



ORIGINAL ARTICLE

Decolourization of Textile Effluent through Indigenous Microorganism

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ABSTRACT

The present research was conducted to exploit the potential of the indigenous microorganism to decolorize the textile industry effluent. Six bacterial and two fungal isolate were obtained from textile effluent. All the isolates were screened at 10, 50, 100 and 150 mg/L of red and green textile dyes. Among the bacterial isolates, Pseudomonas sp. demonstrated a higher percent dye decolourization (67% for red and 73% for green dye) than Bacillus sp. (67% for red and 64% dye) within 24 h. The best decolourizing fungal strain to be isolated was that of Aspergillus that showed decolourization up to 59% for red dye and 70% for green dye within 72h. The indigenous microorganisms also showed 30% decolourization at 2 weeks in the pilot scale experiment when sugar cane and straw extract was used as natural, cheap and sole source of nutrition at ambient temperature.

Key Words: industrial effluent, indigenous microorganism, textile dye, decolourization, bioremediation

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INTRODUCTION

Textile industry effluents are now being considered as an environmental nuisance, gone are the days when people used to think of the sea as a natural digester of all the pollutants. Coloured wastewater from textile industries is rated as the most polluted in almost all industrial sectors [1]. These effluents contain considerable amount of suspended solids, additives, detergents, surfactants, carcinogenic amines, formaldehyde, heavy metals, and dyes that poses serious environmental threats to receiving water bodies [2]. Large amount of synthetic dyes from textile industries are continuously being exhausted in wastewater streams due to their poor adsorption to the fiber [3,4]. This coloured wastewater when discharge into the receiving streams, not only affects the aesthetics of the nature but also reducing photosynthetic activity through interference with the transmission of sunlight. In addition to visual effect, they have also an adverse impact in terms of chemical oxygen demand, toxicity, mutagenesis and carcinogenicity [5].

The treatment of textile waste effluents is still a major environmental concern because of synthetic dyes which are difficult to be removed by conventional physical and chemical technologies [6]. Thus, removal of these synthetic dyes is necessary for the health of receiving body and thereof for the sustainability of whole ecosystems. Various methods (adsorption, sedimentation, flocculation, floatation, coagulation, osmosis, neutralization, reduction, oxidation, electrolysis and ion-exchange) are usually employed for the treatment of textile effluent before discharging into the environment. But these methods cause some limitations as high cost and administration of large quantity of sludge with some toxic waste created during these operations. Therefore, economical and environment friendly techniques are required for the removal or degradation of dye from the textile effluent.

Bioremediation has been proven to be an efficient, eco-friendly and more cost-effective [7] for the treatment of variety of wastes. The microorganisms show very promising ability to decolour, degrade, detoxify and metabolize a number of compounds in various biological treatment processes. Due to the ubiquitous nature, they can be used as invaluable tools for the biological treatment of textile effluent. As a preliminary step in the development of biological treatment for textile effluent, there is a need to isolate bacterial and fungal strains with a potential to decolorize and degrade textile dyes and remove other pollution parameters [8]. A number of bacterial species belonging to genera Bacillus, Micrococcus,

Proteus, Pseudomonas, Sphingomonas and Staphylococcus were reported to have been isolated for biotreatment of textile dyes [9,10,11, 12]. A white rot fungus Phanerochaete chrysosporium has been used extensively for decolourization of dyes in wastewaters [13]. A suitable strain and its use under favorable conditions are requiring realizing its degradation potential and decolourization of textile effluent. Therefore, it is necessary to explore the possibilities of isolating efficient aerobic degraders for use in decolourization and degradation of textile dye waste effluents.

Since Pakistan is a developing country, not much concern is given to the treatment of textile effluent. But on the other hand Pakistan also holds a key position in the textile industry, therefore it is time to pay attention to such problem or else our environment could be damaged irreversibly. The present work investigates the potential of indigenous microorganism to decolorize the textile industry effluent. The main objective of the study was the isolation and characterization of bacterial and fungal strains, which would efficiently decolorize the textile dye and design a pilot plant experiment in which cheap natural substrate is used as a sole source of nutrient for the fungi.

MATERIAL AND METHODS

Sample Collection

Wastewater samples were collected from the textile industry located at North Karachi Industrial Area, Karachi, Pakistan. The samples were collected in sterile air tight bottles with filtering through the ordinary filter paper to remove large suspended particles. Standard procedures (spot and grab) were followed during sampling and samples were transported to the laboratory and stored at 4°C.

Isolation and identification of microorganism

The isolation of indigenous microorganism decolorizing, degrading and detoxifying textile dyes was carried out through serial dilution method [2]. Isolated bacterial strains were screened out by incubating them on nutrient agar medium containing red (6BN) dye and green (mixture of turquoise blue and yellow 2GR) dye 10, 50, 100 and 150 mg/L of each. Fungal strains were isolated on Sabourauds Dextrose Agar (SDA) using same dilution plate technique. The stock cultures of screened bacterial and fungal isolates were maintained routinely on the nutrient agar medium and SDA respectively and stored at 4°C.

The screened bacterial strains were identified on morphological, biochemical and physiological properties up to the level of genus using the protocol given in Bergey's Manual of Determinative Bacteriology [14].

Decolourization of synthetic dyes

The ability of bacterial and fungal strain to decolorize textile dyes was carried out in nutrient broth and Sabourauds dextrose broth amended with red and green dye (150 mg/L). The experiments were run in duplicate and microorganism were allowed to decolorize dyes at 37°C for 24-72 h. Media was centrifuge at max. 4000 rpm for 20 min so that all the cell deposited at the bottom and supernatant was collected to observed decolourization ability by using spectrophotometer at optimum wavelength 540 nm for red and 450 nm for green dyes. The decolourization activity was expressed in terms of percentage decolourization using following formula

$$\text{Decolorization \%} = \frac{A_{t_0} - A_{t_f}}{A_{t_f}} \times 100$$

Where;

A_{t_0} = initial absorbance

A_{t_f} = absorbance at incubation time

Pilot Scale Experiment

Pilot plant experiment has also design to check the decolourization potential of indigenous microorganism in natural environment. For this large Erlenmeyer flasks (250 ml) were used containing coloured effluent (150 mg/L) with 100ml natural substrate or media i.e sugarcane extract and straw extract media (so as to keep the experiment close to the nature). The final concentration of the dye in the flask adjusted to 50 mg/L. Then flask were wrapped with paper and placed in sunlight for a period of 2-3 weeks with occasional shaking. Growth was observed in term of increasing and decreasing turbidity and the pH was also checked.

RESULT AND DISCUSSION

Isolation and Identification of Indigenous Microorganism

A total of six morphologically different bacterial isolate and two fungal isolate were obtained from textile effluent. All the isolates were screened at 10, 50, 100 and 150 mg/L of red and green textile dyes. Out of all three bacterial isolates were screened (Table-01) that are capable of utilizing textile dye as a source of carbon and energy. These were identified as the species of *Bacillus* and *Pseudomonas* (Table-02) on the basis of the gram staining, morphological and biochemical characterization. One fungal isolate showed

promising growth at 10, 50, 100 and 150 mg/L of red and green textile dyes (Table-01) and upon microscopic examination it was identified as *Aspergillus* (Table-03).

Table-01 Screening of bacterial and fungal isolates on different concentrations of textile dyes

| Microbial Isolate | Textile dye colour and concentration (mg/L) | | | | | | | |
|-------------------|---|-------|-----|-------|-----|-------|-----|-------|
| | 10 | | 50 | | 100 | | 150 | |
| | Red | Green | Red | Green | Red | Green | Red | Green |
| B-1 | + | + | + | + | + | + | + | + |
| B-2 | + | + | + | + | + | + | + | + |
| B-3 | - | + | - | + | - | - | - | - |
| B-4 | + | + | + | + | + | + | + | + |
| B-5 | + | + | - | - | - | - | - | - |
| B-6 | + | + | - | + | - | - | - | - |
| F-1 | + | + | + | + | + | + | - | + |
| F-2 | - | + | - | + | - | - | - | - |

(+) Microbial growth; (-) No microbial growth; B=Bacterial; F=Fungal

Table-02 Identification of Bacterial Isolates

| Characteristics | Screened Bacterial Isolate | | |
|----------------------|----------------------------|---------------------|------------------------|
| | B-1 | B-2 | B-4 |
| Gram Reaction | + | + | - |
| Morphology | rod | rod | short rods |
| Motility | + | + | + |
| Glucose Fermentation | - | - | + |
| Lactose Fermentation | + | + | + |
| Indole Production | - | - | - |
| Methyl Red | + | + | - |
| Voges- Proskauer | + | + | - |
| Citrate Utilization | + | - | + |
| Catalase | + | + | + |
| Oxidase | - | - | + |
| Identity | <i>Bacillus subtilis</i> | <i>Bacillus sp.</i> | <i>Pseudomonas sp.</i> |

(+) Positive; (-) Negative

Table-03 Identification of screened fungal isolate

| Fungal Isolate | Colonial Morphology | Microscopic Observation | Identity |
|----------------|--|--|--------------------|
| F-1 | White colonies reverse black, become greenish brown as culture matures | Single celled spores in chain, developing at end of sterigma arising from the terminal bulb of candidophore. | <i>Aspergillus</i> |

Decolourization of Textile Effluent

Figure 1 and 2 showed the percent dye decolourization by the different microbial isolates. It could be seen that all the isolated microorganisms has a percent dye decolourization between 47-67% for red textile dye and 49-73% for green textile dye. Comparatively among the bacterial isolates, *Pseudomonas sp.* demonstrated a higher percent dye decolourization (67% for red and 73% for green dye) than *Bacillus sp.* (67% for red and 64% dye) within 24h. Kalyani et al. (2008) [15] isolated the *Pseudomonas sp.* from contaminated soil near textile industry, with the ability to decolorize 50 ppm of Red BLI 99.28%. In 1998, Jothimani and Prabhakaran has reported 59% dye removal from a dyeing industry effluent using *pseudomonas* after 4 days of inoculation and with *Bacillus sp.* *Aspergillus* showed decolourization up to 59% for red dye and 70% for green dye within 72h. The efficient use of different *Aspergillus* species (*A.*

niger, *A. foetidus*, *A. fumigates* and *A. terreus*) for decolourization of different types of dye has been reported [1, 16, 17, 18].

It was found that at moderate dye concentration (50-100mg/l) decolourization activity is high as compared to higher concentration (Figure 1 and 2). Namdhari et al., (2012) [19] reported that the higher dye concentration strongly inhibits decolorization, which may be due to desorption or toxic effects. Generally, the concentration of color compounds found in the effluent or rivers ranged as low as 12 to 16 mg/L.

Table-04 (a) Decolourization of red textile dye through bacterial Isolates

| Dye Conc. (mg/L) | Bacterial Isolate | | | | | | | | |
|------------------|-------------------|-----------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|------------------|
| | B-1 | | | B-2 | | | B-4 | | |
| | At ₀ | At _f | % D _e | At ₀ | At _f | % D _e | At ₀ | At _f | % D _e |
| 10 | 0.210 | 0.101 | 51.90 | 0.211 | 0.110 | 47.86 | 0.211 | 0.100 | 52.60 |
| 50 | 0.271 | 0.123 | 54.90 | 0.269 | 0.125 | 53.53 | 0.270 | 0.140 | 48.14 |
| 100 | 0.310 | 0.101 | 67.41 | 0.311 | 0.110 | 64.6 | 0.311 | 0.100 | 67.80 |
| 150 | 0.350 | 0.152 | 56.57 | 0.350 | 0.155 | 56.98 | 0.350 | 0.152 | 56.57 |

At₀ = Initial absorbance at 540nm

At_f = Final absorbance at 540nm

%D_e = Percentage decolourization

Table-04 (b) Decolourization of green textile dye through bacterial Isolates

| Dye Conc. (mg/L) | Bacterial Isolates | | | | | | | | |
|------------------|--------------------|-----------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|------------------|
| | B-1 | | | B-2 | | | B-4 | | |
| | At ₀ | At _f | % D _e | At ₀ | At _f | % D _e | At ₀ | At _f | % D _e |
| 10 | 0.376 | 0.152 | 59.57 | 0.375 | 0.155 | 58.66 | 0.376 | 0.101 | 73.13 |
| 50 | 0.463 | 0.191 | 58.74 | 0.462 | 0.185 | 59.95 | 0.462 | 0.155 | 49.51 |
| 100 | 0.545 | 0.221 | 59.44 | 0.545 | 0.195 | 64.22 | 0.545 | 0.189 | 65.32 |
| 150 | 0.602 | 0.260 | 56.81 | 0.601 | 0.301 | 49.91 | 0.601 | 0.298 | 50.41 |

At₀ = Initial absorbance at 450nm

At_f = Final absorbance at 450nm

%D_e = Percentage decolourization

Table-04 (c) Decolourization of red and green textile dye through fungal isolate

| Dye Concentration (mg/L) | Fungal isolate (F-1) | | | | | |
|--------------------------|----------------------|-----------------|------------------|-----------------|-----------------|------------------|
| | Red (540nm) | | | Green (450nm) | | |
| | At ₀ | At _f | % D _e | At ₀ | At _f | % D _e |
| 10 | 0.210 | 0.100 | 52.38 | 0.367 | 0.110 | 70.00 |
| 50 | 0.251 | 0.121 | 61.90 | 0.410 | 0.141 | 65.60 |
| 100 | 0.320 | 0.111 | 65.31 | 0.491 | 0.188 | 61.71 |
| 150 | 0.351 | 0.142 | 59.54 | 0.555 | 0.251 | 54.77 |

At₀ = Initial absorbance

At_f = Final absorbance

%D_e = Percentage decolourization

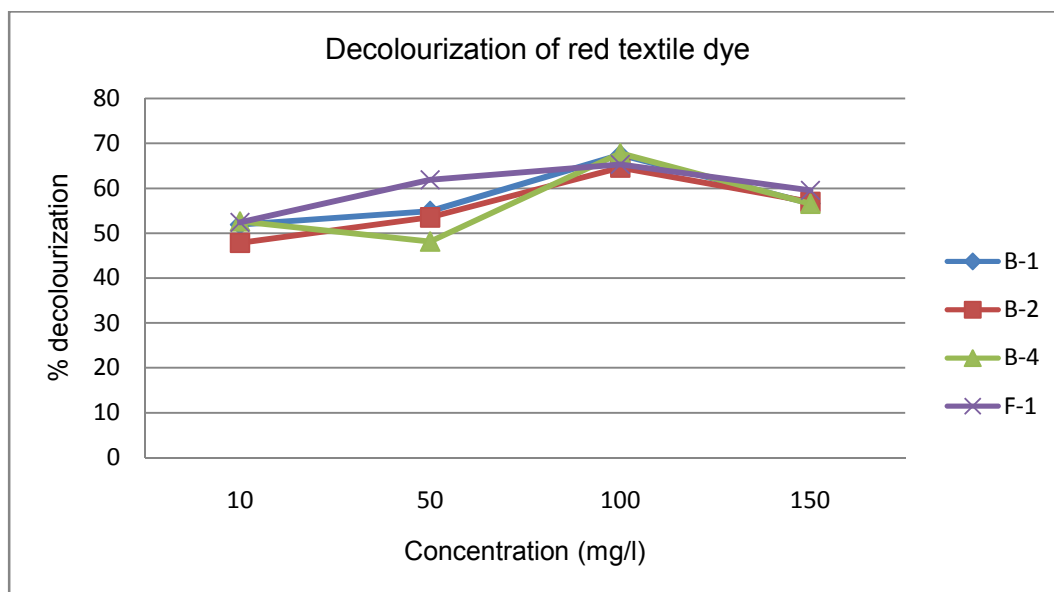


Figure-01 decolourization of red textile dye through indigenous microorganism

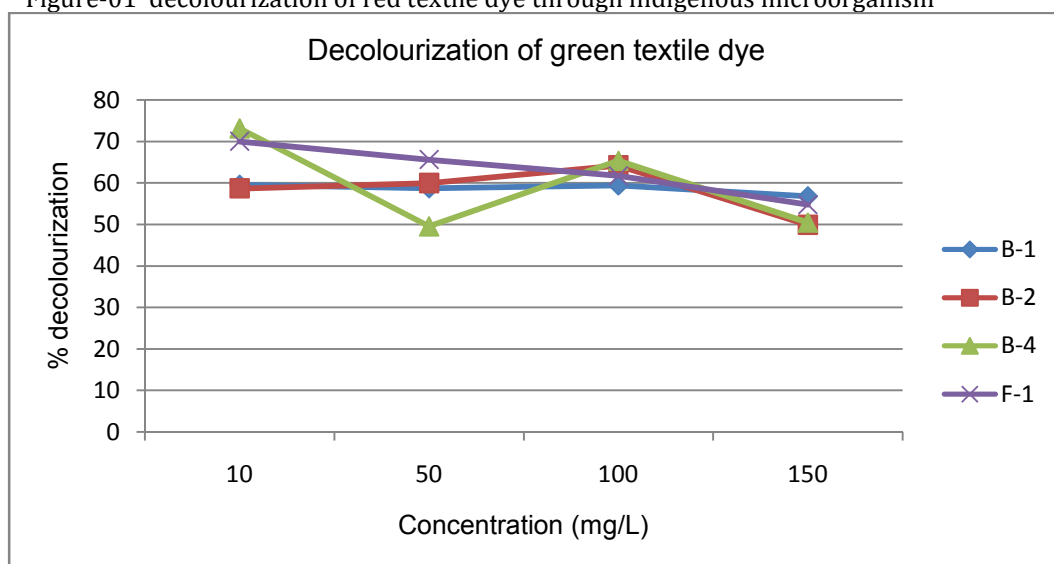


Figure-02 decolourization of green textile dye through indigenous microorganism

Although the decolourization of textile waste is a challenge for the wastewater treatment system, microbiological systems have a great potential to decolourize dye within a few hours. Development of efficient dye degradation requires a suitable strain and its use under favourable condition. The decolourization of textile dye effluents is a result of dye degradation mechanism [20,21, 22]. This degradation mechanism for color removal works in two different simultaneous steps: adsorption of dyes on the surface of the organisms and degradation of dyes by the enzymes produced by these organisms.

Different species use different enzymatic systems to decolourize the synthetic dye. In 2001, it was reported that various enzymatic mechanisms by which diverse categories of microorganisms bring about the degradation and mineralization of dyes [4]. The *Streptomyces* sp. uses extracellular peroxidases, *Pseudomonas* strain K24 uses oxygen-insensitive oxygen reductases system and various anaerobic sp. uses cytoplasmic enzymes for dye degradation.

The primary mechanism of decolorization in fungi is due to dye adsorption by mycelium with reduction of dye intensity in solution [23, 24] and degradation of the dye through their ligninolytic enzyme system. Further type of growth media also enhance the growth and degradation rate by fungi and on addition of carbon or other nutrient sources further increases decolorization process [25]. Moreover, the rate of dye removal can be linked with the available co-substrates [26] and with the exponential growth phase [16]. For this pilot plant experiment has been designed in which sugarcane and straw extract has been used as sole source of nutrient to observe the growth of microorganism and their decolourization potential.

Pilot Scale Experiment

Pilot scale experiment has also designed to evaluate the decolourization potential of indigenous organism in natural environment. After two weeks of incubation, the flask containing sugarcane extract showed significant microbial growth and showed decolourization up to 30% for red dye and 34% for green dye. The *Aspergillus* showed better growth in this media as compared to other bacterial isolate. The form of carbohydrate in sugarcane is sucrose and it is disaccharide, the fungi produce various amounts of enzymes which break disaccharide to monosaccharide i.e glucose to make it available as a source of energy.

CONCLUSION

The present results demonstrated that the indigenous microorganism has the ability to remediate the dye from the textile effluent. Therefore, as a preliminary step in the development of textile waste effluents treatment process using indigenous microbial species, the present study has shown that *Aspergillus*, *Pseudomonas* and *Bacillus sp.* has significant potential for dye decolourization and degradation. Further, it can be suggested that dye contaminated sites can potentially be recovered by a low cost bioremediation process with native bacterial and fungal species isolated from the textile effluent and dye disposal sites.

REFERENCES

1. Andleeb, S.; Atiq, N.; Ali, M. I.; Razi-UL-Hussain, R.; Shafique, M.; Ahmed, B.; Ghumro, P. B.; Hussain, M.; Hameed, A. and Ahmed, S. (2010). Biological treatment of textile effluent in stirred tank bioreactor'. Int. J. Agric. Biol. 12 (2) : 256 – 260.
2. Jadhav, J. P., Phugare, S. S., Dhanve, R. S. & Jadhav, S. B. (2010). Rapid biodegradation and decolorization of direct orange 39 (orange TGLL) by an isolated bacterium *Pseudomonas aeruginosa* strain BCH. Biodegradation. 21: 453-463.
3. Wagner, S. (1993). Improvement in products and processing to diminish environmental impact'. COTTECH conference, Raleigh NC.
4. McMullan G., Robinson T., Marchant R. and Nigam P. (2001). Remediation of dyes in textile effluent: a critical review on current treatment technologies with proposed alternatives, Biosource Technol., 77: 247-255.
5. Chung K.T. and Stevens S.E. (1993). Decolorization of azo dyes by environmental microorganisms and helminthes, Environ. Toxicol. Chem. 12: 2121-2132.
6. Zhang, F.; Yediler, A. Liang, X. and Kettrup, A. (2004). Effects of dye additives on the ozonation process and oxidation by-products: a comparative study using hydrolyzed C1 Reactive red 120'. Dyes pigments. 60:1-7.
7. Phugare, S. S., Kalyani, D.C., Surwase, S. N. & Jadhav, J. P. (2011). Ecofriendly degradation, decolorization and detoxification of textile effluent by a developed bacterial consortium. Ecotox. Environ. Safe. 74: 1288-96.
8. Olukanni O. D., Osuntoki, A. A. & Gbenle, G. O. (2006). Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria. Afr. J. Biotechnol. 5 (20): 1980-1984.
9. Ali, N., Hameed, A. & Ahmed, S. (2009). Physicochemical characterization and bioremediation perspective of textile effluent, dyes and metals by indigenous bacteria. J. Hazard. Mater. 164: 322-328.
10. Zhang, M., Chen, W., Chen, B., Chang, C., Hsueh, C., Ding, Y., Lin, K. & Xu, H. (2010). Comparative study on characteristics of azo dye decolorization by indigenous decolorizers. Bioresource Technol. 101: 2651-2656.
11. Ayed, L., Mahdhi, A., Cheref, A. & Bakhrouf, A. (2011). Decolorization and degradation of azo dye Methyl Red by an isolated *Sphingomonas paucimobilis*: Biototoxicity and metabolites characterization. Desalination. 274: 272-277.
12. Chen, B., Hsueh, C., Chen, W. & Li, W. (2011). Exploring decolorization and halotolerance characteristics by indigenous acclimatized bacteria: Chemical structure of azo dyes and dose-response assessment. J. Taiwan Inst. Chem. E. 42: 816-825.
13. Kirk T.K., Lamar R.T. and Glaser J.A. (1992). The Potential of White rot Fungi in Bioremediation. Biotechnology and Environmental Science Molecular Approaches, Mongkolsuk, S. (Ed.), Plenum Press, UK, 131-138.
14. Hensyl, W. R. (Ed.) (1994). Bergey's Manual of Systematic Bacteriology 9th Edition. Williams and Wilkins, Baltimore, Philadelphia, Hong Kong, London, Munich, Sydney, Tokyo.
15. Kalyani, D. C., Patil, P. S., Jadhav, J. P. & Govindwar, S. P. (2008). Biodegradation of reactive textile dye Red BLI by an isolated bacterium *Pseudomonas sp.* SUK1. Bioresource Technol. 99: 4635-4641.
16. Sumathi S. and Manju B.S. (2000). Uptake of reactive Textile dyes by *Aspergillus foetidus*. Enzyme Microb. Technol. 27(6):347-355
17. Ali, N.; Hameed, A.; Ahmed, S. and Khan, A. G. (2007). Decolorization of structurally different textile dyes by *Aspergillus niger* SA1'. World J. Microbiol. Biotechnol., 24:1067 – 1072.
18. Jin, X. C.; Liu, G. Q.; Xu, Z. H. and Tao, W. Y. (2007). Decolorization of a dye industry effluent by *Aspergillus fumigatus* XC6'. Appl. Microbiol. Biotechnol. 74 : 239 – 243.
19. Namdhari B.S., Rohilla S.K., Salar R.K., Gahlawat S.K., Bansal P. and Saran A.K. (2012). Decolorization of Reactive Blue MR, using *Aspergillus* species Isolated from Textile Waste Water Department of Biotechnology, Chaudhary Devi Lal University, Sirsa, INDIA.
20. Glenn, J. K. and Gold, M. H. (1983). Decolorization of several polymeric dyes by the lignin degrading basidiomycete *phanerochaete chrysoporium*. Appl. Environ. Microbiol. 4: 1741 – 1747.
21. Wesenberg, D.; Kyriakides, I. and Agathos, S. N. (2003). 'White-rot fungi and their enzymes for the treatment of industrial dye effluents'. Biotechnol. Adv., 22: 161-187.

22. Ramya, M.; Anusha, B.; Kalavathy, S. and Devilaksmi, S. (2007). 'Biodecolorization and biodegradation of reactive blue by *Aspergillus* sp', *Afri. J. Biotechnol.* 6 (12):1441 – 1445.
23. Fu Y. and Viraraghvan T. (2002). Dye biosorption sites in *Aspergillus niger*, *Biores. Technol.*, 82: 139-145.
24. Knapp J.S., Newby P.S. and Reece L.P. (1995). Decolorization of dyes by wood rotting basidiomycete fungi, *Enzyme Microbiol. Technol.*, 17: 664-668
25. Swamy, J. and Ramsay J.A. (1999). The evaluation of white rot fungi in the decoloration of textile dyes, *Enzyme Microbiol. Technol.*, 24:130-137
26. Ali N., Ikramullah L.G., Hameed A. and Ahmed S. (2008). Decolorization of Acid red 151 by *Aspergillus niger* SA1 under different physicochemical conditions, *World J. Microbiol.* 24: 1099-1105.

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