Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Technology

Elixir Bio Technology 62 (2013) 17794-17798

ISSN: 2229-712X

Mycoremediation of textile dyes by fungal isolates from dyeing industry effluent

Md. Mahamudul Hassan*, Md. Zobaidul Alam and Md. Nural Anwar

Microbiology Research Laboratory, Department of Microbiology, University of Chittagong, Chittagong – 4331, Bangladesh.

ARTICLE INFO

Article history:

Received: 10 July 2013;

Received in revised form:

25 August 2013;

Accepted: 10 September 2013;

Keywords

Water pollution,

Industrial effluent,

Azo dye,

Mycoremediation.

ABSTRACT

A vast amount of dye effluent from textile industries cause severe water pollution as it comprises of xenobiotic azo dyes that are recalcitrant to biodegradation. Continual research is going on worldwide to develop effective, economical and environment-friendly treatment processes while biological treatment is considered the most promising in all aspects. This study aims at the evaluation of textile dye decolourizing capability of naturally occurring fungi. After screening, 4 fungal species identified as *Aspergillus fumigatus, A. flavipes, A. luchuensis* and *Penicillium rubrum* from dye effluent were found as potential decolourizer, exhibiting strong to mild decolorization of Novacron dyes *viz.* Blue FNR, Red FNR, Yellow FN2R, Orange W3R and Navy WB at habitat concentration of 0.05% after 3, 5 and 7 days of co-incubation in Czepex-Dox broth medium. Maximum decolourization usually found at 7 days. Notably, *A. fumigatus* and *A. flavipes* exhibited 85-99% decolourization of all the dyes. In contrast, *A. luchuensis* selectively decolourized 76-80% of yellow FN2R and blue FNR. *P. rubrum* also caused significant decolourization of red FNR, yellow FN2R and orange W3R. This study thus elucidates that indigenous microorganisms of textile effluent cause remarkable degradation of azo dyes and can be used as potent agent in their bioremediation.

Introduction

Rapid industrialization and urbanization throughout the world have lead to decipher the relationship between environmental pollution and public health (WHO, 1982). Among different environmental pollutions, water pollution is the most threatening one where dyeing industries are playing the key role (Cripps *et al*., 1990). At present, 7×10^5 tons of over 100,000 types of dyes are produced annually (Meyer, 1981; Zollinger, 1987). But, due to inefficient dyeing process, upto 50% of all dyestuff directly discharged in the environment as effluent (Pandey *et al*., 2007). The chemical structure of azo dye consists of substituted aromatic rings joined by one or more azo groups (-N=N-) (Pandey *et al*., 2007) which are unfortunately designed to be resistant to conventional wastewater treatment systems (Shaul *et al*., 1991; Seshadri and Bishop, 1994; Peralta – Zamora *et al*., 1999). Further, some azo dyes are carcinogenic and mutagenic too (Spadaro *et al*., 1992). Usual dye concentration of 10- 200 mg/L in effluent not only aesthetically unacceptable (O'Neil *et al*., 1999); it also affects the aquatic ecosystem by decreasing the light penetration, solubility of gases and interferes with photosynthesis of phytoplankton (Saranaik and Kanekar, 1995). Aquatic biodiversity is also disturbed by the increased alkalinity (Rao and Datta, 1987). Azo bonds in azo dye are highly resistant to breakdown, serving the dye to be persistent in the environment (Mahdavi *et al*., 2001). Though some physico-chemical methods like adsorption, oxidation and precipitation, bleaching, ozone photo degradation or membrane filtration have been proposed for treatment of textile effluent (Robinson *et al*., 2001), they cannot draw much attention due to the production of huge sludge and also for being expensive (Chen *et al*., 2003; Ponraj *et al*., 2011). In contrast, biotreatment of textile effluent have been proven to be the best remedy to overcome such drawbacks of physico-chemical treatment (Salar *et al*., 2012). Since metabolism of synthetic dyes has already been extensively examined for a number of pure bacterial and fungal strains, microbial pollutant treatment system have the advantage of being simple in design, environmentally friendly, publicly accepted and low in cost compared with other conventional treatments (Walker, 1970; Zimmermann *et al*. 1984; Pasti- Grigsby *et al*. 1992). Our study deals with the isolation and identification of indigenous fungi from dyeing industry effluent and evaluation of their dye decolourizing capability in order to find prominent textile dye degrader.

Materials And Methods

Sample

Effluent samples were collected from 4 different discharge locations of Qualitex Industries (BD) Ltd, Export Processing Zone, Chittagong, Bangladesh. After collection, samples were quickly brought to the laboratory in a cooler box and processed within 5 hours.

Dyes and media

Five Novacron dyes *viz.* blue FNR, red FNR, yellow FN2R, orange W3R and navy WB used in our experiment was donated by Qualitex Industries (BD) Ltd, CEPZ, Chittagong, Bangladesh and all the medium except Winstead agar was brought from HiMedia Laboratories. Potato dextrose agar (PDA) medium was used for enumeration and isolation while along with PDA medium, Winstead agar (WA) and Czapek`s Dox agar (CDA) medium used for identification. Decolourization assay was measured in modified Czapek`s Dox Broth (CDB) medium with

Tele:

E-mail addresses: hassanmm@cu.ac.bd

particular dye after isolates being enriched in Potato Dextrose Broth (PDB).

Plating

10 ml of sample was diluted in an Earlenmeyer flask having 90 ml of distilled water. After keeping the mixture in an orbital shaker at 120 RPM for 20 minutes to obtain homogeneity, it was further diluted upto 10^{-4} in distilled water. All the diluted samples were then plated according to pour plate method using PDA medium, pH 5.5 containing filtered Streptomycin solution (50mg/L) to prevent bacterial growth. The number of fungal colonies were counted after incubating at 28°C for 5 days, multiplied with dilution factor and expressed as cfu/ml of sample.

Isolation and screening

From the PDA plates, fungal colonies showing unique growth pattern were isolated in PDA slant on the basis of their colony morphology and cultural characteristics. These were then screened for dye decolourizing capability by inoculating 5 to 7 days old 5mm of diameter fungal disk in 50 ml Czepex-Dox broth supplemented with 0.01% of the respective dye. After incubation at 28°C for 5 to 7 days, visual decolourization in the flasks indicates the potent isolate for further studies. (Moorthi *et al*. 2007)

Identification

Screened fungal species were identified by comparing their Mycellial morphology (branching, colour, septa), sporulation pattern (column or chain), size of the vegetative structures (conidiophores, vesicle, sterigmata, phialides) and cultural properties on PDA, WA and CDA medium with the standard description given in "Manual of Soil Fungi" 2nd ed. (Gilman, 1957).

Dye decolourization assay

Decolourization activity of the screened isolates against 5 commercially important Novacron dyes *viz.* Orange W3R, Red FNR, Yellow FN2R, Blue FNR and Navy WB was measured by following method described by Jothimani and Prabakaran, 2003. Inoculum was prepared by inoculating a loopful of fungal culture in potato dextrose broth and incubating at 200 RPM in a shaker (Model SI50, Stuart Scientific, U.K) for 3 days at 28°C. Experiment was begun by incubating 1 ml culture suspension into each 250 ml Erlenmeyer flask containing 50 ml of Czepex-Dox broth modified with 0.05% of particular Novacron dyes at 200 RPM at room temperature.

Uninoculated flasks were also made which serves as control. After a regular interval of incubation period (3, 5 and 7 days), culture was withdrawn, filtered (Whatman no. 1 filter paper), centrifuged at 5000 RPM for 15 minutes at 4°C and OD (optical density) of the collected supernatant was measured at 594 nm under visible light in spectrophotometer (UV- VIS RS spectrophotometer, LaboMed. Inc.).

OD of the uninoculated (control) flasks also measured by the same way. From these OD values, residual dye concentration was measured from the standard curve that is developed by plotting the absorbance of different concentrations of the respective tested dyes. The rate of decolourization was calculated as percent of decolourization (%) = $(A_i - A_t)/A_i \times 100$, where A_i is the concentration of the initial dye solution and A_t is the concentration at cultivation time (3, 5 and 7 days). Each test was done in triplicate and mean values expressed as result.

Results

Indigenous fungi of the dye effluent

The presence of various microorganisms including fungi in dyeing industry effluent have been known for a long time though azo dye contaminated environment is highly toxic for the growth of many microorganisms. In our study we found an average fungal count of 5×10^4 cfu/mL of sample after performing serial dilution and subsequent plating in PDA medium through pour plate method. From these plates, 10 colonies were isolated for further studies on the basis of their unique colony characteristics. This result revealed that a significant number of fungi could thrive in the effluent by utilizing them as their source of essential growth element.

Fungi capable of decolourizing dyes

Screening was done to sort out the potential fungal isolates capable of decolourizing our tested Novacron dyes *viz.* Blue FNR, Red FNR, Yellow FN2R, Orange W3R and Navy WB in Czepex-Dox broth having 0.01% of particular dye. Through the screening process, a total of 4 fungal species were selected as the potential decolourizer of our tested Novacron dyes.

Identification of potential fungal isolates

Identification of the potential isolates was done by analyzing their growth characteristics, mycellial morphology, sporulation pattern, vegetative structure in Potato Dextrose, Winstead and Czepex-Dox Agar medium. After comparing all these properties with the standard description in "Manual of Soil Fungi" 2nd ed. (Gilman, 1957), screened fungal isolates was found to be the members of Deuteromycotina and identified as *Aspergillus luchuensis* (**Figure 1)**, *Aspergillus fumigatus* **(Figure 2)**, *Aspergillus flavipes* **(Figure 3)** and *Penicillium rubrum* **(Figure 4)**.

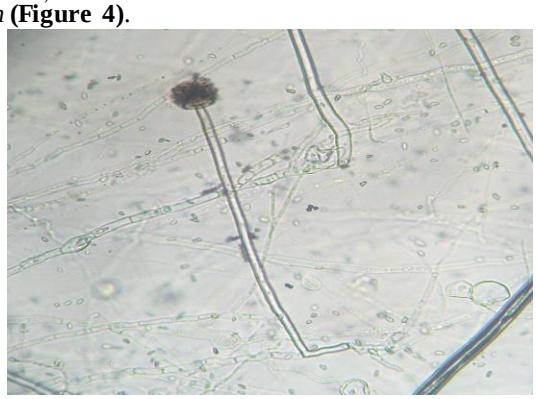

Figure 1: Microscopic view of *Aspergillus luchuensis* (10x view)

Figure 2: Microscopic view of *Aspergillus fumigatus* (40x view)

Figure 3: Microscopic view of *Aspergillus flavipes* (40x view)

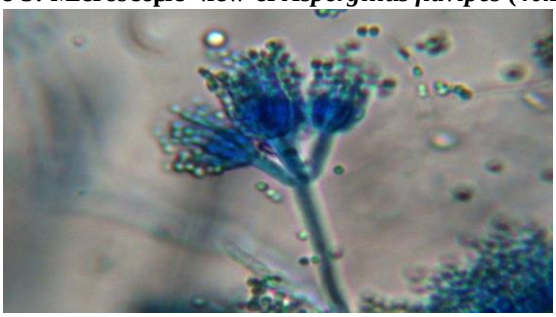

Figure 4: Microscopic view of *Penicillium rubrum* (40x view)

Assesment of dye decolourization

Isolates were subjected to dye decolourization test in 0.05% concentration of each Novacron dyes (Orange W3R, Red FNR, Yellow FN2R, Blue FNR and Navy WB) where assessment was performed on a regular interval (3, 5 and 7 day) to estimate the proceedings of decolourization. All data were then plotted on **Table 1**, from which we can easily identify the most potential fungal isolates for our tested dye degradation.

From the decolourization assay, it is clear that the dye Novacron Blue FNR was extensively degraded by all of the isolates except *Penicillium rubrum*. *Aspergillus luchuensis* decolourised more than 80% of dye while *Aspergillus fumigatus* and *A. flavipes* caused almost complete degradation after 7 days of incubation. But, in all cases decolourization around maximum value was achieved after 5 days of incubation and surprisingly near 80% to more than 85% decolourization of this dye was performed by *Aspergillus fumigatus* and *A. flavipes* respectively after 3 days of incubation **(Figure 5).** Similar case also found in case of Novacron Yellow FN2R decolourization. Here, *Aspergillus luchuensis* and *Penicillium rubrum* performed their maximum decolurisation *i.e.* 75% and 74.1% respectively, after 5 days of incubation and there was only a slight increase of decolourization afterwards. Prompt decolourization during the early stage of incubation was may be due to the young fungal myceliums which are metabolically very active whereas in most cases aged fungal myceliums showed incapability or sluggish decolourization of the dyes even with increase of incubation period. Both *Aspergillus fumigatus* and *A. flavipes* decolourise around 90% of Yellow FN2R after 7 days of incubation and proving them as the most potential degrader of these two dyes **(Figure 6)**.

A. fumigatus and A. flavipes also showed a tremendous decolourization capability against Red FNR and OrangeW3R where both of them decolourised more than 90% of these dyes within 5 days of incubation. Finally, after 7 days of incubation they were found to cause almost complete degradation of Novacron Red FNR and around 95% degradation of Novacron Orange W3R. Besides, *P. rubrum* cause 71.5% decolourization of Orange W3R after 5 days of incubation and there was no further progress of decolourization later on. In addition, it degraded 60% of Red FNR after 7 days of incubation. Surprisingly, *A. luchuensis* couldn't perform any notable degradation of either Novacron Red FNR or OrangeW3R. **(Figure 7 and 8)**

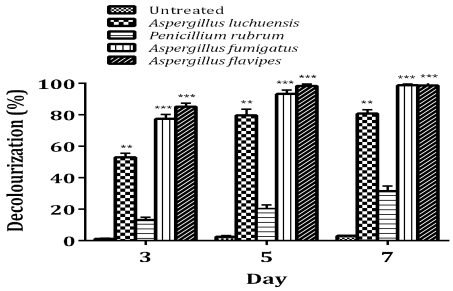

Figure 5: Decolourization of Novacron Blue FNR by fungal isolates. The assay was performed by using isolates *Penicillium rubrum, Aspergillus luchuensis, A. fumigatus* and *A. flavipes* in modified Czepex-Dox broth medium with 0.05% of dye. Decolourization was measured spectrophotometrically after 3, 5 and 7 days of incubation by using the formula: Decolourization (%) = $(A_i - A_t)/A_i \times 100$, where A_i is the concentration of the initial dye solution and A_t is the concentration at cultivation time. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level

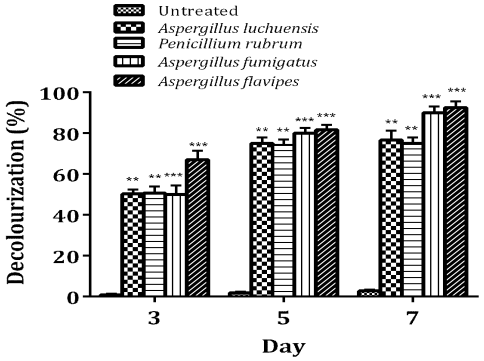

Figure 6: Decolourization of Novacron Yellow FNR by fungal isolates. The assay was performed by using isolates *Penicillium rubrum, Aspergillus luchuensis, A. fumigatus* and *A. flavipes* following the similar process described in Figure 5. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level

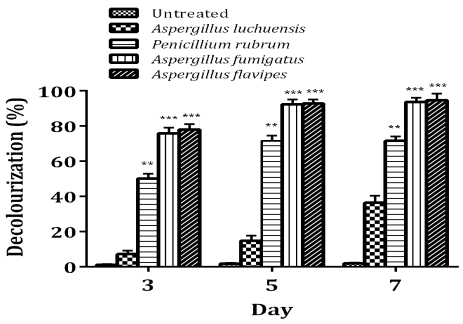

Figure 7: Decolourization of Novacron Orange W3R by fungal isolates. The assay was performed by using isolates *Penicillium rubrum, Aspergillus luchuensis, A. fumigatus* and *A. flavipes* following the similar process described in Figure 5. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level

Table 1: Decolourization (%) of textile dyes by fungal isolates at 0.05% concentration

Fungal Isolates / Textile Dyes	*Aspergillus luchuensis*			*Penicillium rubrum*			*Aspergillus fumigatus*			*Aspergillus flavipes*		
	Decolourization (%)			Decolourization (%)			Decolourization (%)			Decolourization (%)		
	3rd day	5th day	7th day	3rd day	5th day	7th day	3rd day	5th day	7th Day	3rd day	5th day	7th day
Novacron blue FNR	53.0	79.7	80.8	13.2	20.5	31.4	77.4	93.2	98.8	85.2	98.4	98.6
Novacron yellow FN2R	50.2	75	76.6	50.7	74.1	75	50	80	90	67.0	81.7	92.5
Novacron orange W3R	7.25	14.8	36.4	50.2	71.6	71.6	75.8	92.3	93.7	78.0	92.8	94.6
Novacron red FNR	5.02	11.0	26.3	27.2	54.4	60.2	79.2	91.7	98.5	70.2	93.4	97.2
Novacron navy WB	1.28	1.78	24.8	1.4	2.31	5.41	53.1	64.5	86.4	46.3	65.3	85.1

All the tested dyes were provided by Qualitex industries (BD) limited, CEPZ, Chittagong, Bangladesh. Decolourization assay was done in modified Czepex Dox broth media and decolourization (%) was measured spectrophotometrically after 3rd, 5th and 7th day of incubation. Each decolourization (%) value was measured by using the formula: Decolourization (%) = $(A_i - A_t)/A_i \times 100$, where A_i is the concentration of the initial dye solution and A_t is the concentration at cultivation time

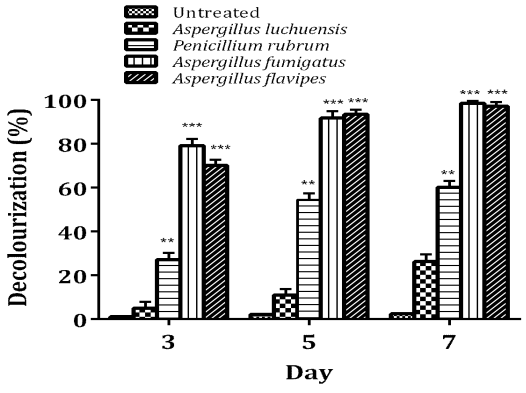

Figure 8: Decolourization of Novacron Red FNR by fungal isolates. The assay was performed by using isolates *Penicillium rubrum, Aspergillus luchuensis, A. fumigatus* and *A. flavipes* following the similar process described in Figure 5. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level.

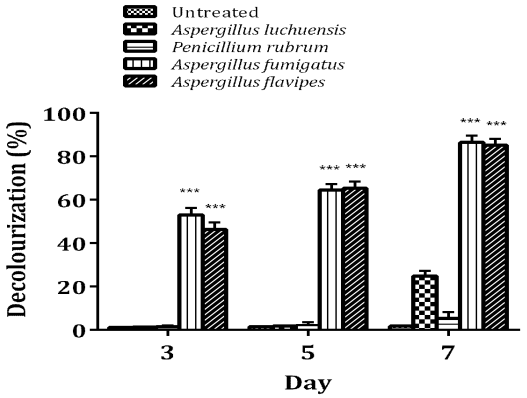

Figure 9: Decolourization of Novacron Navy WB by fungal isolates. The assay was performed by using isolates *Penicillium rubrum, Aspergillus luchuensis, A. fumigatus* and *A. flavipes* following the similar process described in Figure 5. The data is representative of three independent experiments. Here, * = P <0.05, ** = P< 0.01 and *** = P< 0.001, P = significance level.

The dye found to be the most resistant in this study is Novacron Navy WB which was only decolourized by *A. fumigatus and A. flavipes* with a decolourization rate of just more than 85% after 7 days of incubation. This is the lowest among the highest decolourization rates achieved against any dyes. However, there was no significant decolourization of this dye by either *A. luchuensis* or *P. rubrum* **(Figure 9).**

Discussions

From our experiment it is quite clear that *Aspergillus fumigatus* and *Aspergillus flavipes* are the most potential agent for decolourization of all tested Novacron dyes. In the most cases of this study, it was observed that initially dye removal was faster and with time it gradually slowed down. Hence, we can assume in dye decolourization aspect that young fungal biomass is more effective than that of old fungal biomass. This is not unexpected, since dye uptake mostly dependent on metabolic activity of actively growing mycelium.

During 7 days of incubation period, it was found that *Aspergillus fumigatus* and *Aspergillus flavipes* decolourized all of the Novacron dyes to a greater extent. The decolourization (%) was found more effective than that of many previous and recent findings (Ryu and Weon, 1992; Jothimani and Prabakaran, 2003; Muthezhilan *et al.*, 2008 and Saranraj *et. al.* 2010) in terms of time required for maximum decoloutization. In their experiment, Saranraj *et. al.*, found the complete degradation of Direct violet BL and Direct Sky Blue FF by *Aspergillus niger* and *A. flavus* respectively took 9 days while Direct violet BL, Direct Sky Blue FF and Direct Black E by *Trichoderma viride*, *Mucor sp.* and *Penicillium chrysogenum* respectively took 12 days of incubation. In another experiment, highest decolourization (%) of 86.5 after 8 days of incubation was reported by *Aspergillus terreus* (Muthezhilan *et al.*, 2008). In contrast, we found almost complete degradation of Blue FNR by *A. flavipes* within 5 days and Red FNR by *A. fumigatus* within 7 days of incubation. Moreover, the degradation value achieved by *A. flavipes* against Yellow FN2R and Orange W3R

was just below 95% after 7 days of incubation. These results revealed *A. fumigatus* and *A. flavipes* as the most efficient textile dye degrader. In the experiment done for the evaluation of interactive effects of temperature, pH and enzyme concentration by response surface methodology, the maximum colour removal was found 98.9% (Imen *et al*., 2010). Here, we found that both *Aspergillus fumigatus* and *Aspergillus flavipes* can decolourize most of the textile dyes nearly at that extent after 7 days of incubation.

Conclusion

This study clearly reveals that some fungi especially *Aspergillus sp*. and *Penicillium sp*. can thrive in dyeing industry effluent by using dyes as their source of nutrient while some of them found to have tremendous dye decolourizing capability. Among 4 isolates, we found two *Aspergillus sp*. to degrade all of the tested dyes with an excellent decolourization rate, rest two were also satisfactory. So, this mysterious property of fungi as well as other microorganisms could be used as effective arms to fight with dyeing effluent related water pollution that becoming the most concerned topic throughout the world. Further study on their enzymatic property and optimization of their dye degrading process parameter could make them more efficient in textile dye degradation.

References

1. Chen KC, Wu JY, Liu DJ, Hwang SCJ. Decolourization of the textile dyes by newly isolated bacterial strains. J. Biotechnol. 2003; 101: 57 – 68.

2. Cripps C, Bumpus AJ, Aust SD. Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrososporium*. Appl. Env. Microbiol. 1990; 56: 1114 – 1118.

3. Gilman JC. A manual of soil fungi. Oxford and IBH publishing CO. Revised second edition. 1957.

4. Imen K, Benoît M, Raja BA. Decolourization of the reconstituted dye bath effluent by commercial laccase treatment: Optimization through response surface methodology. Chem. Eng. J. 2010; 156 (1): 121 – 133.

5. Jothimani P, Prabakaran J. Dye factory effluent decolourization by fungal cultures under static condition. J. Ecobiol. 2003; 15: 255 – 260.

6. Mahdavi TA, Donnelly T, Anderson GK. Colour removal from a simulated dye wastewater using a two-phase anaerobic packed bed reactor. Water Res. 2001; 35: 425 – 432.

7. Meyer U. Microbial degradation of xenobiotics and recalcitrant compounds, (Leisinger, T., Cook, A. M., Nuesch, J., Hutter, R. eds). Academic Press, London; 1981. p. 371 – 385.

8. Moorthi SP, Selvam PS, Sasikalaveni, Murugesan AK, Kalaichelvan PT. Decolourization of textile dyes and their effluents using white rot fungi. AIr J Biotechnol. 2007; 6(4): 424 –429.

9. Muthezhilan R, Yogananth N, Vidhya S, Jayalakshmi S. Dye degrading mycoflora from industrial effluents. Res. J. Microbiol. 2008; 3(3): 204 – 208.

10. O'neil C, Hawkes FR, Hawkes DL, Lourenco ND, Pinheiro HM, Delee W. Colour in textile effluents, Sources, measurements, discharge consents and simulation a review. J. Chem. Technol. Biotechnol. 1999; 74: 1009 – 1018.

11. Pandey A, Singh P, Iyengar L. Bacterial decolourization and degradation of azo dyes. Int. Biodeter. Biodegr. 2007; 59: 73 – 84.

12. Pasti- Grigsby MB, Paszczynski A, Goszczynski S, Crawford DL, Crawford RL. Influence of aromatic substitution patterns on azo dye degradability by *Streptomyces* sp. and *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 1992; 58: 3605 – 13.

13. Peralta – Zamora P, Kunz A, Demoraes SG, Pelegrini R, Moleiro PD. Degradation of reactive dyes-1, A comparative study of azonation, enzymatic and photochemical processes. Chemosphere. 1999; 38: 835 – 852.

14. Ponraj M, Gokila K, Zambare V. Bacterial decolourization of textile dye-orange 3R. Int j adv biotechnol res. 2011; 2: 168 – 177.

15. Rao MN, Datta AK. Wastewater treatment-Rational methods of design and Industrial practices. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, 2nd edition. 1987.

16. Robinson T, Mcmullan G, Marchant R, Nigam P. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresour. Technol. 2001; 77: 247 – 255.

17. Ryu BH, Weon YD. Decolourization of azodyes by *Aspergillus sojae* B – 10. J. Microbiol. Biotechnol. 1992; 2: 215 – 219.

18. Salar R K, Rohilla S K, Rohilla J K. Decolourization of Reactive Black HFGR by *Aspergillus sulphureus*. Res. J. Recent Sci. 2012; 1(1): 55 – 61.

19. Saranaik S, Kanekar P. Bioremediation of colour of methyl violet and phenol from a dye industry waste effluent using *Pseudomonas* sp. Isolated from factory soil. J. Appl. Bacteriol. 1995; 79: 459 – 469.

20. Saranraj P, Sumathi V, Reetha D, Stella D. Fungal decolourization of Direct azo dyes and biodegradation of textile dye effluent. J. Ecobiotechnol. 2010; 2(7): 12 – 16.

21. Seshadri S, Bishop PL. Anaerobic/Aerobic treatment of selected azo dyes in wastewater. Waste Management. 1994; 14(2): 127 – 137.

22. Shaul GM, Holdsworth TJ, Dempsey CR, Dostal KA. Fate of water soluble azo dyes in activated sludge process. Chemosphere. 1991; 2(1-2): 107 – 119.

23. Spadaro JT, Gold MH, Ranganathan V. Degradation of azo dyes by lignin degrading fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 1992; 58: 2397 – 2401.

24. Walker R. The metabolism of azo compounds: a review of the literature. Food Cosmet. Toxicol. 1970; 8(6): 659 – 676.

25. WHO (World Health Organization). Rapid assesment of sources of air, water and land pollution, offset publication no.62. 1982. p.7.

26. Zimmermann T, Gasser F, Kulla HG, Leisinger T. Comparisons of two bacterial azoreductases acquired during adaptation to growth on azo dyes. Arch. Microbiol. 1984; 138: 37 – 43.

27. Zollinger H. Colour chemistry - syntheses. Properties and applications of organic dyes and pigments. New York: VCH publishers. 1987.