Science World Journal Vol 4 (No 4) 2009
www.scienceworldjournal.org
ISSN 1597-6343

Full length Research Article

DECOLOURISATION OF CRYSTAL VIOLET AND MALACHITE GREEN BY FUNGI

*MOTURI, B.[1] & SINGARA CHARYA, M. A.[2]

[1]Centre for Biotechnology, Jawaharlal Nehru Technological University, Hyderabad – 5000085, AP, India
[2]Department of Microbiology, Kakatiya University, Warangal – 506009, AP, India
moturi.bhargavi@yahoo.co.in

ABSTRACT
Decolourisation of crystal violet and malachite green by white rot fungi, *Polyporus elegans, Trametes versicolor, Lenzites betulina* and soil fungus *Mucor mucedo* isolated from dye effluent amended soils was studied. There was no toxic effect of crystal violet on the growth of the four fungi but malachite green showed retardation of growth. *Mucor mucedo* decolourised 78% of the crystal violet and 65% of malachite green. The white rot fungi showed more than 60% decolourisation of crystal violet and 26 to 57% decolourisation of malachite green. In the process of decolourisation, lignin peroxidase production was high at 15 days incubation by all the organisms. Manganese peroxidase was secreted more after 10 days of incubation and laccase production was high after 15 days of incubation by *Polyporus elegans* and *Trametes versicolor* and after 10 days in the case of *Lengites betulina*. *Mucor mucedo* failed to secrete manganese peroxidase and laccase in all its incubations.

Key words: Triphenyl methane dyes, fungi, lignin peroxidase, manganese peroxidase, laccase, decolourisation.

INTRODUCTION:
The textile industries utilize large volumes of water in its processing operations and generate substantial quantities of wastewater (Hutton, 1972). Dye wastes are usually discarded into water with or without processing. Aesthetic merit, gas solubility and water transparencies are affected by the presence of dyes even in small amounts. The presence of colour in waste water is very important and usually contributes the major fraction of biochemical oxygen demand. Among many classes of synthetic dyes used in the textile and dyeing industries, triphenyl methane group of dyes such as crystal violet and malachite green are the largest and most versatile and play a predominant role in almost every type of application (Bumpus & Brock, 1988). Crystal violet (N, N, N^1, N^1, N^{11}, N^{11} - hexa-methyl-para-rosaniline) has been extensively used in human and veterinary medicine as a biological stain and as a textile dye (Bangert *et al.,* 1977; Eiichi *et al.,* 1985). Some triphenyl methane dyes have been shown to be carcinogenic especially crystal violet which has been proved to be potent carcinogen (Au *et al.,* 1978). Malachite green is not approved by the US Food and Drug Administration and has nominated it to be a priority chemical for carcinogenic testing by the National Toxicology Program in 1993.

To remove dye from waste water, the physical – chemical methods like adsorption, chemical precipitation, flocculation, photolysis, chemical oxidation and reduction, electro-chemical treatment and ion-pair extraction were extensively used (Ansari & Thakur, 2002; Zhang *et al.,* 2002). The importance of fungi and their enzymes in the dye degradation has been well appreciated globally, because of their potential use in detoxification and degradation of dyes (Ferreira *et al.,* 2000; Mielgo *et al.,* 2001; Claus *et al.,* 2002; Assadi *et al.,* 2003), but these methods are costly. Biological methods of treatment combined with physical or chemical methods or both for colour removal are immensely useful and cost effective. It is therefore imperative to study the metabolic and enzymatic systems of fungi in order to gain insight into their degradation metabolisms. Amongst the fungi studied so far, wood degrading white-rot fungi has been reported to have the potential to successfully treat coloured industrial effluents. Hence, in this study two triphenyl methane textile dyes were studied for their removal from textile mill effluents by using the fungal biomass and their enzymes.

Novel procedures for the decolourisation of different dyes by various wood rot fungi have been developed while the role of common soil fungi in the dye amended soils is yet not well understood (Claus *et al.,* 2002). Production of lignolytic enzymes and the role of oxidative enzymes in the bioremediation and biodegradation of pesticides by white rot fungi were well explained (Mielgo *et al.,* 2003, Lopez *et al.,* 2004). Three species of *Phlebia* have been evaluated for their potential to decolourise eight industrial dyes and the study revealed *Phlebia* species to be better dye decolourisers than *Phanerochaete chrysosporium* (Daljit Singh & Mukesh, 2004). The anaerobic treatment of wool dyeing effluents could be improved with a better knowledge of methanogenic dye degradation, but still to be well understood (Bras *et al.,* 2005). Photocatalytic decolourization and degradation of an azo dye Reactive orange 4 was well established using $TiO_{(2)}$ as photocatalyst by UV process (Muruganandham & Swaminathan, 2006). Decolourisation of textile dyes from fungi *Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme* and *Trichoderma harzianum* isolated from the soil samples around the textile distillery industries of Nanjangud, Karnataka, India were tested for their efficacy in decolourisation of textile dyes (Raju *et al.,* 2007). *Phanerochaete chrysosporium* decolourised 6 of the 14 structurally diverse dyes with varying efficiency (Gomaa *et al.,* 2008).

Biodecolourisation of Acid Red 66 using white rot fungi *T. versicolor* was demonstrated by the decolourisation of the culture

medium (Sukumar *et al.,* 2009). The present study is an effort to develop a fungal-based treatment system for the cleaning of dye industrial effluents and for bioremediation of dye-contaminated soils.

MATERIALS AND METHODS

Soil samples were collected from different textile dye industries in Mangalagiri and the fungal organisms were isolated by standard serial dilution plate technique. A total of 20 fungal isolates were made and maintained on Potato Dextrose Agar slants. Of these, one predominant fungus *Mucor mucedo* was selected on the basis of dye agar plate assay and used for this study. Some wild species of white rot fungi were collected from forests as well as from timber depots and identified based on the characteristics of their basidiocarps. Fifteen white rot fungi were collected and among them, *Polyporus elegans, Trametes versicolor*, and *Lenzites betulina* were selected for the present study. Decolourisation studies were carried out in Malt-ammonium chloride broth (Malt extract – 10 g; ammonium chloride – 1 g and distilled water 1 L). Twenty-five milliliters of malt broth was placed in eight 100 ml Erlenmeyer flasks and 0.5 ml of 0.02 % dye solution (200 mg of crystal violet in 100 ml of water and 200 mg of malachite green in 100 ml of water) was added. These flasks were inoculated with each of the fungi after sterilizing the media. After 10 days of incubation period, the mycelial biomass was separated using Whatman No.1 filter paper. The culture extract obtained was centrifuged and used for analysis of decolourisation percentage and the assay of three enzymes lignin peroxidase, manganese peroxidase and laccase (phenol oxidase) after 5, 10 and 15 days of incubation. All the experiments were performed in triplicates. After each day of incubation, the pH of the culture extract and dry weight of mycelial biomass were measured. The enzymatic assays of lignin peroxidase, manganese peroxidase and laccase were performed using standard methods described by Lobos *et al.,* (1994).

Decolourisation assay: Decolourising activity expressed in terms of percent decolourisation was determined following the method described by Yatome *et al.,* (1993). The decrease in absorbance was monitored at A_{590} and A_{616} nm for crystal violet and malachite green respectively. Decolourisation activity was calculated as

$$D\% = 100 \times \frac{(A_{ini} - A_{fin})}{A_{ini}}$$

Where,

D = Decolourisation
A_{ini} = Initial absorbance
A_{fin} = Final absorbance of dye after incubation time.

RESULTS

Effect of pH and growth: From Table 1, it is evident that the fungi *Mucor mucedo*, *Trametes versicolor* and *Lenzites betulina* produced organic acids during their growth, hence the pH of these organisms ranged from 2.5-4.5, while *Polyporus elegans* showed pH of 6.0 during the process of decolourisation. Increase in the incubation time resulted in increased mycelial biomass and no toxic effect of crystal violet was noticed. The growth of all the fungal species was meager in 5 days, moderate in 10 days, and high in

15 days of incubation. The dry weight of the fungal biomass ranged from 220 to 1000 mg.

Enzyme production during decolourisation of crystal violet: The enzymes, lignin peroxidase, manganese peroxidase and laccase estimated during the decolourisation of crystal violet by the four fungi in dye-amended broth are given in Table 2.

Production of lignin peroxidase (LiP): The lignin peroxidase production was high during ten days and reduced in 15 days of incubation. A high range of enzyme production (1.981 U/ml) was recorded in *Polyporus elegans*, while a low range of enzyme production (0.311 U/ml) was recorded in *Mucor mucedo*. *Trametes versicolor* and *Lenzites betulina* showed maximum production of this enzyme (1.886 U/ml, 1.857 U/ml) in 5 and 10 days respectively.

Production of manganese peroxidase (MnP): The high quantity of manganese peroxidase was secreted by *Trametes versicolor* (600 U/ml) in 5 and 10 days of incubation time. *Mucor mucedo* showed minimum range of MnP production (100 U/ml) in 5 days of incubation and failed to secrete the enzyme in 10 and 15 days. *Polyporus elegans* and *Lenzites betulina* showed maximum MnP (400 U/ml and 470 U/ml respectively) in 10 days of incubation.

Production of laccase: *M. mucedo* completely failed to secrete this enzyme in all its incubations. Maximum enzyme production (1100 U/ml) was observed in 15 days of incubation by *T.versicolor*. Moderate quantity (1000 U/ml) of enzyme production was observed in *P. elegans* and lower quantity (800 U/ml) was produced by *L.betulina*.

Decolourisation of crystal violet during growth of fungi: The influence of the four fungi on the decolourisation of crystal violet is presented in Fig. 1. The biodegradation of crystal violet was related to its decolourisation during the growth of the fungi. 63 to 78% crystal violet was decolourised by *P. elegans*, *T. versicolor*, *L. betulina* and *M. mucedo.* Mucor mucedo showed maximum decolourisation of 78 % in 15 days incubation time. There is an increase in decolourisation percentage along with increase in the incubation time. *P.elegans, T. versicolor* and *L. betulina* showed 73 %, 72 % and 75 % decolourisation of crystal violet in 15 days of incubation period.

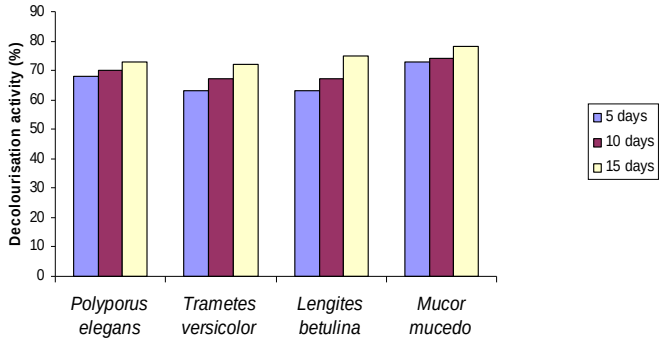

FIG 1. DECOLOURISATION OF CRYSTAL VIOLET BY FUNGI DURING 5, 10 AND 15 DAYS OF INCUBATION

TABLE 1. PH, DRY WEIGHT AND DECOLOURISATION OF CRYSTAL VIOLET BY FUNGI

Fungi	Days of Incubation	pH	Dry weight (mg)	Decolourisation (%)
Polyporus elegans	5	6.0	220	68
	10	6.0	320	70
	15	6.0	520	73
Trametes versicolor	5	4.5	240	63
	10	4.5	300	67
	15	4.5	620	72
Lenzites betulina	5	4.0	240	63
	10	4.0	380	67
	15	4.0	430	75
Mucor mucedo	5	2.5	650	73
	10	2.5	800	74
	15	2.5	1000	78

TABLE 2. PRODUCTION OF LIGNOLYTIC ENZYMES BY FUNGI DURING THE DECOLOURISATION OF CRYSTAL VIOLET

Fungi	Days of Incubation	Lignin peroxidase (U / ml)	Manganese peroxidase (U / ml)	Laccase (U / ml)
Polyporus elegans	5	1.828	300	300
	10	1.978	400	400
	15	1.981	300	1000
Trametes versicolor	5	1.886	600	200
	10	1.776	600	300
	15	1.584	400	1100
Lenzites betulina	5	1.741	200	200
	10	1.857	470	800
	15	1.622	160	0
Mucor mucedo	5	0.442	100	0
	10	0.367	0	0
	15	0.311	0	0

Effect of pH and growth: From Table 3, it is clear that the pH of the culture extracts of all the four fungi remained static throughout the study and the range of biomass variation among different fungal organisms was between 25 to 630 mg indicating the toxic effect of malachite green on the growth of the organisms.

Enzyme production during decolourisation of malachite green: Three enzymes lignin peroxidase, manganese peroxidase and laccase were assayed during decolourisation of malachite green and the results are in Table 3. There was gradual decrease in the production of these three extracellular secretions with the advancement of the time.

Production of lignin peroxidase: The production of the enzyme LiP started on the 5th day, showed high range of production on the 10th day and reached it maximum on 15th day of incubation. Maximum enzyme production was recorded by *T. versicolor* and *L. betulina* (2.000 U/ml), and low enzyme production by *M. mucedo* (0.460 U/ml). *Polyporus elegans* produced maximum enzyme (1.957 U/ml) on 15th day of incubation.

Production of manganese peroxidase: The enzyme MnP was produced in maximum quantity 700 U/ml and 800 U/ml on 5th day of incubation by *L. betulina* and *M. mucedo* and no secretions were reported in 10th and 15th days of incubation. *P. elegans* produced 400 U/ml of the enzyme on 15th day of incubation, while *T. versicolor* secreted maximum enzyme of 600 U/m on 10th day of incubation.

Production of laccase: *P. elegans* and *T. versicolor* showed maximum production (900 U/ml) of laccase on 15th day of incubation. *L. betulina* showed laccase production (400 U/ml) only on 15th day and did not show any secretions on 5th day and 10th day of incubations. *M. mucedo* showed (500 U/ml) laccase production on 10th day of incubation.

Decolourisation of malachite green during growth of fungi: The influence of all the four fungi on the decolourisation of malachite green is presented in Fig. 2. The decolourisation of malachite green was somewhat less when compared to crystal violet. *P. elegans, T. versicolor* and *L. betulina* showed decolourisation activity ranging from 26-65%, whereas, *M. mucedo* was found to be very effective in decolourising malachite green (65%). The decolourising activity increased along with increase in incubation period. *P. elegans* showed 45%, *T. versicolor* 43%, *L. betulina* 57% decolourisation in 15 days of incubation.

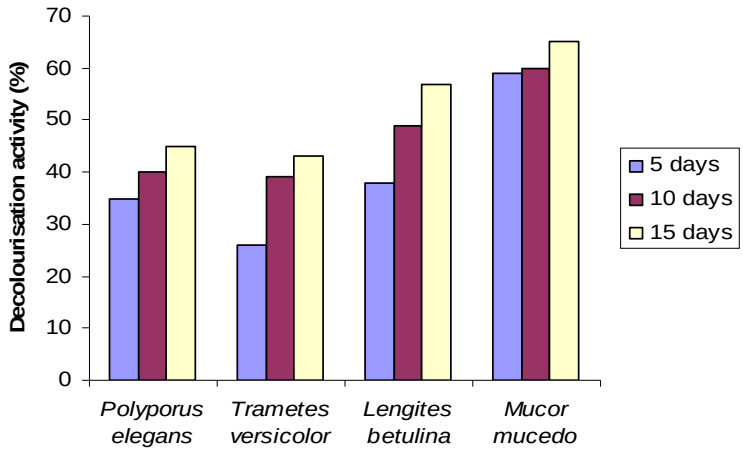

FIG. 2. DECOLOURISATION OF MALACHITE GREEN BY FUNGI DURING 5, 10 AND 15 DAYS OF INCUBATION

TABLE 3. PH, DRY WEIGHT AND DECOLOURISATION OF MALACHITE GREEN BY FUNGI

Fungi	Days of Incubation	pH	Dry weight (mg)	Decolourisation (%)
Polyporus elegans	5	6.0	25	35
	10	6.0	35	40
	15	6.0	165	45
Trametes versicolor	5	5.0	30	26
	10	5.0	170	39
	15	5.0	500	43
Lenzites betulina	5	4.5	90	38
	10	4.5	170	49
	15	5.0	310	57
Mucor mucedo	5	7.0	500	59
	10	7.0	540	60
	15	7.0	630	65

TABLE 4. PRODUCTION OF LIGNOLYTIC ENZYMES BY FUNGI DURING THE DECOLOURISATION OF MALACHITE GREEN

Fungi	Days of Incubation	Lignin peroxidase (U / ml)	Manganese peroxidase (U / ml)	Laccase (U / ml)
Polyporus elegans	5	1.743	0	0
	10	1.899	300	100
	15	1.957	400	900
Trametes versicolor	5	1.908	100	300
	10	1.976	600	300
	15	2.000	0	900
Lenzites betulina	5	1.884	700	0
	10	1.913	0	0
	15	2.000	0	400
Mucor mucedo	5	0.500	800	200
	10	0.581	0	500
	15	0.460	0	0

DISCUSSION

Results revealed that *Mucor mucedo* was found to be very effective in dye decolourisation showing 75 % and 68 % decolourisation of crystal violet and malachite green respectively after 15 days of incubation. The role of lignolytic enzymes useful for decolourisation of textile dyes and for biotechnological applications was thoroughly investigated. Several studies with lignolytic microorganisms demonstrated that the presence of microbial peroxidases seems to be correlated with their abilities to decolourise certain dyes (Glenn

& Gold, 1983). The enzyme systems of the white rot fungi include a group of non–specific extra cellular enzymes, which catalyze degradation of several aromatic and halogenated dye compounds (Bumpus & Aust, 1987). The enzymatic systems of *Phanerocheate chrysosporium* have also been considered responsible for azo dye degradation (Paszczynski *et al.,* 1986, Cripps *et al.,* 1990, Paszczynski & Crawford, 1991). Shin *et al.,* (1997) identified a new enzyme remazol brilliant blue – R (RBBR) decolourising peroxidase produced by *Pleurotus ostreatus* and showed that the enzyme was important in decolourisation of majority of the xenobiotic dyes. *Polyporus elegans, Trametes versicolor* and *Lengites betulina* showed maximum production of lignin peroxidase, manganese peroxidase and laccase. Our results correlated with the previous reports indicating that these enzymatic productions are responsible for the maximum decolourisation activities of crystal violet and malachite green.

Soil fungus *M. mucedo* secreted low range of enzymes lignin peroxidase, manganese peroxidase and laccase proving that the lignolytic system is not that much active as white rot fungi. But *M. mucedo* showed the maximum decolourisation activities of crystal violet and malachite green indicating the role of mycelial biomass responsible for the decolourisation of the dye. Sani *et al.,* (1998), Sani & Banerjee, (1999) extensively searched for triphenyl methane dye decolourising organisms and reported that all of them have great potential to biotransform the crystal violet dye. They further concluded that all the organisms tested in their study do not exhibit extracellular enzymatic activity and the cells are responsible for the decolourisation of the dye. These reports are supporting our results that though *M. mucedo* did not show enzymatic activity, but still efficient in decolourisation of crystal violet and malachite green.

CONCLUSION

Based on the above investigations, proper conclusions were drawn on the development of biotreatment technology for the bioremediation of dye-contaminated soils and also to reclaim the wastewater. Appropriate recommendations can also be made to develop strategies to decrease the pollution loads in dye industries and also to follow certain water conservation practices. In the present investigation, it was found that the isolated soil fungus, *Mucor mucedo* was considered to be the resistant, well – adopted, highly acclimatized to dye contaminated soils and also regarded as natural mutant to survive in the dye amended soils showing the best decolourisation activities of both the triphenyl methane dyes.

A number of organic pollutants pose risks to the health of humans, animals and ecosystem due to their toxicological and carcinogenic properties. The number of reports of contaminated sites has increased recently in developed countries. Due to the environmentally friendly techniques it utilizes, bioremediation has been characterized as a soft technology. Its cost-effectiveness and the little disturbance in the environment render this technology a very attractive alternative. Many soil fungi possess great potential for soil remediation thanks to their ability to degrade a variety of dye pollutants. The identification and research of new fungal strains with the aid of molecular techniques will further improve practical application of fungi and it is anticipated that fungal remediation will be soon a reliable and competitive remediation technology.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. V. Koteswaramma, Principal of Montessori Research Center for providing laboratory facilities.

REFERENCES

Ansari, A. A. & Thakur, B. D. (2006). Biochemical reactor for treatment of concentrated textile effluent. *Colourage 2*: 27-31.

Assadi, M. M., Maryam, M., Taher, S. N., Noohi, A., Shahamat, M. & Levin, M. (2003). Biosorption of Baftkar textile effluent. *Indian Journal of Experimental Biology 41:* 900-905.

Au, W., Pathak, S., Colie, C. L. & Hsu, T. C. (1978). Cytogenetic toxicity of gentian violet and crystal violet on mammalian cells in vitro. *Mutant Research 58*: 269-276.

Banat, I. M., Nigam, P., Singh, D. & Merchant, R. (1996). Microbial decolourisation of textile – dye – containing effluents. A review. *Bioresource Technology 58*: 217 – 227.

Bangert, R., Aichele, W., Schollmeyer, E., Weimann, B. & Hartinger, H. (1977). Photo – oxidation von malachitgrunun Kristall violet. *Melliand Textilber 5*: 399-404.

Bras, R., Gomes, A., Ferra, M. I. A., Pinheiro, H. M. & Goncalves, I. C. (2005). Monoazo and diazo dye decolourisation studies in a methanogenic UASB reactor. *Journal of Biotechnology* 115: 57-66.

Bumpus, J. A & Aust, S. D. (1987). Biodegradation of environmental pollutants by the white rot fungus *P. chrysosporium*: involvement of the lignin degrading system. *Bio Assays 6*: 166-170.

Bumpus, J. A. & Brock, B. J. (1988). Biodegradation of crystal violet by white rot fungus *Phanerochaete chrysosporium*. *Applied Environmental Microbiology 54*: 1143-1150.

Campos, R., Kandelbauer, A., Robra, K. H., Cavaco – Paulo, A. & Gubitz, G. M. (2001). Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclerotium rolfii*. *Journal of Biotechnology 89*: 131 – 139.

Claus, H., Faber, G. & Konig, H. (2002). Redox mediated decolourisation of synthetic dyes by fungal laccases. *Applied Microbial Biotechnology 59*: 672 – 678.

Cripps, C., Bumpus, J. A. & Aust S. D. (1990). Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*. *Applied Environmental Biotechnology 56*: 1114 – 1118.

Daljit Singh, A. & Mukesh, C. (2004). Decolourization of diverse industrial dyes by some *Phlebia* species and their comparison with *Phanerochaete chrysosporium*. *Journal of Basic Microbiology. 44*: 331-338.

Eiichi, I., Ogawa, T., Yatome, T. C. & Horisu, H. (1985). Behavior of activated sludge with dyes. *Bulletin of Environmental Contamination and Toxicology 35*: 729-734.

Ferreira, V. S., Magalhaes, D. B., Kling, S. H., De Silva, J. G. & Bon, E. P. (2000). N-Demethylation of methylene blue by lignin peroxidase from *Phanerochaete chrysosporium*. Stoichiometric relation for H_2O_2 consumption. *Applied Biochemistry and Biotechnology 84*: 255-265.

Glenn, J. K. & Gold, M. H. (1983). Decolourisation of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium. Applied and Enivironmental Microbiology. 45*: 1741-1747.

Gomaa, O. M., Linz, J. E. & Reddy, C. A. (2008). Decolorization of Victoria blue by the white rot fungus, *Phanerochaete chrysosporium*. *World journal of Microbiology and Biotechnology 24*: 2349-2356.

Hutton, D. G. (1972). Improved biological wastewater treatment. *Dupoint Innovation 2*: 6-26.

Lobos, S., Larrain, J., Salas, L., Callem, D. & Vicunia, R. (1994). Isozymes of manganese dependent peroxidase and laccase produced by the lignin degrading *Ceriporiopsis subvermispora. Microbiology 140:* 1691 –1698.

Lopez, C., Moreira, M. T., Feijoo, G. & Lema, J. M. (2004). "Dye decolorization by manganese peroxidase in an enzymatic membrane bioreactor". *Biotechnology Progress 20*: 74-81.

Michaels, G. B. & Lewis, D. L. (1985). Sorption and toxicity of triphenyl methane dyes to aquatic microbial populations. *Environmental Toxicology and Chemistry 4*: 45-50.

Mielgo, L., Moreira, M. T., Feijoo, G. & Lema, J. M. (2001). A packed bed fungal bioreactor for the continuous decolourisation of azo dyes (Orange II). *Journal of Biotechnology 89*: 99-106.

Mielgo, I., Lopez, C., Moreira, M. T., Feijoo, G. & Lema, J. M. (2003). Oxidative degradation of azo dyes by manganese peroxidase under optimized conditions. *Biotechnology Progress 19*: 325-331.

Muruganandham, M. & Swaminathan, M. (2006). Photocatalytic decolourisation and degradation of reactive orange 4 by TiO $_{(2)}$ – UV process. *Dyes and Pigments. 68:* 133-142.

Ogawa, T., Idaka, E., Hirabayashi, K. & Takase, Y. (1974). Biological treatment of acid azo dyes by microbial populations in activated sludge. *Journal of Society for Fibre Science Technology Japan 30*: 153-159.

Paszczynski, A., Huynh, V. B. & Crawford, R. L. (1986). Comparision of ligninase-1 and peroxidase – M2 from the white rot fungi *Phanerocheate chrysoporium. Archives of Biochemistry and Biophysics 244:* 7750-7765.

Paszczynski, A. & Crawford, R. L. (1991). Degradation of azo compounds by ligninase from *Phanerochaete chrysosporium. Biochemical and Biophysical Research Communications 178*:1056-1063.

Raju, N. S., Venkataramana, G. V., Girish, S. T., Raghavendra, V. B. & Shivashankar, P. (2007). Isolation and evaluation of indigenous soil fungi for decolourization of textile dyes. *Journal of Applied Sciences 7*: 298-301.

Sani, R. K., Azmi, W. & Banerjee, U. C. (1998). Comparison of static and shake culture in the decolourisation of textile dyes and dye effluents by *Phanerochaete chrysoporium. Folia Microbiologica 43*: 85-88.

Sani, R. K. & Benerjee, U. C. (1999). Screening for organisms applicable to the decolurisation of triphenylmethane dyes and optimization of biotransformation conditions in stirred tank reactor. *Indian Journal of Enivironment and Ecoplan 2*: 1-9.

Shin, K. S., Oh, I. K. & Kim, C. J. (1997). Production and purification of remazol brilliant blue – R, decolourising peroxidase from the culture filtrate of *Pleurotus ostreatus*. *Applied and Environmental Microbiology 63*:1774-1748.

Sukumar, M., Sivasamy, A. & Swaminathan, G. (2009). In situ biodecolorization kinetics of Acid Red 66 in aqueous solutions by *Trametes versicolor. Journal of Hazardous Materials 167*(1-3):660-3.

Yatome, C., Yamada, S., Ogawa, T. & Metsui, M. (1993). Degradation of crystal violet by *Nocardia corallina. Applied Microbiology and Biotechnology 38*: 565-569.

Zhang, F., Yediler, A., Liang, X. & Kettrup, A. (2002). Ozonation of the purified hydrolyzed azo dye reactive red 120 – CI. *Journal of Environmental Science and Health. Part A. Toxic/Hazardous Substances and Enivironmental Engineering. 37*: 707 -780.