Journal of **A**pplied **B**iotechnology **R**eports

Original Article

Biodecolorization of Textile Effluents by Autochthonous Fungi

Maryam Mazaheri Tehrani[1], Mahnaz Mazaheri Assadi[2*], Hamid Rashedi[3]

Abstract

Textile effluents contain different kinds of chemical dyes and many mutagenic and carcinogenic substances. Discharge of these effluents into terrestrial and aquatic ecosystems causes harmful effects. In this research, decolorization of a textile effluent by nineteen different strains of fungi was investigated. Removal activity of dyes was in order of *Aspergillus* > *Rhizopus* > *Fuzarium* > *Penicillium* > *Saccharomyces*. The selected fungal strain was capable of decolorizing textile effluent at original pH of effluent (8.5- 9) by adding 0.6 and 0.2 g/L of sucrose and NH_4Cl as additional carbon and nitrogen sources respectively at 30°C. 98 % of dyes were absorbed from effluent during the exponential growth phase in the presence of a biodegradable substrate such as sucrose under shake flask conditions. Dyes strongly bound to the fungal biomass required extraction with methanol for their removal. The dyes pigment yellow (PY 74) and reactive blue (RB 38) were totally decolorized after 6 days at initial concentration of 250 ppm by terrestrial *Aspergillus niger*.

1. Nuclear Biotechnology Laboratory, Nuclear Science Research School, Nuclear Science and Technology Research Institute, Tehran, Iran
2. Center of Biotechnology, Iranian Research Organization for science and Technology, Tehran, Iran
3. Biotechnology Group, School of Chemical Engineering ,College of Engineering, University of Tehran, Tehran, Iran

* **Corresponding Author**
Mahnaz Mazaheri Assadi
Center of Biotechnology, Iranian Research Organization for science and Technology, Tehran, Iran
E-mail: mxmazaheriassadi@yahoo.com

Submission Date: 10/10/2014
Accepted Date: 12/20/2014

Keywords: Biodecolorization, Textile Effluent, Carcinogen, *Aspergillus niger*

Introduction

Synthetic dyes have a wide application in the food, pharmaceutical, textile, leather, cosmetics and paper industries due to their ease of production, fast pace, and color variation as compared to natural dyes [1]. Most of these compounds such as azo dyes are toxic, mutagenic, carcinogenic and highly resistant to degradation [2- 4]. It is estimated that from 1 to 15% of the dye is lost during dyeing section of a textile industry process and is released in wastewaters [4- 6].

Most of the azo dyes, which are released into the environment originate from textile industry and dyestuff manufacturing industry [7]. The presence of dyes and chemicals in the waste effluent even at low concentrations is harmful to both aquatic and terrestrial life [8]. Therefore their presence in aquatic systems reduces light penetration which retards the photosynthetic activity and also has a tendency to chelate metal ions producing micro-toxicity to fish and other organisms [9- 10]. A number of physico-chemical methods, such as adsorption, coagulation, precipitation, filtration and oxidation, have been used to treat dye stuff effluents, but these methods have many disadvantages and limitation [11] such as being generally expensive and produce large amounts of sludge [12] that requires special disposal techniques ain accordance with law [4].

In recent years there have been many reports regarding decolourization of various azo dyes to a great extent by the use of various bacterial and fungal cultures [13-16].

Most of the previous studies focused on the dye decolorization by lignin- degrading enzymes of white rot fungi [17- 20]. It was demonstrated in an earlier study by Mazaheri Assadi *et al*. that an autochthonic marine *Aspergillus niger* has efficiently decolorizetextile effluents [21].

In the present study the ability of some terrestrial fungi and a previously studied marine *A. niger* [21] for decolorizing a textile wastewater was evaluated. Furthermore, decolorization ability of this fungus for specific dyes pigment yellow 74 and reactive blue 38 and its growth rate in effluent have been investigated [21].

Materials and Methods

Media and dyes

The dyes pigment yellow (PY-74) and reactive blue (RB-38) used for biodecolorization studies were prepared from textile factories, Tehran, Iran. All media components and chemicals used in this study were of the highest purity or analytical grade, obtained from recognized chemical suppliers (MERK). Thermo-sensitive chemical agents were filter sterilized and aseptically were supplemented to the cultures with determined concentrations.

Effluent source

Highly black effluents of a textile factory utilizing textile dyes such as, reactive blue 38, reactive yellow MERL pigment yellow 74, pigment green 7 etc. and other waste substances due to textile dyeing industry were used. The effluent was collected in 20 L black colored plastic

carboys and immediately was transferred and stored at 4°C in the laboratory for further experiments.

Enrichment and isolation of fungi from effluent

0.1 g glucose, 0.1 g NH_4Cl, 0.1 g yeast extract and 1.5 g agar were added to 100 ml textile effluent, and for the inhibition of the bacterial growth 300 mg/L penicillin G and Kanamycin were added to the medium. After sterilization of the medium in autoclave at 121°C for 15 min the medium was cooled at laboratory temperature and subsequently plated. The petri plates were incubated at 30°C for 4 days and colonies with most distinct morphology were picked up, purified and maintained on potato dextrose agar (PDA- Merck). The isolated fungal strains were identified as *Penicillium sp*. (FSE 1) and *Rhizopus sp.* (FSE 2) by persian type culture collection (PTCC).

Microorganisms and growth

Among the screened fungal strains two were isolated from textile wastewater effluent (FSE1, FSE2) and the rest of autochthonous fungal strains were kindly donated by PTCC (Persian Type Culture Collection, Tehran,Iran). The screened fungi were *A. flavus*, *A. oryzae*, *A. sojae*, *A. niger*, *Fuzarium sp*., *Penicillium sp*., *Cladosporium sphaerospermum*, *Rhizopus sp*., and *Saccharomyces cerevisiae*. All fungal strains were maintained on Potato Dextrose Agar (PDA) and stored at 4°C and periodically sub cultured.

Preparation of inoculums

All fungal strains were initially grown at 28- 30°C on potato dextrose agar (PDA) slants. At the end of the sporulation, 10 ml of sterile distilled water was added to the culture slant of each strain and vortexed. The final concentration of each spore suspension was adjusted to 10^8 spores/ml with sterile distilled water. Homogenized spore suspension of each fungal strain (1 ml) was directly used for inoculation. i.e. for preparation of mycelia biomass; 1 ml of spore suspension (10^8 spores/ml) inoculated in 100 ml potato dextrose broth (PDB) and incubated at 28- 30°C for 4 days on rotary shaker (150 rpm/min [21].

Preliminary batch biodecolorization

All fungal strains were screened for their growth and decolorization ability on a medium that contained: glucose, 10 g; KH_2PO_4, 1 g; NH_4Cl, 5 g; $MgSO_4$. $7H_2O$, 0.05 g; $Fe_2(SO_4)_3$, 0.0002 g; $ZnSO_4$. $7H_2O$, 0.0002 g; $MnSO_4$. H_2O, 0.0002 g in one liter of textile wastewater (pH 4.5-5.5). After inoculating, all flasks were incubated on rotary shaker at 120-150 rpm/min at 30°C. Color removal percentage was measured during necessary time for each fungal strain. The concentration of fungal biomass was determined by measuring the dry weight of mycelium [22-23]. Decolorization efficiency and growth varied among the fungal strains. Based on the results obtained during the first decolorization batch test for screening, a marine *Aspergillus niger* which had been studied previously by Mazaheri Assadi *et al*. [21, 27] was selected for the following decolorization experiments.

Decolorization assay

The degree of decolorization was measured as the change in the absorbance at λ_{max} (590 nm) supernatant of treated samples. Treated samples were centrifuged (6000- 7000 rpm/min, 15 min, IEC HN- Centrifuge, PEMED) and the clear supernatants were analyzed by using a scanning spectrophotometer (Shimadzu UV- 160 A). The decolorization efficiency was expressed as percentage of decolorization [16];

$$\text{Decolorization (\%)} = \frac{I-F}{I} \times 100$$

Where I = initial absorbance and F= absorbance of decolorized medium

Fungal growth studies

The selected fungal strain was also tested for its growth ability and decolorizatin on optimized medium/condition contained: sucrose 5g; KH_2PO_4, 0.5 g; NH_4Cl, 1 g in one liter of effluent without changing pH of effluent (pH 7.5-8) [21]. Inoculation was carried out with 0.1 g (dry weight) fungal mycelia in 100 ml of effluent medium. Culture was incubated in 250 ml Erlenmeyer flask with 100 ml medium on a rotary shaker (120-150 rpm/min) at 30°C. The growth of fungus in the textile effluent and decolorization at λ_{max} 590 nm was checked during 24 h cultivation. Every 2 h, each sample (10 ml) was centrifuged at 5000- 7000 rpm for 15 min, the biomass washed and dried 48 h at 50- 60°C. The samples were weighted and the value expressed as g dry weight/100 ml. The specific growth rate (μ) and the generation time also were computed. The exponential growth phase is the most important phase of the growth cycle when the product you are trying to produce is, either the biomass itself a growth associated product. Quantification of exponential growth rate (i.e. how fast cells grow) is the first fundamental step in the quantification of culture kinetics.

In time interval (dt) increase in biomass (dx) proportional to biomass concentration (X) i.e.

$$\frac{dX}{dt} \propto X \Rightarrow \frac{dX}{dt} = \mu X \qquad (1)$$

were μ is defined as the growth rate (h-1)

and

$$\mu = \frac{1}{X}\frac{dX}{dt}$$

Limit definition

When t = 0 (when exponential growth begins) where X = X_0 (the biomass concentration at the start of the fermentation). When t = t, X = X.

If μ is constant (which in most cases it is), then rearranging Equation (1) and integrating:

$$\int_{X_0}^{X} \frac{dX}{X} = \int_{0}^{t} \mu dt$$

$$Ln\frac{X}{X_0} = \mu t \qquad \text{or}$$

$$X = X_0 e^{\mu t} \qquad (2)$$

Equation (2) applies only to the duration of the exponential growth phase, beyond which either substrate limitation or toxin accumulation become rate determining.

Solutions were prepared aseptically by dissolving 0.25 g/L of each dye individually in 100 ml distilled water and the pH was the initial pH of the solution. The flasks further inoculated by 1 g/L of biomass (dry weight), incubated at rotary shaker (150 rpm/min) at 30°C for 8 days. After incubation, the mycelia homogenized in methanol and the homogenate was centrifuged. The absorbance of supernatant was assayed at maximum wavelength of each dye (PY-74; 490 nm, RB- 38; 640 nm).

Results

Screening of fugal strains for decolorization

Fungal strains were used to investigate their ability to decolorize a textile effluent. Results presented in figure 1 show that decolorization of effluent were very different among the fungal species. It is obvious from Figure 1 that *Aspergillus sp*. can be a good alternativeas it has 84- 96% efficiency in decolorization. On the basis of this observation, there are some studies conducted for decolorization of textile dyes by *Aspergillus* species [26-28].

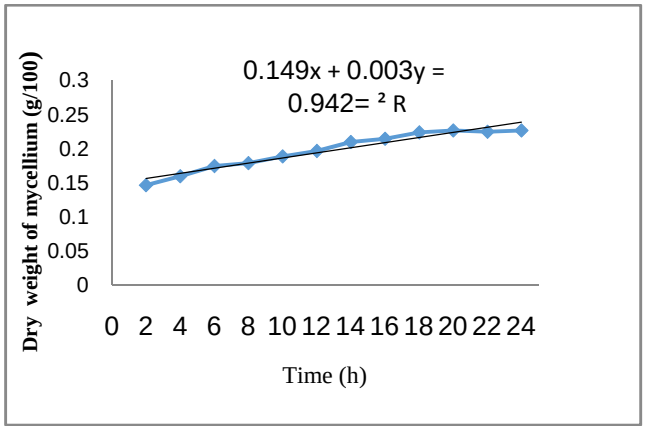

Figure 1. The growth of *Aspergillus niger* in effluent medium.

In addition to, among different *Aspergillus sp*. decolorization was highest by the selected strains from *A. niger* and further marine *A. niger* was found more efficient (95.84% dye removal) than the other strains in decolorizing of effluent.

Study of the fungal growth and decolorization in effluent

The efficiency of the decolorization processes depends on adaptability of the fungus to the textile dyes in the effluent [24]. In our experiments with *Aspergillus niger* strain, already adapted to the textile dyes in effluent, presented a very good growth in the medium at experimental conditions (Fig. 2). It was observed that the dry weight of fungus slowly increases after inoculating in the medium. The growth rate is significant between 4-18 hours of cultivation, compared with control. The logarithmic phase of the growth is corresponding with the maximum rate of decolorization.

Textile dyes decolorization studies

Point inoculation of 0.05 ml of spore suspension (1.0×10^8 spores/ml) of *A. niger* on nutrient agar plates containing individually 250 ppm of PY- 74 and RB- 38 dyes resulted in growth diameter of 1.5-1.75 cm after 7 days incubation at 28- 30°C and no decolorisation zone at the end of 7 days of incubation was observed.

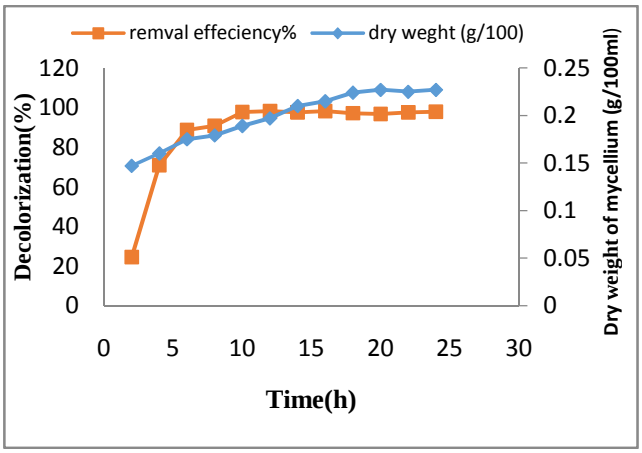

Figure 2. Effect of time on fungal growth and decolorization of textile effluent

This inability of fungus for decolorization in agar plate may be due to the presence agar in medium or high concentration of dyes in medium. It is necessary to perform the process in liquid medium so the absorption of dyes at maxima wavelength of each dye was studied. The spectrophotometric analysis of methanol extracts of *A. niger* showed that more than 80% of both dyes were adsorbed after 4 days incubation in liquid medium containing dye by *A. niger* (Table 1).

Table 1. Comparison of fungal growth in textile effluent during incubation time

Fungi	Dry weight (g/100 ml)	Incubation time (Day)
A. niger 1	0.225	3
A. niger 2	0.237	3
Strain A	0.24	3
A. niger 4	0.22	4
A. niger 5	0.20	4
A. flavus	0.21	4
A. oryzae 1	0.22	4
A. oryzae 2	0.26	4
A. sojae	0.20	4
Rhizopus sp.(FSE2)	0.15	5
*Rhizopus sp.*2	0.16	5
Fusarium sp.	0.11	5
C. sphaerospermum	No growth	7-8
Penicillium sp.(FSE 1)	0.10	6
*Penicillium sp.*2	0.06	7
*Penicillium sp.*3	0.08	7
*Penicillium sp.*4	0.09	7
*Penicillium sp.*5	0.008	6
S. cerevisiae	0.005	5

Dye removal rate for PY- 74 dye was more than RB- 38 at the first 4 days of incubation and further with a slightly difference, the decolorization rate of both dyes was 98 % after 6 days. There after at the end of 7 days incubation, all dyes completely were adsorbed and the fungal mycelia colored. However, by inoculating 1 g/L of fungal biomass (dry weight) to medium containing 250 ppm of dye without any additional carbon and nitrogen source, this fungus was able to decolorize totally the medium within 7 days.

Discussion

A. niger was found more efficient (95.84% dye removal) than other strains in decolorizing of effluent. This fungus had been proved to have high adsorption capacity toward textile dyes [21, 25]. In this study, *Rhizopus* sp. was followed by *Fusarium* sp. with a decolorization of 25- 36 % (FSE 2, 35.87% dye removal) and 24% respectively. These two fungi were detected as the second and third most successful textile effluent bioremediator (Fig. 1) 2-14.5%, 8.85% and 5% colour removal were obtained for FSE 1, *Penicillium* sp. and *Saccharomyces cerevisiea*, respectively. In our study the decolorization capacity of *Penicillium* sp. is against the other reports by Erdal et al. [22], Seyis *et al*. [29] and Gou *et al*. [30]. The textile decolorization of *Cladosporium sphaerospermum* in present study was not successful (Fig. 1). The growth of fungi was also compared during the textile effluent decolorization. It was found that the growth of *Aspergillus* species, which showed successful decolorizatin, were high (0.2-0.26 g/100ml) during 3-4 days of incubation. *Fusarium* sp. and *Rhizopus* sp. as the second active fungi in decolorization in our study had the growth rate between 0.11-0.16 g/100ml, respectively. *Penicillium* sp. and *Saccharomyces cerevisiea* had the lowest growth in effluent (Fig. 2). Furthermore, the results of fungal growth in Table 1 showed *Cladosporium sphaerospermum* had no growth and efficiency for dye removal during 7- 8 days of incubation. According to our insufficient knowledge there is no study published on dye removal by this species. The screening experiment concluded that the optimum decolorization was achieved with the fungal strains that had the high growth in the effluent. This finding shows that growth and mycelia production affect on the decolorization to a great extent and also indicates that the mechanism of decolorization by these fungi is absorption in comparison with enzyme production. However Fu and Viraraghavan suggested that a biosorption mechanism might also play an important role in the decolorization of dyes by living fungi in addition to biodegradation [36]. Brahimi *et al*. added *Myrothecium verrucaria* into azo dyes solution and found that 70-95 % of the dyes were removed by absorption [25]. Also in previously study reported by Mazaheri *et al*. proved that the mechanism of decolorization by our tested fungus is via surface and internal cell adsorption [21].

Maximum decolorization happened after 12 hours incubation (98.19% decolorization) with biomass production of 0.2 g % dry weight and at this case the decolorization rate remained approximately constant whereas the growth of fungus continually increased by 0.23 g % dry weight.

In the similar study Revankar and Lele [17] recorded maximum decolorization of 96 % of 100 ppm amaranth in 8 h by *Ganoderma* sp. WR-1 In optimized medium. Ramsay and Goode [17] also reported similar fundings of 95% decolorization of a carpet effluent in shake flask after 10 h by *Trametes versicolor*.

Our results are better or in good agreement with those claimed by Ali *et al*. [28] for decolorization malachite green by *Aspergillus flavus* and *Alternaria solani*. They reported both the species were able to decolorize different concentrations of malachite green (10 to 50 µm) almost completely 96% within 6 days. Harazono *et al*. also in the same study observed that a medium containing 3% malt extract and 200 mg/L of Reactive Red 120 was decolorized by *Phanerochaete sordida* strain YK-624 up to 90.6 % after 7 days [20]. As mentioned above about 98 % of bound dyes to the fungal mycelia was recovered with methanol, it is clear that biosorption/bioadsorption mechanism contributed an important part to the decolorization of textile dyes by fungal biomass.

Conclusions

The applied fungus (*Aspergillus niger*) adsorbs most of textile dyes in plant effluent and do not produces any enzyme due to biodegradation of dyes. From the data can be estimated the 1 g of dry weight mycelia biomass to have a removal capacity greater than 90% within 22 h. The results in this report suggest the fungal biomass of *A. niger* may be employed in an efficient manner to remove textile dyes from plant effluent and aqueous solutions.

References

1. Adedayo, O., Javadpour, S., Taylor, C., Anderson, W.A. and Moo-Young, M., Decolorization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant. *World J Microbiol Biot*, 2004. Vol. 20, pp. 545–550.

2. Brown, M.A., De Uito, S.C., "Predicting azo dye toxicity", *Crit Rev Environ Sci Technol*., 1993. Vol. 23, pp. 249-324.

3. Sharma, D.K., Saini, H.S., Singh, M., Chimniandb, S.S., chadha, S., To develop a cost effective and efficient biological treatment process for small scale textile processing industries (TPI) releasing Letters in Applied Microbiology. *Lett Appl Microbiol*, 2004, Vol. 38(5), pp. 345-350.

4. Neill, C.O., Hawkes, F.R., Lourenço, N.L., Colour in textile effluents – sources, measurement, discharge consents and simulation: a review. *J Chemical Tech Biotech*, 1999, Vol. 74 (11), pp. 1009–1018.

5. Galindo, C., Jacques, P., Kalt, A., Photo oxidation of the phenylazonaphthol AO20 on TIO2: kinetic and mechanistic investigations. *Chemosphere*, 2001, Vol. 45, pp. 997-1005.

6. Sarnaik, S., and Kanekar, P., Bioremediation of colour of methyl violet and phenol from a dye-industry waste effluent using *Pseudomonas* spp. isolated from factory soil. *J Appl Bact*, 1995, Vol. 79 (4), pp. 459–469.

7. MacKay, G., Otterburn, M.S., Sweeney, A.G., The removal of colour from efluent using varios adsorbents. III. silica: rate process. *Water Res*, 1980, Vol. 14, pp. 15–20.

8. Barka, N., Assabbane, A., Nounah, A., Removal of textile dyes from aqueous solutions by natural phosphate as a new adsorbent. *Desalination*, 2009, Vol. 1, pp. 264-275.

9. Bhatt, M., Patel, M., Rawal, B., Novotný, H.P., Sasek, V., Biological decolorization of the synthetic dye RBBR in contaminated soil. *World J Microbiol Biotechnol*, 2000, Vol. 16, pp. 195 -198.
10. Song, Z.Y., Zhou, J.T., Wang, J., Yan, B. Du, C.H., Decolourization of azo dyes by Rhodobacter spheroids. *Biotechnol*, 2003, Vol. 25, pp. 1815–1818.
11. 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, Vol. 77(3), pp. 247-55.
12. Stolz, A., Basic and applied aspects in the microbial degradation of azo dyes. *Appl Microbiol Biotechnol*, 2001, Vol. 56, pp. 69–80.
13. Mielgo, I., Moreira, M.T., Feijoo, G., Lema, J.M., Biodegradation of a polymeric dye in a pulsed bed bioreactor by immobilised *Phanerochaete chrysosporium*. *Water Res*, 2002, Vol. 36, pp. 1896–1901.
14. Dave, S.R., Dave, R.H., Isolation and characterization of *Bacillus thuringiensis* for acid red 119 dye decolourisation, *Bioresource Technol*, 2008, Vol. 100 (1), pp. 249-253.
15. Revankar, M.S., Lele, S.S., Synthetic dye decolorization by white rot fungus, *Ganoderma* sp. WR-1. *Bioresource Technol*, 2007, Vol. 98 (4), pp. 775-780.
16. Eichlerova, I., Homolka, L., Nerud, F., Synthetic dye decolorization capacity of white rot fungus *Dichomitus squalens*. *Bioresource Technol*, 2006, Vol. 97 (16), pp. 2153–2159.
17. Kapdan, I., Kargi, F., McMullan, G., Marchant, R., Comparison of white-rot fungi cultures for decolorization of textile dyestuffs. *Bioprocess Eng*, 2000, Vol. 22, pp. 374-351.
18. Harazono, K., Watanabe, Y., Nakamura, K., Decolorization of azo dye by the white-rot basidiomycete *Phanerochaete sordida* and by its manganese peroxidase. J Biosci Bioeng, 2003, Vol. 95, pp. 455-459.
19. Mazaheri-Assadi, M., Mazaheri, M., Nejad-Satari, T., Noohi, A., Shahamat, M., Levin, M., Biosorption of baftkar textile effluent. *Indian J Exp Biol*, 2003, Vol. 41, pp. 900–904.
20. Genisel, M., Erdal, S., Turk, H., Dumlupinar, R., The availability of bone powder as inorganic element source on growth and development in wheat seedlings. *Toxicol Ind Health*, 2012, Vol. 28, pp. 458-462.
21. Atlas and Bartha., Degradation and mineralization of petroleum in sea water: limitation by nitrogen and phosphorous. *J Biotech Bioeng*, 1972, Vol. 14, pp. 309–318.
22. Brahimi-Horn, M.C., Lim, K.K., Liang, S.L., Binding of textile azo dyes by *Mirothecium verrucaria*. *J Ind Microbiol Biotechnol*, 1992, Vol.10, pp. 31−36.
23. Xc, J., Gq, L., Zh, X., Wy. T., Decolorization of a dye industry effluent by *Aspergillus fumigatus* XC6. *Appl Microbiol Biotechnol*, 2007, Vol. 74(1), pp. 239-43.
24. Mazaheri-Assadi, M., and Jahangiri, M.R., Effect of *Aspergillus niger* on textile wastewater treatment. *Desalination*, 2001, Vol. 141, pp. 1-6.
25. Ali, H., Ahmad, W., Haq, T., Decolorization and degradation of malachite green by Aspergillus flavus and *Alternaria solani*. Afri J Biotech, 2009, Vol. 8.
26. Seyis, I., Subasioglu, T., Screening of different fungi for decolorization of molasses. *Brazilian J Microbiol*, 2009, Vol. 40, pp. 61–65.
27. Gou, M., Qu, Y., Zhou, J., Ma, F., Tan, L., Azo dye decolorization by a new fungal isolate. *Penicillium* sp. QQ and fungal-bacterial co-cultures. *J Hazard Mater*, 2009, Vol. 170, pp. 314-319.
28. Ikramullah, A.N., Lutfullah, G., Hameed, A., Ahmed, S., Decolorization of acid red 151 by *Aspergillus niger* SA1 under different physicochemical conditions. *World J Microbiol Biotech*, 2008, Vol. 24, pp. 1099-1105.
29. Mazmanci, M.A., Unyayar, A., Decolorization efficiency of *Funalia trogii* under static condition: Effect of C: N ratios. *Afri J Biotech*, 2010, Vol. 9 (39), pp. 6539-6544.
30. Dahiya, J., Singh, D., Nigam, P., Decolourisation of molasses wastewater by cells of *Pseudomonas fluorescens* immobilised on porous cellulose carrier. *Bioresource Tech*, 2001, Vol. 78(1), pp. 111-114.
31. Ramsay, J.A., and Goode, C., Decoloration of a carpet dye effluent using *Trametes versicolor*. *Biotechnol Lett*, 2004, Vol. 26(3), pp. 197-201.
32. Y1, F., Fungal decolorization of dye wastewaters. *Bioresour Technol*, 2001, Vol. 79(3), pp. 251-262.
33. Hadibarata, T., and Tachibana, S., Decolorization of remazol briliant blue R by laccase from white Rot fungus *Polyporus* sp. S133. *Indonesian J Biotechnol*, 2009, Vol. 14, pp. 1162-1168.
34. Kumar, K., and Abraham, T.E., Biosorption of anionic textile dyes by nonviable biomass of fungi and yeast. *Bioresource Technol*, 2007, Vol. 98, pp. 1704-1710.
35. Bhole, B.D., Ganguly, B., Madhuram, A., Deshpande, D., Joshi, J., Biosorptionof methyl violet, basic fuchsin and their mixture using dead fungal biomass. *Curr Sci*, 2004, Vol 86(12), pp. 1641-1645.
36. Somasiri, W., Ruan, W., Xiufen, L., Jian, C., Decolourization of textile wastewater containing acid dyes in UASB reactor system under mixed anaerobic granular sludge, *Electron J Environ Agric Food Chem*, 2006, Vol. 5, pp.1224-1234.
37. Khehra, M.M.S., Saini, H.S., Sharma, D.K., Chadha, B.S., Chimni, S.S., Decolorization of various azo dyes by bacterial consortium. *Dyes and Pigments*, 2005, Vol. 67 (1), pp. 55-61.
38. Andleeb, S., Atiq, N., Ali, M.I, Ur-Rehman, F., Hameed, A., Ahmad, S., Biodegradation of anthraquinone dye by *Aspergillus niger* SA1 in self designed fluidized bed bioreactor. *Iran J Environ Health Sci Eng*, 2010, Vol. 7, pp. 371-376
39. Kabbouta, R., Taha, S., Biodecolorization of textile dye effluent by biosorption on fungal biomass materials. *Physics Procedia*, 2014, Vol. 55, pp. 437–444.