water samples from Buddha Nala, effluent sample from alcohol, dye shops of Patiala and textile dyeing plant in Ludhiana) were collected and preserved at 4°C till further use. Soil/sludge samples were air dried and sieved after grinding followed by serial dilutions of the samples prepared in the saline (0.85% NaCl). Different dilutions were spread over potato dextrose agar (PDA) followed by incubation at 28°C for 72°C. Isolated fungal colonies were repeatedly sub cultured on fresh PDA plates to ensure the axenic nature of the isolates. Pure cultures were preserved at 4°C till further use.

Physiochemical analysis

Temperature, pH, colour and odour of the various wastewater samples were recorded on the spot. Samples collected from the discharge sites were filtered through Whatman No. 1 filter paper and their chemical oxygen demand and biological oxygen demand wasdetermined using standard procedures [12].

Decolorization assay

The ability of fungal strains to decolorize textile dyes was carried out in C-limited Czapek Dox broth (all in g L^{-1}) (sucrose, 5; $NaNO_3$, 2; K_2HPO_4 1; $MgSO_4 \cdot 7H_2O$, 0.5; KCL, 0.5; $FeSO_4 \cdot 7H_2O$, 0.01) according to Yatome *et al*. (1993). Textile dyes, i.e., Reactive blue MR (Z_{max} 585 nm), Orange M2R (Z_{max} 494 nm), Yellow M4G (Z_{max} 425 nm), Black HFGR (Z_{max} 594 nm) and Red M8B (Z_{max} 545 nm) were used at 200 mg/L. Agitated cultures of fungal spe- cies were grown for 10 days in a shake incubator at 30 ± 2°C and 120 rpm. Samples were withdrawn aseptically on alternate days, centrifuged at 5000 rpm for 10 min and supernatant was scanned in a spectrophotometer at Z_{max} of the respective dye.[12] Control flasks without dye were also maintained. Percent decolorization was calculated using the formula:

$$\text{Decolorization (\%)} = [(A_0 - A_f)/A_0] \times 100$$

where A_0 is the initial dye absorbance at the dye's λ_{max} and A_f is the final absorbance at the dye's λ_{max} [13]

Statistical analysis

The data were analyzed as mean of triplicates ± standard deviation (SD). Duncan's multiple range test (DMRT) was employed to test the level of significance at $P < 0.05$.

4. RESULTS

Physicochemical characterization of effluents

The data pertaining to physicochemical characterization of effluent samples from various sites is presented in **Table 1**.

Table 1: Physicochemical properties of textile waste water of different dyeing units.

nit	-L	-L	
ashion Kolkata (MFK)			
ile (SMT)			
xtile Processing (MTP)			

Note: different letters within a column are significantly different (DMRT; $P < 0.05$); n = 5

Wastewater was highly colored showing high concentrations of unused dye in the effluents. The effluents were found to be highly alkaline with a pH value ranging from 8.0 to 9.5. COD values were also found to be very high (985 – 1215 mg L^{-1}) at various sites. BOD levels ranged from 365 – 480 mg L^{-1} at various sampling sites. This data is also consistent with Devi and Kaushik (2005), who also observed a high BOD (560 mg L^{-1}), COD (1418 mg L^{-1}) and pH (9.4) of effluents taken from a textile industry, SGL industry, Faridabad (Haryana). High BOD and COD levels are indicative of the fact that substances present in the effluents can be biologically degraded (Pathe *et al*. 1995). Increased pH of the effluents is due to the use of carbonate, bicarbonate, H_2O_2 and NaOH during the bleaching process in the textile industry [14].

Isolation and identification of dye decolorizing fungi

Dye decolorizing fungi have been frequently isolated from textile effluents and soils exposed to dye wastes. In the present investigation, soil samples collected from waste disposal sites of three different textile industries were screened for the occurrence of fungi. Fungal species native to the sampling sites were isolated on CYA medium using dilution plate method. The species were identified by their morphological characteristics using a steriobinocular microscope and with the help of taxonomic keys and standard manuals. A total of 10 fungal species belonging to five genera were isolated and identified (**Table2**). Out of these, four species *viz*., *Aspergillus niger*, *A. terrus*, *A. flavus* and

Rhizopus sp. were ubiquitous and recovered from all the screened soil samples. The incidence of fungi in the polluted water and soil depends on the availability of nutrient, oxygen and biological, physical and chemical features of the pollutant.[15].

Table 2: Fungal strains identified in different industrially polluted soil

ns			
us niger			
us terrus			
us nidulans			
us flavus			
us fumigatus			
insolens			
brevis			
a hawaiiensis			
rbarum			
sp.			

MFK: Maddy Fashion Kolkata, SMT: S M Textile, MTP: Maya Textile Processing; + = Present, - = Absent

Decolorization of textile dyes

In the present investigation, all the ten isolated fungal strains were used to study decolorization of textile dyes *viz*., Reactive Blue MR, Orange M2R, Yellow M4G, Black HFGR and Red M8B at 200 mg L^{-1} in a C-limited Czapek Dox broth *in vitro*. The decolorization efficiencies were measured after 10 days of incubation under controlled conditions. The results revealed that all the isolated fungal species had variable potential to decolorize different textile dyes (**Table 3**). The most potent fungal strain in decolouri- zing Reactive Blue MR was *A. niger* followed by *A. terreus*> *A. fumigatus* > *A. nidulans* > *A. flavus* > *Rhizopus* sp. > *D. hawaiiensis* > *H. insolens* > *T. herbarum* > *H. brevis*. Orange M2R was most efficiently degraded by *A. flavus* followed by *A. terreus* > *A. niger* > *A. nidulans* > *A. fumigatus* > *T. herbarum* > *Rhizopus* sp. Yellow M4G was the most recalcitrant among all the dyes studied for degradation by the isolated fungi. The degradation efficiencies by different fungal strains varied from 5.14 to 39.38% (**Table 3**). From the results it was also observed that Black HFGR dye was highly degradable by all the fungal strains and the degradation efficiency varied from 28.19 to 90.26% (**Table 3**). Similarly Red M8B was degraded to 88.51% by *H. brevis*. The level of significance $P < 0.05$ using DMRT for each dye and against all test organisms is presented in **Table 3**.

Table 3: Per cent decolorization of textile dyes by isolated fungal strains after 10 days of incubation

	Decolorization (%)					
	Blue MR	**2R**	**4G**	**GR**		**f all dyes**
s niger	34 a	56 b	33 c	49 a	33 d	38 d
s terrus	55 a	71 b		72 a	55 a	38 c
s nidulans	20 a	43 b	9 c	49 a	25 d	35 b
s flavus	75 a	75 b	24 c	74 b	70 a	29 c
s fumigatus	30 a	36 b	35 c	35 a	19 d	35 c
insolens	37 a		26 b	70 c	3 b	21 b
brevis	34 a		30 a	52 b	68 b	31 a
hawaiiensis	43 a		21 b	38 c	35 a	38 c
barum	34 a	39 b	0 c	30 d	20 c	27 a
p.	68 a	34 b	35 b	43 c	7 d	52 e
sortium*	23 a	24 a	72 b	53 c	36 d	52 c

Note: ± = standard deviation, - = no decolorization, *Fungal consortium consisted of all 10 fungal strains; different letters within a row are significantly different (DMRT; $P < 0.05$)

In waste water effluents, we seldom get a single dye rather a mixture of dyes is present. Similarly pure cultures of microorganisms are never present in natural ecosystems. Thus, in the present investigation a fungal consortium consisting of all the ten fungal strains and a mixture of all the five dyes was also studied to evaluate the degradation efficiencies of isolated strains *in vitro*. It was noted that the fungal consortium was able to degrade Black HFGR (79.09%) and Red M8B (64.27%) most efficiently.

Degradation kinetics of fungal consortium revealed that maxi- mum degradation was achieved after 8 days of incubation and further incubation had no effect on the degradation of dyes (**Fig. 1**).

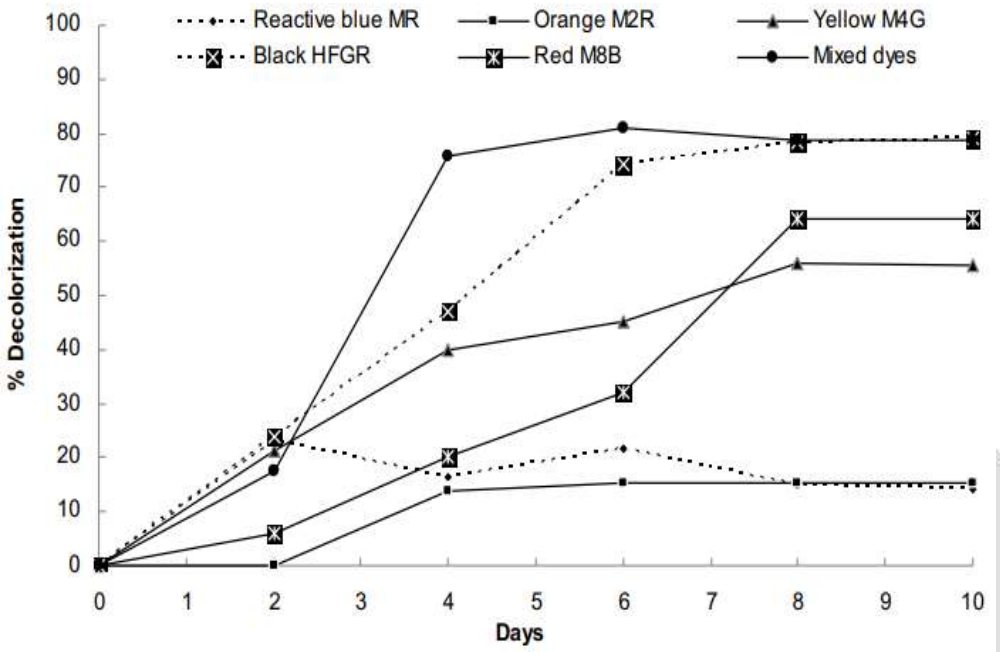

Fig. 1: Decolorization kinetic of various dyes by mixed microbial consortia

Similarly with regard to pure cultures degrading mixture of dyes, it was observed that *Rhizopus* sp. was able decolorize the mixture of dyes more efficiently (81.01%) as compared to individual dye (**Table 3**). Further-more, the recalcitrant Yellow M4G was also more efficiently degraded by fungal consortium (55.76%) as compared to pure cultures (**Table 3**).

5. DISCUSSION

Decolorization of textile dye effluents is a serious environmental problem. While physical and chemical methods of dye removal are expensive and result in high sludge problem, biological methods are environmentally safe and convert organic compounds completely into water and carbon dioxide, are cost effective and easy to use (Ponraj *et al*. 2011). In the present investigation, textile effluents from Maddy Fashion Kolkata was highly coloured and had BOD, COD and pH of 480 mg^{-L}, 1215 mg^{-L} and 9.5, respectively. These results are in agreement with earlier reports from textile effluents (Devi and Kaushik 2005). High pH is mainly due to the use of carbonate, bicarbonate, H_2O_2 and NaOH during bleeching of the textile (Wood and Kellog 1988). *Aspergillus* spp. are well adapted to textile waste water and are frequently isolated from effluents and dye contaminated soils (Devi and Kaushik 2005; Faryal and Hameed 2005; Raju *et al*. 2007; Ponraj *et al*. 2011). However, as far as *Humicola insolens*, *H. brevis* and *Rhizopus* species are concerned there is hardly any literature available about their use for decolorization of dyes. Ten fungal species were successfully identified using taxonomic guides and standard procedures. *Aspergillus niger*, *A. terrus*, *A. flavus* and *Rhizopus* sp. were the most important species and recovered from all the sites studied (**Table 2**). From the results of the present study, it is also clear that these species were also found to be efficient degrader of textile dyes. Most of the isolated fungal species were able to decolorize textile dyes (200 mg^{-L}) within 6-10 days under static culture condition. In our study, it was observed that fungal consortium consisting of all the ten fungal isolates was able efficiently degrade a mixture of dyes as compared to individual dyes (**Table 3**).

6. CONCLUSION

The possible mechanism of decolorization may be biosorption by fungal biomass or oxidative degradation/reduction

of dye (Fu and Viraraghvan 2002). Ronkarappa *et al*. (2006)used *A. niger* and *A. nidulans* for biosorption of Congo Red. They observed that dead biomass of *A. niger* is most efficient in biosorption compared to the living biomass. Further- more, process optimization may result in enhanced dye removal (Kaushik and Malik 2011). Based on these findings, it can be suggested that azo dye contaminated sites can potentially be reclaimed by low cost bioremediation process using a consortia of native fungal species isolated from the dye disposal sites. These fungal strains may also have a potential for use in bioreactors for industrial discharge treatment, through biotechnological approaches to colour removal.

7. REFERENCES

1. Vasudev, K (2011) Decolorization of triphenylmethane dyes by six white rot fungi isolated from nature. J Bioremed Biodegrad 2(5):61-66.
2. Raghukumar, C., D. D'Souza-Ticlo and A.K. Verma, 2008. Treatment of colored effluents with lignin-degrading enzymes: An emerging role of marine-derived fungi. Crit. Rev. Microbiol., 34: 189-206.
3. Aan, M.P.S., M. Krishna, H.N.N. Murthy and S.K. Rai, "Thermal, mechanical, morphological and flame retardance properties of DABA modified novolac/CFRP composites", Asian J. Mater. Sci., 2011, 3: 20-30.
4. Vasudev, K (2011), "Decolorization of triphenylmethane dyes by six white rot fungi isolated from nature", J Bioremed Biodegrad 2(5):61-66.
5. Saroj, S., Dubey, S., Agarwal, P. *et al.* Evaluation of the efficacy of a fungal consortium for degradation of azo dyes and simulated textile dye effluents. *Sustain. Water Resour. Manag.* **1,** 233–243 (2015).
6. Babita Rani, Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability, Braz. J. Microbiol. 45 (3) • Sept 2014
7. Babita Rani, Vivek Kumar, Jagvijay Singh, Sandeep Bisht, Priyanku Teotia, Shivesh Sharma, Ritu Kela, "Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability", Brazilian Journal of Microbiology 45, 3, 1055-1063 (2014), ISSN 1678-4405
8. Hinsch, E.M.; Chen, H.L.; Weber, G.; Robinson, S.C. Colorfastness of extracted wood staining fungal pigments on fabrics: A new potential for textile dyes. J. Text. Appar. Technol. Manag. 2015, 3.
9. Yeddou-Mezenner N (2010), "Kinetics and mechanism of dye biosorption onto an untreated antibiotic waste", Desalination 262:251-259.
10. Ali, H. and S.H. Muhammad, 2008. Biodecolorization of acid violet 19 by Alternaria solani. Afr. J. Biotechnol., 7: 831-833.
11. Mohorčič M, Teodorovič S, Golob V, Friedrich J (2006) Fungal and enzymatic decolourisation of artificial textile dye baths. Chemosphere 63:1709– 1717. doi:10.1016/j.chemosphere.2005.09.063
12. Raja, A.S.M. and G. Thilagavathi, 2011. Influence of enzyme and mordant treatments on the antimicrobial efficacy of natural dyes on wool materials. Asian J. Textile, 1: 138-144.
13. Novotný C, Dias N, Kapanen A, Malachová K, Vándrovcová M, Itävaara M, Lima N (2006) Comparative use of bacterial, algal and protozoan tests to study toxicity of azo- and anthraquinone dyes. Chemosphere 63:1436–1442