



ECO-FRIENDLY COLOUR REMOVAL POTENTIAL OF FUNGAL SPECIES ISOLATED FROM VEGETABLE AND FRUIT WASTES

R. Palani velan^{a,*} and P.M. Ayyasamy^a, Subashni B^b, Suresh S.S. Raja^c

^aDepartment of Microbiology, Periyar University, Salem, Tamil Nadu, India

Tel. +91 9952517712; email: starvel2005@yahoo.co.in (R. Palani velan)*,

Tel. +91 9486327103; email: pmayyasamy@gmail.com (P.M. Ayyasamy)

^bDepartment of Biotechnology, Sri Ramachandra University, Chennai, Tamil Nadu, India

Tel. +91 9840061848; email: subashni91@gmail.com (**Subashni. B**)

^cDepartment of Microbiology, Bharathidasan University College, Perambalur 621107, Tamil Nadu, India,

Tel. +91 9486160107; email: sudalaimuthuraja@yahoo.co.in (S.S.S. Raja)

ABSTRACT: Coloured effluents discharging from different types of industries are a major hazardous problem in the environment. Various physico-chemical methods have been employed in effluent treatment plants, which have certain limitations in the colour removal process. The present investigation deals with multiple dye decolorization ability of fungal species isolated from vegetable and fruit waste materials. Among the fungal strains, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* sp. were screened for decolorizing ability of Orange 2R, Green HE4BD, Yellow FG, Brown GR and Red RGB. *A. niger* and *A. flavus* exhibited higher dye decolorization ability which ranged from 95 to 97% and 65 to 96% respectively. Fungal growth and pH analysis showed that the chemistry of dye molecule influences the fungal biomass and dye removal capacity in an aqueous solution. The present study suggests that fungal species from vegetable and fruit wastes would be a prospective candidate for the treatment of coloured effluents in future.

Key words: Coloured effluents, vegetable and fruit wastes, azo dye, decolorization, fungi.

Abbreviations: PDA - Potato Dextrose Agar LPCB - Lacto Phenol Cotton Blue

OD - Optical Density Lip - Lignin peroxidase MnP - Manganese Peroxidase

INTRODUCTION

Synthetic dyes are coloured compounds, widely used in food, textile dyeing, cosmetic and pharmaceutical industries. Worldwide, more than 10,000 different dyes are commercially available. Among the various chemical groups, azo dyes are the largest class extensively utilized in diverse industries [1]. It is estimated that 10% of dyes released in the environment by textile processing units is due to partial binding nature with the fibers [2]. The textile sector generates a noteworthy quantity of waste water along with multi-coloured dyes, which in turn has serious impact on the eco system [3]. Similarly sugarcane molasses-based distillery effluent is dark brown in colour due to high concentration of complex biopolymer: melanoidin [4]. The chemical structure of azo dyes are complex with one or more N=N bonds, that are not easily degraded in conventional effluent treatment method. Apart from that azo dyes are designed to resist chemical attack in order to be light-resistant and stable during washing. It is estimated that 90% of dyes from industrial effluents could not be degraded by physical and chemical processes. Consequently, it produces a wide range of toxic intermediates and metabolites. Biological method of dye removal by using microorganisms is an eco-friendly and cost-competitive mineralization process [5]. The microbial decolorization and degradation of dyes have gained much attention and are also widely reported in bacteria, yeasts and filamentous white rot fungus [6].

The fungal community is one of the suitable candidates for the treatment of coloured effluents through biodegradation, biosorption, bioaccumulation and enzymatic mineralization [7]. Fungal mycelium has an advantage of converting the insoluble substrate into soluble products by production of extracellular enzymes, even at higher concentration of toxicants [8]. Fungi have greater cell surface, therefore it enhances physical and enzymatic contact with the environment. The decolorizing ability of fungal population from vegetable and fruit wastes is not well documented. The present study hypothesize that the fungal communities bound to vegetable and fruit wastes have greater colour removal ability, due to presence of enzymes possessing ligninolytic activity, which are responsible for organic waste decomposition. The aim of the present investigation was to isolate and characterize the fungal isolates from vegetable and fruit wastes, in order to decolorize multi azo dye and to analyze the impact of pH on fungal growth against its decolorizing potential.

MATERIALS AND METHODS

Isolation of Fungal Isolates from Vegetable and Fruit Wastes

The vegetable and fruit waste samples were collected from the local market of Coimbatore (Tamil Nadu, India) in sterile polythene bags and sealed tightly. About 10 grams of mixed sample (vegetables and fruits) was added to 90 ml of sterile distilled water and it was shaken for half an hour. After serial dilution, 0.1 ml of aliquot was spread-plated on potato dextrose agar (PDA) plates, purchased from Himedia, which contained 200 g/l peeled potato, 20 g/l dextrose, 15 g/l agar and pH of 5.5. Following six days of incubation at 28°C, morphologically diverged isolates were transferred into fresh PDA plates. The isolated cultures were sub-cultured on PDA slants regularly every 30 to 60 days and stored at 4°C.

Identification of Fungal Isolates

The selected fungal isolates were microscopically identified by Lacto Phenol Cotton Blue (LPCB) method. The chemical reagent composed of 20 ml phenol, 20 ml lactic acid, 40 ml glycerol, 20 ml distilled water and 0.50 g cotton blue. A loop full of mycelium of each fungal isolates was mounted on clean slide containing a drop of lacto phenol cotton blue solution. A small portion of the aerial mycelium was inoculated on a slide containing a fraction of LPCB reagent. The smear was covered with clean cover slip and viewed under the light microscope under 10X and 40X objective lens to detect special structures. The fungal isolates were characterized based on morphology: texture, colour and diameter of the colony; the rate of growth and reverse pigmentation were also taken into account for identification.

Preparation of spore Suspension

The fungal isolates were separately cultured in 100 ml of potato dextrose broth at room temperature for 4 to 5 days. At the end of incubation, 30 ml of sterile distilled water was added to the cultures and incubated in orbital shaker (50 rpm) for 30 minutes. After incubation, the culture broth was filtered using sterile muslin cloth. For further experiments, filtered spores diluted with distilled water with a concentration of 10^5 spore/ml were used [9].

Dye Decolourization Assay

Decolourizing ability of fungal strains were separately experimented in 250 ml Erlenmeyer's flask containing 100 ml of potato dextrose broth. The flasks were also amended with 100 mg/l of Orange 2R and inoculated with 1 ml of fungal spore suspension (10^5 /ml concentration). Similar setup was adopted for other dyes (Green HE4BD, Yellow FG, Brown GR and Red RGB), which were tested at 100 mg/l concentration. All the flasks were incubated for 15 days in orbital shaker at 250 rpm [10]. Experiments were carried out in triplicate with abiotic controls. At the end of 5, 10, 15 days of incubation, the decolourizing broth samples were aseptically transferred and centrifuged at 5000 rpm for 15 min. Concurrently, pH of the decolourizing broth was also determined using pH meter. The optical density (OD) value of cell free supernatant was analyzed by using UV Visible spectrophotometer at 418 nm for Yellow FG, 520nm for Red RGB, 621nm for Orange 2R, 401nm for Green HE4BD, and 475 nm for Brown GR respectively. The decolourization percentage was determined by implementing the following formula [11]:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

MEASUREMENT OF FUNGAL BIOMASS

At the intervals of 5, 10 and 15 days of incubation period, culture broth was filtered by No. 1. Whatmann filter paper (pre-weighed size) and it was dried in an oven at 105 °C for 48 hours. The dried filter paper along with mycelium was re-weighed. Fungal biomasses were calculated using the following formula:

$$\text{Weight of mycelium} = \text{weight of filter paper} + \text{weight of mycelium} - \text{weight of filter paper}$$

RESULTS

Four morphologically different fungal colonies were isolated and purified on Potato Dextrose Agar plates from vegetable and fruit wastes. Macroscopic and microscopic examinations confirmed that the following fungal isolates viz., *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* sp. were widespread in vegetable and fruit wastes. The diverse colours of synthetic dyes employed in this study and the decolorization results varied based on utility of the fungi. The fungal cultures showed maximum dye removal capacity (95 to 97%) in the decolorization of Orange 2R (Figure 1). Influence of the fungi cultures on decolorization of Green HE4BD is comparatively less, presented in Figure 2. The impact of fungi on Yellow FG decolorization was recorded in the range of 87 to 96% (Figure 3). At the end of 15th day, *Aspergillus niger* and *Fusarium* sp. showed effective decolorization than *A. flavus*. Maximum decolorization of Brown GR was accomplished by *Aspergillus niger* (96%) and *Aspergillus flavus* (82%) at 15th day of incubation (Figure 4). In the same way, various fungi portrayed decolorization of Red RGB with a capacity range between 70 to 96% (Figure 5). The weight of dry fungal biomass varied depending upon the fungi. All the fungi that decolorized the broth samples exhibited acidic pH level (Table 1). The dry fungal biomass content, measured at the end of 15th day incubation, ranged between 204 to 285 mg.

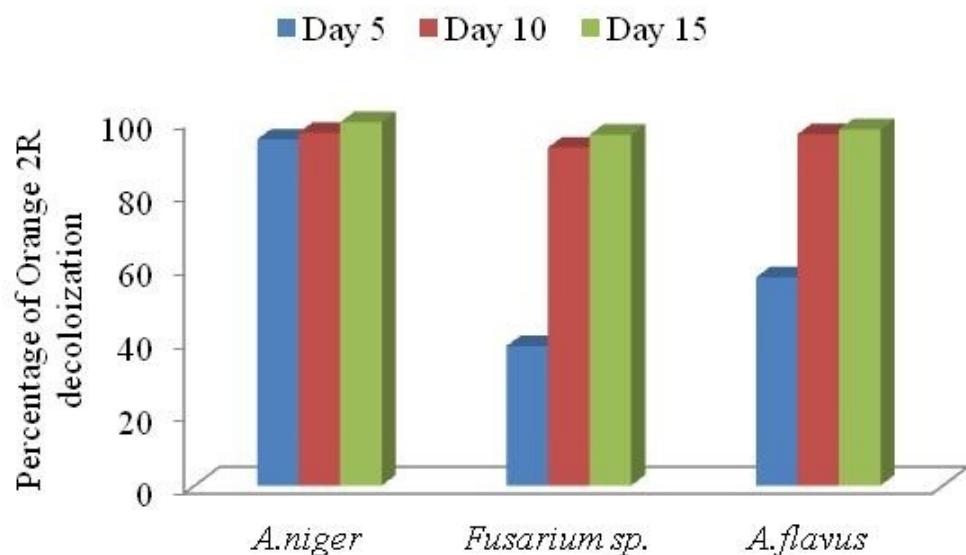


Figure 1: Decolorization of orange 2R

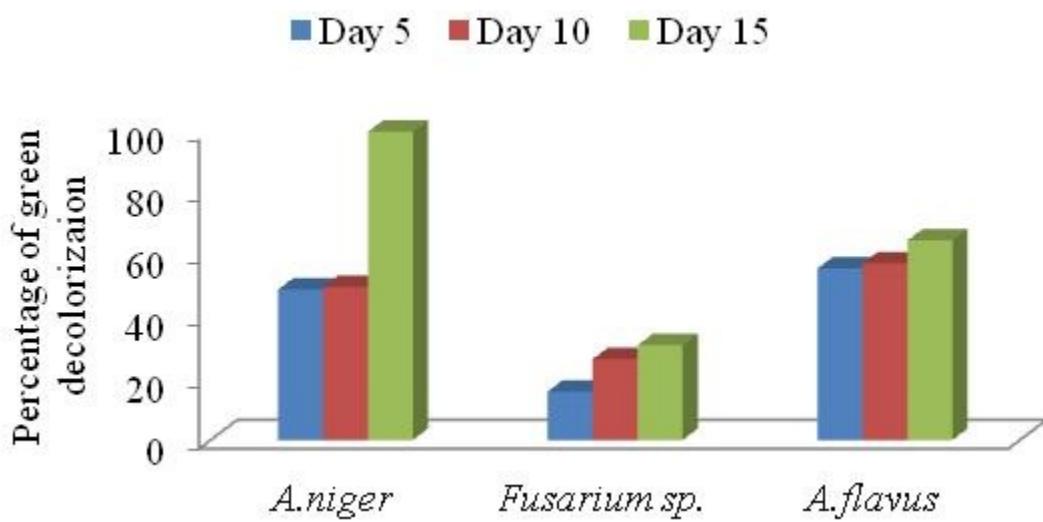


Figure 2: Decolorization of green HE4BD

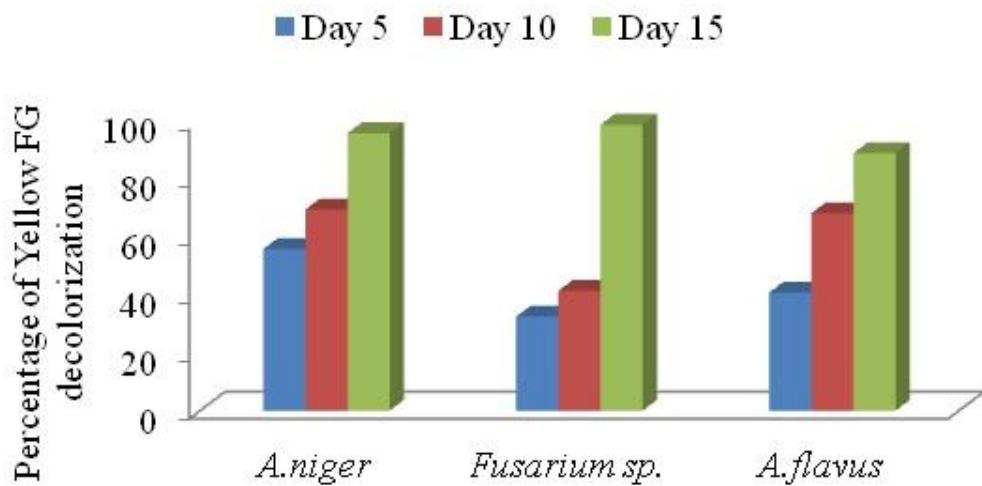
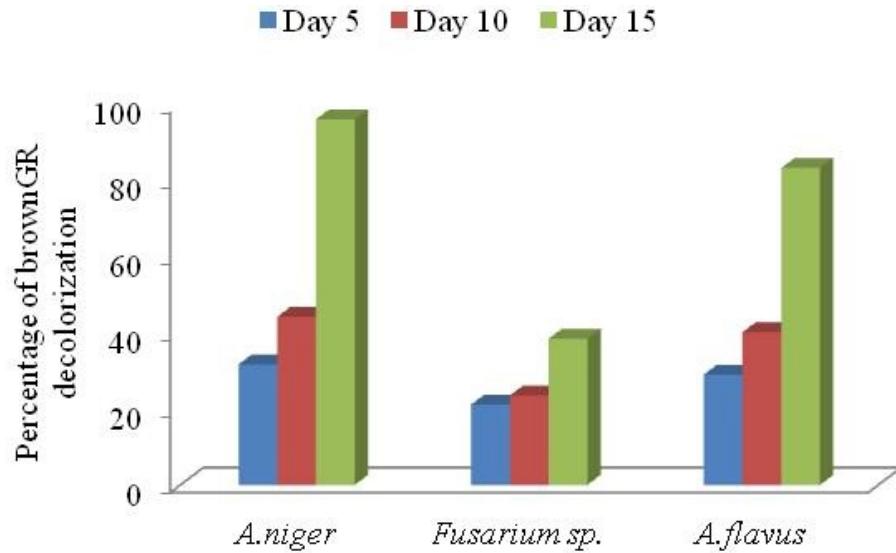
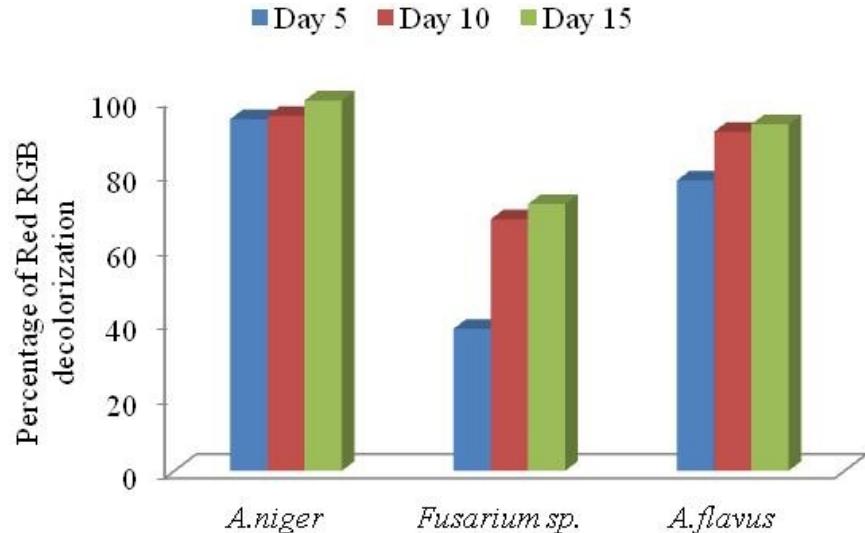
**Figure 3: Decolourization of Yellow FG****Figure 4: Decolourization of brown GR****Figure 5: Decolourization of Red RGB**

Table 1: Impact of fungal biomass and pH in dye decolourization

| S.No | Dyes decolourized | Name of the Fungus | Day 5 incubation | | Day 10 incubation | | Day 15 incubation | |
|------|-------------------|---------------------|------------------|-----|-------------------|-----|-------------------|-----|
| | | | Dry weight | pH | Dry weight | pH | Dry weight | pH |
| 1. | Orange2R | <i>A.niger</i> | 183 | 4.4 | 223 | 4.2 | 275 | 4.2 |
| | | <i>Fusarium sp.</i> | 169 | 5.3 | 195 | 5.1 | 210 | 4.7 |
| | | <i>A.flavus</i> | 180 | 5.2 | 222 | 5.3 | 253 | 5.0 |
| 2. | Green HE4BD | <i>A.niger</i> | 201 | 5.1 | 235 | 4.8 | 268 | 4.4 |
| | | <i>Fusarium sp.</i> | 172 | 5.3 | 195 | 4.4 | 205 | 4.4 |
| | | <i>A.flavus</i> | 159 | 5.4 | 176 | 4.9 | 210 | 4.5 |
| 3. | Yellow FG | <i>A.niger</i> | 215 | 5.5 | 238 | 3.7 | 285 | 3.6 |
| | | <i>Fusarium sp.</i> | 165 | 5.1 | 200 | 5.0 | 209 | 4.4 |
| | | <i>A.flavus</i> | 180 | 4.9 | 207 | 5.2 | 285 | 4.7 |
| 4. | Brown GR | <i>A.niger</i> | 195 | 5.1 | 232 | 3.7 | 215 | 3.6 |
| | | <i>Fusarium sp.</i> | 156 | 5.2 | 192 | 4.2 | 204 | 4.2 |
| | | <i>A.flavus</i> | 172 | 5.6 | 228 | 4.3 | 245 | 4.3 |
| 5. | Red RGB | <i>A.niger</i> | 190 | 4.6 | 225 | 4.8 | 281 | 4.7 |
| | | <i>Fusarium sp.</i> | 172 | 5.2 | 190 | 5.0 | 204 | 4.9 |
| | | <i>A.flavus</i> | 180 | 5.3 | 224 | 5.1 | 248 | 4.5 |

DISCUSSION

Abundant quantities of dyes are discharged through various industrial effluents which contribute to deleterious aquatic pollution. White rot fungi have the ability to degrade and mineralize this wide range of recalcitrant toxic compounds by producing an array of ligninolytic enzyme system [12]. To our knowledge, only few reports have been documented about the decolourizing potential of fungi from vegetable and fruit wastes. *Aspergillus* sp. and *Penicillium* sp. from wastes of potato and its epidermis showed ability to decolorize Isolan Red and coloured waste water by exhibiting oxidase activity [13]. *A. flavus* and *A. niger* effectively decolorizes the azo dyes when compared to the other fungal genus viz., *Mucor* and *Penicillium* sp. *Aspergillus niger* showed decolorization activity, with a range of 89 to 97% after 15 days of incubation was reported in similar study by Ponraj *et al.*, 2011 [14]. In this study, the effectiveness of colour removal was shown to increase with increase in incubation time and this was subsequently found to be in agreement with the study documented by Spadaro *et al.*, 1992 [15], where maximum decolorization was obtained after 15 days of incubation. *A. niger* showed to possess potential decolorization capacity of Orange 2R. *Fusarium* sp. and *A. flavus* exhibited lower green dye decolorization namely 27% and 60% respectively, after 5 days of incubation. Among these *Fusarium* sp. in particular did not exhibit significant decolorization. These results correlated in previous reports of azo dye decolorization by *A. niger* and *Fusarium* sp [10]. Similarly, precedent study also recorded the decolorization of vat dye by 5 different fungal genus [11], in particular *Coriolous vesiculifer*.

Maximum amount of biomass contents were produced in the decolorization cultures of *A. flavus* and *A. niger*. The lowest amount of biomass was produced by *Fusarium* sp. in the decolorization of Brown GR and Red RGB. Diverse kinds of mechanisms mediated the dye removal process in fungi, such as biodegradation, biosorption, and bioaccumulation. The decolorization occurring via biosorption takes place when an electrostatic force is formed between positive ions of the cell wall (hyphae) and negative ions of dyes [5]. In other scenarios, the extracellular lignin-degrading enzymes act on the dye and it is then adsorbed into cell wall of fungi [17]. It is also well known that the group of enzymes namely, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase favours degradation of dyes and hence become accessible to intracellular mineralization [16, 18].

CONCLUSION

In conclusion, it is evident that fungal species from vegetable and fruit wastes can decolorize dyes from coloured effluents. This occurs without prior fungal adaptation to the dye compounds in an aqueous solution. The present investigation provides a new source for the development of an effective fungal dye removal process. Further this study also involves the inception of exploring the enzymes produced by these fungal species in order to carry out decolorization of dyes in industrial effluents.

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REFERENCES

- [1] Cameron, M.D., Timofeevski, S. and Aust, S.D. 2000. Enzymology of *Phanerochaete chrysosporium* with respect to the degradation of recalcitrant compounds and xenobiotics. *Appl. Microbiol. Biotechnol.* 54: 751-758.
- [2] Adedayo,O. Javadpour,S. Taylor,C. Anderson,W.A. and Moo-Young. 2004. Decolourization and detoxification of methyl red by aerobic bacteria from a waste water treatment plant. *World J. Microbiol. Biotechnol.* 20: 545-550.
- [3] Palanivelan, R. Rajakumar, S. and Ayyasamy, P.M. 2014. Effect of various carbon and nitrogen sources on decolourization of textile dye remazol golden yellow using bacterial species. *J. Environ. Biol.* 35(5):781-787.
- [4] Chaturvedi, S. Chandra, R. and Rai, V. 2002. Isolation and characterization of *Phragmites australis* (L.) rhizosphere bacteria from contaminated site for bioremediation of coloured distillery effluent. *Ecol. Eng.* 27: 202-207.
- [5] Gogate, P.R. and Pandi, A. B. 2004. A review of imperative technologies for wastewater treatment II: hybrid methods *Advan. Environ. Res.* 8: 553-597.
- [6] Kudlich, M. Keck, A. Klein, J. and Stolz, A. 1997. Localization of the enzyme system involved in anaerobic reduction of azo dyes by *Sphingomonas* sp strain BN6 and effect of artificial redox mediators on the rate of azo dyes reduction. *Appl. Environ. Microbiol.* 61: 4374-4377.
- [7] Iqbal, Z. Qureshi, T.M. Akhtar, M. and Akhtar, J. 2010. Evaluation of industrial effluents for reclaiming salt affected soil and achieving economic crop productivity. *Soil. Environ.* 29(1): 87-90.
- [8] Kaushik, P. and Malik, A. 2009. Effect of the nutrient composition on dye decolourization and extra cellular enzyme production by *Lentinus edodes* on solid medium. *Enzyme Microb. Technol.* 30: 381-386.
- [9] Sivakumar, D. Wilson Wijeratnam, R.S. Wijesunder, R.L.C Marikar F.M.T. and Abeysekere, M. 2002. Antagonistic effect of *Trichoderma harzianum* on post harvest pathogens of Rambutan (*Nephelium lappaceum*). *Phyto Parasitica.* 28: 240-247.
- [10] Kousar, N. Seshikala, D. and Singara Charya, M.A. 2000. Decolourization of textile dyes by fungi. *Indian J. Microbiol.* 40: 191-197.
- [11] Palanivelan, R. Rajakumar, S. Suresh S.S. Raja and Ayyasamy, P.M. 2014. Optimization of prime parameters for textile dye decolourization using *Lysinibacillus fusiformis* M1 by design of experiments (DOEs). *Desalination and water treatment (DOI:10.1080/19443994.2014.944583).*
- [12] Jothimani, P. and Prabakaran, J. 2003. Dye factory effluent decolourization by fungal cultures under static condition. *J. Ecobiol.* 15: 255-260.
- [13] Gomaa, O.M. Momtaz, O.A. Kareem, H.A.E. and Fathy, R. 2011. Isolation, identification, and biochemical characterization of a brown rot fungus capable of textile dye decolourization. *World J Microbiol Biotechnol.* 27: 1641-1648.
- [14] Ponraj, M. Jamunarani, I.P. and Zambare, V. 2011. Isolation and optimization of culture conditions for decolourization of true blue using dye decolourizing fungi. *Asian J. Exp. Biol. Sci.* 2(2): 270-277
- [15] Spadaro, J.T. Michael, H.G. and Renganathan, V. 1992. Degradation of azo dyes by the lignin degrading fungus *Phanerocheate chrysosporium*. *Appl. Enviro. Microbial.* 58: 2397-2401.
- [16] Asgher, M. Batool, S. Nawaz Bhatti, H. Razia Noreen, Rahman, S.U. and Javaid Asad, M. 2008. Laccase mediated decolourization of vat dyes by *Coriolus versicolor* IBL-04. *Int. Biodeterior. Biodegrad.* 62: 465-470.
- [17] Jaspers, C.J. Jimenez, G. and Penninckx, M.J. 1994. Evidence for a role of manganese peroxidase in the decolourization of kraft pulp bleach plant effluent by *Phanerochaete chrysosporium*: Effects of initial culture conditions of enzyme production. *J. Biotechnol.* 37: 229-234.
- [18] Lin, J. Wang H.Y. and Hickey, R.F. 1989. Degradation kinetics of pentachlorophenol by *Phanerochaete chrysosporium*. *Biotechnol Bio. Engg.* 35: 1125-1134.