© 2011 Triveni Enterprises
Vikas Nagar, Lucknow, INDIA
editor@jeb.co.in
Full paper available on: www.jeb.co.in

J. Environ. Biol.
33, 667-671 (2012)
ISSN: 0254-8704
CODEN: JEBIDP

Biological decolorization of textile dyes from isolated microfungi

Author Details

Halil Biyik	Adnan Menderes University, Faculty of Science and Arts, Department of Biology, Aydin, 09100, Turkey
Gamze Basbulbul	Adnan Menderes University, Faculty of Science and Arts, Department of Biology, Aydin, 09100, Turkey
Fatih Kalyoncu (*Corresponding author*)	Celal Bayar University, Faculty of Science and Arts, Department of Biology, Manisa, 45140, Turkey e-mail: fatihkalyoncu@hotmail.com
Erbil Kalmis	Ege University, Faculty of Engineering, Department of Bioengineering, Izmir, 35100, Turkey
Erman Oryasin	Adnan Menderes University, Faculty of Science and Arts, Department of Biology, Aydin, 09100, Turkey

Publication Data

Paper received:
11 January 2011

Revised received:
20 May 2011

Accepted:
14 June 2011

Abstract

In this study, biological decolorization of two textile dyestuff (Benazol black ZN and Cibacron black W-NN) was comparatively studied using 22 microfungi strains isolated from polluted industrial soil areas. The initial dye concentrations in the medium were 250 and 500 mg l^{-1}. Benazol black ZN was the best decolorized by *Haematonectria haematococca* (HH1) (36.0%) and Cibacron black W-NN was the best decolorized by *Aspergillus niger* (AN1) (33.0%) at 250 mg l^{-1} dye concentration. At 500 mg l^{-1} dye concentration for two different dyes all microfungi strains used showed weak decolorization rates, maximum 13.0% for Benazol black ZN and 6.0% for Cibacron Black W-NN.

Key words

Microfungi, Biological decolorization, Benazol black ZN, Cibacron black W-NN

Introduction

Synthetic dyes are extensively used in a number of industries, such as textile dyeing or paper printing. The treatment of wastewater from textile and dyestuff industries is one of the most challenging. Recently, new and tighter regulations coupled with increased enforcement concerning wastewater discharges have been forced in many countries. This tight legislation, in conjunction with international trade pressures, such as increasing competition and the introduction of eco-labels for textile products on the European and US markets, has been threatening the very survival of the textile industry in many industrialized countries.

Commonly applied treatment methods for colour removal from coloured effluents consist of integrated processes involving various combinations of biological, physical and chemical decolorization methods (Galindo and Kalt, 1999; Robinson *et al.,* 2001; Azbar *et al.,* 2004). These integrated treatment methods have limited efficiency and suffered from several drawbacks such as high amounts of chemical usage and/or sludge generation, costly infrastructure requirements and/or high operating expenses. Conventional wastewater treatment plants relying on activated sludge systems are not adequate for the treatment of textile mill effluents, since the use of bacteria in the biological treatment of dye effluents may result in the generation of colourless, dead-end aromatic amines, which are generally more toxic than the parent compounds (Banat *et al.,* 1996). In view of the need for a technically and economically satisfying treatment technology, a flurry of emerging technologies (*e.g.* biological processes, granular activated carbon filtration, foam floatation, electrolysis, photocatalysis, biosorption and Fenton oxidation) are being proposed and tested at different stages of commercialization (Kalmis *et al.,* 2008).

Although the decolorization is a challenge for textile industry as well as for wastewater treatment systems, the literature suggest that there is a great potential for developing microbiological decolorization systems with total colour removal in some cases within few hours (Balan and Monterio, 2001). Development of efficient dye degradation requires a suitable strain and its use under favourable conditions to realize the degradation potential. In recent years there has been an intensive research on fungal decolorization of dye wastewater. It is turning into a promising alternative to replace or supplement present treatment processes. The most studied fungus

is the white rot fungus *Phanerochate chrysosporium*, which is able to decolorize various dyes (Ramya *et al.,* 2007). The use of macrofungus species of the genera *Pleurotus*, *Bjerkandera*, *Trametes*, *Polyporus* and *Phellinus* and microfungus species of the genera *Aspergillus*, *Trichoderma*, *Penicillium* and *Rhizopus* have been also investigated (Zheng *et al.,* 1999; O'Mahony *et al.,* 2002; Revankar *et al.,* 2006; Ramya *et al.,* 2007). In fungal decolorization of dye wastewater, these fungi can be classified into two kinds according to their life state: living cells to biodegrade and biosorb dyes and dead cells (fungal biomass) to adsorb dyes. For living cells, the major mechanism is biodegradation because they can produce the lignin modifying enzymes, laccase, manganese peroxidase and lignin peroxidase to mineralize synthetic lignin or dyes. For dead cells, the mechanism is biosorption, which involves physico-chemical interactions, such as adsorption, deposition and ion exchange (Fu and Viraraghavan, 2001).

The present study aimed at using some microfungi species that isolated from polluted industrial areas in three cities from Turkey for decolorization of Benazol black ZN and Cibacron black W-NN in liquid system.

Materials and Methods

Fungal strains, culture and analysis: The decolorization study was carried out with 22 strains of microfungi. All the strains used in this study were isolated from soil that polluted by industrial wastewaters in Aydin, Izmir and Manisa city in Turkey. All the strains were deposited in the Department of Biology, Faculty of Science and Arts, Adnan Menderes University, Turkey.

All strains used in this study were cultured on potato dextrose agar (PDA) for one week at 25°C. Mycelial plugs (diameter 6 mm) were used as inoculum when the plates were fully covered with the mycelia. Three mycelial plugs were transferred to 250 ml Erlenmeyer flasks containing 50 ml of liquid Kirk's basal media (1.25 g glucose, 0.036 g urea, 2 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.099 g $CaCl_2$ in 1000 ml distilled water) (Tien and Kirk 1988) and Benazol black ZN and Cibacron black W-NN dyes. The dye concentrations chosen were 250 and 500 mg l^{-1}. Liquid media were sealed by cotton plugs and autoclaved at 121°C for 15 min. They were kept in static culture in an incubator at 25°C for at least 30 days (Kalmis *et al.,* 2008). During the incubation period, static culture was shaken gently once a day to avoid fungal mat formation. Decolorization of

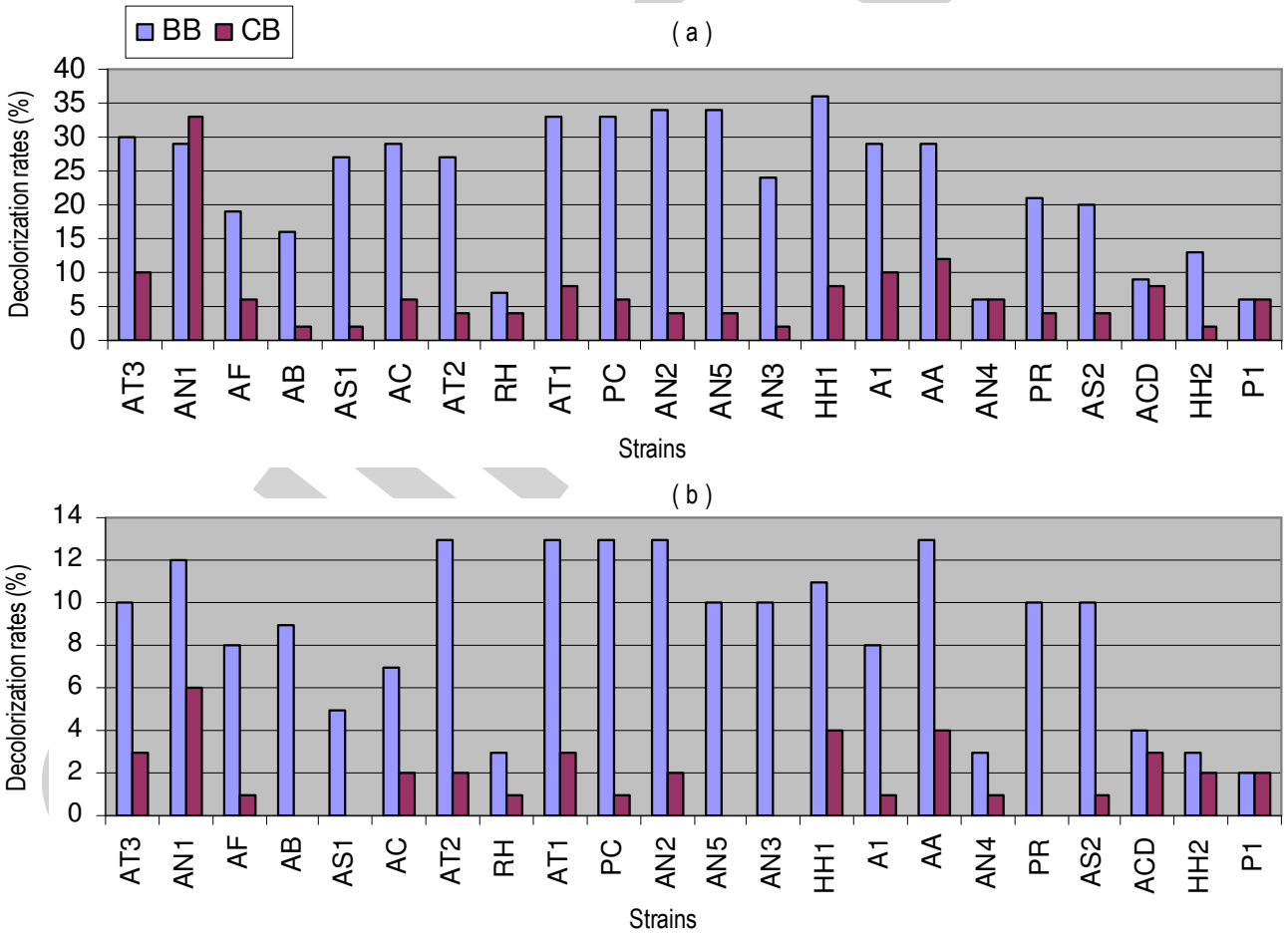

Fig. 1 (a) and (b): Decolorization rates of two different textile dyes Benazol Black ZN (BB) and Cibacron Black W-NN (CB) at (a) 250 mg l^{-1} and (b) 500 mg l^{-1} concentration

Table - 1: Effect of various dye concentrations on biomass, glucose concentrations and pH for Benazol black ZN

	Dye concentrations (mg l^{-1})								
	0	250	500	0	250	500	0	250	500
	Biomass* ¨(mg)			Residual glucose concentration*¨			pH* α		
Strains									
Absidia sp.	8.0	8.0	9.0	6.134	5.005	7.800	4.96	4.68	4.62
Acremonium sp.	8.0	10.0	13.0	3.510	4.620	5.380	4.48	4.20	4.08
A. alternata	8.0	11.0	9.0	4.100	5.197	5.172	4.50	4.50	4.33
Alternaria sp.	7.0	9.0	7.0	3.550	5.540	6.810	4.83	4.70	4.51
A. candidus	9.0	14.0	15.0	5.400	7.012	7.120	5.04	4.79	4.75
A. fumigatus	9.0	10.0	14.0	2.020	5.755	6.989	4.90	4.62	4.47
A. niger (AN1)	8.0	9.0	8.0	5.180	5.210	7.675	4.68	4.61	4.40
A. niger (AN2)	9.0	13.0	10.0	5.030	4.000	5.620	4.73	4.43	4.32
A. niger (AN3)	8.0	11.0	9.0	5.255	3.218	6.409	4.74	4.58	4.40
A. niger (AN4)	12.0	18.0	16.0	3.840	6.690	6.820	4.34	4.55	4.11
A. niger (AN5)	10.0	14.0	10.0	4.220	3.129	3.670	4.73	4.60	4.40
(AS1)	8.0	10.0	9.0	5.000	4.106	5.690	4.65	4.37	4.41
(AS2)	10.0	11.0	15.0	1.890	3.879	5.450	3.70	3.60	3.50
A. terreus (AT1)	9.0	11.0	9.0	3.140	3.850	3.900	4.76	4.63	4.43
A. terreus (AT2)	8.0	12.0	10.0	5.160	4.750	4.875	4.87	4.69	4.51
A. terreus (AT3)	7.0	10.0	9.0	4.673	6.445	6.995	4.52	4.07	4.00
(HH1)	10.0	15.0	13.0	2.810	4.310	4.630	4.35	4.40	4.27
(HH2)	5.0	5.0	6.0	4.010	5.558	7.000	5.00	4.49	4.36
P. citrinum	10.0	13.0	11.0	3.000	4.235	5.990	4.00	3.40	4.10
P. rugulosum	7.0	12.0	11.0	2.115	3.110	3.980	4.81	4.51	4.42
Penicillium sp.	7.0	11.0	13.0	5.001	5.440	6.705	5.01	4.78	4.62
Rhizopus sp.	7.0	11.0	11.0	3.720	5.730	6.020	5.07	4.58	4.56

* = Data are from 30-day-old cultures; ¨ dry weight from 60 ml liquid media

¨ = Initial glucose concentration at day 0 was 1000 mg l^{-1}, 1/100 diluted values

α = initial pH values; 0: 4.90, 250: 4.75, 500: 4.55

dyes in the culture medium was monitored at regular intervals during the experimental study. The systems without the fungus served as abiotic controls.

Liquid samples (0.5 ml) were taken from each reaction flask at regular time intervals and residual colour was measured immediately by a UV-vis double-beam spectrophotometer (Unicon) at the maximum wavelength of absorbance (610 nm). Absorbance values were used for the calculations of decolorization efficiencies. Distilled water containing Kirk's basal medium was used as reference (Kalmis *et al.,* 2008).

Biomass growth in liquid media was determined as a dry mass of mycelia at 10, 20 and 30 days periodically. Mycelia were harvested from the cultivation liquid by filtration through filter paper and washed with distilled water, and biomass was dried at 105°C for one day (Kalmis *et al.,* 2008). Glucose concentration was measured by colorimetric methods using the phenol-sulphuric acid method (Dubois *et al.,* 1956). All measurements were repeated three times and the results are reported as the average of these replicates.

Results and Discussion

Azo and anthraquinone dyes are the most commonly used dyes in the textile industry. Benazol black ZN and Cibacron black W-NN are widely preferred diazo-type dyes, were used for the determination of decolorization potential of 22 microfungus strains in this study.

pH was controlled (3.70 to 5.07) throughout the study. The lower pH values at the end of the study could be explained by the accumulation of organic acids derived from catabolism of glucose and oxidative degradation process during the decolorization. Final pH values at the end of the experiments were not found to be low enough to negatively affect the organisms used.

Fig. 1 and Fig. 2 depict the colour removal efficiency of each organism used for two textile dyes at 250 and 500 mg l^{-1} dye concentrations, respectively. As it can be seen in Fig. 2, the decolorization efficiency of the organisms affected negatively by increasing dye concentrations to varying degrees.

For Benazol black ZN textile dye, organisms could be enumerated as best decolorization efficiencies when they were exposed to 250 mg l^{-1} dye concentration in the growth media as follows: *Haematonectria haematococca* (HH1) (36%) > *A. niger* (AN2) (34%) = *A. niger* (AN5) (34%) > *A. terreus* (AT1) (33%) = *Penicillium citrinum* (33%) > *A. terreus* (AT3) (30%). An order could be given when the organisms were exposed to 500 mg l^{-1} dye concentration as follows: *Alternaria alternata* (14%) = *A. niger*

Table - 2: Effect of various dye concentrations on biomass, glucose concentrations and pH for Cibacron black W-NN

	Dye concentrations (mg l^{-1})								
	0	250	500	0	250	500	0	250	500
	Biomass* ¨(mg)			Residual Glucose concentration*ⁱ			pH* ª		
Strains									
Absidia sp.	9.0	10.0	9.0	6.134	4.830	5.005	4.96	4.68	4.63
Acremonium sp.	9.0	10.0	10.0	3.510	5.630	5.500	4.48	4.39	4.08
A. alternata	8.0	11.0	8.0	4.100	6.335	6.620	4.50	4.48	4.39
Alternaria sp.	7.0	9.0	6.0	3.550	6.660	7.380	4.81	4.51	4.57
A. candidus	9.0	12.0	10.0	5.400	5.550	6.000	5.04	4.79	4.75
A. fumigatus	10.0	9.0	9.0	2.020	6.980	7.550	4.90	4.68	4.67
A. niger (AN1)	8.0	9.0	8.0	5.180	5.356	5.800	4.68	4.58	4.38
A. niger (AN2)	9.0	11.0	8.0	5.030	7.000	8.760	4.73	4.40	4.62
A. niger (AN3)	8.0	7.0	5.0	5.255	8.820	8.996	4.74	4.52	4.54
A. niger (AN4)	12.0	13.0	15.0	3.840	4.700	4.630	4.34	4.48	4.23
A. niger (AN5)	10.0	10.0	7.0	4.220	7.930	9.270	4.73	4.52	4.51
(AS1)	9.0	9.0	9.0	5.000	7.670	7.600	4.65	4.34	4.39
(AS2)	110	10.0	7.0	1.890	4.870	8.500	3.70	3.60	3.95
A. terreus (AT1)	8.0	8.0	6.0	3.140	8.700	9.101	4.76	4.60	4.63
A. terreus (AT2)	9.0	10.	9.0	5.160	8.230	8.940	4.87	4.69	4.67
A. terreus (AT3)	7.0	9.0	7.0	4.673	6.560	6.950	4.55	4.27	4.20
(HH1)	10.0	11.0	8.0	2.810	6.700	6.660	4.35	4.38	4.49
(HH2)	5.0	5.0	4.0	4.010	7.990	8.960	5.00	4.39	4.48
P. citrinum	10.0	10.0	9.0	3.000	6.330	7.900	4.00	3.87	4.30
P. rugulosum	7.0	12.0	6.0	2.115	7.720	8.830	4.81	4.48	4.62
Penicillium sp.	8.0	11.0	11.0	5.001	5.930	5.950	5.01	4.60	4.64
Rhizopus sp.	7.0	10.0	9.0	3.720	6.500	6.660	5.07	4.58	4.67

* Data are from 30-day-old cultures; ¨ dry weight from 60 ml liquid media

ⁱ = Initial glucose concentration at day 0 was 1000 mg l^{-1}, 1/100 diluted values

ª = Initial pH values; 0: 4.90, 250: 4.75, 500: 4.55

(AN2) (14%) > *A. terreus* (AT1) (13%) = *A. terreus* (AT2) (13%) = *Penicillium citrinum* (13%) > *A. niger* (AN1) (12%) (Fig. 1, 2). It was observed that the organisms showed a decolorization efficiency varying between 5 and 36% in 250 mg l^{-1}, 2 and 14% in 500 mg l^{-1}, respectively.

For Cibacron black W-NN textile dye, Fig. 1 and 2 show the decolorization efficiencies of each strain for Cibacron black W-NN at 250 and 500 mg l^{-1} dye concentrations, respectively. *Aspergillus niger* (AN1) showed the best decolorization efficiency in both 250 and 500 mg l^{-1} dye concentrations at 33% and 6%, respectively. This strain was followed by *Alternaria alternata* (12%), *Alternaria sp*. (10%) and *Aspergillus terreus* (AT3) (10%). When the organisms were exposed to 500 mg l^{-1} dye concentration *A. niger* (AN1) followed by *Alternaria alternata* and *Haematonectria haematococca* (HH1) as 4%.

Ramya *et al.* (2007) reported that an *Aspergillus* strain demonstrated high decolorization efficiency against Reactive blue (99%), R. black (75%), R. yellow (70%), R. red (33%) and Coloran violet (66%). According to Saratale *et al.* (2006), *Aspergillus ochraceus* NCIM-1146 showed significant ability to decolorize malachite green (98%), cotton blue (92%), methyl violet (61%) and crystal violet (57%) at 0.5, 0.5, 0.2 and 0.2 g l^{-1} dye concentration, respectively. In present study, *Aspergillus niger* (AN1) had average decolorization efficiency against both Benazol black ZN and Cibacron W-NN at 250 mg l^{-1} dye concentration. As the concentrations of two textile dyes were increased to 500 mg l^{-1}, the decolorization capacity of all strains was found to be decreased. Also, all strains showed lower decolorization efficiency against Cibacron black when it compared with Benazol black. This may be due to complexity in chemical structures. A slower rate of decolorization was attributed to higher molecular weight, structural complexicity and the presence of inhibitory groups like $–NO_2$ and $–SO_3Na$ in the dyes (Hu and Wu, 2001).

Effect on growth: For Benazol black ZN textile dye, all organisms, except for *Absidia sp*., *Acremonium sp*., *Aspergillus candidus*, *A. fumigatus*, *A. sclerotiorum* (AS2), *Haematonectria haematococca* (HH2) and *Penicillium sp*. showed best growth at 250 mg l^{-1} dye concentration while others showed were best growth at 500 mg l^{-1} dye concentration, resulting in higher biomass growth. All strains had lower biomass growth in no dye added media (Table 1). This shows that all strains were able to use the Benazol black ZN for their growth. Similar results have been reported in the study were malachite green dye was used (Ali *et al.,* 2009).

For Cibacron black textile dye, biomass concentration of all strains used in this study were not affected by increasing amount of dye in growth media. No significantly differences appear between dye concentrations and biomass amount of all organisms (Table 2).

Glucose as a carbon source was previously shown to be crucial for decolorization studies (Swamy and Ramsay, 1999). Table 1 and 2 show the glucose consumption rates during the decolorization assays, including the condition where no dye was added as the control assay. With glucose as the main substrate, its consumption was also negatively affected because of the increasing dye concentrations. Because of the toxic effect of increasing dye concentration (500 mg l^{-1}) on organisms, glucose consumptions decreased for all organisms. Zhang *et al.* (1999) reported that glucose concentration affected the decolorization rates and the most suitable concentration of glucose was about 5 g l^{-1}. But, Mou *et al.* (1991) studied the effects of glucose concentration on decolorization of dyes by *Myrothecium verrucaria* and observed that the glucose concentration did not significantly influence the bio-decolorization process.

It is more important to elucidate the different properties of wild-type organisms that were isolated from nature as it is the biggest source. This study demonstrated the comparing lower decolorization capacity of 22 wild-type strains of microfungi belonging to 7 genera for widely used textile dyes, Benazol black ZN and Cibacron black W-NN.

Dye molecules have many different and complicated structures. Decolorization by living cells involves more complex mechanisms such as extracellular oxidases and biosorption. The process is closely related to the operational conditions, such as nutrition requirements and influent concentration. Also, there are various factors influencing fungal dye degradation related to fungal growth and the characteristics of wastewater. Fungi may be exposed to a wide variety of organic and inorganic pollutants in the environment, thus, it is obviously desirable that more is known about the impact of pollutants on these organisms.

There are many microfungi capable of degrading dye molecules. There is a need to develop these fungi which can grow in basic, cheap medium and have high production rate and possess high biosorption capacity. Future research should be targeted towards search for fungal strains with producing dye-degrading enzymes. Such organisms could be used for treatment of wastewaters or scaling up of enzyme productions. Biofilms for immobilization of dye-degrading fungi or their enzymes for continuous use in wastewater treatment is yet another challenge for the future.

Fungal decolorization is a promising alternative to present treatment processes. However, using fungal strains to remove colour in dye wastewaters is still in the research stage. Further research is needed to establish the relationships between dye molecule structure and fungal decolorization. Also, more studies are needed to develop practical applications.

Acknowledgments

The authors wish to thank the Scientific and Technological Research Council of Turkey for providing financial support to this study under grant 104Y393.

References

Ali, H., W. Ahmad and T. Haq: Decolorization and degradation of malachite green by *Aspergillus flavus* and *Alternaria solani*. *Afr. J. Biotechnol.*, **8**, 1574-1576 (2009).

Azbar, N., T. Yonar and K. Kestioglu: Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fiber dyeing effluent. *Chemosphere*, **55**, 35-43 (2004).

Balan, D.S.L. and R.T.R. Monteiro: Decolorization of textile indigo dye by ligninolytic fungi. *J. Biotechnol.*, **89**, 141-145 (2001).

Banat, I.M., P. Nigam, D. Singh and R. Marchant: Microbial decolorisation of textile dye containing effluents: A review. *Bioresource Technol.*, **58**, 217-227 (1996).

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith: Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350-356 (1956).

Fu, Y. and T. Viraraghavan: Fungal decolorization of dye wastewater. *Bioresource Technol.*, **79**, 251-262 (2001).

Galindo, C. and T. Kalt: UV/H2O oxidation of azo dyes in aqueous media: evidence of a structure – degradability relationship. *Dyes Pigments*, **42**, 199-207 (1999).

Hu, Tai-lee and S.C. Wu: Assessment of the effect of azo dye Rp2B on the growth of nitrogen fixing cyanobacterium, *Anabena sp*. *Bioresource Technol.*, **77**, 3-95 (2001).

Kalmis, E., N. Azbar and F. Kalyoncu: Evaluation of two wild types of *Pleurotus ostreatus* (MCC07 and MCC20) isolated from nature for their ability to decolorize Benazol black ZN textile dye in comparison to some commercial types of white rot fungi, *Pleurotus ostreatus*, *Pleurotus djamor* and *Pleurotus citrinopileatus*. *Can. J. Microbiol.*, **54**, 366-370 (2008).

Mou, D.G., K.K. Lim and H.P. Shen: Microbial agents for decolorization of dye wastewater. *Biotechnol. Adv.*, **9**, 613-622 (1991).

O'Mahony, T., E. Guibal and J.M. Tobin: Reactive dye biosorption by *Rhizopus arrhizus* biomass. *Enzyme Microb Technol.*, **31**, 456-463 (2002).

Ramya, M., B. Anusha, S. Kalavathy and S. Devilaksmi: Biodecolorization and biodegradation of Reactive blue by *Aspergillus sp*. *Afr. J. Biotechnol.*, **6**, 1441-1445 (2007).

Revankar, S.M. and S.S Lele: Synthetic decolorization by white rot fungi, *Ganoderma sp*. WR-1. *Biores. Technol.*, **4**, 729-970 (2006).

Robinson, T., G. McMullan, R. Marchant and P. Nigam: Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. *Biores. Technol.*, **77**, 247-255 (2001).

Saratale, G.D., S.D. Kalme and S.P. Govindwar: Decolorisation of textile dyes by *Aspergillus ochraceus* (NCIM-1146). *Ind. J. Biotechnol.*, **5**, 407-410 (2006).

Swamy, J. and J. Ramsay: Effects of glucose and NH_4^+ concentrations on sequential dye decolorization by *Trametes versicolor*. *Enz. Microb. Technol.*, **25**, 278-284 (1999).

Tien, M. and T.K. Kirk: Lignin peroxidase of *Phanerochaete chrysosporium*. *Methods Enzymol.*, **161**, 238-249 (1988).

Zhang, F., J.S. Knapp and K.N. Tapley: Decolourisation of cotton bleaching effluent with wood rotting fungus. *Water Res.*, **33**, 919-928 (1999).

Zheng, Z., R.E. Levin, J.L. Pinkham and K. Shetty: Decolorization of polymeric dyes by a novel *Penicillium* isolate. *Process Bochem.*, **34**, 31-37 (1999).