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Effect of carbon and nitrogen source amendment on synthetic dyes decolourizing efficiency of white-rot fungus, *Phanerochaete chrysosporium*

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Abstract: *Decolourization activity of Phanerochaete chrysosporium for three synthetic dyes viz., congo red, malachite green and crystal violet and impact of additional carbon and nitrogen supply on decolourization capacity of fungus were investigated. Maximum decolourizing capacity was observed up to 15 ppm. Addition of urea as nitrogen source and glucose as carbon source significantly enhanced decolourizing capacity (up to 87%) of fungus. In all the cases, both colour and COD were reduced more in non-sterilized treatments as compared to sterilized ones. Significant reductions in COD content of dye solutions (79-84%) were recorded by fungus supplied with additional carbon and nitrogen. A highly significant correlation ($r = 0.78$, $p<0.001$) between colour and COD of dye solutions was recorded. Thus, a readily available carbon and nitrogen source is imperative to enhance the bioremediation activity of this fungus which has been the most suitable for synthetic dyes and textile industry wastewater treatment.*

Key words: *Textile dyes, Decolourization, COD, Phanerochaete chrysosporium*
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Introduction

Synthetic dyes, including several structural varieties of dyes such acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal–complex dyes (Banat *et al*., 1996), have been increasingly used in the textile, paper, cosmetics, pharmaceutical and food industries because of their ease of use, cost effectiveness in synthesis, stability and variety of colour compared with natural dyes (Marmion, 1991; Singh *et al.,* 2007). Most of them are recalcitrant to biodegradation, thereby creating an aesthetic problem in the currently used biological treatment (Swamy and Ramsay, 1999).

Around 10^6 tonnes and more than 10,000 different synthetic dyes and pigments are produced annually world wide and used extensively in dye and printing industries. It is estimated that about 10% of these are lost in industrial effluents (Young and Yu, 1997). These dyes are difficult to degrade once released into aquatic systems, and are typically not removed from water by conventional waste water treatment systems (Pazarlioglu *et al*., 2005). The current state of the art for the treatment of wastewaters containing dyes is physicochemical techniques, such as adsorption, precipitation, chemical oxidation, photodegradation, or membrane filtration (Churchley, 1994; Yeh and Thomas, 1995; Demir *et al.,* 2007).

Conventional treatments of textile effluents are either ineffective, costly, complicated or have sludge disposal problems. Therefore, the potential exists for the breakdown of dyes in effluent by extra cellular enzymes produced by white-rot fungi (such as *Phanerochaete chrysosporium*) during fermentation (Robinson *et al*., 2001). The low substrate specificity of their lignin degradation system (Tien and Kirk, 1983) suggests that white rot fungi could be used for the degradation of other complex compounds such as those present in wastewaters or polluted soils (Dott *et al*., 1995).

Here, a treatment study is presented using the decolourizing ability of white-rot fungi, *P. chrysosporium*, with glucose and urea as nutrient amendments.

Materials and Methods

Dyes: Three commercially most utilized dyes in various industries were selected for the present investigation.

Congo red: Azo dyes, the largest class of synthetic dyes, are characterized by the presence of one or more azo bonds (-N=N-) in association with one or more aromatic systems, which may also carry sulfonic acid groups responsible for allergic and asthmatic reactions in sensitive individuals (Combes and Haveland-Smith, 1982).

Malachite green: A N-methylated diaminotriphenylmethane dye, used as a fungicide and antiseptic in fish cultures (Schnick, 1988); its high toxicity to bacteria and mammalian cells has been demonstrated (Fessard *et al*., 1999).

Crystal violet: The triphenylmethane dye crystal violet (N,N,N′,N′, N′′, N′′ - hexamethylpararosanilin) has seen in extensive use in human and veterinary medicine, as a biological stain, and as a textile dye (Kean and Haskins, 1978). It has been suggested to be responsible for promotion of tumor growth in some fishes (Nelson and Hites, 1980).

Table - 1: Decolourizing effect of *P. chrysosporium* on colour of dyes after 5 days of treatment (Values in parentheses shows SE)

Dye concentration (ppm)	Initial	After 5 days
	Congo red	
5	41.50 (1.14)	0.00 (0.00)
10	111.31 (1.35)	47.3 (4.12)
15	682.37 (1.96)	346.50 (11.12)
20	884.00 (1.69)	808.06 (12.86)
50	1260.40 (3.98)	1230.30 (10.91)
	Malachite green	
5	1531.82 (2.80)	207.08 (9.64)
10	1540.63 (1.73)	578.6 (12.36)
15	2237.64 (2.24)	976.00 (15.60)
20	2421.42 (3.36)	2240.90 (12.62)
50	3561.31 (4.22)	3492.42 (16.35)
	Crystal violet	
5	219.21 (1.10)	39.39 (4.69)
10	918.63 (1.15)	373.64 (12.66)
15	1850.90 (2.12)	851.57 (17.98)
20	2047.77 (2.36)	1903.03 (10.63)
50	2974.67 (2.92)	2833.34 (15.87)

Fungal strain: The culture *P. chrysosporium* (MTCC-787), a white-rot fungus used in this investigation was obtained from microbial culture collection maintained in the department. The stock culture was sub cultured on media as specified by Institute of Microbial Technology, Chandigarh (India). The medium consisted of malt extract 20 g, glucose 20 g, peptone 1 g, agar 20 g in 1 litre of distilled water with pH adjusted to 5.8 and growth at 39°C.

Selection of optimum dye concentration: A five-day experiment was conducted with five concentrations (*i.e.* 5, 10, 15, 20 and 50 ppm) of each dye. 100 ml of each dye solution of different concentrations was taken separately in a set of four 250 ml Erlenmeyer flasks. Fungus was inoculated in the form of four mycelial discs of 1 cm diameter in each flask, which were cut out from the zone of active growth on a petri dish (100 × 15 mm). All the flasks were kept on a rotary shaker for 5 days at 39±1°C (150 rpm). The solution from each flask was employed for the measurement of colour (APHA, 1992) on starting of the experiment and after 5 days.

Table - 2: Description of different treatments

Notation	Treatment
T_0	Control having no fungus
T_C	With fungus but without any substrate
T_U	With fungus and 2 g urea
T_G	With fungus and 5g glucose
T_{U+G}	With fungus and 2 g urea and 5 g glucose

Table - 3: Variance ratio for pH, colour and COD of dyes treated with *P. chrysosporium* along with different combinations of substrates

Variance	pH	Colour	COD
Dye (D)	3377.7***	136707.4***	6538.9***
Incubation period (IP)	41645.2***	83876.8***	12819.2***
Sterilization (S)	2176.7***	3087.8***	967.4***
Co-substrate (CS)	32181.5***	38493.9***	2880.9***
D × IP	217.8***	7200.8***	676.7***
D × S	544.8***	42.5***	171.8***
D × CS	1433.8***	3722.8***	303.1***
IP × S	376.7***	95.3***	2.7*
IP × CS	4424.5***	4876.7***	684.4***
S × CS	172.8***	107.8***	31.8***
D × IP × S	99.3***	24.9***	4.7***
D × IP × CS	203.4***	462.4***	45.8***
D × S × CS	273.4***	40.5***	18.5***
IP × S × CS	182.3***	19.1***	9.1***
D × IP × S × CS	40.5***	13.2***	6.7***

Level of significance: * = p<0.05 and *** = p<0.001

Effect of co-substrate amendment in the medium: *P. chrysosporium* showed its ability to decolourize dyes up to 15-ppm concentration after which a sharp reduction in decolourization was found (Table 2). Thus, 15-ppm concentration was used for further study. 100 ml of each dye solution (15 ppm) was taken into 10 sets (3 flasks for each set) of 250 ml Erlenmeyer flasks. Five set of flasks for each dye solution was autoclaved and the remaining sets left unsterilized. Four discs of *P. chrysosporium* (1 cm diameter) were added in 8 sets of flask (4 sets of sterilized and 4 sets of non-sterilized). A detailed description of various treatments is mentioned in Table 2. All the flasks were tightly plugged with cotton and kept on rotary shaker (150 rpm) at 39±1°C. Observations for pH colour and COD of dye solution were taken at initial stage and then with an interval of 5 days and experiment was concluded on 15th day.

Table - 4: Correlation matrix of substrate addition and physico-chemical characteristics of dyes treated with *P. chrysosporium*

	Incubation period	Co-substrate addition	pH	Colour	COD
Incubation period	1.00	0.000^{NS}	-0.595***	-0.507***	-0.635***
Co-substrate addition		1.00	-0.381***	-0.313***	-0.253***
pH			1.00	0.580***	0.517***
Colour				1.00	0.777***
COD					1.00

Level of significance: *** = p <0.001 and ^{NS} = Not significant

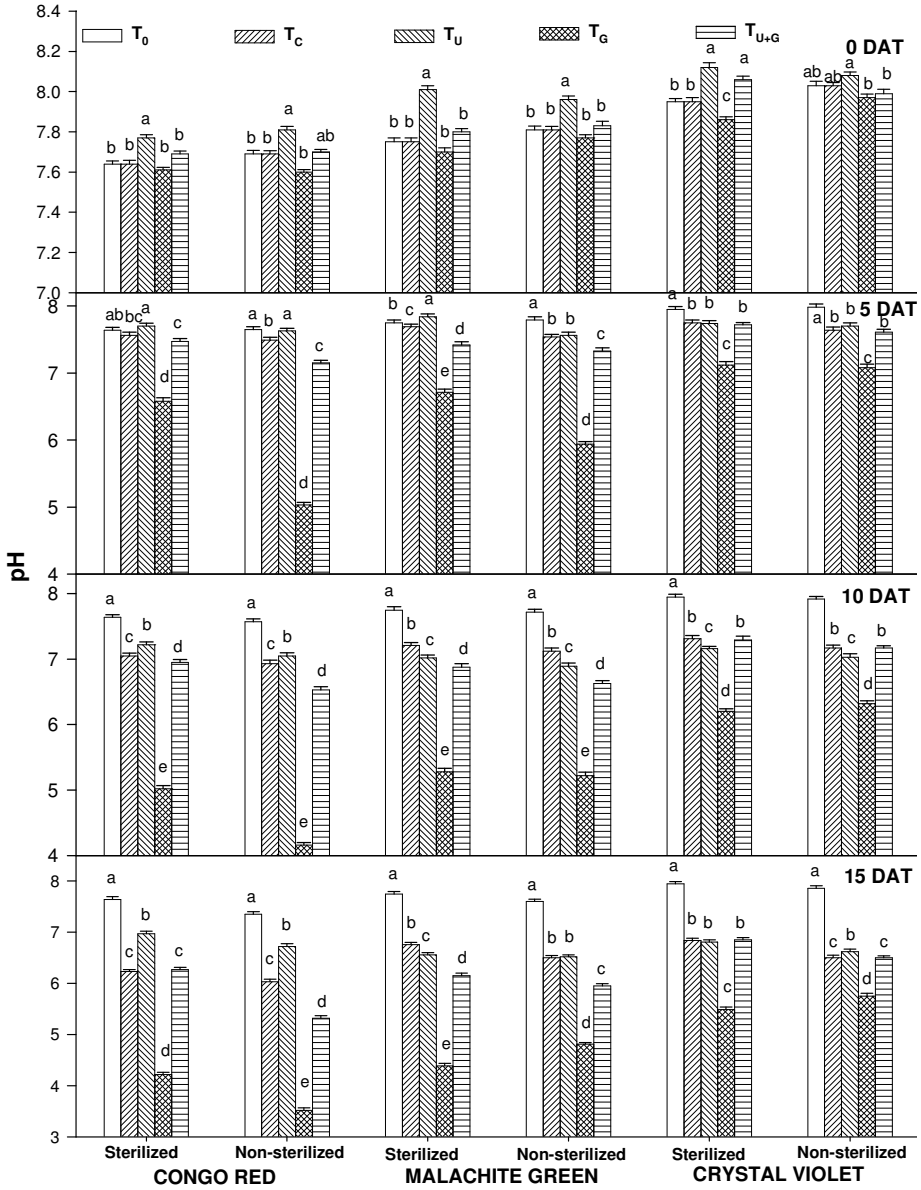

Fig. 1: Effect of co-substrate addition with *P. chrysosporium* on pH of 15 ppm dyes. Within each grouping, bars not followed by the same letter are significantly different at $p \leq 0.05$

pH and colour was measured by following standard methods mentioned in APHA (2005) and COD was determined by using the method of Moore *et al*. (1949).

Statistical analysis: Data recorded was analyzed through SPSS Inc. (v. 10.0) soft ware for multiple analysis of variance to observe the significant effect of addition of co-substrate and sterilization and their interaction on decolourization of dye solution by *P. chrysosporium*. The quantitative changes observed for various parameters due to addition of different co-substrate were evaluated for the level of significance at 5% by Duncan's multiple range test (DMRT).

Results and Discussion

The effect of *P. chrysosporium* on different concentrations of three dyes is presented in Table 1. In all the observations at 5 days after treatment (DAT), decolourization of dyes decreased with the increasing concentration of dyes. Papinutti and Forchiassin (2004) observed that higher concentration of dye malachite green inhibited both growth and decolourizing ability of *P. chrysosporium* and *Fomes sclerodermeus*. Similarly, in present study a slight reduction in decolourization of dyes by fungus was observed as concentration of dyes increased to 15 ppm (more than 50% reduction), while above 15 ppm dye decolourization by fungus reduced drastically (less than 10% reduction) on further increase in dyes concentration. Recently, El-Naggar *et al*. (2004), reported that the maximum rate of crystal violet decolourization using a *Pseudomonas aeruginosa* bioreactor was obtained when crystal violet was applied in a concentration of 15 mg l^{-1}. Beyond this concentration decolourization rate reduced drastically. Thus, to observe the effect of sterilization and co-substrate

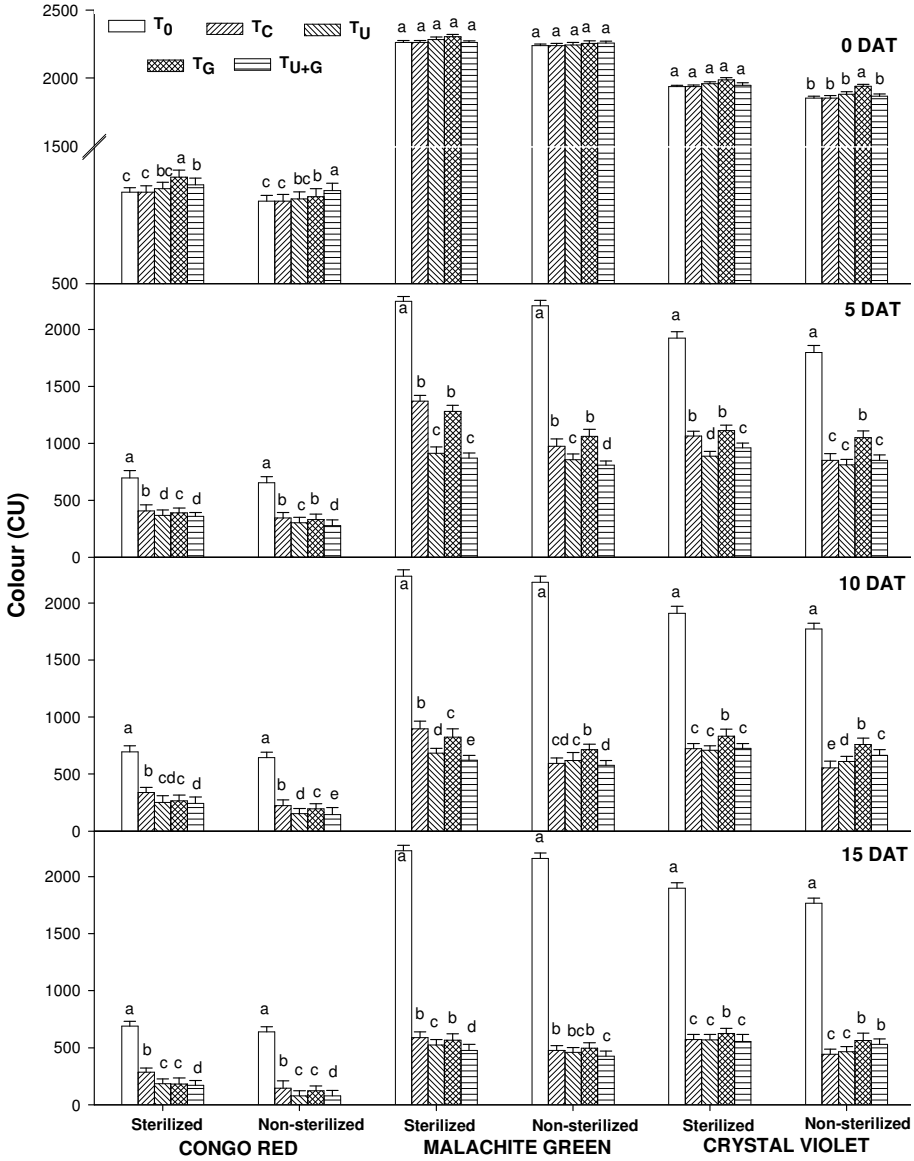

Fig. 2: Effect of co-substrate addition with *P. chrysosporium* on colour of 15 ppm dyes. Within each grouping, bars not followed by the same letter are significantly different at $p \leq 0.05$

addition on decolourization ability of *P. chrysosporium*, 15 ppm dye solution was chosen for further study.

The ability of *P. chrysosporium* to degrade azo dyes is generally correlated with the ability of these organisms to synthesize lignin-degrading exoenzymes such as lignin and manganese peroxidases and laccases (Cripps *et al*., 1990). The white rot fungus, *P. chrysosporium*, grown under ligninolytic conditions, was shown to metabolize crystal violet to three metabolites by sequential N demethylation of the parent compound, which was catalyzed by lignin peroxidases (Bumpus and Brock, 1988). They also reported that nonligninolytic culture of *P. chrysosporium* could also degrade crystal violet.

At initial stage, sterilization and addition of urea increased pH of dye solutions while glucose addition decreased it (Fig. 1). Reduction in pH was observed in *P. chrysosporium* treated dye solutions and recorded maximum (3.52) at 15 DAT in non-sterilized and glucose added solutions (T_U). This might be due to the formation of organic acids by the fungus. Correlation matrix showed significant negative correlation between pH and time ($r = -0.59$, $p < 0.001$) (Table 4). Analysis of variance illustrated significant effect of dye, incubation period, sterilization, co-substrate addition and their interactions on pH of *P. chrysosporium* treated dye solution (Table 3).

Addition of co-substrates and sterilization increased colour of dye solution. *P. chrysosporium* significantly decolourized dye solution and co-substrates increased its decolourization capacity. DMRT showed significant differences in colour of differentially treated dye solutions (Fig. 2). More reduction in colour was observed in non-sterilized dye solutions in comparison to sterilized ones. It might be due to the fact that in the sterilized samples, other microorganisms, especially bacteria are not involved. Whereas, in non-sterilized

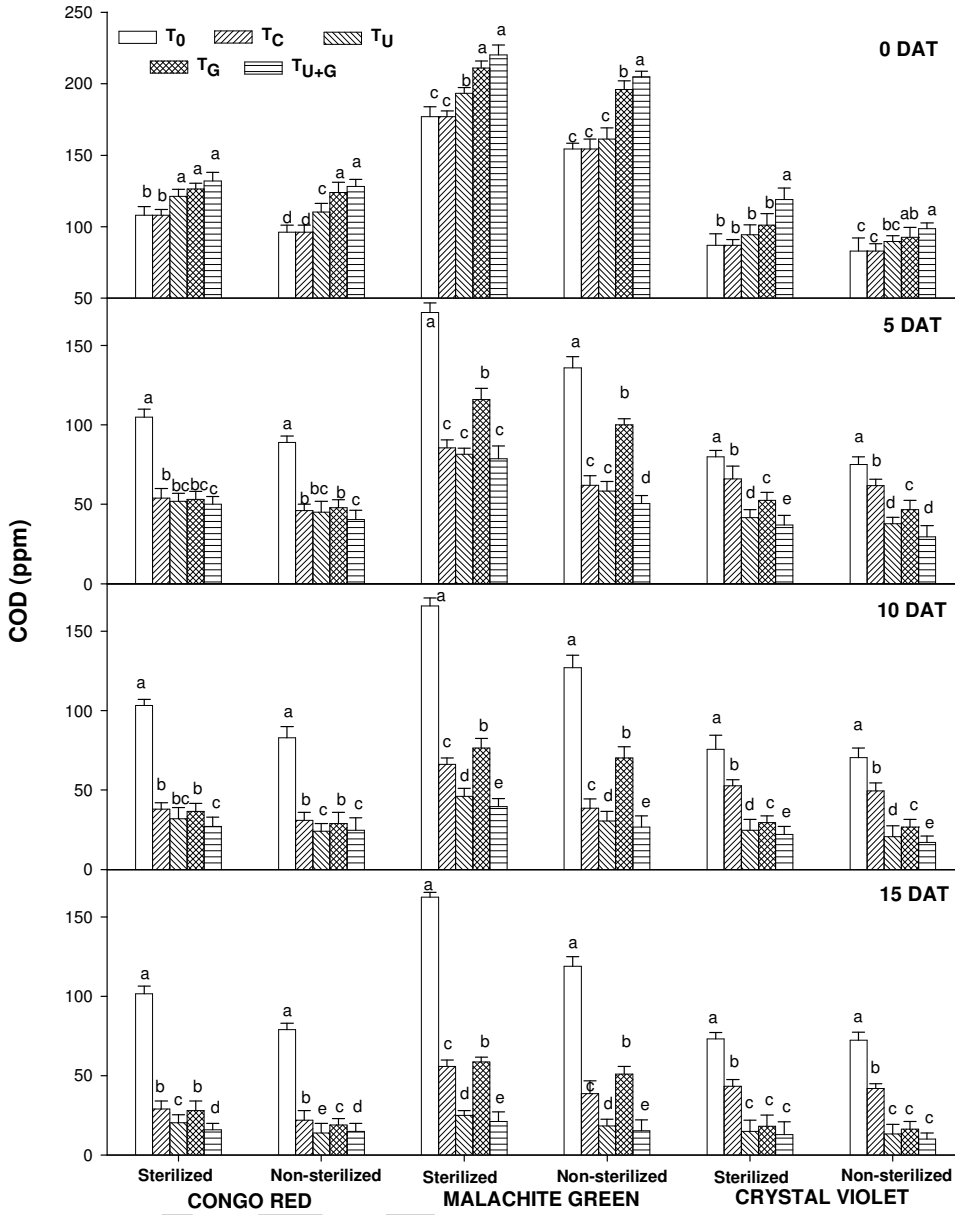

Fig. 3: Effect of co-substrate addition with *P. chrysosporium* on COD of 15 ppm dyes. Within each grouping, bars not followed by the same letter are significantly different at $p \leq 0.05$

samples, reduction in colour and COD might be due to both inoculated fungus, *P. chrysosporium* and bacteria. Decolourization activity of fungus was significantly enhanced by the addition of urea as nitrogen source. These results are corroborated by the study of Jain *et al*. (2000), who found that when no nitrogen source was supplied to the culture media, only 35% decolourization was found after four days of incubation but on addition of 0.5% nitrogen source, the organism showed appreciable amount of dye decolourization. Chao and Lee (1994), also reported similar findings on N-source concentration in culture media. Addition of glucose as easily assimilable carbon source had a positive effect on decolourization efficiency of fungus. The presence of added assimilable carbon (as glucose) in the medium was critical to initiate fungal growth and the decolorisation process, consistent with the findings of other researchers (Swamy and Ramsay, 1999; Zhang *et al*., 1999). Hossain *et al*. (2001), also showed that in case of microbial bleaching of pulp and paper mill effluent with *P. chrysosporium*, the specific growth rate of organism increased with increasing glucose concentration. Maximum reduction in colour of non-sterilized dye solutions of congo red (88.8%), malachite green (81.2%) and crystal violet (71.6%) by *P. chrysosporium* was recorded in T_{U+G} treatment, while for sterilized dye solutions reductions were 76.1, 78.9 and 71.5%, respectively. This is due to availability of carbon source (glucose) and nitrogen source (urea) together in the medium.

Colour showed significant and negative correlation with incubation period ($r = -0.51$, $p<0.001$) and co-substrate addition ($r =$ -

0.31, p<0.001) and positive correlation with pH (r= 0.58, p<0.001) of *P. chrysosporium* treated dye solution (Table 4). Variance ratio clearly showed significant effect of dye, incubation period, sterilization, co-substrate addition and their interactions on colour of *P. chrysosporium* treated dye solutions (Table 3). The present study clearly demonstrates that the presence of N-source is necessary for effective dye decolourization; however in lower concentration similar results have been obtained by other workers in various studies of influence on N–source on decolourizing capability of *P. chrysosporium*.

Chemical oxygen demand (COD) of *P. chrysosporium* treated dye solutions showed significant negative correlation with incubation period (r= -0.64, p<0.001) and positive correlation with pH (r= 0.52, p<0.001). Darah and Ibrahim (1997), reported that the colour reduction of textile wastewater increased with the decrease in COD level of the wastewater and in the present study a positive correlation was observed between COD and colour (r= 0.78, p<0.001) of dye solutions (Table 3). COD of dye solutions was increased by addition of co-substrates (Fig. 4). Similar trend was observed for reduction of COD by *P. chrysosporium* with different co-substrates as recorded for decolourization of dye solutions. Reduction in COD was observed more in non-sterilized in comparison to sterilized *P. chrysosporium* treated dye solutions. Maximum reduction in COD was recorded in non-sterilized solution with T_{U+G} treatment for malachite green (92.5%) followed by crystal violet (90.2%) and congo red (88.3%).

Based on the observations of the present study, it can be suggested that application of white-rot fungus, *Phanerochaete chrysosporium* plays an important role in removal of colour and COD of dye-containing industrial effluents and presence of carbon and nitrogen source as co-substrates greatly enhance the rate of decolourization. However, in place of chemical nutrient amendments, cheaper carbon and nitrogen sources such as agricultural residues will not only enhance the efficiency of the process but also greatly reduce the cost of treatment. The use of above mentioned treatment could be an effective method of treating dye wastewater which is suitable for application in field as irrigation water.

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References

APHA.: Standard methods for the examination of water and wastewater. 21st Edn. Washington, D.C. (2005).

Banat, I.M., P. Nigam, D. Singh and R. Marchant: Microbial decolorization of textile-dye-containing effluents: A review. *Biores. Technol.*, **58**, 217-227 (1996).

Bumpus, J.A. and B.J. Brock: Biodegradation of crystal violet by the white rot fungus, *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **54**, 1143-1150 (1988).

Chao, W.L. and S.L. Lee: Decolourization of azo dyes by three white-rot fungi: Influence of carbon source. *World J. Microbiol. Biotechnol.,* **10**, 556-559 (1994).

Churchley, J.H.: Removal of dye waste colour from sewage effluent- the use of a full scale ozone plant. *Water Sci. Tech.,* **30**, 275-284 (1994).

Combes, R.D. and R.B. Haveland-Smith: A review of the genotoxicity of food, drug, and cosmetic colours, and other azo, triphenylmethane, and xanthene dyes. *Mut. Res.,* **98**, 101-248 (1982).

Cripps, C., J.A. Bumpus and S.D. Aust: Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.,* **56**, 1114-1118 (1990).

Darah, I. and C.O. Ibrahim: Decolourization of methylene blue by culture of *Phanerochaete chrysosporium*. *Biosci.,* **8**, 94-100 (1997).

Demir, Goksel, H. Kurtulus Ozcan, Nese Tufekci and Mehmet Borat: Decolorization of Remazol Yellow RR Gran by white rot fungus *Phanerochaete chrysosporium*. *J. Environ. Biol.,* **28,** 741-745 (2007).

Dott, W., D. Feidieker, M. Steiof, P.M. Becker and P. Koampfer: Comparison of *Ex situ* and *In situ* techniques for bioremediation of hydrocarbon polluted soils. *Int. Biodeter. Biodegr.,* **35**, 301-316 (1995).

El-Naggar, M.A., S.A. El-Aasar and K.I. Barakat: Bioremediation of crystal violet using air bubble bioreactor packed with *Pseudomonas aeruginosa*. *Water Res.,* **38**, 4313-4322 (2004).

Fessard, V., T. Godard, S. Huet, A. Mourot and J.M. Poul: Mutagenicity of malachite green and leucomalachite green *in vitro* tests. *J. Appl. Toxicol.,* **19**, 421-430 (1999).

Hossain, S.K.M., M. Das and S.H. Ibrahim: Aerobic studies on pollution-abatrment of sulfite pulp bleaching effluent using *Phanerochaete chrysosporium* (MTCC-787). *J. Ind. Pollut. Cont.,* **17**, 191-200 (2001).

Jain, N., A. Kaur, D. Singh and S. Dahiya: Degradation of acrylic Red 2 B dye by *Phanerochaete chrysosporium*: Involvement of carbon and nitrogen source. *J. Environ. Biol.,* **21**, 179-183 (2000).

Kean, B.H. and D.W. Haskins: Drugs for intestinal parasitism *In*: Drugs of choice (*Ed.*:W. Modell). C. V. Mosby Co., St. Louis. pp. 371-381 (1978).

Marmion, D.M.: Handbook of U. S. Colorants. 3rd Edn., John Wiley and Sons, Inc. New York. p. 573 (1991).

Moore, W.A., R.C. Kroner and C.C. Ruchhoft: Dichromate reflux method for determination of chemical oxygen demand. *Annl. Chem.,* **21**, 953 (1949).

Nelson, C. R. and R.A. Hites: Aromatic amines in and near the buffalo river. *Environ. Sci. Technol.,* **14**, 1147-1149 (1980).

Papinutti, V.L. and F. Forchiassin: Modification of malachite green by *Fomes sclerodermeus* and reduction of toxicity to *Phanerochaete chrysosporium*. *FEMS Microbiol. Lett.,* **231**, 205-209 (2004).

Pazarlioglu, N.K., R.O. Urek and F. Ergun: Biodecolourization of direct blue 15 by immobilized *Phanerochaete chrysosporium*. *Process Biochem.* **40**, 1923-1929 (2005).

Robinson, T., B. Chandran and P. Nigam: Studies on the production of enzymes by white-rot fungi for the decolourization of textile dyes. *Enzyme Microbiol. Technol.,* **29**, 575-579 (2001).

Schnick, R.A.: The impetus to register new therapeutics for aquaculture. *Prog. Fish-Cult.,* **50**, 190-196 (1988).

Singh, K.D., S. Sharma, A. Dwivedi, P. Pandey, R.L. Thakur and V. Kumar: Microbial decolorization and bioremediation of melanoidin containing molasses spent wash**.** *J. Environ. Biol.,* **28**, 675-677 (2007).

Swamy, J. and J.A. Ramsay: The evaluation of white rot fungi in the decoloration of textile dyes. *Enzyme Microbiol. Technol.,* **24**, 130-137 (1999).

Tien, M. and T.K. Kirk: Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* burds. *Science,* **221**, 661-663 (1983).

Yeh, R.Y.L. and A. Thomas: Color difference measurement and color removal from dye wastewaters using different adsorbents. *J. Chem. Tech. Biotechnol.,* **63**, 55-59 (1995).

Yound, L. and J. Yu: Ligninase catalysed decolarization of synthetic dyes. *Water Res.,* **31**, 1187-1193 (1997).

Zhang, F.M., J.S. Knapp and K.N. Tapley: Decolourization of cotton bleaching effluent with wood rotting fungus. *Water Res.,* **33**, 919-928 (1999).