

BIODEGRADATION AND PHYSICO-CHEMICAL CHANGES OF TEXTILE EFFLUENT BY VARIOUS FUNGAL SPECIES

N. RAMAMURTHY*, S. BALASARASWATHY*, P. SIVASAKTHIVELAN**

*Department of Physics, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India
e-mail: drnramamurthy@gmail.com

**Department of Agricultural Microbiology, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India

Abstract. Six different fungal species were selected to study the biodegradation of textile effluent. The physico-chemical properties of the textile effluent showed drastic changes during the fungal treatment. The pH of all the treated effluents attained neutrality; the total dissolved solids and Electrical conductivity reduced at the end of the fungal treatment. The turbidity of the treated effluents increased due to biomass production. UV-VIS spectrophotometric studies were carried over a range 200–800 nm and the results confirmed the biodegradation of textile effluent on fungal treatment as evidenced by the disappearance of the peak at visible region.

Key words: Textile effluent, physico-chemical parameters, biodegradation, fungal treatment, UV-VIS.

INTRODUCTION

Industrial effluents are more complex, containing a mixture of dyes and other chemicals. In a typical dyeing plant, the major chemical usage includes dyes/pigments, finishing agents, acids/alkali, surfactants and auxiliaries [4, 18]. Textile industries consume nearly two-thirds of the total dyestuff market and huge volumes of water and chemicals are utilized for various processing of textiles [5, 23]. The textile and dyestuff manufacturing industries are two major polluting sources for the release of synthetic dyes. Almost 100 liters of water are required to process 1kg of dyed fabrics [1, 22]. Moreover up to 15% of applied dyestuffs are lost to the effluents due to an inefficient dyeing process [10, 15].

More than 100,000 dyes are commercially available with a production of over 7×10^5 metric tons per year [5, 7, 24]. The discharge of these highly colored synthetic dye effluents results in serious environmental problems. Even a very

Received: January 2011;
in final form April 2011.

small concentration of dye in water is highly visible. It affects not only the aesthetic merit of water, it also reduces water transparency which in turn affects the photosynthetic activity, thus causing severe damage to the ecosystem [2, 12, 21]. Industrial dyes have been designed and synthesized to be highly resistant to washing, chemical agents including solvents and environmental factors such as the action of sunlight, water and microbial attack [14, 16, 25]. In aquatic systems, the dyes undergo various reactions and the variations in their chemical structures result in the formation of new xenobiotic compounds, which may be more or less toxic than their parental compounds [11, 17].

Several physico-chemical methods such as adsorption, irradiation, ion-exchange, oxidative process, ozonation, coagulation and so on have been used to decolorize textile effluent but these methods are rather costly, inefficient and sometimes produce hazardous by-products and sludge [6, 9, 20, 23]. Although decolorization is a challenging process to the textile industry, the great potential of microbial decolorizing can be adopted as an effective tool and they are considered to be sustainable and eco-friendly [3].

In biological removal of color from effluents, the use of fungi or their oxidative enzymes constitute an alternative mode of treatment in aerobic conditions. The decolorization can be achieved by two mechanisms, either by adsorption of the dye to the fungal mycelium or by oxidative degradation of the dye molecules [16]. The decolorization by fungi using an oxidative mechanism has the advantage of giving products that are less toxic than the initial dye. Fungal degradation of dyes was reported to be catalyzed by extracellular enzymes called as ligninolytic peroxidases [26]. Fungi from basidiomycetes group, known as white rot fungi, are a heterogeneous group of microorganisms but they have in common the capacity to degrade lignin as well as other wood components [21]. Ligninolytic fungi can mineralize xenobiotics to CO₂ and water through their highly oxidative and non-specific ligninolytic systems, which are also responsible for the decolorization and degradation of a wide range of dyes [3, 9]. Many studies were devoted to biodecolorization of the textile dyes, but less attention has been paid to textile dye baths in which the presence of salts and high dye concentration may be inhibitory to biological agents.

MATERIAL AND METHODS

SAMPLE COLLECTION

The textile effluent was collected from the discharge tanks of a textile mill located in Madurai district, Tamil Nadu, India. The effluent was sampled in dry, sterile, plastic cans and stored in the incubator at 15°C.

FUNGAL CULTURES

Six fungi, namely *Rhizopus spp.*, *Pencillium spp.*, *Aspergillus niger*, *Trichoderma viride*, *Trametes hirsuta* and *Trametes versicolor*, were selected for textile effluent degradation. *Trametes hirsuta* and *Trametes versicolor* were procured from microbial type culture collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Other fungi were obtained from the Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

CULTURE MEDIA

Stock cultures of *T. hirsuta* and *T. versicolor* were maintained on yeast glucose agar at 30 °C as mentioned by MTCC. Other four fungi were maintained on nutrient agar at 30 °C. Subcultures were made periodically. Fresh cultures were grown separately in 80 mL of sabouraud's dextrose broth (liquid medium without agar). The medium contains 40 g of dextrose and 10 g of peptone per liter of distilled water. The inoculated PET bottles were incubated at 25 °C ± 1°C for 10 days.

PHYSICO-CHEMICAL AND DECOLORIZATION STUDIES

After 10 days of incubation, 50 mL of each culture broth were drawn and added separately to six different PET bottles, each containing 1 liter of textile effluent. Raw effluent was taken as control. The bottles were aerated for 3 hours a day at room temperature. To know the effects of fungi on the physico-chemical parameters of textile effluent, periodic observations (at 24 h interval) on pH, Electrical conductivity (EC), Total Dissolved Solids (TDS), turbidity and dissolved oxygen (DO) were made. At intervals of 48 hours, 5 mL of samples were drawn and analyzed spectrophotometrically using UV-VIS spectrophotometer over a range of 200–800 nm.

RESULTS AND DISCUSSION

The physico-chemical parameters were carried out at an interval of 24 hours. The results were plotted as graph and discussed. Drastic changes were observed on the 6th and 7th day of fungal treatment. In the figures, the samples 1, 2, 3, 4, 5, 6, 7 indicate raw effluent, *Rhizopus spp.*, *Pencillium spp.*, *A. niger*, *T. viride*, *T. hirsuta*, *T. versicolor* treated effluents. Raw effluent was used as the control sample.

EFFECTS OF FUNGI ON pH OF THE EFFLUENT

The pH of the effluent affects the physico-chemical properties of receiving water which in turn adversely affects the aquatic life and human beings. This also changes soil permeability which results in polluting underground resources of water. In this present work, all the fungi tolerated the alkaline nature of the textile effluent (pH 11.5), decreased the pH to neutral and almost maintained the neutrality till the degradation process completes. In the beginning the pH varies gradually from 11.5 to 9 up to 6th day of the treatment. On the 7th day of treatment, *Pencillium spp.*, *Aspergillus niger* and *Trametes hirsuta* treated effluent showed a drastic fall in the pH. *Pencillium spp.* reduced the pH from 9.10 to 7.10. *A. niger* decreased the pH from 9.46 to 6.92 whereas *T. hirsuta* altered the pH from 9.39 to 6.62. *Rhizopus spp.*, *Trichoderma viride* and *Trametes versicolor* showed sudden change in the pH of the effluent on the 8th day of treatment. *Rhizopus spp.* decreased the pH from 9.73 to 7.36, the pH of *T. viride* treated effluent dropped from 9.41 to 7.09 and *T. versicolor* reduced the pH from 9.36 to 7.06. Almost on 7th and 8th day of treatment, the effluent attained neutrality.

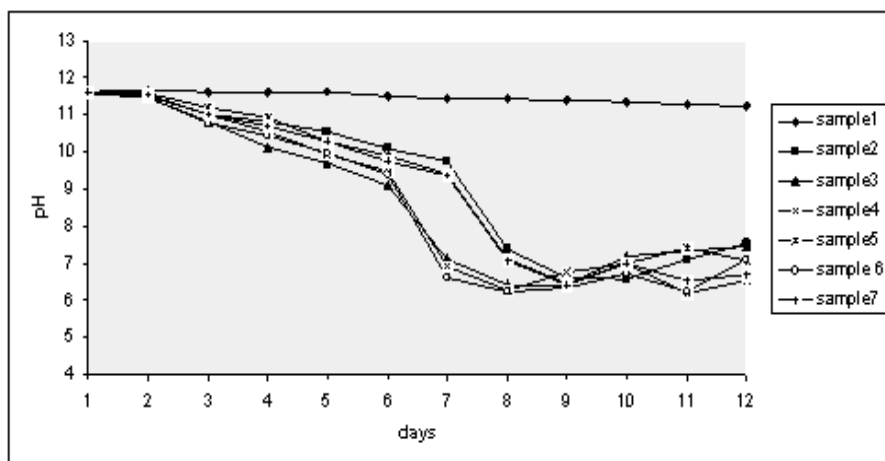


Fig. 1. Variation of pH of the effluent on different fungal treatments.

At the completion of degradation treatment, the pH of *Rhizopus spp.*, *Pencillium spp.*, *A. niger*, *T. viride*, *T. hirsuta* and *T. versicolor* treated effluent were 7.54, 7.43, 6.54, 7.06, 7.12 and 6.69 respectively. Aeration did not cause any change in the pH of control sample. The alkalinity of the effluent was neutralized by the extracellular enzymes secreted by the fungi which have greater affinity towards electron acceptors.

Rani Faryal *et al.* reported that the *Rhizopus spp.*, and *Aspergillus spp.*, were predominant genera found in highly alkaline effluent [19].

EFFECTS OF FUNGI ON ELECTRICAL CONDUCTIVITY OF THE EFFLUENT

Electrical conductivity gives the measure of water conductivity as well as the indication of the level of inorganic constituents in water. The electrical conductivity (EC) of the effluent decreased initially up to day 6 of the treatment and showed a slight increase at the end. The EC of the control sample showed a gradual decrease from the initial value of 5170 $\mu\text{S}/\text{cm}$ to 4490 $\mu\text{S}/\text{cm}$. *Rhizopus* reduced the EC to 3840 $\mu\text{S}/\text{cm}$ (minimum) on the 6th day and the value increased to 4000 $\mu\text{S}/\text{cm}$ at the end of the treatment.

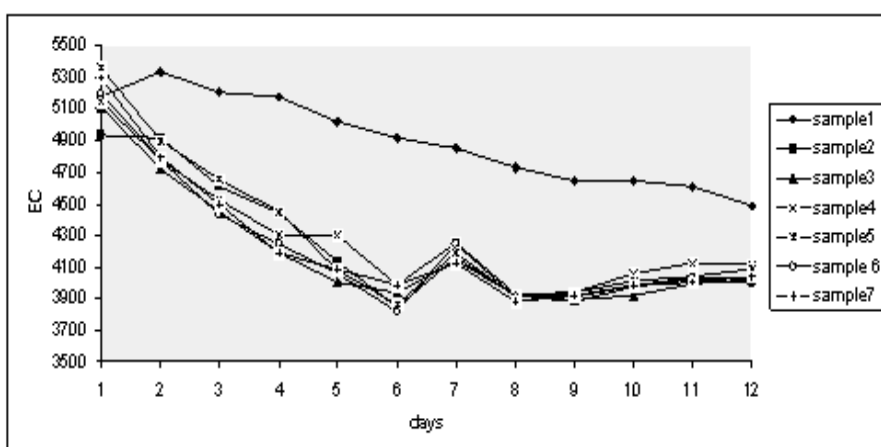


Fig. 2. Changes in EC on different fungal treatments.

Pencillium spp. treated effluent reached its minimum of 3940 $\mu\text{S}/\text{cm}$ and then increased to 4020 $\mu\text{S}/\text{cm}$. The EC of *A. niger* treated effluent decreased up to 6th day of treatment to a value of 3980 $\mu\text{S}/\text{cm}$ and at the completion period, it increased to 4110 $\mu\text{S}/\text{cm}$. *T. viride* reduced the EC to 3860 $\mu\text{S}/\text{cm}$ during the treatment and later increased to 4090 $\mu\text{S}/\text{cm}$. The conductivity of *T. hirsuta* treated effluent reached its minimum of 3820 $\mu\text{S}/\text{cm}$ on 6th day and the value increased to 4030 $\mu\text{S}/\text{cm}$. *T. versicolor* decreased the EC value to 3980 $\mu\text{S}/\text{cm}$ on 6th day and it increased to 4040 $\mu\text{S}/\text{cm}$ at the end of the treatment.

EFFECTS OF FUNGI ON TOTAL DISSOLVED SOLIDS

High total dissolved solids (TDS) are one of the major sources of sediments which reduce the light penetration into water and ultimately decrease the photosynthesis process of aquatic flora. TDS followed the same trend as EC. On the 6th day of treatment, the TDS (mg/L) of *Rhizopus*, *Pencillium*, *A. niger*, *T. viride*, *T. hirsuta* and *T. versicolor* treated effluent reached their minimum of 2688, 2618, 2646, 2702, 2674 and 2646. At the end of the treatment, the values

arose to 2800, 2814, 2877, 2863, 2821 and 2828 mg/L respectively. The decrease in TDS was due to the utilization of dissolved solids by the degrading fungi.

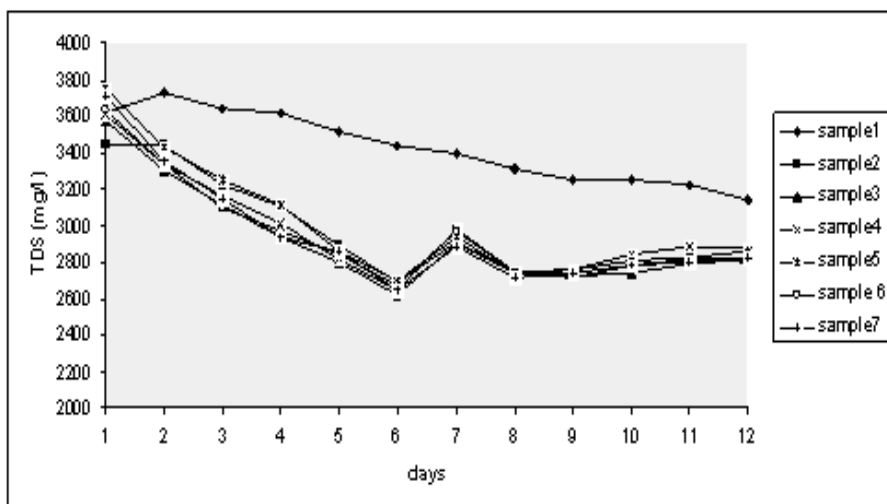


Fig. 3. Changes in TDS of the effluent on different fungal treatments.

EFFECTS OF FUNGI ON TURBIDITY OF THE EFFLUENT

During the degradation process, the turbidity showed a drastic increase in all the fungal treatment. *Rhizopus spp.* increased the turbidity of the effluent from an initial value of 30 NTU to 58.8 NTU on the final day of treatment. The turbidity of *Pencillium* treated effluent increased from 33.8 NTU to 62.0 NTU. *Pencillium spp.* treated effluent showed a steep increase on the final day, i.e. from 41.9 to 62.0 NTU. *A. niger* treated effluent showed its maximum turbidity of 64.6 NTU on 9th day when the pH changed from 7 to 6.26 and then the value of turbidity fluctuated. The turbidity of the effluent treated with *T. viride* showed a gradual increase from 27.7 to 49 NTU. *T. hirsuta* showed a maximum turbidity of 83.5 NTU at the end whereas it showed a rapid increase from 35.9 NTU to 63.0 NTU on 7th day when the pH changes from 9.39 to 6.62. *T. versicolor* increased the turbidity to a maximum of 62.8 NTU on day 9 when the pH was 6.4. On the final day of treatment, the turbidity reduced to 43.5 NTU.

Increase in turbidity was due to many factors such as biomass and extracellular enzymes production, production of metabolites on degradation and also as the result of microemulsion formation [18]. Sukumar *et al.*, 2009 reported that the biomass production of *T. versicolor* was maximum at pH 6.5 when compared to neutral pH [24]. In all the cases when the pH reached the slight acidic nature, $\text{pH} \leq 6.6$, the turbidity got increased. This indicates that pH 6–6.5 gives a favorable condition for the biomass production (increase in turbidity).

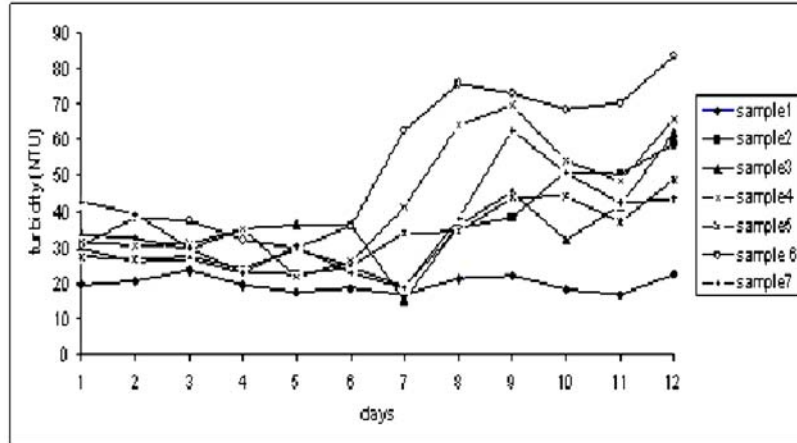


Fig. 4. Changes in turbidity of the effluent on different fungal treatments.

EFFECTS OF FUNGI ON DISSOLVED OXYGEN

The amount of oxygen currently dissolved in a body of water is termed as dissolved oxygen (DO). This gas is constantly entering the water from two main sources: the atmosphere and photosynthesis. Decay of organic waste consumes a lot of oxygen. Lina *et al.*, [13], reported that the dissolved oxygen concentration is very low in the effluent which ranges from 1 to 3 mg/L. These concentrations are associated with high temperature and high concentration of dissolved solids. Thus, this indicates the need of aeration to prevent septic condition. In the present study, aeration was applied to avoid depletion of oxygen in the effluent. The dissolved oxygen ranged from 6 to 8 mg/L in all treated effluents.

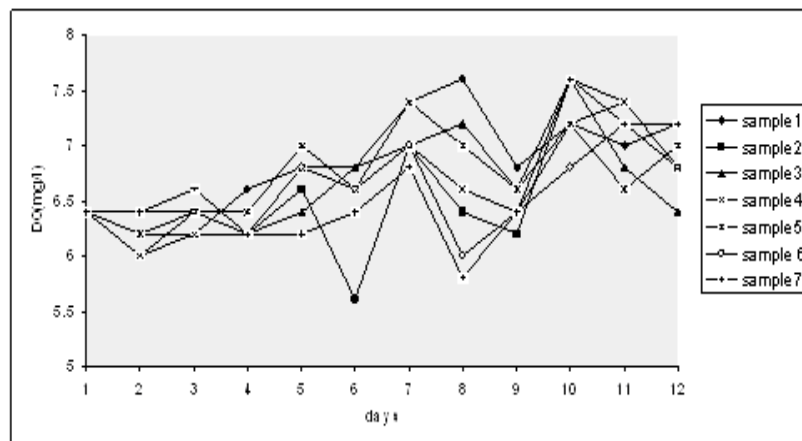


Fig. 5. Variations in DO of the effluent on different fungal treatments.

DECOLORIZATION STUDIES

The samples obtained at an interval of 48 hours were subjected to spectral scan between 200–800 nm. The scan of the raw effluent showed two peaks, one at visible region (480 nm) and another peak at UV region (219 nm).

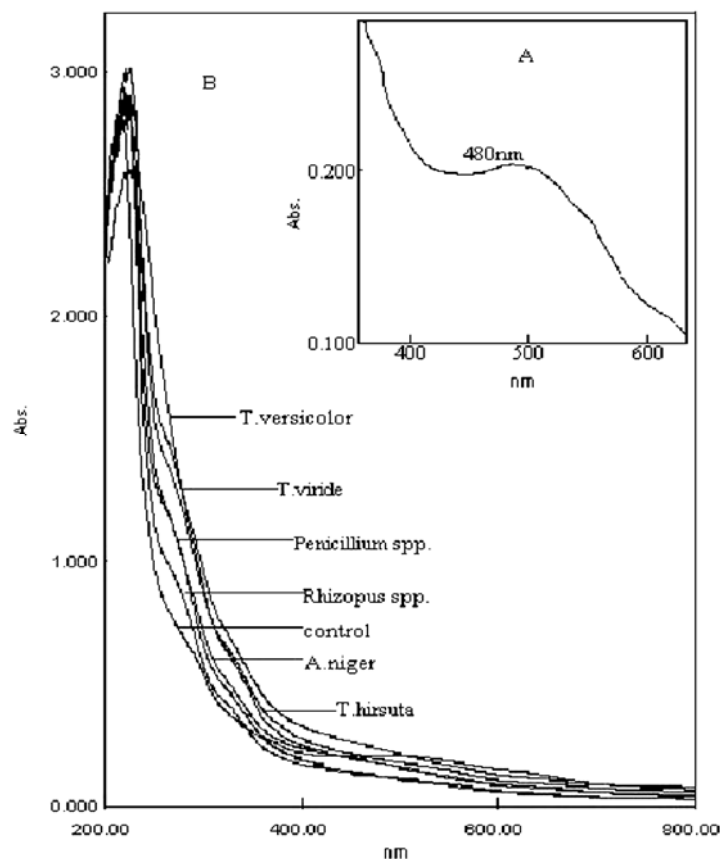


Fig. 6. A. Peak at 480 nm; B. Spectral scan of control and different fungal treated effluents at 48 h.

Within 48 hours of fungal treatment, the peak at 480 nm disappeared; the peak at 219 nm in the UV region remained as such and as the degradation progresses, a shoulder was obtained near 270 nm in all the cases except for *T. versicolor* treated effluent. On the final day of treatment, the overall absorbance increased due to the darkening of enzymatic treatment of the effluent [27], but no peak was obtained at 480 nm. Gurulakshmi *et al.* [8] and Patil *et al.* [17] reported that if dye removal is attributed to dye biodegradation, either the major light absorbance peak will completely disappear or a new peak will appear. The present

study reflects the degrading ability of the six fungi and the dye removal was due to biodegradation.

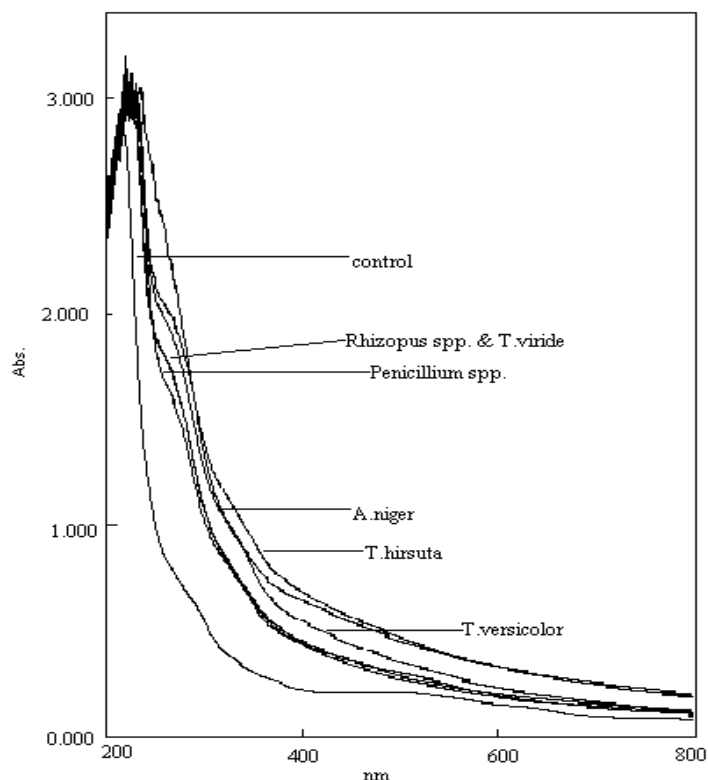


Fig. 7. Spectral scan of control and different fungal treated effluent at the end of the treatment.

CONCLUSION

The physico-chemical characteristics of the effluent such as pH, EC and TDS were much reduced after the fungal treatment. After the fungal treatment, the effluents attained neutrality and almost maintained the condition till the end of the treatment. The turbidity of the effluent (all cases) showed a steep increase due to biomass production in the pH range of 6 to 6.6. As all the samples were aerated, the dissolved oxygen content was more or less between 6 and 8 mg/L. Thus the changes in the pH and other parameters show that the acidic pH favors fungal reproduction and the large production of enzymes. These enzymes are the cause of color removal in the effluent. UV-VIS spectroscopic studies clearly indicated that the dye removal was due to biodegradation. Further studies have to be carried out to identify the metabolites present in the effluent after degradation.

REFERENCES

1. ABADULLA, E., T. TZANOV, S. COSTA, K. ROBRA, A. CAVACO-PAULO, G.M. GUBITZ, Decolorization and detoxification of textile dyes with a Laccase from *Trametes hirsuta*, *Applied and Environmental Microbiology*, 2000, **66**, No.8, 3357–3362.
2. BAFANA, A., T. CHAKRABARTI, S. SARAVANADEVI, Azoreductase and dye detoxification activities of bacillus *Velezensis* strain AB, *Appl. Microbiol. Biotechnol.*, 2008, **77**, 1139–1144.
3. BHATTI, H.N., N. AKRAM, M. ASGHER, Optimization of culture conditions for enhanced decolorization of Cibaron Red FN-2BL by *Schizophyllum commune* IBL-6, *Appl. Biochem. Biotechnol.*, 2008, **149**, 255–264.
4. COUTO, S.R., J.L. TOCA-HERRERA, Laccases in the textile industry, *Biotechnology and Molecular Biology Review*, 2006, **1**(4), 115–120.
5. COUTO, S.R., J.L. TOCA-HERRERA, Industrial and biotechnological applications of Laccases: A review, *Biotechnology Advances*, 2006, **24**, 500–513.
6. ENAYATZAMIR, K., H.A. ALIKHANI, B. YAKHCHALI, F. TABANDEH, S.R. COUTO, Decoloration of azo-dyes by *Phanerochaete chrysosporium* immobilized into alginate beads, *Environ. Sci. Pollut. Res.*, 2009, **17**, 145–153.
7. FU, Y., T. VIRARAGHAVAN, Fungal decolorization of dye wastewaters: a review, *Bioresource Technology*, 2001, **79**, 251–262.
8. GURULAKSHMI, M., D.N.P. SUDARMANI, R. VENBA, Biodegradation of leather acid dye by *Bacillus subtilis*, *Advanced Biotech.*, 2008, 12–18.
9. JIN, X., G. LIU, Z. XU, W. TAO, Decolorization of a dye industry effluent by *Aspergillus fumigatus* XC6, *Appl. Microbiol. Biotechnol.*, 2007, **74**, 239–243.
10. KEHARIA, H., D. MADAAMWAR, Transformation of textile dyes by white rot fungus *Trametes versicolor*, *Applied Biochemistry and Biotechnology*, 2002, **103**, 99–108.
11. KHELIFI, E., H. GANNOUN, Y. TOUHAMI, H. BOUALLAGUI, M. HAMDY, Aerobic decolorization of the indigo dye-containing textile wastewater using continuous combined bioreactors, *Journal of Hazardous Materials*, 2008, **152**, 683–689.
12. KHELIFI, E., H. BOUALLAGUI, Y. TOUHAMI, J. GODON, M. MAMDY, Bacterial monitoring by tools of a continuous stirred tank reactor treating textile wastewater, *Bioresource Technology*, 2009, **100**, 629–633.
13. LINA, N., ABU-GHUNMI, A.I. JAMRAH, Biological treatment of textile wastewater using sequencing batch technology, *Environmental Modeling and Assessment*, 2006, **11**, 333–343.
14. MEENU CHHABRA, SAROJ MISHRA, T.R. SREEKRISHNAN, Mediator-assisted decolorization and of textile dyes/dye mixture by *Cyathus bulleri* Laccase, *Appl. Biochem. Biotechnol.*, 2008, **151**, 587–598.
15. MISHRA, S.S., V.S. BISARIA, Production and characterization of Laccase from *Cyathus bulleri* and its use in decolorization of recalcitrant textile dyes, *Appl. Microbiol. Biotechnol.*, 2006, **71**, 646–653.
16. MOHORCIC, M., S. TEODOROVIC, V. GOLOB, JOZEFA FRIEDRICH, Fungal and enzymatic decolorization of artificial textile dye baths, *Chemosphere*, 2006, **63**, 1709–1717.
17. PATIL, P.S., U.U. SHEDBALKAR, D.C. KALYANI, J.P. JADHAV, Biodegradation of reactive blue 59 by isolated bacterial consortium PMB11, *J. Ind. Microbiol. Biotechnol.*, 2008, **35**, 1181–1190.
18. RAMSAY, A.J., C. GOODE, Decoloration of a carpet dye effluent using *Trametes versicolor*, *Biotechnology Letters*, 2004, **26**, 197–201.
19. RANI, F., A. HAMEED, Isolation and characterization of various fungal strains from textile effluent for their use in bioremediation, *Pak. J. Bot.*, 2005, **37**(4), 1003–1008.
20. REN, S., J. GUO, G. ZENG, G. SUN, Decolorization of triphenylmethane, azo and anthroquinone dyes by a newly isolated *Aeromonas hydrophila* strain, *Appl. Microbiol. Biotechnol.*, 2006., **72**, 1316–1321.

21. SATHIYAMOORTHY, P., S. PERIYARSELVAM, A. SASIKALAVENI, K. MURUGESAN, P.T. KALAICHELVAN, Decolorization of textile dyes and their effluents using white rot fungi, *African journal of Biotechnology*, 2007, **6**(4), 424–429.
22. SELVAM, K., K. SWAMINATHAN, K. CHAE, Microbial decolorization of azo dyes and dye industry effluent by *Fomes lividus*, *World Journal of Microbiology*, 2003, **19**, 591–593.
23. SIRIANUNTAPIBOON, S., P. SRISORNAK, Removal of disperse dyes from textile wastewater using bio-sludge, *Bioresource Technology*, 2007, **98**, 1057–1066.
24. SUKUMAR, M., A. SIVASAMY, G. SWAMINATHAN, *In situ* biodecolorization kinetics of Acid Red 66 in aqueous solutions by *Trametes versicolor*, *Journal of Hazardous Materials*, 2009, **167**(1–3), 660–663.
25. WESENBERG, D., I. KYRIAKIDES, S.N. AGATHOS, White rot fungi and their enzymes for the treatment of industrial dye effluents, *Biotechnology Advances*, 2003, **22**, 161–187.
26. ZEROUAL, Y., B.S. KIM, M.W. YANG, M. BLAGHEN, K.M. LEE, Decolorization of some azo-dyes by immobilized *Geotrichum sp.* Biomass in fluidized bed reactor, *Appl. Biochem. Biotechnol.*, 2007, **142**, 307–316.
27. ZILLE, A., B. GORNACKA, A. REHOREK, A. CAVACO-PAULO, Degradation of azo dyes by *Trametes villosa* Laccase over long periods of oxidative conditions, *Applied and Environmental Microbiology*, 2005, **71**(11), 6711–6718.