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ISOLATION OF A MESOPHILIC STRAIN OF *ASPERGILLUS WENTII* CAPABLE OF DECOLOURIZING TEXTILE DYEING MILL EFFLUENT

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ABSTRACT

In all nine fungal isolates were obtained from various sources such as soil, deteriorated paints and decaying wood collected from Karad (Maharashtra, India) locality, by enrichment culture technique followed by isolation by dilution plate technique, using Sabouraud Glucose Agar and incubating the plates at R. T. All nine isolates were subjected to primary screening by spot inoculation – Agar plate method using modified Sabouraud Glucose Agar (with various concentrations of textile effluent). Primary screening yielded four isolates designated as F - II, F - III, F - IV and D-1 capable of decolourizing the waste at 20% conc., V/V. These four isolates were subjected to secondary screening by liquid culture method. Secondary screening revealed that fungal isolate F-III was the best amongst the isolates as it showed maximum decolourization (91.38%) of 1:5 diluted effluents at 37 ^{0}C within eight days. The fungal isolate being of interest was then studied for its morphological and cultural characteristics and was identified as a strain of *Aspergillus wentii* Wehmer. The identity of the culture was confirmed by referring to the Fungus Identification Service Center.

KEY WORDS : Decoluorization, Textile dyeing waste, Fungus, *Aspergillus wentii*

INTRODUCTION

Textile wastes are coloured, highly alkaline, high in BOD and suspended solids and high in temperature (Nemerow, 1978; Nosheen *et al.,* 2000). Physicochemical characteristics of textile mills have been described in details by many workers (Rao and Datta 1979; Nosheen *et al.,* 2000). Synthetic dyes such as azo dyes, xanthenes dyes and anthraquinone dyes are very toxic to living organisms (Khadijah *et al.,* 2009). During and after dyeing. Large amount of dyestuffs are directly lost to the waste water and impart colour to the waste water in the industry which in turn imparts it to natural water body in which it is disposed off. Dyes present in the water on contact can cause variety of health problems.

Generally, these dyestuffs are designed to resist chemical fading and light induced oxidative fading (Nigam *et al.,* 2000). These dyes are highly resistant to microbial degradation under aerobic conditions; hence, wastes containing them are not much amenable to aerobic treatment as for as decolourization is concerned. This mainly makes them more resistant to biodegradation. Amongst the other factors that contribute to reduction in their biodegradability includes high water solubility, high molecular weights and fused aromatic ring structures which inhibit penetration through biological membranes (Keharia and Medamwar, 2003). Azo dyes are the largest class of dyes, which are not readily degraded by microorganisms. Microorganisms those are able to degrade azo dyes anaerobically, have been isolated (Growther & Minakshi 2009). Wastewater treatment facilities are often unable to completely remove commercial dyestuffs, thus contributing to the pollution of aqueous habitats. There are number of reports indicating that fungi can play a role in the decolorization in the textile industry waste (Fukuzumi, T. 1980; Thomas *et al.,* 1981; Livernoche *et al.,* 1983; Datta *et al.,* 1985; Belsare & Prasad, 1988,

Sartale *et al.,* 2006; Zope *et al.,* 2007; Murthi *et al.,* 2007; Emrah *et al.,* 2007; Kaushik and Malik, 2009; Gomathi *et al.,* 2012; Imran *et al.,* 2015; Pokharia and Ahuwalia, 2017).

There is need of isolation and systematic screening of larger number of microbial species capable of degrading the effluent and to study optimization of dye decolourization. Hence, present study included isolation and screening of fungi from various sources for decolourization of a textile mill effluent.

MATERIALS AND METHODS

Collection of effluent from Textile mill

The textile dyeing mill effluent was collected from 'Vasanti Dyeing Mill',MIDC area, Solapur (MS), India. A sterile plastic carboy of 5L capacity was used for the collection of the sample and brought to the laboratory. It was preserved at 4^0C. During transportation the carboy was protected from direct sunlight by putting the carboy in bag (Greenberg *et al.,* 1998).

Collection of samples for isolation of Fungi

The soil samples were collected in sterile polythene bags from the nearby area of sludge tank and effluent treatment plant of mill. Both deteriorated paint sample (walls of building) and decaying wood sample were collected from the campus of Yashwantrao Chavan College of Science, Karad Dist Satara (MS), India.

Physiochemical characterization of Textile mill effluent

Physiochemical characterization of the textile mill effluent was examined for colour, odour, pH, total suspended solids, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) by the using standard methods (Greenberg *et al.,* 1998).

Isolation, screening and identification of dye decolourizing fungi

For isolation of fungi, enrichment culture technique followed by spread plate technique was used (Atlas, 1973; Cappuccino and Sherman, 2005). Two soils samples, deteriorated paints and decaying wood sample were subjected to serial dilutions up to 10^{-7} by using sterile distilled water. Each dilution was then inoculated on modified Sabouraud Glucose Agar (instead of distilled water textile mill effluent was used) by spread plate method.

All these plates were incubated at room temperature up to seven days. After incubation period was over isolates were subjected to purification. The purified isolates were partially characterized and preserved on PDA slants at 4 °C for further studies. All these isolates were assigned by code numbers. Morphology of molds was studied by wet mount preparation using lactophenol cotton blue, as the mounting medium (Cappuccino and Sherman, 2005 and Gilman, 1957).

Primary screening for decolourization (Agar Plate method)

The textile dyeing mill effluent itself was used for media preparation. Different dilutions as 1:1, 1:5 and 1:10 of textile effluent were prepared by using distilled water. Each dilution was prepared in separate flask and each flask supplemented with nutrient as; 1% peptone and 4 % glucose and 2.5 % agar agar (modified Sabouraud dextrose Agar) and pH was adjusted to 5.4 using pH meter. All nine fungal isolates were spot inoculated separately on each diluted and undiluted textile effluent-containing medium plate. One plate from each diluted and undiluted medium was kept uninoculated as negative control.

All these plates were kept for incubation at room temperature for 5 days. All these plates were observed for growth of fungal isolates and decolourization of effluent. The fungal isolates which showed growth with decolourization of effluent were selected for further studies.

Secondary screening for decolourization (Broth Dilution method)

Preparation of medium

The modified Sabouraud dextrose broth was prepared for different dilutions as 1:1 and 1:5 same as above. All these media were sterilized by autoclaving and distributed into separate flasks.

Inoculum preparation

Initially, all selected fungal isolates were grown till sporulation occurred on Sabouraud Glucose Agar. For inoculation of these fungal isolates into above prepared liquid medium, 10 mm diameter agar disc with mycelial growth was inoculated into each dilution of broth and undiluted broth. One flask from each dilution and one undiluted broth were kept uninoculated as negative control. All flasks were kept for incubation at 37 °C for five days. After the incubation, all flasks were observed for

decolourization by comparing with negative control. The percent decolourization was determined using decolourization assay method (Gupta and Goel, 2004; Moorthi *et al.*, 2007).

After broth culture study, the fungal isolate, F-III which showed maximum percent decolourization was selected for identification and optimization study.

Optimization study for decolourization

For optimization study, F-III culture was selected which showed maximum percent decolourization of textile dyeing mill effluent at 1:1 and 1:5 dilutions, at 37 °C after 8 days incubation.

Modified Sabouraud dextrose broth using 1:5 dilution of textile waste (instead of distilled water) was prepared and used for optimization study. For optimization, 1% concentration of carbon sources (glucose, maltose, lactose, glycerol, sucrose and starch); 0.5 % concentration nitrogen source at (peptone, yeast extract, urea, ammonium chloride (NH_4Cl) and ammonium sulphate ($(NH_4)_2SO_4$); different levels of inocula as 0.1%, 0.25%, 0.5%, 1.0% and 1.5% and different temperature ranges as 20 °C, 30^0C, 37 °C and 50 °C these parameters were selected. A 10 mm diameter of agar disc containing mycelial growth of F-III culture was used as inoculum for carbon source, nitrogen source and temperature optimization study. For inoculation of different levels of inocula, uniform inoculum suspension was prepared by inoculating spores of F-III isolates in sterile saline. Then, % of inoculums was inoculated by using sterile pipette into separate flasks. The same amount of saline as each % inoculum level was added in separate flask for control and kept uninoculated. All flasks were incubated at 37 °C for five days along with control uninoculated flask.All flasks inoculated for temperature optimization study were kept at respective temperature for incubation.

After incubation all flasks were observed for decolourization and percent decolourization was determined by decolourization assay method (Gupta and Goel, 2004; Moorthi *et al.*, 2007).

Identification of promising fungal isolate

The identification of the promising fungal isolate to the species level was done by studying colonial characteristics, sporulation pattern, spore nature and microscopic observation of the wet mounts (Gilman, 1957; Cappuccino and Sherman, 2005). Then, identity of the promising culture was confirmed by referring to the Fungus Identification Service Center.

RESULTS AND DISCUSSION

Physicochemical characteristics of Textile Dyeing mill effluent

Physicochemical characteristics of textile dyeing mill effluents were studied. The results obtained during the present study for the characterization of the textile mill effluent for some important parameters are as shown in Table 1. It showed high COD and BOD values. Apart from being coloured, it showed offensive odour and alkaline pH.

Table 1. Physicochemical characteristics of textile dyeing mill effluent

Sr. No.	Parameters	Average Values
1	pH	10.3
2	Odour	Offensive odour
3	Colour	Greenish black
4	Total Suspended solids	701.6 mg/L
5	COD	6745 mg/L
6	BOD, 5 days at 20 °C	100 mg/L

Isolation of microorganisms from various sources

During the present study, total nine fungal isolates were obtained from various sources. All these isolates were assigned code numbers as A-1, A-4, B-1, B-2 and D-1 (Deteriorated paint) F-I, F-II (from decaying wood), F-III and F-IV (Soil).

Primary screening for decolourization of textile dyeing mill

Out of nine isolates inoculated none of fungal isolates showed growth on undiluted textile effluent, while fungal isolates as F-I, F-II, F-III, F-IV and D-1 showed growth on 1:1, 1:5 and 1:10 diluted textile effluent containing media. While decolourization zones were observed on 1:5 and 1:10 diluted effluent containing media by F-II, F-III, F-IV and D-1 after 5 days incubation at room temperature. In case of 1:1 diluted and undiluted effluents no decolourization was observed. Total four fungal isolates as F-II, F-III, F-IV and D-1 were selected for secondary screening.

During the liquid (broth) culture study, four fungal isolates showed decolourization at 1:1 and 1:5 diluted textile effluent. The percent decolourization for each fungal isolate showing decolourization of textile dyeing mill waste was

determined. The results were obtained at dilution 1:1 and 1:5 after 8 days incubation at 37 °C as shown in Figure 1. F-III isolate showed maximum percent decolourization 53.60 % and 91.38 % at 1:1 dilution and 1:5 dilutions respectively in comparison with other fungal isolates.

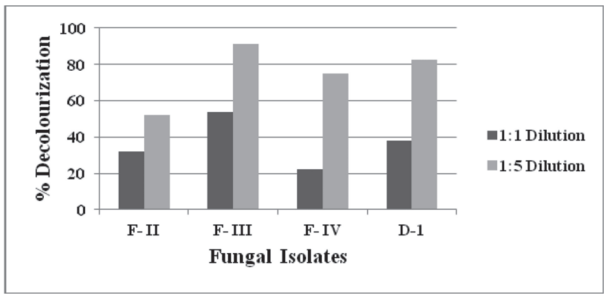

Fig. 1. % Decolourization of textile mill effluent by fungal isolates after 8 days incubation

Optimization of textile effluent decolourization

As maximum decolourization of the textile dye was showed by fungal isolate F-III, it was used for optimization of carbon source, nitrogen source, inoculums level and temperature.

Effect of carbon source

The results of the effect of carbon sources on decolourization by F- III isolate are shown in Figure 2. It can be seen from the figure that amongst the carbon sources tested at 1% concentration maximum decolourization was found to be with sucrose

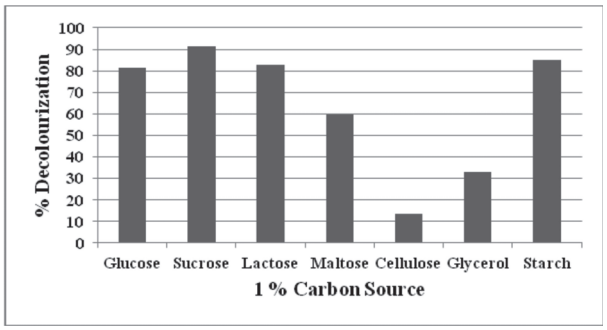

Fig. 2. Effect of 1 % carbon source on % decolourization of textile mill effluent by F-III Isolate

Effect of nitrogen source

The results of the effect of nitrogen sources on decolourization are as shown in Figure 3. It becomes evident from Figure 3 that there was maximum decolourization when yeast extract was used as nitrogen source that is 62.64%. Whereas, when

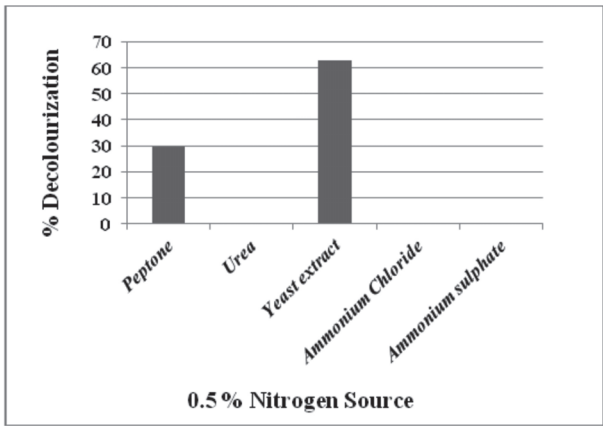

Fig. 3. Effect of 0.5% of nitrogen source on % decolourization of textile mill effluent by F-III isolate

NH_4Cl, $(NH_4)_2SO_4$ and urea were used as nitrogen source there was no growth and decolourization observed.

Similar effect of yeast extract as nitrogen source is also observed by other workers with decolourization of True blue dye by *Aspergillus flavus* (Ponraj *et al.*, 2011)

Effect of inoculum

The results of the effect of inoculum levels on decolourization of textile dyeing mill waste by using F-III isolate are as shown Figure 4 that the maximum percent decolourization was observed 66.74 % at 1.5 % inoculum level. It was observed that inoculum levels in the range 0.5 % to 1.5 % showed more than 60% decolourization.

Effects of temperature

Effects of temperature on decolourization of textile dyeing mill waste are as shown in Table 2. It is clear from table that percent decolourization was

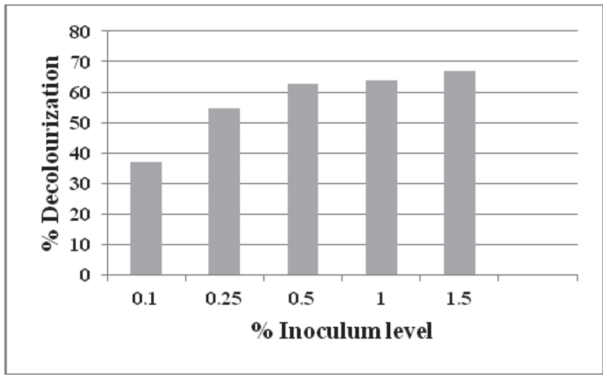

Fig. 4. Effect of inoculum levels on% decolourization of textile mill effluent by F-III isolate

Table 2. Effect of temperature on decolourization of textile dyeing mill effluent

Sr. No.	Temperature range (°C)	% Decolourization
1	20	20.25
2	30	53.93
3	RT	70.82
4	37	72.38
5	50	-

maximum at 37 °C followed by at room temperature and minimum at 20 °C.

Identification of F-III isolate

Fungal isolate F-III, which was found to be a promising strain for decolourization of textile dyeing mill waste, was tentatively identified as *Aspergillus species* and further confirmed as a strain of *Aspergillus wentii whelmer* by referring to the Fungus Identification Service Center (National Fungal Culture Collection, ARI, Pune).

It is seen from fig.1 that percent decolourization of textile dyeing mill effluent by this isolate is 91.38 % at 1:5 dilution within eight days incubation and 53.60% at 1:1 dilution within five days incubation at 37 °C respectively, flask culture, level. Optimization study by *A. wentii* showed that 1 % sucrose as carbon source, 0.5% yeast extract as nitrogen source, 0.5-1.5 % inoculum levels and 30 °C to 37 °C incubation temperature at pH 5.4 with the incubation period of five days is best for maximum decolourization of 1:5 diluted textile effluents.

CONCLUSION

The fungal strain isolated from soil and identified as *Aspergillus wentii whelmer* is promising one as it is capable of decolorizing the 1:5 diluted textile mill effluent to the extent of 91% and 1:10 diluted waste to the extent of 53% within eight days. It could be particularly suitable for the treatment of textile mill waste which are located at places were ample of amount of water is readily available for dilution of the waste . The fungal strain is worth considering for further studies such as strain improvement.

REFERENCES

Atlas, R. M. 1973. *Handbook of Microbiological Media,* (ed. Lawrence, C. Parks), CRC Press, London.

Belsare, D. K. and Prasad, D. Y. 1988. Decolourization of effluent from the bagasse-based pulp mills by white-rot fungus, Schizophyllum commune. *Appl. Microbiol. Biotechnol.* 28: 301-4.

Bergbauer, M.,Eggert, C. and Kraepelin, G. 1991. Degradation of chlorinated lignin compounds in a bleach plant effluent by White rot fungus *Tremetes versicolor. Appl. Microbiol. Biotechnol.* 35 : 105.

Bergbauer, M.,Eggert, C. and Kalnowski, G. 1992. Biotreatment of pulp bleachery effluents with the coelomycetos fungus *Stagnospora gigaspera. Biotech Lett.* 14 : 317-322.

Cappuccino, J. M. and Sherman, N. 2005. *Identification of Unknown Fungi, Microbiology a Laboratory Manual,* 7th ed., Pearson Benjamin Cummings, New York: 239-42.

Datta, S. A., Prasad, N.M. and Joshi, S. R. 1985. Decolourization of lignin bearing waste by *Aspergillus sp.*, *IAWPAC Technol. Annu.* 12 : 32-37.

Deshmukh, S.K. and Rathod A.P. 2008. Adsorption of dyes from waste water using coconut shell as bio-adsorbent. *Poll. Res.* 27 (3) : 569-573.

Emrah, A., Erkurt Ali Unyayar, Halil Kumbur 2007. Decolourization of synthetic dyes by white rot fungi involving Laccase enzyme in the process. *Biochemistry*. 42 : 1429-1435.

Fukuzumi, T. 1980. Microbial decolorization and defoaming of pulping waste liquors, In *Lignin Biodegradation: Microbiology, Chemistry and Potential Applications,* Vol.2, (ed. Krik, T.K., Higuchi, T. and Chang, H.M.), CRC Press, Boca Raton, FL: 161-71.

Gilman, J.C. 1957. *A Manual of Soil Fungi Revised.* 2nd edition, Oxford and IBH publishing co.: 215-32.

Gomathi, V. 2012. Decolourization of paper mill effluent by immobilized cells of *Phenerochate chrysosporium, IJPAES*. 2(1) : 141-46.

Greenberg 1998. *Standard Methods for the Examination of Water and Wastewater,* 20th ed. American Public Health Association [APHA], American Water Works Association [AWWA], Water Environmental Federation [WFE]; Washington, DC.

Growther, L. and Meenakshi, M. 2009. Biotechnological approaches to combat textile effluents. *The Internet J. Microbiol.* 7(1) : 1-7.

Gupta, P. and Goyal, D. 2004. Decolourization of synthetic dyes by *Aspergillus* sp., *Indian Journal of Microbiology*. 44 : 191-95.

Husseiny, S. M. 2008. Biodegradation of the reactive and direct dyes using egyptian isolates. *J. Appl. Sci. Res.* 4(6): 599-606.

Imran, M., Crowley, DE., Khalid, A., Hussain, S., Mumtaz, M.W. and Arshad, M. 2015. Microbial biotechnology for decolorization of textile wastewaters, reviews in *Environmental Science and Bio/Technology*. 14(1) : 73-92.

Kaushik, P. and Malik, A. 2009. Fungal dye decolorization: recent advances and future potential. *Environmental International.* 35 (1) : 127-41.

Keharia, H. and Madamwar, D. 2003. Bioremediation

concepts for treatment of dye containing wastewater: A review. *Indian J. of Microbiol.* 41: 1068 -1075.

Khadijah, O., Lee, KK. Faiz, M. and Abdullah, F. 2009. Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability. *Mal. J. Microbiol.* 5 (1) : 25-32.

Livernoche, D., Jurasek, L., Desrochers, M. and Veliky, Z. A. 1981. Decolourization of kraft mill effluent with fungal mycelium immobilized in calcium alginate gel. *Biotechnol. Lett.* 3 : 701-706.

Livernoche, D., Jurasek, L., Desrochers, M., Dorica, J. and Veliky, I. A. 1983. Removal of colour from kraft mill wastewater with culture of white-rot fungi and with immobilized mycelium of *Corious versicolor. Biotechnol. Bioeng.* 25 : 2055-2065.

Moorthi, S.P., Selvam, P.S., Sasikalaveni, Murugesan, A.K. and Kalaichelvan, P.T. 2007. Decolorization of textile dyes and their effluents using white rot fungi. *Afr. J. Biotechnol.* 6 (4) : 424-29.

Nemerow, N.L. 1978. *Industrial Waste Water Pollution-origins, Characteristics and Treatment*. Addison-Wesley Publishing Company.

Nigam, P., Armour, G., Banat, I.M., Singh, D., and Marchant, R. 2000. Physical removal of textile dyes from effluents and solid state fermentation of dye based agricultural residues. *Biores. Technol.* 72 : 219.

Nosheen, S., Nawaz, H. and Rehman, K. U. 2000. Physico-chemical characterization of effluents of local textile industries of Faisalabad Pakistan. *Int. J. Agri. Biol.* 2 (3) : 232-33.

Pokharia, A. and Ahuwalia, S.S. 2017. *Bioremidation Technologies for Decolorization of Effluent in Advances in Environmental Biotechnology,* Springer, Singapore (pp. 93-123).

Rao, M.N. and Datta, A.K. 1979. *Waste Water Treatment-rational Methods of Design and Industrial Practices,* Oxford and IBH publishing Co., Calcutta.

Royer, G., Desrochers, M., Jurasek, L., Roulean, D. and Meyer, R. 1985. Batch and continuous decolourization of bleached kraft effluents by a white rot fungus. *J. Chem. Technol.* 3513 : 14-22.

Saratale, G. D., Kalme, S. D. and Govindwar, S. P. 2006. Decolourisation of textile dyes by *Aspergillus ochraceus* (NCIM- 1146). *Indian J. Biotechnol.* 5: 407-10.

Sehanat, P., Pongtharin, L., Tsuyoshi, I. and Hunsa, P. 2009. Decolourization of pulp mill wastewater using thermotolerant white rot fungi. *Science Asia.* 35 : 37-41.

Thomas, W., Chang, H.M., Alton, G., Kirk, D. and Kirk, K. 1981. Removal of kraft bleach plant colour by a ligninolytic fungus. *Proceedings of the Applied Environmental Conferences* USA: 225-228.

Zope, V., Kulkarni, M. and Chavan, M. 2007. Biodegradation of synthetic textile dyes reactive red 195 and reactive green 11 by *Aspergillus niger grp*: An alternative approach. J. *Scientific Industrial Res.* 66 : 411-414.