INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–196/MIG/2010/12–2–256–260
http://www.fspublishers.org

Full Length Article

Biological Treatment of Textile Effluent in Stirred Tank Bioreactor

SAADIA ANDLEEB[1], NAIMA ATIQ, MUHAMMAD ISHTIAQ ALI, RAJA RAZI-UL-HUSSNAIN†, MARYAM SHAFIQUE, BASHIR AHMAD, PIR BUX GHUMRO, MASROOR HUSSAIN, ABDUL HAMEED AND SAFIA AHMAD
Department of Microbiology, Quaid-i-Azam University, Islamabad 45320, Pakistan
†*Pakistan Scientific and Technological Centre Islamabad, Pakistan*
[1]Corresponding author's e-mail: saadiamarwat@yahoo.com

ABSTRACT

A fungal isolate *Aspergillus terreus* SA3 previously isolated from the waste water of a local textile industry was efficiently utilized for the removal of dye (Sulfur black) from textile effluent. The treatment was performed in a self designed lab scale stirred tank bioreactor. The reactor with 5 L capacity (working volume 2 L) were operated at room temperature and pH 5.0 in continuous flow mode with different dye concentrations (50, 100, 150, 200, 300 & 500 ppm) in simulated textile effluent (STE). The reactors were run on fill, react, settle and draw mode with hydraulic retention time (HRT) of 24-72 h, depending upon the concentration of dye. Overall color, BOD and COD in the Stirred tank reactor system (STR) were removed by 84.53, 66.50 and 75.24%, respectively with 50 ppm dye concentration and HRT of 24 h. The removal efficiency of the reactor decreased as the concentration of the dye was increased. This STR system was found very effective for efficient treatment of textile waste water (up to 200 ppm Sulfur black dye) by the fungal strain *A. terreus* SA3. © 2010 Friends Science Publishers

Key Words: Drimarene blue dye; BOD; COD; Stirred tank bioreactor

INTRODUCTION

In Pakistan, 670 textile units are discharging their wastes into water bodies without any effective waste water treatment. Physical and chemical methods for treatments of dyes are discouraged due to high cost, inefficiency and chemical pollution. Biological treatment is safe and cost effective way for control of pollution. Colored waste water from textile industry is rated as the most polluted in almost all industrial sectors. It has been estimated that over 10,000 different textile dyes and pigments are in common use and the total world organic colorant productions are more than 100,000 tons/year (Easton, 1995; McMullan *et al.*, 2001). Huge amount of dyes in textile sectors are continuously being exhausted in wastewater streams due to their poor adsorbability to the fiber (Wagner, 1993; McMullan *et al*., 2001).

Dyes have environmental implications, because up to 50% of the initial dye mass (up to or exceeding 800 mg L^{-1}) used in the dyeing process remains in the spent dye bath in its hydrolyzed form which no longer has an affinity for the fabric and thus cannot be reused in the dyeing process (Laszlo, 1995). The presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water-bodies (McKay, 1979). This renders them more stable and less amenable to biodegradation (Seshadri *et al.,* 1994).

Synthetic dyes specially, sulfonated and their related biodegradation products contain structural elements, which are un-known or rare in nature, they not only have a negative aesthetic effect but also resist microbial attack and contribute to aquatic and soil toxicity (Wang & Yu, 1998). Recent research shows that although the level of the anthraquinone dye in the environment is expected to be orders of magnitude lower than that found in commercial, spent reactive dyebaths the effect of long-term, low-level dye exposure needs to be evaluated (Epolito *et al.,* 2005).

The treatment of textile waste water is still a major environmental concern, because of synthetic dyes, which are difficult to be removed by conventional treatment systems (Zhang *et al*., 2004). The majority of physical, chemical and biological color removal techniques work either by concentrating the color into sludge, solid supports, or by the complete destruction of the dye molecule. Currently the major methods of textile wastewater treatment involve physical and/or chemical processes as membrane filtration, coagulation/flocculation, precipitation, flotation, adsorption, ion exchange, ion pair extraction, ultrasonic mineralization, electrolysis, chemical reduction and advanced chemical oxidation (Gogate & Pandit, 2004). The biological removal processes including microbial remediation strategies has recently proved to be the most efficient treatment system.

The most widely researched fungi in regard to dye degradation are the ligninolytic fungi. White-rot fungi in

To cite this paper: Andleeb, S., N. Atiq, M.I. Ali, R. Razi-Ul-Hussnain, M. Shafique, B. Ahmad, P.B. Ghumro, M. Hussain, A. Hameed and S. Ahmad, 2010. biological treatment of textile effluent in stirred tank bioreactor. *Int. J. Agric. Biol.,* 12: 256–260

particular produced enzymes as lignin peroxidase, manganese peroxidase and laccase that degrade many aromatic compounds due to their non-specific activity (Toh *et al.,* 2005). Large literature exists on the potential of these fungi (White-rot) to oxidize phenolic, non-phenolic, soluble and non-soluble dyes (Libra *et al.,* 2003). In particular laccase from *Pleurotus ostreatus, Schizophyllum commune, Sclerotium rolfsii* and *Neurospora crassa*, seemed to increase up to 25% the degree of decolorization of individual commercial triarylmethane, anthraquinonic and indigoid textile dyes using enzyme preparations (Abadulla *et al.,* 2000).

Bioreactors are the core of any biotechnology based production processes for vaccine, proteins, enzymatic or microbial biotransformation, bioremediation and biodegradation (Chishthi & Young, 2001). Concerning the most widespread reactors used for culturing fungi, stirred tank reactors constitute the largest group. Many examples describing processes based on immobilized fungi are also available at lab and industrial scale in fixed-bed reactors (Zhang *et al*., 1999), rotating biological contactors (where the biofilm develops on the surface of vertical disks that rotate within the liquid) (Kapdan & Kargi, 2002) and trickling filter reactors (where the biofilm is slightly humidified by water or another liquid) (Messner *et al.,* 1990). Hollow fiber or membrane biofilm reactors, where the microbial layer is attached to a porous gas permeable membrane, are promising technologies since they can provide an efficient gas supply to the base of the biofilm (Lema *et al.,* 2001).

The isolated strain *A. terreus* SA3, efficiently involved in the decolorization of sulfur black dye, belongs to genus Aspergillus and main division Ascomycota. The role of *Aspergillus* sp. and other fungal strains to decolorize synthetically different dyes has been studied recently (Ali *et al*., 2007; Parshetti *et al*., 2007; Ramya *et al*., 2007). Dyes are removed by fungi by biosorption (Fu & Viraraghavan, 2000) and enzymatic mineralization (degradation) by Lignin peroxidase (LiP), manganese peroxidase (MnP), manganese independent peroxidase (MIP) and laccases (Laccs) (Raghukumar *et al.,* 1996; Duran *et al*., 2002; Wesenberg *et al*., 2003; Svobodova *et al*., 2006).

The objective was to develop a bio-treatment process using fungal strain *A. terreus* SA3 to treat sulfur Black dye containing Simulated Textile Effluent (STE) in lab scale self designed bioreactor. The dye removal efficiency of stirred tank bioreactor system (STR) was monitored in terms of color, BOD and COD.

MATERIALS AND METHODS

Chemicals: The majority of chemicals and media component were obtained from Sigma-Aldrich (USA). The investigated commercial dye sulphur black was obtained from Kohinoor Textile Mill, Rawalpindi, Pakistan. Simulated textile effluent (STE) was made by adding per litre of distilled water; Acetic Acid (99.9%) 0.15 mL, $(NH_2)_2CO$ 108.0 mg, KH_2PO_4 67.0 mg, $NaHCO_3$ 840.0 mg, $MgSO_4.7H_2O$ 38.0 mg, $CaCl_2$ 21.0 mg, $FeCl_3.6H_2O$ 7.0 mg and glucose 860 mg (Luangdilok & Panswad, 2000).

Selection and identification of fungi: The fungal (*A. terreus*) isolate SA3 able to biodegrade and decolorize dyes was obtained from the culture collection of Microbiology Research Laboratory Quaid-i-Azam University Islamabad. The strain was previously isolated from the sludge, collected from waste water storage pond of Kohinoor Textile Mill, Rawalpindi. It was identified on the basis of the vegetative and reproductive structure as *A. terreus* SA3 (Fig. 1).

Inoculum preparation: Spore suspension of fungal cultures was prepared. Fungal cultures were refreshed on saboraud dextrose agar (Merck), in tissue culture bottles. 20 mL of 0.05% Tween20 solution was used as a spore suspension medium in sterile collection tubes. Concentration of spores/mL was determined by MOD-FUCH'S ROSETHAL haemocytometer (Depth 0.2 mm, 1/116 mm^2 WEBER England). Spore suspension was added to medium (100 mL) in conical flasks so that final concentration was 10^3spores/mL. Streptomycin (0.05%) was used as antibacterial agent. pH of STE was adjusted by pH meter (Horiba M.8 E) at 5 using 0.1 M HCl and 0.1 M NaOH.

Stirred tank bioreactor: A lab scale stirred tank bioreactor of 5000 cm^3 capacity with the instrumentation necessary for the working of system was used. The reactor set up consisted of an overhead stirrer (company) and a (5000 cm^3 volume) vessel body. The working volume was 2000 cm^3. The effluent and influent flow was maintained by the peristaltic pump (Vera varistaltic pump plus by manostat® Division of Barnant). The reactor was operated at room temperature, pH 5.0 and continuous flow mode. The bioreactor was filled wit 5% spore suspension as inoculum, STE medium with 50 mg L^{-1} of dye concentration was fed into the reactor. The hydraulic retention time was 24-72 h depending upon the conc. of dye in the feed. It was run on fill, react, settle and draw mode. The effluent was drawn after 24-72 h retention time and the reactors were fed again with the higher concentration of dye (100, 150, 200, 300 mg L^{-1}) each time. When almost complete adaptation of the dye to the fungi was achieved, the reactor was turned to the batch mode. Fixed dye concentration (500 mg L^{-1}) was added to the reactor and operated untill maximum decolorization was achieved. Samples were drawn periodically (depending upon the HRT of the treatment) throughout all experimental set up for the estimation of different parameters. All samples were filtered though whatman paper no. 1 and centrifuged at 10,000 rpm for 10 min (Fig. 2).

Color removal: Decolorization was monitored by UV–Vis spectroscopic analysis. Decolorization of dye was followed by monitoring, changes in its absorption spectrum (λ610 nm for sulfur black) and comparing the results, to those of the respective controls. The pellet was discarded after centrifugation and clear solution was analyzed using the AGILENT UV visible recording spectrophotometer.

% Decolorization = Initial conc. of dye – Final conc. of dye/Initial conc. of dye X 100.

Chemical oxygen demand (COD): COD analyses of the un-treated and treated samples from bioreactors were performed by using closed reflex, colorimetric method (APHA 5220 D standard method). Glass ampoules with cap were washed with 20% sulfuric acid to prevent contamination. Calibration standards were prepared in series using different volume of potassium hydrogen phthalate (KHP) and from these ampoules 1.33 mL KHP was taken and 1 mL digestion solution and 2.66 mL H_2SO_4 reagent was added. Blank and samples were treated in the same way. Reference tubes were also made for confirmation of calibration curve. Glass ampoules were then kept in thermo-reactor (ECO 25, VELPA Scientifica, Italy) at 150ºC for 2 h and allowed the processed calibration standards, blank and sample ampoules to cool down to room temperature. COD was estimated taking absorbance at 600 nm. COD was measured as COD mg O_2 L^{-1}.

Biological oxygen demand (BOD): BOD analysis of the un-treated and treated sample from bioreactors was performed by using 5-Day BOD Test (APHA standard method).

RESULTS

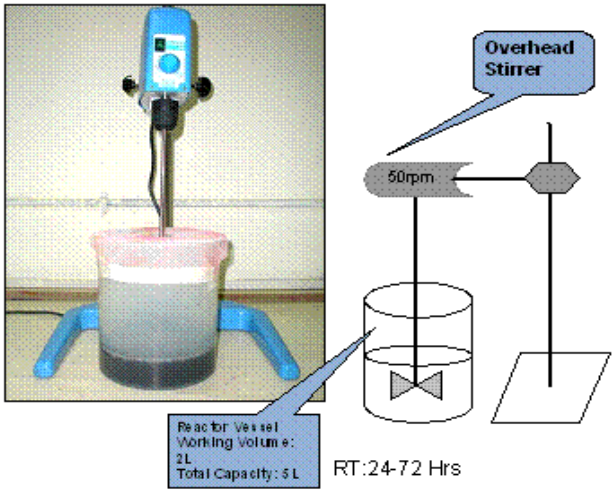
Color removal: Maximum color removal of 84.53% was observed, when the reactor was operated for 1 day (HRT 24 h) for 50 ppm sulfur black dye removal in the STE. The rate of color removal was shown to be in inverse correlation with the dye loading rate of the reactor. Thus, it was assumed that increasing the concentration of dye in the input feed of the reactor, decreased the dye removal performance of the reactor. 80.06, 71.55, 54.55, 41.89 and 30.09% dye was removed during 100, 150, 200, 300 and 500 ppm dye treatments in the reactor operation of 72 h HRT (Fig. 3).

COD removal: The same trend of decreasing removal levels with increasing COD load during each operation was also observed in case of COD. A maximum COD removal of 75.24% was obtained at low influent dye concentration of 50 ppm. The reduction level was quite significant up to 200 ppm dye treatment. The poor COD reduction (44.23 & 44.15%) was obtained at high influent COD and dye concentration (300 & 500 ppm), indicating that COD removal was affected by dye COD (Fig. 4).

BOD removal: The rate of BOD removal was found to be decreasing with increasing dye concentration up to a certain limit (150 ppm dye in put). When the same reactor operation was carried out in continuation but with increasing dye concentration (200 ppm), an increase in removal rate was observed. With 300 ppm and 500 ppm dye in the reactor input, BOD removal again followed the same trend i.e., higher the dye loading rate, lower the removal efficiency. Maximum BOD was reduced at 24 h HRT (66.50%), with significant reduction up to 200 ppm dye treatment (Fig. 5).

Fig. 1: Morphology of colonies of fungal isolate SA3 on Saboraud dextrose agar plate

Fig. 2: Stirred tank bioreactor system for decolorization of Sulfur black dye in Simulated textile effluent



DISCUSSION

Stirred tank bioreactor (STR) treatment implied for biological removal of sulfur black dye from simulated textile effluent (STE) effectively removed the dye by fungal strain *A. terreus* SA3. This strain belong to a main division of fungi i.e., Ascomycota. Ramya *et al.* (2007) isolated seven different morphologically distinct fungi from the soil collected from a textile industry. Among them one fungal isolate showed higher decolorization and it was identified as *Aspergillus sp.* and decolorized chemically different dyes such as reactive black (75%), reactive yellow (70%), reactive red (33%) and coloron violet (66%).

Role of different species of *Aspergillus* for decolorization of different types of dyes has been reported earlier. Sumathi and Manju (2000) isolated *A. foetidus* and

found its effective role in the decolorization of Drimarene range of fibre reactive dyes. The entire color was found to be strongly bioadsorbed to the fungal biomass pellets without undergoing significant biotransformation. Free and Immobilized *A. fumigatus* has also shown its role for the effective decolorization of dye industry effluent and reactive brilliant blue KN-R dye, respectively (Jin *et al.*, 2007; Wang & Hu, 2008). Ali *et al.* (2007) showed the Apparent decolorization of AR 151 (Reactive diazo) was also found to be associated with cellular uptake mechanism of *A. niger* SA1, which was confirmed by the detailed microscopic examination. However, this dye removal mechanism was not merely due to biosorption/bioadsorption.

The results of HPLC at different physicochemical conditions proved the role of degradation mechanism in the dye removal phenomenon. The dye removal ability was reduced each time the reactor was implied with high concentrations of dye. Decrease in the color intensity of the output of the reactor and dye removal rates indicated that the fungal strains were showing removal capabilities even with much higher concentration of the dye. Ali *et al.* (2007) reported the similar decolorization pattern in shake flask experiments conducted from lower to higher (10–500 mg L^{-1}) concentrations of acid red dye (AR 151). Results showed that increase in concentration of dye proved to have an inverse effect on decolorization and biomass production, which were positively correlated with each other during the experiments.

In a previous study of decolorization of sulfur black dye by a bacterial strain, the similar decolorization pattern was reported. Efficiency of W3 (a bacterial strain) increased slightly to a maximum (about 99%) when the concentration of sulfur black increased from 0.01 to 0.2 mg mL^{-1} and then decreased when the sulfur black concentration rose to 1.0 mg L^{-1} (Keung, 1997). Increased retention time enabled the fungal system to acclimatize the sudden increase of dye loading rate and helps the system to cope with chemical shock. The effect of hydraulic retention time (HRT) on the Orange II and COD removal efficiencies was investigated in a study. It was concluded that the COD and Orange II removal efficiencies were enhanced from 27 to 35% and 82 to 97%, respectively by the increase of HRT from 24 to 48 h (Ong *et al.*, 2005). Pourbabaee *et al.* (2006) observed similar reductions in BOD and COD during the study of aerobic decolorization and detoxification of a disperse dye (Terasil Black) in textile effluent by a newly isolated Bacillus sp. Reductions in BOD and COD level indicates the formation of new metabolites during the process of decolorization. The metabolic product formation during the course of reactor operation may contribute to the total COD and BOD loads, thus decreasing capabilities of removals.

CONCLUSION

Fungal cells represent an inexpensive, readily available source of biomass that has a significant potential for dye decolourization and thus is an important and promising material for the removal of dyes from textile dye effluents. The present bioreactor system due to its cost effectiveness and easy maintenance has great potential to be used as a biotechnological approach for the *in situ* bioremediation for textile dyes.

Fig. 3: Effect of increased dye concentration on dye removal rates by *A. terreus* SA3 in Stirred tank bioreactor system

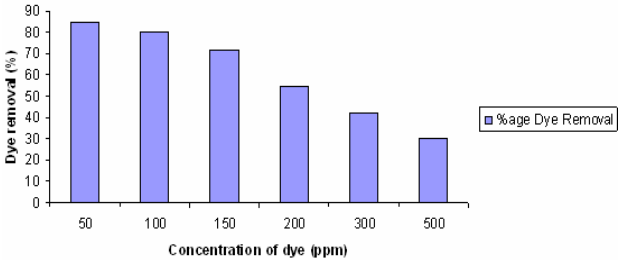


Fig. 4: Initial (before treatment) and final COD (mg O_2/L) (after treatment), with COD reductions efficiencies by *A. terreus* SA3 with increasing dye loading rate Stirred tank bioreactor

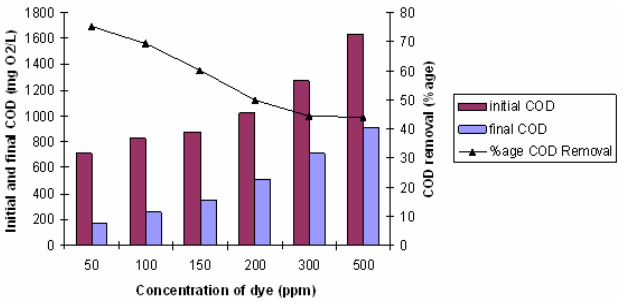
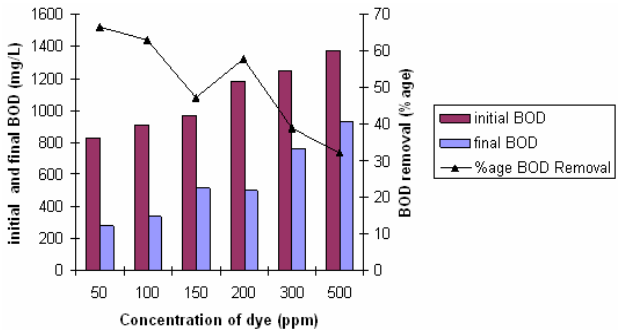


Fig. 5: Initial (before treatment) and final BOD (mg O_2/L) (after treatment), with BOD reductions (%) efficiencies of *A. terreus* SA3 with increasing dye loading rate in Stirred tank bioreactor



REFERENCES

Abadullah, E., T. Tzanov, S. Costa, K.H. Robra, A. Cavaco-Paulo and G.M. Gübitz, 2000. Decolourisation and detoxification of textile dyes with laccase from *Trametes hirsuta*. *Appl. Environ. Microbial*., 66: 3357–3362

Ali, N., A. Hameed, S. Ahmed and A.G. Khan, 2007. Decolorization of structurally different textile dyes by *Aspergillus niger* SA1. *World J. Microbiol. Biotechnol*., 24: 1067–1072

Chishthi and Moo-Young, 2001. Bioreactor design, Chapter-7, Fundamentals of Biotechnology *chromofuscus. J. Bacteriol.*, 176: 1339–1347

Duran, N., M.A. Rosa, A.D. Annibale and L. Gianfreda, 2002. Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: A review. *Enzym. Microb. Technol*., 31: 907–931

Easton, J.R., 1995. The dye maker's view. *In*: Cooper, P. (ed.), *Colour in Dyehouse Effluent,* pp: 9–21. Society of Dyehouse and Color, Bradford, England

Epolito, W.J., Y.H. Leea, L.A. Bottomley and S.G. Pavlostathis, 2005. Characterization of the textile anthraquinone dye Reactive Blue 4. *Dyes Pigments*, 67: 35–46

Fu, Y. and T. Viraraghavan, 2002. Dye biosorption sites in *Aspergillus niger*. *Bioresource Technol.*, 82: 139–145

Gogate, P.R. and A.B. Pandit, 2004. A review of imperative technologies for wastewater treatment II: hybrid methods. *Advan. Environ. Res*., 8: 553–597

Jin, X.C., G.Q. Liu, Z.H. Xu and W.Y. Tao, 2007. Decolorization of a dye industry effluent by *Aspergillus fumigatus* XC6. *Appl. Microbiol. Biotechnol*., 74: 239–243

Kapdan, I.K. and F. Kargi, 2002. Biological decolorization of textile dyestuff containing wastewater by Coriolus versicolor in a rotating biological contactor. *Enzyme Microb. Technol.*, 30: 195–199

Keung, M.K., 1997. *Bacterium Useful in the Removal of Sulphur Black Dye from a Substrate Document Type and Number*. United States Patent 5610064

Laszlo, J.A., 1995. Electrolyte effects on hydrolyzed reactive dye binding to quaternized cellulose. *Textile Chem. Colorist,* 27: 25–27

Lema, J.M., E. Roca, A. Sanroman, M.J. Nun˜ ez, M.T. Moreira and G. Feijoo, 2001. Pulsing bioreactors. *In*: Cabral, J.M.S., M. Mota and J. Tramper (eds.), *Multiphase Bioreact. Design,* pp: 309–329

Libra, J.A., M. Borchert and S. Banit, 2003. Competition strategies for the decolorization of a textile-reactive dye with the white-rot fungi *Trametes versicolor* under non-sterile conditions. *Biotechnol. Bioeng*., 82: 736–744

Luangdilok, W. and T. Panswad, 2000. Effect of chemical structures of reactive dyes on color removal by an anaerobic–aerobic process. *Wat. Sci. Technol*., 42: 377–382

McKay, G., 1979. Waste colour removal from textile effluents. *American Dyestuff. Rep.,* 68: 29–36

Mcmullan, G., C. Meehan, A. Conneely, N. Nirby, P. Robinson, I. Nigam, M. Bannat and S.W.F. Marchant, 2001. Mini review: Microbial decolorization and degradation of textile dyes. *Appl. Microbiol. Biotechnol.,* 56: 81–87

Messner, K., G. Ertler, S. Jakling-Farcher, P. Bosbovsky, U. Regensberger and A. Blaha, 1990. Treatment of bleach effluents by the MyCoPOR system. *In*: Kirk, T.K. and H.M. Chang (eds.), *Biotechnology in Pulp and Peper Manufacture,* pp: 245–253. Butterworth-Heinemann, Stoneham, Massachusetts

Ong, S.A., E. Toorisaka, M. Hirata and T. Hano, 2005. Treatment of azo dye Orange II in aerobic and anaerobic-SBR systems. *Process Biochem.*, 40: 2907–2914

Parshetti, G.K., S.D. Kalme, S.S. Gomare and S.P. Govindwar, 2007. Biodegradation of Reactive blue-25 by *Aspergillus ochraceus* NCIM-1146. *Bioresource Technol.*, 98: 3638–3642

Pourbabaee, A.A., F. Malekzadeh, M.N. Sarbolouki and F. Najafi, 2006. *Aerobic Decolorization and Detoxification of a Disperse Dye in Textile Effluent by a New Isolate of Bacillus sp. Wiley Inter Science.* (www.interscience.wiley.com), DOI: 10.1002/bit.20732

Raghukumar, G., D. Chandramohan, F.C. Michael and C.A. Reddy, 1996. Degradation of lignin and decolorization of paper-mill bleach plant effluent (Bpe) by marine fungi. *Biotech. Lett*., 18: 105–106

Ramya, M., B. Anusha, S. Kalavathy and S. Devilaksmi, 2007. Biodecolorization and biodegradation of Reactive Blue by Aspergillus spp. *African J. Biotechnol.,* 6: 1441–1445

Seshadri, S., P.L. Bishop and A.M. Agha, 1994. Anaerobic/aerobic treatment of selected azo dyes in wastewater. *Waste Manag*., 15: 127–137

Sumathi, S. and B.S. Manju, 2000. Uptake of reactive textile dyes by *Aspergillus foetidus*. *Enzyme Microb. Technol.,* 27: 347–355

Svobodova, K., P. Erbanova, J. Sklenar and C. Novotny, 2006. The role of Mn-dependent peroxidase in dye decolorization by static and agitated culture of *Irpex lacteus*. *Folia Microbiol.*, 51: 573–578

Wagner, S., 1993. *Improvement in Products and Processing to Diminish Environmental Impact*. COTTECH Conference, Raleigh NC

Wang, B.E. and Y.Y. Hu, 2008. Bioaccumulation versus adsorption of reactive dye by immobilized growing *Aspergillus fumigatus* beads. *J. Hazard. Mater.*, 157: 1–7

Wang, Y. and J. Yu, 1998. Adsorption and degradation of synthetic dyes on the mycelium of Trametes versicolor. *Water Sci. Technol.,* 38: 232–237

Wesenberg, D., I. Kyriakides and S.N. Agathos, 2003. White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnol. Adv*., 22: 161–187

Zhang, F., A. Yediler, X. Liang and A. Kettrup, 2004. Effects of dye additives on the ozonation process and oxidation by products: a comparative study using hydrolyzed CI Reactive red 120. *Dyes Pigments*, 60: 1–7

Zhang, Z., R.E. Levin, J.L. Pinkham and K. Shetty, 1999. Decolorization of polymeric dyes by a novel *Penicillium* isolate. *Process Biochem.*, 34: 31–37

(Received 04 June 2009; Accepted 24 June 2009)