



## Physicochemical Characteristics, Identification of Fungi and Optimization of Different Parameters for Degradation of Dye from Tannery Effluent

ASHLEY BEN, B; AMUTHA, E; PUSHPALAKSMI, E; JENSON SAMRAJ, J;  
\*ANNADURAI, G

<sup>1</sup>Division of Nanoscience, Sri Paramakalyani Centre of Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi - 627412, India

\*Corresponding author: [gannadurai@msuniv.ac.in](mailto:gannadurai@msuniv.ac.in)

**ABSTRACT:** This study was carried out to find out the qualities of tannery effluents with the assessment of physicochemical parameters of effluent, isolation, and identification of fungi and their optimization of different parameters on dye decolorization. In the present study, various physicochemical parameters such as Color, Odor, pH, EC, TSS, TDS, BOD, COD, Chromium, Copper, Chloride, and Sodium of untreated tannery effluent was studied. The results of the parameters showed that the effluent was blackish with a disagreeable odor, alkaline in pH with a high organic and inorganic loads such as EC, TDS, BOD, COD, TSS, Chromium, Copper, Chloride and Sodium. The physicochemical parameters were determined as per the standards prescribed by CPCB. Four fungal species were isolated and identified by LPCB staining namely *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum*, and *Curvularia lunata*. To test the activity of these fungi on different dyes, experiments were carried out for the optimization of different parameters. The maximum decolorization of dye was achieved by *Aspergillus niger*. From this study, it was found that the maximum biotransformation of dye effluent can help to solve the pollution problem.

DOI: <https://dx.doi.org/10.4314/jasem.v24i7.12>

**Copyright:** Copyright © 2020 Ashley *et al.* This is an open access article distributed under the Creative Commons Attribution License (CCL), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Dates:** Received: 16 May 2020; Revised: 29 June 2020; Accepted: 07 July 2020

**Keywords:** physicochemical parameters, fungal isolation, and identification, *Aspergillus niger*,

Nowadays water resource forms crucial assets in our day-day life not only for humans but also for other living organisms to survive Wang *et al.*, (2020). Tannery wastewater encloses an extensive amount of perilous pollutants in which heavy metals are very common Saranraj *et al.*, (2013). There are several physical and chemical treatment methods such as electrochemical methods employed to remove heavy metals in the effluent. The primary and pretreatment methods were used for the removal of suspended organic and inorganic solids. It is cost-effective and therefore microorganisms are used for oxidizing the organic and inorganic components (Chaturvedi, 1992). Therefore, Dye decolorization may be economical to develop alternative means of dye decolorization such as bioremediation due to its reputation as an environmentally friendly acceptable treatment technology Supaka *et al.*, (2004). The tannery effluent is considered as high pollutants among all other industrial effluents Eye *et al.*, (1971). A variety of physicochemical treatments have been devised previously for the dyes and textile wastewater. However, these suffered from some serious drawbacks in terms of their limited applications or their high cost. The wastewater is highly polluted in terms of

biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, nitrogen, electrical conductivity, sulphate, sulphide, and chromium Mondal *et al.*, (2005). Hexavalent chromium present in tannery effluent can cause nausea, vomiting, skin irritation, and problem-related to the respiratory tract can cause lung carcinoma due to chromium toxicity Palmer *et al.*, (1994). *Chaetomium globosum*, *Fusarium solani*, and *Aspergillus niger* utilize tannin as a carbon source. A wide variety of microorganisms are reported to be capable of decolorization of dyes and it can be used in effluent treatment plant for removal of these dyes Ponraj *et al.*, (2011). In the present study, an attempt was made to analyze the physicochemical characteristics of untreated tannery effluent, isolation and identification of fungi from the tannery effluent by LPCB staining, the potential of fungi strains for decolorization of tannery effluent containing dyes, optimization of various parameters such as pH, temperature, carbon source, and nitrogen source.

### MATERIALS AND METHODS

*Collection and transport of tannery effluent:* The tannery effluent was collected from a common

\*Corresponding author: [gannadurai@msuniv.ac.in](mailto:gannadurai@msuniv.ac.in)

effluent treatment plant (CETP), Pallavaram during March 2019. The sample was collected in a sterile plastic container and transported to the laboratory for bacteriological analysis.

*Physical and chemical analysis of tannery effluent:*

The physicochemical parameters of the effluent such as color, odor, pH, Electrical conductivity (EC), Total dissolved solids (TDS), Total suspended solids, BOD, COD, Chromium, copper, chloride, and sodium were determined as per the standard prescribed by CPCB (1995).

*Isolation of dye degrading fungal isolates from tannery effluent:* Fungal isolation was carried out by serially diluting the effluent and plated on SDA medium by pour plate technique and incubated at 30°C for 3-5 days. After incubation, colonies were isolated and identified by LPCB staining. The identified fungi were used for screening techniques.

*Screening of dye degrading fungal isolates:* The potential strain for decolorization of dye was selected by inoculating the isolates in 100ml of SD broth with 10ml of dye effluent and incubated in shaking condition at 30°C for 3-5 days. After incubation, the OD value was measured at 470nm. The isolate which showed more than 70% decolorization was selected for further studies Khatid *et al.*, (2008).

*Optimization of parameters:* The decolonization process was optimized by studying the effect of different physical and nutritional factors. Decolorization of dye in 15ml of SD broth with 3.5ml effluent was optimized by using 5ml of potential strain

concerning the effect of 1% carbon sources (glucose, rice, sucrose, starch, wheat), 1% nitrogen sources (yeast, ammonium sulphate, ammonium chloride, peptone, urea), pH (6, 6.5, 7, 7.5, 8) and temperature (20°C, 25°C, 30°C, 35°C, 40°C). The initial absorbance was taken and all the flask were incubated at 30°C under shaking condition for 3-5 days. After incubation, final OD was measured by centrifuging at 8000 rpm for 10 minutes and the OD value was measured by using the supernatant.

## RESULTS AND DISCUSSIONS

A significant proportion of dyes enter the environment through wastewater. Microbial decolorization has been proposed as a less expensive and less environmentally intrusive alternative.

*Physical and chemical analysis of tannery effluent:*

The physicochemical characteristics of the untreated tannery effluent were analyzed for March month 2019. The result of the study shows that it is alkaline with high BOD, COD, organic particulate matter, disagreeable odor, and blackish color. An unpleasant odor may be due to microbial growth or H<sub>2</sub>S gas. The pH of the tannery effluent was highly alkaline of about 8.9. The electrical conductivity was found to be high and ranges about 11625  $\mu$ mhos/cm. The total dissolved solid was found to be 2984 mg/ml. The total suspended solids were found to be 142 mg/ml. The value of BOD was found to be 846 mg/l and COD was found to be 1098 mg/l. The concentration of total chromium was found to be 5 mg/l and copper 6 mg/l as shown in Table 1.

**Table 1.** Analysis of physicochemical parameters of untreated tannery effluent

S. No	Parameters	CPCB 1995	Observations
1	Color	Colorless	Blackish
2	Odor	Odorless	Disagreeable odor
3	pH	5.5-9.0	8.9
4	EC	400 $\mu$ s/cm	11625
5	TDS	2100 mg/l	2984
6	TSS	100 mg/l	142
7	BOD	30 mg/l	846
8	COD	250 mg/l	1098
9	Chromium	2 mg/l	5
10	Copper	3 mg/l	6

**Table 2.** Percentage of dye decolorization by fungal isolates

Fungal Isolates	<i>A. niger</i>	<i>A. flavus</i>	<i>P. citrinum</i>	<i>C. lunata</i>
% of Dye decolorization	75.4	64.4	50.2	58.1

**Table 3.** Effect of different carbon source in dye decolorization

Different carbon source	Glucose	Rice	Sucrose	Starch	Wheat
% of decolorization	59.7	30.5	45.0	22.3	22.7

**Table 4.** Effect of different nitrogen source in Dye decolorization

Different Nitrogen source	Yeast	Ammonium Sulphate	Ammonium Chloride	Peptone	Urea
% of Decolorization	62.9	52.1	77.3	71.9	32.2

*Isolation of fungal isolates from tannery effluent:* The effluent is rich in organic and inorganic nutrients which would have supported the growth of the fungal population. The fungal strains were isolated from untreated tannery effluent by serial dilution technique. The results of the microbial analysis of the effluent showed four species of fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citrinum*, and *Curvularia lunata*.

*Screening of dye degrading fungal isolates:* These fungal isolates were screened for the decolorization of dye effluents by using a spectrophotometer. *Aspergillus niger* was screened out which showed more than 70% decolorization. The percentage of decolorization was shown in Figure 1 and Table 2.

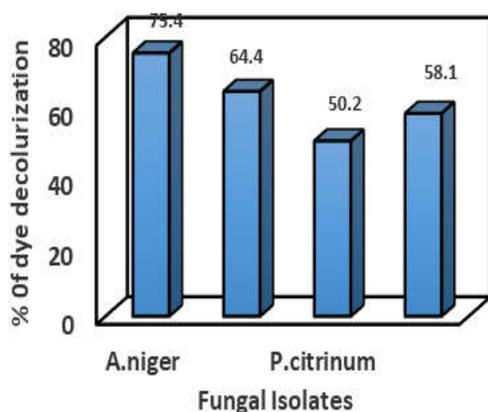


Fig 1. Percentage of dye decolorization

*Optimization of Parameter: Effect of different carbon sources:* The increase in decolorization percentage after the addition of carbon resources is attributed to the reality that the dyes are deficient in carbon content and biodegradation without any more carbon assets is difficult Georgiou *et al.*, (2005). The lower in decolorization percent after the addition of a few carbon sources and the potential of a few carbon sources to induce boom without growth in decolorization might be attributed to that, the sugars can also inhibit the decolorization of azo dyes because of its effect as catabolite repression. Different carbon sources were evaluated for dye decolorization. Among various carbon sources, glucose (59.7%) showed maximum activity for the decolorization of dye by using *Aspergillus niger*. The percentage of decolorization at different carbon sources as shown in Figure. 2. The effect of different carbon sources on decolorization of dye was investigated and the results were furnished in Table. 3. Five different carbon sources viz., yeast extract, ammonium chloride, and urea were tested in the present decolorization study. Among the microorganism isolated tested, maximum

decolorization of dye was observed by *Aspergillus niger* in the medium supplemented with wheat (22.7 %). The bacterial isolate *Aspergillus niger* showed minimum decolorization of dye (59.7 %). Next to glucose, maximum *Aspergillus niger* decolorization of dye observed in the medium supplemented with Glucose, rice, sucrose, starch wheat.

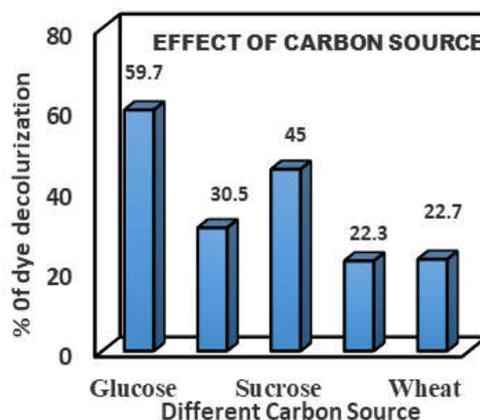


Fig 2. Effect of Carbon Source

*Effect of different nitrogen sources:* Nitrogen source supplementation in medium plays a major role in dye decolorization activity. The highest percentage of decolorization of effluent with ammonium chloride was found to be 77.3%. Lowest decolorization percentage were observed using urea as nitrogen sources. The effect of different nitrogen sources on decolorization by *A. niger* was illustrated in Figure. 3. The effect of different nitrogen sources on decolorization of dye was assessed and the results were furnished in Table. 4. Five different nitrogen sources viz., yeast extract, ammonium chloride, and urea were tested in the present decolorization study. Among the *Aspergillus niger*, bacterial isolates tested, maximum decolorization of dye was observed by *Bacillus odyseus* in the medium supplemented with ammonium chloride (77.3 %) (Goel, 1997). The bacterial *Aspergillus niger* showed minimum decolorization of dye as observed in the medium supplemented with urea. Nigam *et al* reported that isolated PDW did not show decolorization when yeast extract omitted from the medium. Growth of *Pseudomonas luteola* was directly related to the concentration of yeast extract and when the concentration of yeast extract was reducing growth and color removal was decreased. Nigam *et al.*, (2020) have also reported maximum decolorization of azo dyes in the presence of yeast extract (5 g/l) in PDW consortium. The color removal percentage of most dyes increased sharply after the addition of yeast extract and this is by other reports (Pearson, 1980).

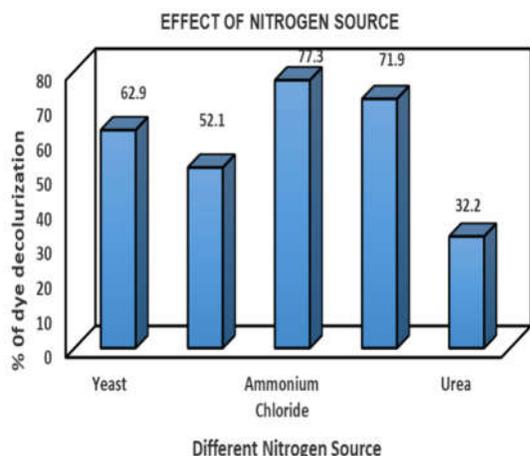


Fig 3. Effect of Nitrogen Source

**Effect of pH:** The results show that the organism was capable of decolorizing dyes over the pH range of 6-8. The best decolorization (77.3%) was achieved at pH 6 in 5 days. This could be because the optimum pH for the growth of *Aspergillus niger* was acidic. Whereas the rate of color removal was much lower at the strongly alkaline condition. The effect of different pH on the decolorization of dyes by *Aspergillus niger* was

shown in Figure. 4. The effect of the pH value on the decolorization studies has a significant role as it determines the surface charge of the bio sorbent and the degree of ionization and speciation of the dye solution. Figure. 4 showed that as the pH increased the removal percent increased and reached the maximum removal efficiency (77.3%) at pH 6.0. Then by increasing pH value, the decolorization process was decreased as shown in Table 5.

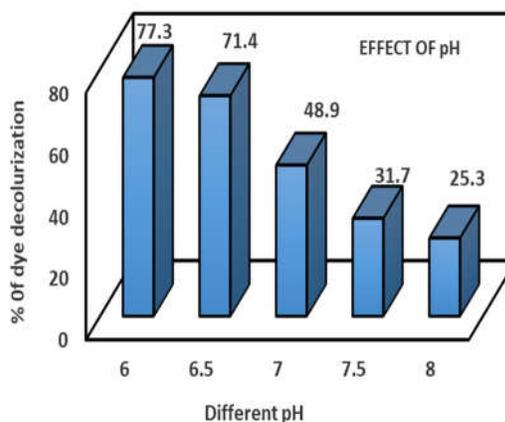


Fig 4. Effect of pH

Table 5. Effect of different pH in dye decolourization

Different pH	6	6.5	7	7.5	8
% of decolorization	77.3	71.4	48.9	31.7	25.3

Table 6. Effect of different temperature in dye decolorization

Different Temperature	20°C	25°C	30°C	35°C	40°C
% of decolorization	31.7	78.7	73.7	46.7	23.5

**Effect of temperature:** It was found that within an increase in temperature from 20-40°C the decolorization rate increased. The maximum decolorization (78.7%) was observed at 25°C and a further increase in temperature to 40°C drastically affected the decolorization activity of fungal isolates. The effect of different temperatures on decolorization was shown in Figure 5. Although the optimum temperature for dyes Colour removal by *Aspergillus niger*. was 25°C, our analysis showed that the *Aspergillus niger* capacity for decolorization of azo dyes reached the minimum values at temperature degrees more than 40°C as shown in Table 6. The reason for the negative effect of high temperature on dyes removal might be because of the injury of cell viability and suppress the bacterial enzymes responsible for azo dyes degradation Wang *et al.*, (2009). However, microbial decolorization of dyes may refer to various processes e.g. biodegradation, adsorption, precipitation, and/or biosorption. All these processes may be acting together as interdisciplinary processes or maybe one or two of them could be the major decolorization process effected in various

degrees by pH, temperature, time, and inoculum concentration.

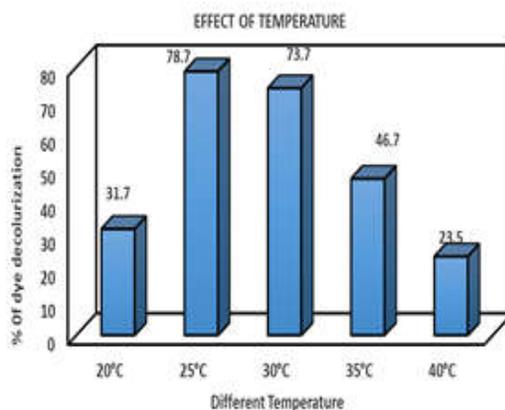


Fig 5. Effect of Temperature

The results of physicochemical parameters were compared with CPCB 1995. pH is alkaline due to the presence of carbonates and bicarbonates present in the effluent Saxena *et al.*, (2002). The electrical conductivity was found to be very high which may be due to the presence of inorganic substances and soil

that show good conductivity. High electrical conductivity levels may be due to a higher concentration of acid-base and salt in water. A high level of TDS may be due to high salt content and it is unsuitable for irrigation Moosvi *et al.*, (2005). A high level of TSS may be due to the insoluble organic and inorganic present in the effluent Nagarajan *et al.*, (2005). A high level of BOD indicates that the tannery effluent has a high organic load. Increased BOD leads to the depletion of dissolved oxygen Poole *et al.*, (2009). The COD level of tannery effluent was higher than the standard level. COD is used for the determination of total oxygen demand by organic material present in the effluent Raj *et al.*, (2002) recorded higher values of COD in the tannery effluent. The concentration of chromium and copper is higher than the standard level. The concentration of heavy metals varies depending upon the process of tanning in various industries Vidya *et al.*, (2007). The results indicated that some native fungi are adapted to heavy metals under constant metal stress for a long time and the toxic metals are used as micronutrients by the growth of fungi Zhang *et al.*, (2008). The growth of the fungi may be affected by the presence of dyes at toxic concentration. This also affects the dye decolorization efficiency of the fungal species Kaushik *et al.*, (2009). Glucose is needed for azo dye decolorization by different microorganisms. Sugars also serve as reducing agents for dye decolorization Ali *et al.*, (2010). The best decolorization was achieved at acidic pH 3 and PH 4 in 4 days by using *Aspergillus niger* (Sabrien, 2016). *Aspergillus niger* and *Mucor* sp were the most effective decolorizer at 27°C and 37°C Ponraj *et al.*, (2011). At a higher 35°C or lower 25°C, the decolorization activity of the fungus is reduced because the fungus can unable to produce peroxidases for decolorization or peroxidases denature Radha *et al.*, (2005).

**Conclusion:** Our findings revealed that *A. niger* removed from fabric wastewater has a high affinity for the decolorization procedure. The results obtained show that *Aspergillus niger* isolated from the tannery effluent is the efficient strain to decolorize the dye present in the effluent. Temperature, pH, carbon, and nitrogen source were found to be an important parameter in biodegradation of tannery effluent. Removal of dye was due to the production of extracellular enzymes produced by fungi. Pollution due to dye present in tannery effluent can be reduced by fungi.

**Acknowledgements:** I am Ashley Ben. B (Register No: 17231232272002) acknowledge the Research Centre, Sri Paramakalyani Centre for Excellence in Environmental Science, Manonmaniam Sundaranar

University, Alwarkurichi, providing the support for this research work.

## REFERENCES

- Ali, N; Hameed, A; and Ahmed, S (2010). Role of brown - rot fungi in the bioremoval of azo dyes under different conditions. *Braz. J. Microbio.* 41: 907-915.
- Chaturvedi, MK (1992). Biodegradation of tannery effluent, isolation and characterization of microbial consortium. *Indian J. Env. Pro.* 12(35): 335-340
- Eye, JD; Lawrence, L (1971). Treatment of waste water from a sole leather industry. *J. Wat. Pollut. Cont. Fed.* 43: 2291-2302.
- Georgiou, D; Hatiras, J; Aivasidis, A (2005). Microbial immobilization in a two stage fixed bed-reactor pilot plant for on-site anaerobic decolorization of textile wastewater. *Enzyme and Microbial Technology.* 4: 4184-4186.
- Goel, PK (1997). Water pollution, causes, effects and control, New Age International (P) Ltd., publishers. New Delhi. 269.
- Kaushik, P; Malik, A (2009). Fungal dye decolorization, recent advances & future potential. *Environ in.* 35: 127-141.
- Khatid, A; Arshad, M; Crowley, DE (2008). Accelerated decolonization of structurally different azo dyes by newly isolated bacterial strains. *Appl. Microbiol.* 78: 361-369.
- Mondal, NC; Saxena, VK; Singh, VS (2005). Impact of pollution due to tanneries on groundwater regime." *Curr. Sci.*, 88: 25-27.
- Moosvi, S; Keharia, H; Madamawar, D (2005). Decolourization of textile dye reactive violet by a newly isolated bacterial consortium. *World Journal of Microbiology and Biotechnology.* 21: 667- 672.
- Nagarajan, P; Moorthy, TR; Raja, RE; Raj, AP (2005). Physicochemical characteristics of water and soil at Senthaniapuram, Thiruchirapalli and their influence on germination of green gram and cow pea. *J. Ecotoxicol. Environ Monito.* 15: 229-234.
- Palmer, D; Puis, W (1994). Natural attenuation of hexavalent chromium in groundwater and soils. *EPA Ground Water Issue.* 87: 90-94.

- Pearson, TH (1980). Marine pollution effects of pulp and paper industry wastes. *Helgolander Meeresunters*, 33: 340-365.
- Ponraj, M; Jamunarani, P; Zambare, V (2011). Isolation and Optimization of culture conditions for decolorization of true-blue using dye decolorizing fungi. *Bio Sci*. 2(2): 270-277.
- Poole, NJ; Wildish, DJ; Kristman, DD (2009). The effect of the pulp of paper industry on the aquatic environment. *Cur.Rev.Environ.con* 8: 159-195.
- Radha, K; Regupati, I; Arunagiri, A; Murugesan, T (2005). Decolorization studies of Synthetic dyes using *Phanerochaete chrysosporium* and their kinetics. *Process Biochemistry*. 40: 3337-3345.
- Raj, EM; Sankaran, SK; Kuamran, S; Mohan, M (2002) Studies on the treated effluent characteristics of a few tanneries at chrompet Madras. *Ind. J. Environ. Prot.* 6: 252-254.
- Sabrien, A (2016). Decolonization of different textile dyes by isolated *Aspergillus niger*. *Journal of Environmental Science and Technology*. 9: 149-156.
- Saranraj, P; Sujitha, D (2013). Microbial bioremediation of chromium in tannery effluent. *Intl. J. Microbiol.* 4(3): 305-320.
- Saxena, S; Shrivastava, P (2002) Bioremediation of tannery waste water by *Aspergillus niger* SPFSL2-an isolated from tannery sludge. *Int. J. Basic Appl. Sc.* 2: 88-93.
- Supaka, NL; Juntagjin, S; Damrobglerd, ML; Strehaiano, D; Stephano, S (2004). Microbial Decolorization of Reactive Azo Dyes in a Sequential Anaerobic–Aerobic System. *Chemical Engineering Journal*. 99(2): 169-176.
- Vidya, S; Usha, K (2007). Remediation potential of *Ocimum Basilicum* against tannery wastes. *Pollut. Res*, 26: 42-425.
- Wang, H; Su, JQ; Zheng, XW; Tian, Y; Xiong, XJ; Zheng, TL (2009). Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3q. *Int. Biodeterioration Biodegradation*. 63: 395-399.
- Wang, X; Deng, B; Yu, L; Cui, E; Xiang, Z; Lu, W (2020). Degradation of azo dyes Congo red by MnBi alloy powders: Performance, kinetics and mechanism. *Mater. Chem. Phys.* 251: 123096
- Zhang, Y; Liu, M; Shi, X; Zhao, Z (2008). Dark septate endophyte (DSE) fungi isolated from metal polluted soils, their taxonomic position, tolerance and accumulation of heavy metal invitro. *J. Microbiol.* 46: 624-632.