Int. J. Environ. Res., 7(4):957-962,Autumn 2013
ISSN: 1735-6865

Optimization of Reactive Blue 19 Biodegradation by Phanerochaetechrysosporium

Hemmat, J. and MazaheriAssadi, M.*

Environmental and Industrial Biotechnology Group, Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran

Received 23 Oct. 2012; Revised 7 July 2013; Accepted 17 July 2013

ABSTRACT: Problematic dyes extensively used in different industries such as textile, paper, food, plastics and cosmetics have undesirable environmental effects. White rot fungi demonstrating effective extracellular enzyme system, which is capable of degrading dyes and various xenobiotics. The aim of this study was to optimize decolorization of reactive blue 19 (RB19) dye using *Phanerochaetechrysosporium*. A Box-Behnkendesign and Response Surface Methodology (RSM) were used to study the effect of independent variables, namely glycerol concentration (15, 20 and 25 g/L), temperature (27, 30 and 40°C) and pH (5.5, 6.0 and 6.5) on color removal efficiency in aqueous solution. From RSM-generated model, the optimum conditions for RB19 decolorization were identified to be at temperature of 20°C, glycerol concentration of 120/L and pH: 6.5. At the optimum conditions, predicted decolorization was 98 percent. The confirmatory experiments were conducted, which confirmed the results by 99.8 percent color removal. Thus, the experimental investigation and statistical approach enabled us to improve reactive blue 19 biodegradation process by *Phanerochaetechrysosporium* up to 1.25 times higher than non-optimized conditions.

Key words: White rot, Decolorization, Response Surface Methodology (RSM)

INTRODUCTION

Large amount of dyes, approximately 10,000 different types and pigments are used per year, by various industrial applications such as textile and printing industries. It is estimated that about 10% are lost in industrial effluents (Aksu *et al.*, 2007). As a result, a significant proportion of these dyes are released to the environment in wastewater (Castro-Gutierrez *et al.*, 2012; Zahed *et al.*, 2010; Owabor *et al.*, 2010; Nagda and Ghole, 2008; Torkian *et al.*, 2007). White rot fungi (WRF) are the most efficient lignonlytic organisms capable of degrading various types of dyes such as azo, heterocyclic, reactive and polymeric. The capability is due to extracellular non-specific enzyme systems composed of laccases, lignin peroxidase and manganese peroxidase. Laccase, which catalyze the oxidation of both phenolic and non-phenolic compounds. This ligninolytic system of WRF is directly involved in the degradation of various xenobiotic compounds and dyes. Use of WRF is the most unique technology of bioremediation as its ability to degrade structurally diverse xenobiotic organo-pollutants is more conciderable. Biological treatment systems have been widely used for color removal from wastewater, which have edge over physical and chemical methods, flocculation, coagulation adsorption, oxidation, filtration and electrochemical methods (MazaheriAssadi *et al.*,2001; Fazlia *et al.*, 2010; Ofomaja *et al.*, 2009; Palmieri *et al.*, 2005). Nowadays, the most common and standard treatments applied to textile wastewater involve biological and chemical methods (MazaheriAssadi *et al.*,2001; Fazli *et al.*, 2010; Merwe *et al.*, 2002; 2009; Palmieri *et al.*, 2005). While various physical and chemical methods provide high color removal, they are disadvantageous, since color removal efficiency varies with dye type contained in the wastewater and these are expensive methods. Adsorption seems to be an efficient method among the color removal methods. The most commonly used adsorbent is active carbon dust. However, active carbon dust method is expensive, when the ratio of the amounts of removed color to consumed adsorbent amount is taken into consideration. Overall, a combination of physical, chemical and biological processes is most efficient for dye decolourization but can be expensive (Hai *et al.*, 2007; Robinson *et al.*, 2001). Therefore, a more efficient and cost-effective treatment is needed.

*Corresponding author E-mail:mxmazaheriassadi@yahoo.com

Phanerchaetechrysosporium (PC) is a ligninolytic fungus which produces the oxidative enzyme, lignin peroxidase. PC has been shown to degrade a wide variety of environmentally hazardous compounds, including nitroaromatics, polycyclic aromatic hyrdrocarbons, chlorinate organics, and azo dyes to carbon dioxide (Swamy *et al.*, 99). Under nutrient limiting conditions, PC secretes lignin peroxidase that is capable of catalyzing the initial oxidation of several xenobiotics (Toh *et al.*, 2003 - Teerapatsakul *et al.*, 2007).. Not much information is available on ability of PC in degrading Reactive Blue 19. Studies indicate that these dyes are toxic and also become harmful to the environment. They are considered non-degradable under aerobic conditions with Phanerochaetechrysosporium. Response Surface Methology(RSM) is a very useful biostatical tool for different research purpose as it provides statistical models which helps in understanding the interactions among the parameters that have been optimized (Azin *et al.*, 2008; Khataeea *et al.*, 2011; Sharma *et al.*, 2012).The advantages of using RSM have been reported to include reduction in number of experimental trials needed to evaluate multiple parameters and the ability of the statistical tool to identity interactions. In addition, to analyzing the effects of the independent variables, the experimental methodology also generates a mathematical model that describes the overall process (Fazli *et al.*, 2010). The objective of this study was to optimize decolorization of reactive blue 19 (RB19) dye by *Phanerochaete chrysosporium* using RSM.

MATERIALS & METHODS

A culture of white-rot fungus *Phanerochaete chrysosporium* , obtained from Persian type culture collection, was used in this study. The fungal stock culture was maintained through periodic transfer on Potato Dextrose Agar (PDA) at 4°C until use. To prepare the inoculums, the fungus was transferred onto a fresh PDA plate and incubated at 24° C for 7 days, and was used for further experimental studies (Demain *et al.*, 99).The reactive dyes used in this study were obtained from the Biotechnology Department, IROST , which was Reactive Blue 19 (RBBR) RBBR is a synthetic anthraquinone-based reactive dye (Fazlia *et al.*, 2010).

In present study the response surface methodology (RSM) was used in order to determine the optimum conditions for the color removal. The experimental design and statistical analysis were performed using MiniTab software. The experiments were based on a Box-Behnken design with a quadratic model in order to study the combined effects of three independent variables (glycerol concentration, temperature and pH). The proposed factors and levels obtained from screening experiments of 10 initial variables (i.e. type of carbon source, carbon source concentration, nitrogen source concentration, $CuSO_4$ concentration, temperature, ethanol concentration, inoculum volume, pH, shaker speed and dye concentration) using 2-level fractional factorial experimental design for color removal in preliminary stage of research. The three selected variables were represented by X_1, X_2 and X_3, respectively. Each independent variable was coded 3 levels which were -1, 0 and +1, as shown in Table 2. The optimization experiments were based on 15 combinations with two replicates. Table 3 represents the design matrix of the trials experiments. The experimental designs were randomized to exclude any bias(Azin *et al.*, 2007; Fazli *et al.*, 2010).

Decolorization experiments were carried out in flasks. The dye solutions were prepared with the supplement of (g/L): glucose 10.0; K_2HPO_4 1.0; $MgSO_4 7H_2O$ 0.5; KCl 0.5; $FeSO_4 7H_2O$ 0.01; NH_4NO_3 1.75; and pH 5.5. To prepare inocula for liquid cultures, 20 agar plugs (7mm from the edge of a 7-day-old agar culture) of *Phanerochaete chrysosporium* growing mycelia were inoculated into 250 mL Potato Dextrose medium and then incubated with 150 rpm shaking at 24 °C for 7 days (MazaheriAssadi *et al.*,2001– Merwe *et al.*, 2002). After that, they were filtered through cheese cloth to obtain fungal pellets. The bio-oxidation experiment was carried out in 500-mL flasks containing 300 mL dye solution. These were inoculated with 2.5% (w/v) wet *Phanerochaete chrysosporium* pellets and incubated with 150 rpm shaking at 28 °C for 7 days. The color units and lignin-degrading enzymes production were monitored periodically in order to evaluate the performance of fungal cells in decolorization.. The related parameters, including the concentration of dyes (200, 400, 600, 800, and 1,000 mg/L), fungal inoculum size (1, 2, and 3g/100ml), and pH (3, 5, 7, and 9) were studied. All treatments were performed in triplicates Lignin peroxidase (LiP) activity was measured by monitoring the oxidation of veratryl alcohol in the presence of H_2O_2 at 310 nm (molar extinction coefficient = 9,300 $M^{-1}cm^{-1}$) according to report of MazaheriAssadi *et al.*, one unit of LiP activity was defined as the amount of enzyme catalyzing the formation of 1mol of veratraldehyde per minute (MazaheriAssadi *et al.*,2001). Determination of Manganese peroxidase (MnP) activity using MBTH and DMAB was based on previous studies (Chedchant *et al.*, 2009; D'souza *et al.*, 99). MBTH and DMAB were oxidatively coupled by the action of the enzyme in the presence of added H_2O_2 and Mn^{2+} ions to give a purple indamine dye product. One unit of MnP activity was defined as an amount of catalyzing the production of 1mol of green or purple product per ml per min.

Color as American Dye Manufacturer Institute (ADMI) value was measured according to EPA Method 110.1. This method is an extension of the Tristimulus

Filter Method. Tristimulus values are converted to an ADMI single number color difference, of the same magnitude assigned to platinum-cobalt standards, using the Adams Nickerson Color Difference (DE). Hach DR5000 spectrophotometer was used for ADMI values because standard curves and complex equations have been installed in this instrument (Fazlia *et al.*, 2010). Percentage of decolorization was calculated:

$$\text{Decolorization (\%)} = (1 - \frac{\mathbf{ADMI}}{\mathbf{ADMI}}) \times 100$$

Where ADMI0 is initial color solution and ADMI is final color solution..

RESULTS & DISCUSSION

The effects of Reactive Blue 19 concentrations on per cent decolorization, MnP activities by *Phanerochaete chrysosporium*were conducted. Dye in solutions was varied from100 to 600 and finally 1,000 mg/L. The results indicated a dramatic decrease (99 and 99.8%) in color reduction of RB19 solutions at every concentration. The results (99.8% decolorization in 72 hours) also indicated the rate and extent of decolorization of RB19 (Table 1) compared favourably with those by other white-rot fungi (83% decolorization in 264 hours; *Bjerkandera* sp. BOS55) 65% decolorization in 480 hours;(Swamy *et al.*, 99; Palmieri *et al.*, 2005; Park *et al.*, 2007), and *Trametestrogii*(85% decolorization in 72 hours (Zouari-Mechichi *et al.*, 2006). Furthermore, the result demonstrated that dye concentration did affect the time period to reach maximum decolorization. A general tendency was that more concentrated dye solution caused slower rate and longer time period for decolorization. For example *Phanerochaetechrysosporium* reached maximum 99.7% color reduction from 200mg L^{-1} RB19 solution within only 72 hours of treatment. About 99% decolorization 1,000 mg L^{-1} RB19 solution was achieved after 72 hours of treatment (Table 1). This was consistent with the study by Aksu et al.(2007). The white-rot *T. versicolor* took 8 days to reach maximum 95% color reduction from 58.4 mg L^{-1} RB5 starting solution, whereas maximum 77 percent color reduction from 358.6 mg L^{-1} RB5 starting solution was achieved within 14 days. Interestingly, RB19 seemed to be degraded much better with *Phanerochate chrysosporium* than Ganoderma as observed higher decolorization reported by MohamadianFazeli et al. (2010). However, different reports (Gayazov *et al.*, 96 ; Fu *et al.*, 2001; Kao *et al.*, 2001; Chedchant *et al.*, 2009) stated that the difference between decolorization of structurally different dyes was not easy to explain. This is because the process requires some time to degrade the chromophore, thus, the slow decolorization rate of some dyes could be attributed to the complexity of their chromophores. However, the overall complexity alone was not an indicator of the difficulty of decolorization of a particular dye. Decolorization rate of RB19 based on first order rate equation resulted k=-0.009 h^{-1} that means half-life 3.2 days at 28 ^{o}C. It has been reported that the half-life of hydrolyzed RB19 is about 46 years at pH 7 and 25^{o}C (Demain *et al.*, 99). However, approximately 90 mg/L of 100 mg/L RB19 was decolorized by *F. trogii* at the end of 48 hs (Palmieri *et al.*, 2005; Sarnthima *et al.*, 2004). Toh et al. also clearly showed the difference in decolorization ability of different fungi for different dyes (2003). Kirby et al. reported that *Phlebiatremellosa* taken periods of between 7 and 20 days to achieve 90 percent decolorization in diverse range of synthetic dyes (2000). In our previous studies, we have shown that the enzyme production starts in secondary growth phase of fungus and various cultivation parameters that influence LiP production and activity. These factors include carbon limitation, nitrogen source and concentration and microelements (MazaheriAssadi *et al.*, 2001).

The statistical combination of the factors and the decolorization measurements are shown in Table 1 with the total 32 set of experiments. The color removal assay performed after 7 days. The minimum and maximum decolorization is 76 and 99.8 percent, respectively. It means that this fungus has good potential of color removal in 72 hours.the aim of these experiments was to investigate main effects and two factor interactions. By the design of Box-Behnken (Azin *et al.*, 2007),Table, 2, followed with 30 trial experiments (Table 3), color removal efficiency varied from 87.1 percent to 99.8 percent in the 15 different combinations with duplication. Thus, the best conditions in conducted experiments were the process with 20 g/L glycerol, 28 ^{o}C temperature and pH 6.0. The maximum decolorization achieved for 200 mg/L dye after 72hs.Table 4 shows the analysis of variance (ANOVA) of the results for the decolorization. The linear and quadratic effects of variables was significant ($p<0.0001$), while there was no significant interaction ($p<0.798$). In order to optimize the experiment by statistically significant factors and interactions, a second order (quadratic) polynomial equation was fitted to the experimental data for decolorization by *Phanerochaete chrysosporium* and a correlation was constructed with R^2 of 0.94.

$$\text{Decolorization (\%)} = 92.717 - 2.7\,X_2 + 1.338\,X_3 - 0.115\,X_1^2 - 0.515\,X_2^2 - 1.465\,X_3^2 + 0.113\,X_1X_2 + 0.138\,X_1X_3 - 0.188\,X_2X_3.$$

Where X is the coded value (between -1 and +1) for the factor indicated by attached subscript in Table 2. The coefficients of temperature (linear), pH (linear and quadratic) were statistically significant at a level of $P<0.0001$, however, no interactions were statistically

Table1. Mn peroxidase, LiP activities and percent decolorization by *Phanerochaete chrysosporium after 72 hs*

RB19(mg/L) Concentrations	Mn Peroxidase U/L	LiP U/L	% decolorization
0	0.11	0.1	0
100	976	768.4	99.8
200	956.76	892.64	99.7
400	923.04	880	99.6
600	786.92	767.07	97.0
800	675.67	765	99
1000	675.00	765	99

Table 2. Independent variables and their coded levels used for the optimization of RB19 decolorization by Phanerochaete chrysosporium

Key	Factor	Levels		
		Low	Medium	High
		-1	0	+1
X_1	Glycerol concentration	13	23	28
X_2	Temperature	27	29	37
X_3	pH	5.5	6.0	6.5

Table 3. Box-Behnken design (Three factors in three levels) used for the optimization of RB19 decolorization by Phanerochaete chrysosporium

Run	X_1	X_2	X_3	Decolorization*, %	Run	X_1	X_2	X_3	Decolorization, %
1	-1	-1	0	96.0	16	0	-1	+1	96.3
2	0	0	0	98.9	17	-1	0	+1	97.7
3	0	-1	+1	99.4	18	0	+1	+1	97.1
4	+1	-1	0	99.6	19	0	+1	+1	98.2
5	+1	0	+1	99.5	20	+1	+1	0	99.8
6	+1	-1	0	98.9	21	0	0	0	99.4
7	+1	0	-1	89.8	22	-1	-1	0	94.4
8	-1	+1	0	89.8	23	0	+1	-1	97.1
9	+1	0	+1	89.8	24	0	0	0	93.0
10	0	-1	-1	92.6	25	0	0	0	92.6
11	0	+1	-1	87.9	26	-1	+1	0	89.4
12	0	0	0	92.1	27	+1	+1	0	89.1
13	-1	0	-1	91.0	28	0	-1	-1	99.3
14	-1	0	-1	86.8	29	0	0	0	90.3
15	+1	0	-1	86.3	30	-1	0	+1	95.3

X_1: glycerol concentration, X_2: temperature, X_3: pH;
* Decolorization of 200 mg/L dye after 72hs.

Table 4. Factors and levels used for screening variables affecting decolorization of RB19 by Phanerochaete chrysosporium

Factor	Unit	Level	
		Low	High
Type of carbon source	-	glycerol	Starch
Carbon source concentration	g/L	20	39
Nitrogen source concentration (Yeast extract)	g/L	0.2	0.4
Temperature	^oC	25	30
$CuSO_4$ concentration	g/L	0.001	0.004
Ethanol	V/V	0	2
Inoculum volume	mL	5	10
pH	-	5	6
Shaker speed	Rpm	100	150
Dye concentration	mg/L	100	200

significant.The increase of temperature and pH improve the color removal efficiency, and decreasing of carbon source increases decolorization. However, results showed that using glycerol resulted in higher color removal than starch.

These findings were similar to some before reports (Mahmoodi *et al.,* 2009; Murugesan *et al.,* 2007; Fazlia *et al.,* 2010) but, in contrast to those scientists that have reported starch is better (Park *et al.,* 2007; Sarnthima et al. 2008). Ethanol as inducer improved decolorization but was not significant. While, Merwe has reported ethanol under 4 percent level resulted in a significant increase in laccase production by *Pycnoporussanguineus* (Merwe *et al.,* 2002). In the course of using *Phanerochaete chrysosporium*weobtained similar results using the same dye and Ganoderma species i.e eight interactions were significant ($P<0.01$) that are depictedbut in shorter period of time. Interactions are both positive (e.g. carbon type×temperature) and negative (e.g. nitrogen concentration×shaking speed). Negative effect of interaction means high levels of factors cause lower decolorization (Fazlia *et al.,* 2010). Results obtained in this study emphasized on observations of those who reported these factors and interactions are important in color removal (Gayazov *et al.,* 96; Fu *et al.,* 2001; Murugesan *et al.,* 2007; Montgomery *et al.,* 2008; Toh *et al.,* 2003; Wesenberg *et al.,* 2003). Analyzing data depicted from decolorizationof RB19 by *Phanerochaetechrysosporium* performed using fractional factorial design experiment, it is concluded that four factors and eight interactions are important for improving and optimizing color removal process. Statistical analysis method showed that factors including temperature, type of carbon and energy source, carbon source concentration and pH were significant variables and optimization of process should be investigated based on them. Proper media components and operating conditions for optimization is suggested, type of carbon source: glycerol, carbon source concentration: around 20g/L, nitrogen source concentration: 0.5g/L, temperature: around 28°C, pH: around 6, copper sulfate concentration: 0.004 g/L, dye concentration: 200 mg/L, ethanol: 2 volumetric percent, volume of inoculation: 5% and shaking speed: 150 rpm.

CONCLUSION

A significant proportion of textile wastewater which is composed of different dyes are released to the environment. White rot fungi (WRF) are the most efficient lignonlytic organisms capable of degrading various types of dyes such as azo, heterocyclic, reactive and polymeric dyes. In this study decolorization of reactive blue 19 (RB19) dye using *Phanerochaetechrysosporium*. Optimised by Response Surface Methodology (RSM) investigated. The effect of independent variables, namely glycerol concentration, temperature and pH on color removal efficiency in aqueous solution were monitored. From RSM-generated model, the optimum conditions for RB19 decolorization were identified to be at temperature of 20°C, glycerol concentration of 120/L and pH: 6.5. At the optimum conditions, predicted decolorization was 98 percentwithin 72 hs.

REFERENCES

Aksu,Z., Kilic, N. K.,Ertugrul, S.andDonmez, G. (2007). Inhibitory effects of chromium (VI) andRemazol Black B on chromium (VI) and dyestuff removals by *Trametesversicolor*, Enzyme and Microbial Technology,**40**, 1167–1174.

Azin, M., Moravej, R. andZareh, D.(2008). Production of xylanase by *Trichodermalongibrachiatum* on a mixture of wheat bran and wheat straw: Optimization of culture condition by Taguchi method, Enzyme and Microbial Technology,**40**, 801-805.

Castro-Gutiérrez, V. M., Rodríguez-Rodríguez, C. E. and Vargas-Azofeifa, I. (2012). Hydrocarbon Degrading Microflora in a Tropical fuel-Contaminated Aquifer: Assessing the Feasibility of PAH Bioremediation. Int. J. Environ. Res., **6 (1)**, 345-352.

Chedchant, J., Petchoy, O., Vaithanomsat, P., Apiwatanapiwat, W.,Kreetachat, T.andChantranurak, S. (2009).Decolorization of lignin-containing effluent by white-rot fungus Datronia sp. KAPI0039, (Paper presented at the 47th Kasetsart University Annual Conference, Bangkok).

Demain, A. L.and Davis, J. E. (1999). Manual of industrial microbiology and biotechnology.Washington: ASM press.

Dos Santos, A. B., Cervantes, F. J. and Lier, J. B. V. (2007). Review paper on current technologies for decolourisation of textile wastewater: perspectives for anaerobic biotechnology.Bioresource Technology, **98**, 2369-2385.

D'souza, T. M., Merritt, C. S. and Reddy, C. A. (1999). Lignin-Modifying enzymes of the white rot basidiomyceteGanodermalucidium, Applied and Environmental Microbiolgy, **65**, 5307-5313.

Erkurt, E. A., Unyayar, A. andKumbur, H. (2007).Decolorization of synthetic dyes by white rot fungi, involving laccase enzyme in the process. Process Biochemistry,**42,** 1429–1435.

Fu, Y. andViraraghavan, T. (2001). Fungal decolorization of dye wastewaters: a review. Bioresource Technology, **79,** 251-262.

Gayazov, R. andRodakiewicz-Nowak, J. (1996). Semi-continuous production of laccase by *Phelebia radiate* in different culture media, Folia Microbiol, **41**, 480-484.

Heinzkill, M., Bech L., Halkier, T., Schneider, P.andAnke, T. (1998).Characterization of laccase and peroxidase from wood-rotting fungi. Appl. Environ. Microbiol.,**64**, 1601-1606.

Kao, C. M., Chou, M. S., Fang, W. L., Liu, B. W. and Huang, B. R. (2001). Regulating colored textile wastewater by 3/31 wavelength ADMImethods in Taiwan. Chemosphere, **44**, 1055-1063.

Khataeea, A. R., Kasirib, M. B.andAlidokht, L.(2011). Application of response surface methodology in the optimization of photocatalytic removal of environmental pollutants using nanocatalysts.Environ. Technol.,**33**, 1669-84.

Kirby, N., Marchant, R. and McMullan, G. (2000).Decolourisation of synthetic textile dyes by Phlebiatremellosa. FEMS Microbiology Letters,**188**, 93-96.

Mahmoodi, N. M., Arami, M. andGharanjig, K. (2009). Laboratory studies and CFD modeling of photocatalytic degradation of colored textile wastewater by titania nanoparticles. Desalination and Water Treatment, **1**, 312–317.

MazaheriAssadi, M., Rostami, K., Shahvali, M. and Azin, M. (2001).Decolorization of textile wastewater by Phanerochaetechrysosporium. Desalination, **141**, 331-336.

MohammadianFazlia, M., Mesdaghiniaa, A. R., Naddafia, K., Nasseria, S.,Yunesiana, M.,MazaheriAssadi, M.,Rezaie, S. andHamzehei, H. (2010). Screening of factors affecting reactive blue 19 decolorization by *Ganoderma*sp.using fractional factorial experimental design. Desalination and Water Treatment, **22**, 1-8.

Merwe, J. J. V. (2002). Production of laccase by the white-rot fungus Pycnoporussaguinus.Thesis of Master Sciences.16-17.

Murugesan, K., Nam, I., Kim, Y.and Chang, Y. (2007) Decolorization of reactive dyes by a thermostablelaccase produced by Ganodermalucidum in solid state culture. Enzyme and Microbial Technology, **40**, 1662-1672.

Montgomery, D. C.(Eds.) (2001).Design and analysis of experiments.New York: John Wiley andSons.

Nagda, G. K. and Ghole, V. S. (2008).Utilization of Lignocellulosic Waste from Bidi Industry for Removal of Dye from Aqueous Solution.Int. J. Environ. Res., **2 (4)**, 385-390.

Ofomaja, A. E. (2009) Equilibrium sorption of methylene blue using mansonia wood sawdust as biosorbent. Desalination and Water Treatment, **3**, 1–10.

Owabor, C. N., Ogbeide, S. E. andSusu, A. A. (2010). Model Simulation of Biodegradation of Polycyclic aromatic Hydrocarbon in a Microcosm.Int. J. Environ. Res., **4 (4)**, 807-816.

Palmieri, G.,Cennamo, G.andSannia, G. (2005).Remazol brilliant blue R decolourisation by the fungus Pleurotusostreatus and its oxidative enzymatic system.Enzyme Microb. Technol., **36**,17–24.

Park, Ch., Lim, J., Lee, Y., Lee, B., Kim, S., Lee, J.and Kim, S. (2007). Optimization and morphology for decolorization of reactive black 5 by Funaliatrogii. Enzyme and Microbial Technology, **40**, 1758-1764.

Revankar, M. S. and Lele, S. S. (2007). Synthetic dye decolorization by white rote fungus, Ganoderma sp. WR-1.Bioresource Technology,**98**, 775-780.

Rezaee A., Ghaneian, M. T., Khavanin, A., Hashemian, S. J., Moussavi, Gh.,Ghanizadeh, Gh. andHajizadeh, E. (2008). Photochemical oxidation of reactive blue 19 dye in textile wastewater by UV/$K_2S_2O_8$ process. Iranian Journal of Environmental Health, Science and Engineering, **5,**95-100.

Sarnthima, S. andKhammuang, S., (2008).Evaluation of dyes decolourisation by the crude enzyme from Pleurotussajor-caju grown on sorghum seed media. Pakistan Journal of Biological Sciences,**11**, 2-67.

Sharma, P.and Singh, L. (2012) Application of response surface analysis for biodegradation of azo reactive textile dye using Aspergillusfoetidus.,**52**, 314-23.

Silva, C. M. M., Melo, I. S. and Oliveira, P. R. (2005).Ligninolytic enzyme production by Ganoderma spp. Enzyme and Microbial Technology, **37**, 324-329.

Swamy, J. and Ramsay, J. A. (1999). The evaluation of white rot fungi in the decoloration of textile dyes,Enzyme and Microbial Technology,**24**, 130–137.

Tavcar, M., Svobodova, K., Kuplenk, J., Novotny, C. andPavko, A. (2006). Biodegradation of azo dye R016 in different reactors by immobilized Irpexlacteus. ActaChim.Slov.,**53**, 338-343.

Tachibana, S., Kiyota, Y. and Koga, M. (2007). Bioremediation of dioxin-contaminated soil by fungi screened from nature. Pakistan Journal of Biological Sciences, **10**, 486-491.

Toh, Y., Yen, J.J.Y., Obbard, J.P. and Ting, Y. (2003).Decolorisation of azo dyes by white-rot fungi (WRF) isolated in Singapore. Enzyme and Microbial Technology.**33,** 569–575.

Torkian, A., KeshavarziShirazi, H. and Mehrdadi, N. (2007).Fate of intermediate biodegradation products of triethyl amine in a compost-based biofiltration system. Int. J. Environ. Res., **1 (2)**, 163-169.

Tripathi, A. K., Harsh, N. S. K. and Gupta, N. (2007). Fungal treatment of industrial effluents: a mini-review. Life Science Journal, **4**, 78-81.

Teerapatsakul, Ch., Parra, R., Bucke, Ch. and Chitradon, L. (2007).Improvement of laccase production from Ganoderma sp. KU-ALK4 by medium engineering.World J. Microbiol.Biotechnol.,**23**, 1519-1527.

Wesenberg D.,Kyriakides I. and Agathos S. N. (2003).White-rot fungi and their enzymes for the treatment of industrial dye effluents.BiotechnologyAdvances, **22**, 161-187.

Zahed, M. A., Aziz , H. A., Isa, M. H. and Mohajeri, L. (2010). Enhancement Biodegradation of n-alkanes from Crude Oil Contaminated Seawater. Int. J. Environ. Res., **4 (4)**, 655-664.

Zanirun Z., Abd-Aziz S., Ling F. T. and Hassan M. A. (2009). Optimization of lignin peroxidase production using locally isolated Pycnoporus sp. through fractional design. Biotechnology.**8 (3)**, 296-305.

Zouari-Mechichi H., Mechichi, T., Dhouib, A., Sayadi, S., Mart´ýnez,A. T.andMart´ýnez,M. J. (2006).Laccase purification and characterization from Trametestrogii isolated in Tunisia: Decolorization of textile dyes by the purified enzyme, Enzyme and Microbial Technology, **39**, 141–148.