

Effect of Nutritional Requirement in the Medium for Growth and Decolorization of Textile Dye by Indigenous Fungal Isolates

¹Shazia Erum, ¹Safia Ahmed, ²Zubair Anwar and ²Jabar Zaman Khan Khattak

¹Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

²Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan

Abstract: The effects of nutritional requirement for growth and decolorization of textile dye by indigenous fungal isolates have been studied using Duncan multiple range test and analysis of variance. Maximum decolorization was observed at 0.5-1 M nitrogen concentration by the selected strains, where higher concentration had a negative effect. Growth also showed their maximal (0.35 g/100 mL by SA 1, 0.27 g/100 mL by SA 2 and 0.31 g/100 mL by SA 3) in this range with all tested strains. All the fungal strains showed significant correlation with nitrogen concentration (R² 0.82, 0.77 and 0.88 of *Aspergillus Niger* SA 1, *Aspergillus flavus* SA 2 and *Aspergillus terreus* SA 3 respectively). Similar results observed for increased in concentration of carbon source as there was increasing trend of decolorization efficiencies of all the selected fungal strains (R² 0.85, 0.76 and 0.97 of SA1, SA2 and SA3, respectively). The comparison of the fungal response obtained, suggests that *Aspergillus flavus* SA2 yielded the best result of 80.72% which was followed by *Aspergillus Niger* SA1 behaving 78.29% decolorization *Aspergillus terreus* SA3 rated third within selected strains by rendering a percentage of 78.29.

Keywords: *Aspergillus*, carbon, DMRT, decolorization, nitrogen

INTRODUCTION

Toxic effluents containing azo dyes are discharged from various industries and they adversely affect water resources, soil fertility, aquatic organisms and ecosystem integrity. Approximately 10-15% of the dyes are released into the environment (Singh, 2006). Azo dyes are the main chemical class of dyes with the greatest variety of colors; therefore they have been extensively used in tattooing, cosmetics, foods and consumer products (Chen, 2006).

Biological remediation is considered more efficient in terms of its long lasting benefits and for having almost no harmful effects on environment. In addition, it would be economical as compared to other different physicochemical techniques tested (Balan and Monterio, 2001; Wallace, 2001). Various fungal strains that have proved more efficient in decolorization of textile dyes mostly belonged to the group of White-rot. Dyes are removed by fungi by biosorption (Conatao and Corso, 1996; Paymann and Mehnaz, 1998; Fu and Viraraghavan, 2000) and enzymatic mineralization (degradation).

Moreover, biologically mediated decolorization of different textile dyes has been found to be influenced under different physicochemical conditions, including; pH (Baldrian, 2004) temperature (Masud Hossain and Anantharaman, 2006), additional carbon (Mester and

Tien, 2000) and nitrogen source (Dong *et al.*, 2005) and type and concentration of dyes (Arora and Chander, 2004). In addition it is also affected by salts and inhibitory molecules like sulphur compounds, surfactants, heavy metals, bleaching chemicals, carbon and nitrogen sources and other nutrients (Mester and Tien, 2000). Hence, biotic and abiotic need to be given top consideration in biological remediation of textile dyes at any scale.

Fungal degradation of aromatic structures (produced after dye decolorization and breaking of azo bond) is a secondary metabolic event that starts when nutrients (C, N and S) become limiting (Kirk and Farrell, 1987). The objective of this study was to evaluate the relative importance of various nutrients for Acid red dye decolorization in submerged fermentation. Effect of nitrogen and carbon source (glucose) on dye decolorization abilities of fungal isolates was investigated.

MATERIALS AND METHODS

Micro organism: Different fungal strains belonged to class Ascomycetes were acquired from Microbiology Research Laboratory, Quaid-i-Azam University Islamabad, Pakistan in 2010. These strains were previously isolated from textile water pond of Kohinoor Textile Mill Rawalpindi, Pakistan (Ali *et al.*, 2008).

They include *Aspergillus Niger* SA1, *Aspergillus flavus* SA2, *Aspergillus terreus* SA3 the cultures were regularly maintained on Sabraud Dextrose agar (SDA).

Culture media: *Aspergillus nidulans* minimal medium was prepared by mixing three solutions (A, B and C). Solution 'A' was made by adding grams per 500ml of distilled water, NaNO₃ 6.0, KH₂PO₄ 1.52 and KCl 1.52. pH was adjusted to 6.5 with 2N NaOH. Solution 'B' contained MgSO₄.7H₂O 0.52 g, FeSO₄. 7H₂O trace (0.001 g), ZnSO₄. 7H₂O trace, while the solution 'C' was made only by adding 10 g of glucose in 200ml of distilled water. All the solutions were separately autoclaved for 15 min at 121°C with 15 psi pressure and mixed.

Culture conditions: Incubation was carried out at 30±1°C under shaking conditions in 250 mL Erlenmeyer flasks containing 100 mL of medium, which were inoculated with 2% v/v of homogenized culture. Individual fungal strains were grown on *Aspergillus nidulans* minimal agar plate for a week at 30°C. After a full growth of fungal colony, the spores were scratched with L-shaped needle and the needle was dipped in sterilized 2 L Erlenmeyer flasks containing 50ml ANMM broth and incubated under static condition at 30°C for 7 days. After that whole mass was mixed in a blender to make a homogenous mixture. On day 7, fungal cultures were filtered through a filter paper using a Buchner funnel and culture supernatants were used for decolorization studies.

Experimental design and statistical analysis: The different concentration of nitrogen (0.5, 1, 1.5, 2 M) using sodium nitrate as additional nitrogen source and carbon using glucose as a carbon source in different molar concentrations (0.4, 0.8, 1.6, 2.4, 3.2, 4, 4.8, 5.4, 6.2 and 7M) were analyzed for the decolorization of AR

Aspergillus flavus SA 2 and *Aspergillus terreus* SA 3). 151 by selected fungal strains (*Aspergillus Niger* SA 1, For studying each single parameter, Batch Shake Flask experiment (BSF) was carried out at neutral pH of 100 mL *Aspergillus Nidulans* Minimal (ANM) media. Experiments were replicated three times. The statistical analyses were performed by use of Duncan Multiple Range Test (DMRT). Analysis of Variance tool was applied for calculating Means with significant difference and they were compared by using multiple regressions and ANOVA.

Analytical methods: Spectrophotometric method was used to monitor the optical density at λ512nm for acid red 151 dyes by Shimadzu UV visible Spectrophotometer. Fungal growth was measured by taking dry cell mass of oven dried samples at 40°C after 24 h.

Chemicals: The chemical compounds and media components were obtained from BDH laboratory chemical division (Poole, Dorset, England), DIFCO Lab. (Detroit, Michigan, USA). Acid red 151 dyes (AR 151) and all other chemicals were from Sigma (St. Louis, Mo, USA).

RESULTS

All 3 selected *Aspergillus* strains (*Aspergillus Niger* SA 1, *Aspergillus flavus* SA 2 and *Aspergillus terreus* SA 3) were showing growth and decolorization of AR 151 dye on solid agar medium as well as in liquid medium (ANM). The effects of nutritional requirement (nitrogen and carbon) for the removal of AR 151 dyes were studied in batch shake flask experiments. Statistical analysis (DMRT) of effect of carbon source concentration on dye decolorization by different fungal strains is discussed in tabular form in Table 1.

Table 1: Statistical Analysis (DMRT) of effect of carbon source concentration on dye depolarization by different fungal strains

Strains	Strains means	Treatments (M)	Treatments means
A. Niger SA1	78.29 b	0.4	64.86 D
A. flavus SA2	80.72 a	0.8	67.29 D
A. terreus SA3	71.88 c	1.6	71.44 C
		2.4	76.68 B
		3.2	77.63 B
		4	77.14 B
		4.8	78.08 B
		5.4	84.13 A
		6.2	87.28 A
		7	85.11 A
LSD at 0.05	2.269		4.143

Any two means not sharing a letter differ significantly at p<0.05; Treatments means of dry weights are highly significant which are given below; 0.244 c, 0.273 bc, 0.260 bc, 0.299 ab, 0.298 ab, 0.30 4ab, 0.30 4ab, 0.309 ab, 0.309 ab, 0.323 a, 0.323a which is significantly differ from all others; LSD = 0.0422 at 5%

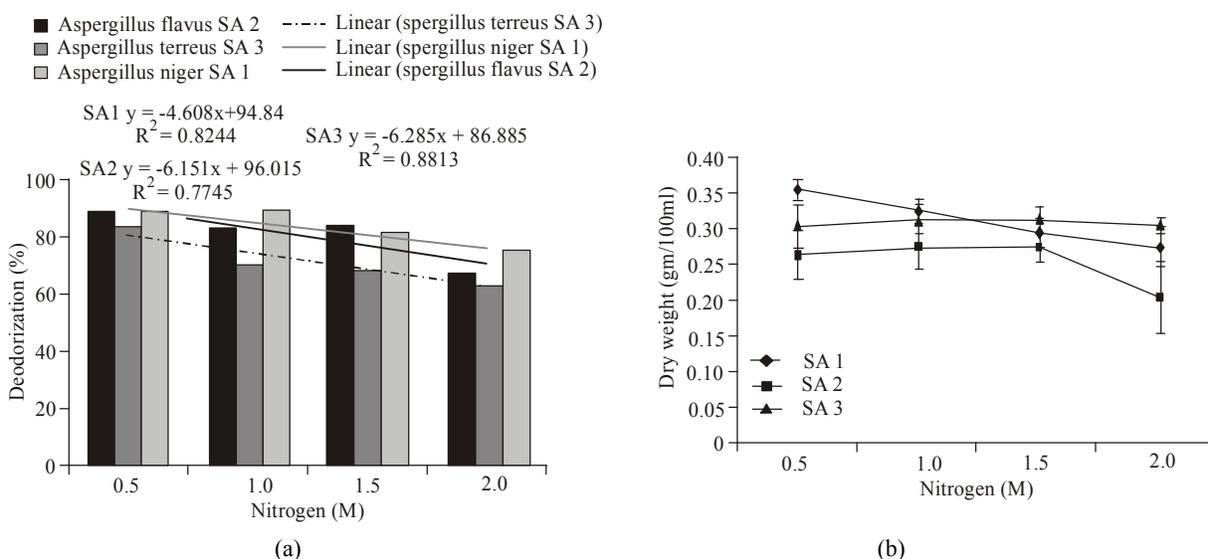


Fig. 1: Effect of Nitrogen concentration on AR 151 decolorization, (a) and growth, (b) by selected fungal strains

Table 2: Statistical Analysis (DMRT) of effect of Nitrogen concentration on dye decolonization by different fungal strains

Strains	0.5 M	1 M	1.5 M	2 M	Strains mean
A. Niger SA1	87.67 ab	89.37 a	81.27 bc	75.00 cd	83.33 A
A. Flavus SA2	88.53 ab	82.63 ab	83.73 ab	67.67 e	80.64 A
A. Terreus SA3	83.40 ab	70.30 de	67.67 e	63.33 e	71.18 B
Treatments mean	86.53 A	80.77 B	77.56 C	68.67 D	
	Strains	Treatments		Interaction (SxT)	
LSD at 0.05	3.419			3.948	6.838

Any two means not sharing a letter differ significantly at $p < 0.05$. Strains dry weight is significant. Aspergillus flavus SA2 mean value i.e., 0.254b is significantly differ from the other two strains (Aspergillus terreus SA3 and Aspergillus Niger SA1) i.e. 0.311 a, 0.308 at 5%. LSD 0.0377 at 5%; Treatments means for dry weights are non significant

Effect of nitrogen concentration: Maximum decolorization was observed at 0.5-1 M nitrogen concentration by all the 3 strains, where higher concentration had negative effect (Fig. 1a). Growth also showed their maximal (0.35 gm, 100 mL⁻¹ by SA1, 0.27 gm, 100mL⁻¹ by SA2 and 0.31 gm 100 mL⁻¹ by SA3) in this range with all tested strains (Fig. 1b). All the fungal strains showed significant correlation with nitrogen concentration (R^2 0.82, 0.77 and 0.88 of Aspergillus Niger SA1, Aspergillus flavus SA2 and Aspergillus terreus SA3 respectively).

Statistical data Table 2 reveals significant differences among treatments (Nitrogen concentration), strains and for their interaction as well. Overall, the good results were yielded by lowest concentration of 0.5 M in interaction with different strains, however; the best interaction was developed between Aspergillus Niger SA1 and 1M nitrogen concentration which gave us a super ceding rate of decolorization in this study i.e., 89.37%. Oppositely, quite poor interaction was observed in case of Aspergillus flavus SA2 and Aspergillus terreus SA3 with 2M concentration where

unfair consequences were obtained in terms of 67.67 and 63.33% decolorization tendency. Elevated concentration of nitrogen exerted a negative impact on this particular studied as Table 2 clarify that there is a gradual fall in percentage data with most stumpy outcome (68.67) at the highest level of 2 M whereas 0.5 M was the best treatment from statistical point of view yielding a good score of 86.53%. Fungal strains, Aspergillus flavus SA2 and Aspergillus Niger SA1 were also ranked first at $p = 0.05$ in this parameters. Results achieved by these strains were 80.64 and 83.33% respectively, comparative to Aspergillus terreus SA3 which had a bare achievement of 71.18% decolonization (Table 2, Fig. 1).

Effect of carbon source concentration: Glucose as carbon source in the media was tested at different concentration (0.4-7M) for its effect on AR 151 dyes decolorization by the selected fungal strains. As the concentration of carbon source increased there was increasing trend of decolorization efficiencies of all the fungal strains (Fig. 2a).

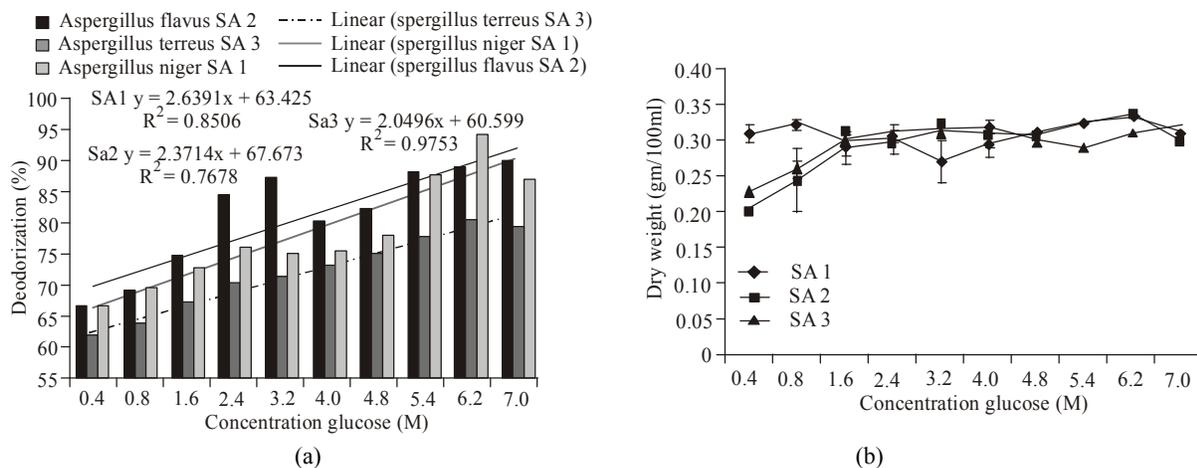


Fig. 2: Effect of Carbon concentration on AR 151 decolorization, (a) and growth, (b) by selected fungal strains

Table 3: Analysis of variance (ANOVA) for the effect of nitrogen concentration on dye depolarization (A) and dry cell weight (B) by selected fungal strains

K value	Source	df	SS	MS	F-value	Probability
A: Dye depolarization						
2	Strains (S)	2	977.762	488.881	29.6891	0.0000 **
4	Treatments (T)	3	1504.814	501.605	30.4618	0.0000 **
6	SxT	6	286.380	47.730	2.8986	0.0286 *
7	Error	24	395.200	16.467		
	Total	35	3164.156			
B: The dry weights						
2	Strains (S)	2	0.025	0.012	4.9764	0.0156 *
4	Treatments (T)	3	0.013	0.004	1.6899	0.0156 *
6	SxT	6	0.010	0.002	0.6798	0.1958 NS
7	Error	24	0.059	0.002		
	Total	35	0.107			

A: Coefficient of Variation: 5.18%; B: Coefficient of variation: 17.08 %

Table 4: Analysis of variance (ANOVA) for the effect of carbon source concentration on dye depolarization (A) and dry cell weight (B) by selected fungal strains

K value	Source	df	SS	MS	F-value	Probability
A: Carbon concentration for AR 151 depolarization						
2	Strains (S)	2	1252.935	626.467	32.4535	0.0000 **
4	Treatments (T)	9	4469.755	496.639	25.7279	0.0000 **
6	SxT	18	406.503	22.583	1.1699	0.3142 NS
7	Error	60	1158.214	19.304		
	Total	89	7287.406			
B: The dry weights for Carbon concentration						
2	Strains (S)	2	0.009	0.004	2.6512	0.0788 NS
4	Treatments (T)	9	0.050	0.006	3.3787	0.0021 *
6	SxT	18	0.045	0.003	1.5199	0.1149 NS
7	Error	60	0.099	0.002		
	Total	89	0.203			

A: Coefficient of variation: 5.71%; B: Coefficient of variation: 13.89%

All the three fungal strains showed highly significant correlated with carbon concentration and decolorization (R2 0.85, 0.76 and 0.97 of SA1, SA2 and SA3, respectively).

Means values for the study (Table 3) showed disparity in treatment response and vary significantly from each other. 6.2M and 7M treatments were most

outstanding with an upshot of 87.28 and 85.11%. Furthermore analysis of treatments exhibits an ascending trend, where optimum results were obtained at highest concentrations while poor riposte was obtained at reduced concentrations. ANOVA (Table 4) elucidates that strains and treatments are highly significant but there interaction was non-significant at $p = 0.05$.

Evaluation of strains gives us an idea that *Aspergillus flavus* SA2 afforded the best result of 80.72% which was followed by *Aspergillus Niger* SA1 bearing 78.29% decolorization. Contrary to this, *Aspergillus terreus* SA3 ranked third among these strains by yielding 78.29%.

In spite of decolorization, maximum fungal biomass production (>0.31 gm 100 mL⁻¹) was also shown similar trend of carbon concentration i.e., 6.2 M that was correlated with decolorization efficiency of all three fungal strains (Fig. 2b).

DISCUSSION

The effect of different concentrations of nitrogen (NaNO₃) was tested in the present study on the dye removal efficiency of fungal isolates. It was observed that lower concentration of nitrogen (0.5M to 1.0 M) proved itself aid towards AR 151 decolorization and the rate of dye removal decreased from 86.53% to 68.67% with increase in sodium nitrate concentration from 0.5 to 2 M, respectively (Fig. 1). The fungal response in enzyme production support the previous study as the deprivation of nitrogen and carbon sources was considered as a major factor in triggering ligninolytic system of white rot fungi (Leatham and Kirk, 1983; Mester *et al.*, 1996). Further increase in NaNO₃ showed decreased color removal might be due to accumulation of nitrogen waste/toxicity of metabolic products such as NO₃, NO₂, NH₃ etc. (Panswad and Luangdilok, 2000). The concentration of urea as nitrogen source below 0.01 gmL⁻¹ and above 1gmL⁻¹ proved to be quite limiting for the decolorization of AR 151 dye, Orange II and DbK2R1 and related biomass production in different fungi (Ali *et al.*, 2008). Nitrogen limiting condition showed increased dye removal than N-rich (Mehna and Bajpai, 1995; Moreira *et al.*, 2004; Robinson *et al.*, 2001a; Raghukumar, 2000). The role of nitrogen in dye removal can never be ignored as it enhanced the strains activity of azo dye decomposition significantly. This activity was due to increase in enzymatic activity, not to cell growth in the presence of growth nutrients (Yu *et al.*, 2001). Nitrogen supplementation improved enzyme activities and dye decolorization (Robinson *et al.*, 2001b). The concentration of glucose as a carbon source below optimum concentration (6.2 M) and above 7M proved to be quite limiting for the decolorization of AR 151 dyes and related biomass production in different fungi (Fig. 2). The removal was clearly metabolism dependent as indicated by glucose consumption and biomass production with increased decolorization (Rojek *et al.*, 2004). Optimizing the culture medium by different co-substrate (as N and additional C) can

improve the biomass quality which results in better color removal abilities of fungi (Kumar *et al.*, 1998; Nagarathamma and Bajpai, 1999; Fujita *et al.*, 2000; Bras *et al.*, 2001; Ali *et al.*, 2008). Decolorizing capacity significantly enhanced up to 87% of the fungus by the addition of nitrogen (urea as a source) and carbon (glucose as a source) for three synthetic dyes were Congo red, malachite green and crystal violet (Pant *et al.*, 2008).

REFERENCES

- Ali, N., A. Hameed, S. Ahmed, A.G. Khan, 2008. Decolorization of structurally different textile dyes by *Aspergillus niger* SA1. *World J. Microb. Biot.*, 24: 1067-1072.
- Arora, D.S. and M. Chander, 2004. Decolorization of diverse industrial dyes by some *Phlebia* spp and their comparison with *Phanerochaete chrysosporium*. *J. Basic. Microb.*, 44(5): 331-338.
- Balan, D.S.C. and R.T.R. Monterio, 2001. Decolorization of textile dye indigo by lignolytic fungi. *J. Biotechnol.*, 89: 141-145.
- Baldrian, P., 2004. Purification and characterization of laccase from the white-rot fungus *Daedalea quercina* and decolorization of synthetic dyes by the enzyme. *Appl. Microbiol. Biot.*, 63(5): 560-563.
- Bras, R., I.A. Ferra, H.M. Pinheiro and I.C. Goncalves, 2001. Batch tests for assessing decolourisation of azo dyes by methanogenic and mixed cultures. *J. Biotechnol.*, 89: 155-162.
- Chen, H., 2006. Recent advances in azo dye degrading enzyme research. *Curr. Protein Peptide Sci.*, 7(2): 101-111.
- Conatao, M. and C.R. Corso, 1996. Studies of adsorptive interaction between *Aspergillus niger* and the reactive azo dye procion blue MX-G. *Ecler. Chem.*, 21: 97-102.
- Dong, J.L., Y.W. Zhang and R.H. Zhang, 2005. Influence of culture conditions on laccase production and isozyme patterns in the white-rot fungus *Trametes gallica*. *J. Basic Microb.*, 45(3): 190-198.
- Fu, Y.Z. and T. Viraraghavan, 2000. Removal of a dye from aqueous solution by the fungus *Aspergillus niger*. *Water Qual. Res. J. Canada*, 35: 95-111.
- Fujita, M., A. Era, M. Ike, S. Soda, N. Miyata and T. Hirao, 2000. Decolorization of heat treatment liquor of waste sludge by a bioreactor using polyurethane foam immobilized white rot fungi equipped with an ultra membrane filtration unit. *J. Biosci. Bioeng.*, 90: 387-394.

- Kirk, T.K. and R.L. Farrell, 1987. Enzymatic 'combustion': The microbial degradation of lignin. *Ann. of Microbiol.*, 41: 465-505.
- Kumar, M.N., V.R. Sridhari and K.D. Bhavani, 1998. Trends in color removal from textile mill effluents. *Colorage*, 40: 25-34.
- Leatham, G.F. and T.K. Kirk, 1983. Regulation of ligninolytic activity by nutrient nitrogen in white-rot basidiomycetes. *FEMS Microbiol. Lett.*, 16: 65-67.
- Masud Hossain, S.K. and N. Anantharaman, 2006. Activity enhancement of ligninolytic enzymes of *Trametes versicolor* with bagasse powder. *Afr. J. Biotechnol.*, 5(1): 189-194.
- Mehna, A. and P. Bajpai, 1995. Studies on decolorization of effluent from a small pulp mill utilizing agriresidues with *Tremetes versicolor*. *Enzyme Microb. Tech.*, 17: 18-22.
- Mester, T., M. Pena, J.A. Field, 1996. Nutrient regulation of extracellular peroxidases in the white-rot fungus *Bjerkandera* spp. strain BOS55. *Appl. Microbiol. Biot.*, 44: 778-784.
- Mester, T. and M. Tien, 2000. Oxidation mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants. *Int. Biodeter. Biodegr.*, 46: 51-59.
- Moreira, M.T., C. Viacava and G. Vidal, 2004. Fed-batch decolorization of poly R-478 by *Trametes versicolor*. *Braz. Arc. Biol. Techn.*, 47(2): 179-183.
- Nagarathnamma, R. and P. Bajpai, 1999. Decolorization and detoxification of extraction-stage effluent from chlorine bleaching of Kraft Pulp by *Rhizopus oryzae*. *Appl. Environ. Microb.*, 65(3): 1078-1082.
- Panswad, T. and W. Luangdilok, 2000. Decolorization of reactive dyes with different molecular structures under different environmental conditions. *Water Res.*, 34: 4177-4184.
- Pant, D., A. Singh, Y. Satyawali, R.K. Gupta, 2008. Effect of carbon and nitrogen source amendment on synthetic dyes decolorizing efficiency of white-rot fungus, *Phanerochaete chrysosporium*. *J. Env. Biol.*, 29(1): 79-84.
- Paymann, M.A. and M.A. Mehnaz, 1998. Decolorization of textile effluent by *Aspergillus niger* (marine & terrestrial). *Fresen. Env. Bull.*, 7: 1-7.
- Raghukumar, C., 2000. Fungi from marine habitats: an application of bioremediation. *Mycol. Res.*, 104: 1222-1226.
- Robinson, T., B. Chandran and P. Nigam, 2001a. Studies on the production of enzymes by white-rot fungi for the decolorization of textile dyes. *Enzyme Microb. Tech.*, 29: 575-579.
- Robinson, T., G. McMullan, R. Marchant, 2001b. Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. *Bioresource Technol.*, 77: 247-255.
- Rojek, K., F.A. Roddick and A. Parkinson, 2004. Decolorisation of natural organic matter by *Phanerochaete chrysosporium*: The effect of environmental conditions. *Water Sci. Technol.*, 4(4): 175-182.
- Singh, H., 2006. *Mycoremediation: Fungal Bioremediation*. John Wiley & Sons, Hoboken, New Jersey, pp: 592, ISBN: 047175501X.
- Wallace, T.H., 2001. Biological treatment of synthetic dye water and an industrial Textile Wastewater containing azo dye compounds. MSc. Thesis. Faculty of Virginia Polytechnic Institute and State University, USA.
- Yu, J., X. Wang and P.L. Yue, 2001. Optimal decolorization and kinetic modeling of synthetic dyes by *Pseudomonas* strains. *Water Res.*, 35(15): 3579-3586.