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BIOREMEDIATION OF WASTEWATER CONTAINING REACTIVE DYES BY *Agaricus bisporus* A21: EFFECT OF SUPPLEMENTS AND REDOX MEDIATORS

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This study involves the development of bioremediation process for simulated solutions of reactive dyes by *Agaricus bisporus* A21. Initially screening trial with four Reactive dyes, viz. NOVACRON Reactive black, NOVACRON Reactive blue, NOVACRON Reactive red and NOVACRON Reactive G. Yellow was carried out using *Agaricus bisporus* A21. Among four dyes tested the fungus showed better decoloration efficiency (67.7 %) with NOVACRON Reactive black as compared to other dyes during a 10^{th} day period. The effect of various operational parameters like pH, incubation temperature, carbon & nitrogen additives, surfactant as well as dye concentration was optimized for enhanced decolorization of the dye under study. The effect of various redox mediators like ABTS, veratryl alcohol, $MnSO_4$, guaiacol, glyoxlate on enzyme activity and removal of color was also investigated. After optimization of process conditions the rate of removal was enhanced up to 84 % just within 5 h .Enzymes involved in the degradation of the dye have been investigated and it was found that MnP (820 IU/mL) was the major enzyme responsible for the dye degradation. It was shown that there was a direct correlation between the observed enzyme activity and the investigated process effectiveness. The results indicated that *Agaricus bisporus* A21 could be used to treat dyes containing wastewater from various industries.
Keywords: *Agaricus bisporus* A21, manganese peroxidase, decolorization, NOVACRON reactive black, redox mediators

INTRODUCTION

Reactive dyes are extensively used in the textile industry due to wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimum energy consumption (Lee and Pavlostathis, 2004; Asgher and Bhatti, 2012). Reactive dyes are typically azo-based chromophore combined with different types of reactive groups such as vinyl sulfone, chlorotriazine, trichloropyrimidine and difluorochloropyrimidine (Asgher and Bhatti, 2010). Due to the poor fixation rates on the fabrics these dyes are discharged into aqueous streams with other pollutants. Direct discharge of such wastewaters containing reactive dyes in the ecosystem is a dramatic source of esthetic pollution, eutrophication and perturbations in the aquatic life (Lachheb *et al.*, 2002; Allege *et al.*, 2006). Moreover, it causes ulceration of skin and mucous membrane, dermatitis, perforation of nasal septum and severer irritation of respiratory track (Deshmukh *et al.*, 2008).The treatment of dyes containing wastewater is usually carried out by various physico-chemical methods such as adsorption, coagulation-flocculation, oxidation, reduction, filtration and electrochemical methods (Lins and Pengs, 1996; Karcher *et al.*, 2002). These methods are quite expensive and have operational problems. In recent years, biological decolourization methods have been considered as an alternative and eco-friendly methods to dye degradation and colour removal (Zhang *et al.*, 2007; Asgher *et al.*, 2008; Asgher *et al.*, 2009; Asgher *et al.*, 2010; Bibi *et al.*, 2009; Bibi *et al.*, 2010; Bibi *et al.*, 2011).

White-rot fungi (WRF) are wood decomposing basidiomycetes capable of degrading several toxic organic pollutants using a complex extracellular lignin-modifying enzymes system (Asgher *et al.*, 2008). The main ligninolytic enzymes are oxidoreductases which consist of three types of peroxidase, namely lignin peroxidases (LiP) manganese peroxidases (MnP) and phenoloxidase or laccase (Miyazaki *et al*., 2005). White rot fungi have a high tolerance to toxic and stressful environments and have been indicated as potent bioremediator for use in the bioremediation of dye polluted environments. There is thus a need to screen white rot fungal strains which can grow in simple, inexpensive media and which have a high decolorization rate as well as production of ligninolytic enzymes.

The aim of this study is to observe the bioremediation potential of least studied fungus *Agaricus bisporus* A21 for decolorization of some reactive textile dye. This study may form the basis of using *Agaricus bisporus* A21 for

development of effective decolorization process for textile industry real effluents. The effects of various process parameters and medium supplements on the decolorization of reactive dyes have been reported. The results would help to select the right medium for an effective decolorization by this novel *Agaricus bisporus* A21.

MATERIALS AND METHODS

Dyestuffs and chemical: All the reagents and chemicals used in this study were of analytical grade and mainly purchased from Sigma-Aldrich Chemical Co, U.S.A. The reactive textile dyes NOVACRON Reactive Black, NOVACRON Reactive Blue, NOVACRON Reactive Red and NOVACRON Golden Yellow were very generously provided free of cost by Huntsman private Ltd. Faisalabad Pakistan for research purpose.

***Microorganism and maintenance of culture*:** Pure culture of *Agaricus bisporus* A21 was obtained from Institute of Horticultural Sciences, University of Agriculture Faisalabad Pakistan. The culture was grown on potato dextrose agar (PDA) slants at 30°C for 5-7 days and preserved at 4 °C in a refrigerator (Bibi *et al*., 2010) and served as stock culture. Subcultures were routinely made after every 10 to 15 days. The inoculum was prepared by adding fungal spores to the sterilized inoculum medium (pH 4.5) having composition of modified Kirk's medium (Asgher *et al*., 2010). The inoculum flasks were incubated (30 °C) in an orbital shaker (Gallen-Kemp, UK) at 120 rpm to get homogenous spore suspension ($1x10^8$ conidia ml^{-1}).

***Dye decolorization protocol*:** Experiments were performed using triplicate flasks (250 ml) in a temperature controlled incubator/shaker (Gallen-Kemp, Loughborough, UK). Each flask contained 100 ml of 100 ppm of respective reactive dye solution prepared in Krik's basal medium (pH 4.5). The flasks were sterilized for 15 min at 121°C, allowed to cool and then inoculated with 2 ml homogenous conidia of *Agaricus bisporus* A21. The inoculated flasks were incubated for 10 days at 30˚C in an orbital shaker at 120 rpm. The control contained only dye solution and nutrients but did not receive inoculum. The supernatants were used to determine the extent of dye decolorization and enzyme activities.

***Media composition for screening*:** Four different media were used to select the most appropriate medium for decolorization study of the dye. Dyestuff (0.01 %) was added to each medium and pH was adjusted to 4.5.

Medium I: It was Kirk's basal salt medium having the composition (g L^{-1}): ammonium tartarate, 0.22; KH_2PO_4, 0.21: $MgSO_4 \cdot 7H_2O$ 0.05; $CaCl_2.2H_2O$, 0.01, Thiamine, 0.001, Tween-80 solution (10%) solution, 10 ml L^{-1}; veratryl alcohol (100 mM), 10 ml L^{-1} and trace elements solution, 10 ml L^{-1}.

Medium II: It has the same composition as that of medium I except veratryl alcohol and Tween-80.

Medium III: This medium has the composition (g L^{-1}): glucose, 20; urea, 0.03; KH_2PO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.1; $CaCl_2.2H_2O$, 0.01

Medium IV: This medium has the composition (g L^{-1}): glucose, 20; urea, 0.04; KH_2PO_4, 0.2; K_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2.2H_2O$, 0.05.

Dyestuff analysis: The extent of dyestuff decolorization was determined by measuring the absorbance of each dye by UV/Visible spectrophotometer (Hitachi U2001, Tokyo, Japan). Decolorization activity in terms of percent decolorization was determined by taking out sample from the flasks. Samples (2 ml) of each dye were removed after every 24 h and centrifuged at 10,000 rpm for 15 min. The supernatants were used to determine the extent of dye decolorization and enzyme assay. The decrease in optical density was monitored at 607 nm for NOVACRON Reactive Black, 630 nm for NOVACRON Reactive Blue, 520 nm for NOVACRON Reactive Red and 415 nm for NOVACRON Reactive G. Yellow. Decolourisation efficiency was determined as decolourised dye concentration and calculated according to the following formulation (Eq. 1):

$$\text{Percentage of decolourisation} = \frac{Ab - Aa}{Ab} \times 100 \qquad (1)$$

Ab is the absorbance at the maximum absorption wavelength of dye before decolourisation and Aa the absorbance at the maximum absorption wavelength of dye after decolourisation. Control contained only dyes. The most decolorized dye by the fungus was selected for the optimization of decolorization processes (Bibi *et al.*, 2011).

***Ligninase assays*:** The ligninolytic enzymes profile in the culture supernatants was determined by standard methods. Manganese peroxidase (MnP) activity was determined by monitoring the oxidation of 1mM $MnSO_4$ in 50 mM sodium melonate buffer (pH 4.5) in the presence of 0.1 mM hydrogen peroxide (Wariishi *et al.*, 1992), manganic ions Mn^{3+} form a complex with malonate that absorbs at 270nm. (ϵ_{270} =11590 $M^{-1}cm^{-1}$). One unit of MnP activity was defined as the amount of enzyme required to produce a unit increase in absorbance per minute per ml of the reaction mixture. Lignin peroxidase (LiP) was assayed by determining the oxidation rate of veratryl alcohol to veratraldehyde in the presence of 10 mM sodium tartarate buffer (pH 3.0) and H_2O_2 (Tien and Kirk, 1988). The oxidation rate of veratryl alcohol to veratraldehyde was determined at 310 nm in sodium acetate buffer (pH 3) in the presence of H_2O_2. (ϵ_{310} = 9300). One unit of LiP activity was defined as the amount of enzyme required to catalyze the formation of 1µmol of veratraldehyde per min under the reaction conditions. Laccase activity was determined following the oxidation of 2, 2́-azinobis (3-ethyl-benzo-thiazoline-6-sulphonic acid (ABTS) at 420 nm (Shin and Lee, 2000) (ε_{420}, 36000 M^{-1} cm^{-1}). One unit of laccase

activity was defined as the amount of enzyme required to produce one unit increase in absorbance per min per ml of reaction mixture.

***Dye decolorization optimization*:** From screening trials, it was noted that from all the reactive dyes, the NOVACRON Reactive black was maximally decolorized dye by *Agaricus bisporus* A21 and therefore decolorization of NOVACRON Reactive black was optimized to find out most suitable culture conditions like pH (4-8), temperature (25-45 ˚C), carbon source (glucose, molasses, maltose, fructose, sucrose), nitrogen source (ammonium sulphate, ammonium nitrate, urea, ammonium tartarate and peptone). The effects of surfactant, redox mediators (veratryl alcohol, $MnSO_4$, ABTS, guaiacol, glyoxlate) as well as dye concentration (100-200 ppm) in a series of experiments were also investigated. The classical approach was adopted for the optimization of different conditions by varying one parameter at a time and maintaining the previously optimized parameters at constant level (Hafiz *et al*., 2008; Kanwal *et al.*, 2010) a shake flask without culture was served as control.

All experiments were conducted in triplicate flasks and analyses were carried out on triplicate samples. The data values have been presented as means of three replicates with standard error (mean ± SE) and SE values are shown as Y error bars in figures.

RESULTS AND DISCUSSION

***Effect of incubation time on decolorization of reactive dyes by Agaricus bisporus A21*:** Decolorization of textile dye effluents is serious environmental problem, which is evident from the magnitude of research done in this field during the last decade. Treatment of textile dye effluent by physical and chemical methods have a high cost potential and a high sludge problem, whereas biological process convert organic compounds completely into water and carbon dioxide with simple and low cost technology (Ramsay et al., 2004; Wang *et al.*, 2008; Asgher *et al.*, 2009; Bibi *et al.*, 2011). In the present study microbial decolourization of some commonly used reactive textile dyes was carried out with *Agaricus bisporus* A21. Initial screening trials for the decolorization of different reactive dyes viz. NOVACRON Reactive black, NOVACRON Reactive blue, NOVACRON Reactive red and NOVACRON Reactive G.Yellow by *Agaricus bisporus* A21 were carried out for 10 days using Kirk basal medium. Results of preliminary investigation of dyes decolorization showed that decolorization of NOVACRON Reactive black was very sluggish in the start and it increased gradually up to 67.7 % at the end of 10^{th} days. However after 10^{th} day of incubation the rate of decolorization was slightly decreased (data not shown), while minimum decolorization was observed for NOVACRON reactive red (Fig.1). The decolorization rates of dyes show that color removal increased with an increase in the incubation period.

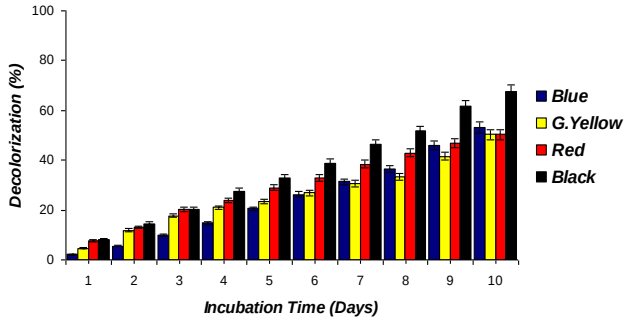

Figure 1. Effect of incubation time on the decolorization of different reactive dyes by *Agaricus bisporus* A21

The lignin modifying enzymes (LMEs) profile of *Agaricus bisporus* A21 was also studied during the dyes decolorization process and the results are shown in Fig. 2. The results reveal that maximum activity (441 IU mL^{-1}) of manganese peroxide (MnP) was observed in flask containing NOVACRON Reactive black with minimum production of laccase and lignin peroxidase (LiP) after 10^{th} days of incubation.

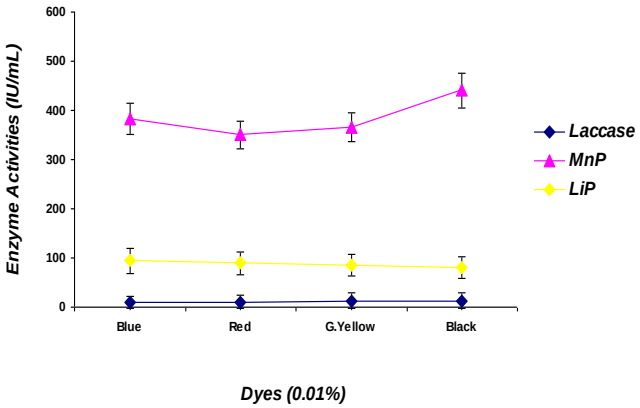

Figure 2. Production of LMEs during the decolorization of reactive dyes by *Agaricus bisporus* A21

White rot fungi (WRF) have been explored for their biodecolorization ability through the ligninolytic enzymes. The variation in decolorization trend of dyes by *Agaricus bitorqus* A21 might be due to variation in structure of the dyes as the decolorization ability of white rot fungi also depend on structures of dyes (Wesenberg *et al.*, 2003; Santos *et al.*, 2006; Asgher *et al.*, 2008).The degree of dye decolorization is mainly due the breakdown of aromatic ring of dye which relies on the substituents of ring including amino, 2 methoxyphenol, acetamide, phenol as well as other

easily degradable functional groups (Chander *et al.*, 2004). The initial pH of medium was adjusted at 5.0 in all flasks that remained fairly constant except a small variation in case of decolorization of Reactive blue dye; in this case the final pH was 5.3 (data not shown) that may be attributed to the production of basic metabolites by the fungus during growth on the said dye. On the basis of the results of screening trial, NOVACRON Reactive black was selected for further process optimization to acquire enhanced decolorization by *Agaricus bisporus* A21.

***Process optimization for enhanced dye decolorization*:** Culture conditions affect the fungal physiology, expression and activity of ligninolytic enzymes which ultimately affect the rate of decolorization. Process optimization was carried out for enhanced decolourization of the NOVACRON Reactive black. In order to improve ligninolytic enzymes production and to keep the culture in the productive state for prolonged time, we developed a process in relation to various other physiological parameters like media composition, pH, temperature, carbon sources, nitrogen sources, redox mediators and initial dye concentration.

***Effect of media composition on the efficiency of color removal*:** Effect of media composition on decolorization of NOVACRON Reactive black as well as the production of ligninolytic enzymes by *Agaricus bisporus* A21 was investigated by varying the contents of Kirk's basal medium (M-I) and the results are shown in Fig. 3. The results revealed that maximum decolorization (69.12 %) of Reactive black with MnP activity (449.86 IU mL^{-1}) was observed with medium M-1 while minimum decolorization and enzyme activity was recorded with M-IV. The major enzyme secreted by *Agaricus bisporus* A21 during the decolorization of Reactive black was MnP with minor activities of LiP and laccase. Thus MnP played the key role during dye decolorization.

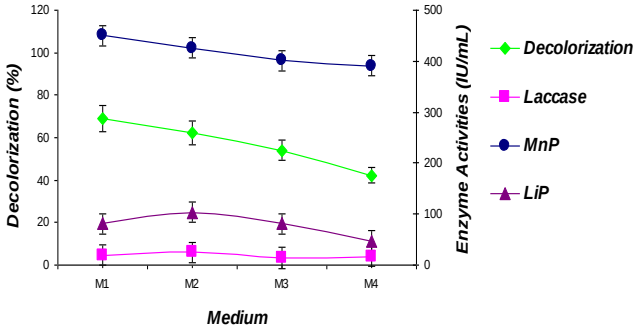

Figure 3. Effect of medium composition on the production of (LMEs) and decolorization of NOVACRON Reactive black by *Agaricus bisporus* A21

Agaricus bisporus A21 could grow well on simple Kirk's medium without any additional nutrients. These finding suggested that less color removal in other media might be due to deficiency or excess of some essential elements in rest of the media. Baldrain *et al.* (2005) reported that the additional supplements added to the medium could provide energy to micro-organisms and enhance the production of enzymes which ultimately increase the dye decolorization. Growth pattern of WRF, enzyme production and biodegradation of different dyes varies with composition of culture medium (Kapdan *et al.*, 2000; Hafiz *et al.*, 2008; Kanwal *et al.*, 2010). Medium M-1 was used for the decolorization of Reactive black dye by *Agaricus bisporus A21* in the subsequent experiments.

***Effect of medium pH on the efficiency of color removal*:** It is an established fact that the medium pH is an important parameter influencing the performance of the color removal process. To examine the effect of pH, the dye solution was adjusted to the desired pH for each experiment by adding few drops of 0.1 N sodium hydroxide or succinic acid solution. The effect of medium pH on the color removal of Reactive black dye is shown in Fig. 4. The results revealed that dye decolorization increased with increase in initial pH of the dye solution and reached maximum (71.46%) at pH 4.5. Similarly higher activity of MnP was observed at this pH. Further increase in pH caused a decrease in decolorization efficiency of the fungus as well as MnP synthesis.

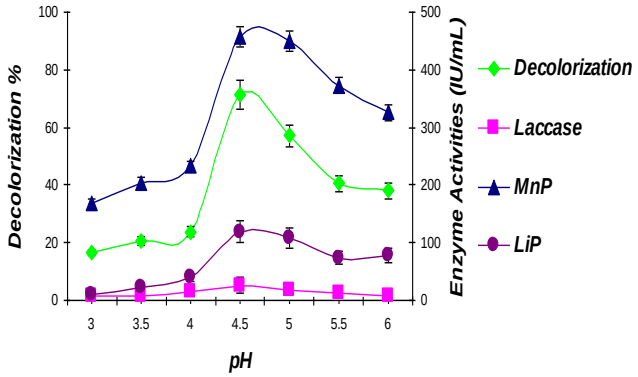

Figure 4. Effect of pH on the production of (LME) and decolorization of NOVACRON Reactive black dye by *Agaricus bisporus* A21

The initial pH of the medium significantly influences the ligninolytic enzyme synthesis/activities and dye colorization capacity of WRF and it is therefore, necessary to adjust the pH at optimum level (Zhang *et al.*, 2007). The pH of the medium plays a key role for the appropriate functioning of all the enzymes, acidic pH is favorable for growth and the ligninolytic enzymes formation of *Agaricus* species (Swamay and Rasmay, 1998).The optimum pH varied from 4-6 for decolorization of various textile dyes by different WRF depending upon the structure of dyes as well as the

medium composition (Kapdan *et al.*, 2000). Maximum color removal of various dyes including Red 114, methyl orange and acid orange was observed in the range of pH 4-5 (Bhatti *et al.*, 2008). Color removal was higher at lower pH and it drastically decreased as the pH was raised to 6.0. No dye decolorization or enzyme activity was observed in the alkaline pH range (Nigam *et al.*, 2000).

***Effect of incubation temperature*:** The effect of incubation temperature on the decolorization of NOVACRON Reactive black by *Agaricus bisporus* A21 was studied by incubating the flasks containing dye solution (100 ml of 0.01 % in Kirk basal medium with 2 ml inoculum size) at different temperatures (20-45 ˚C) and the results are shown in Fig. 5.

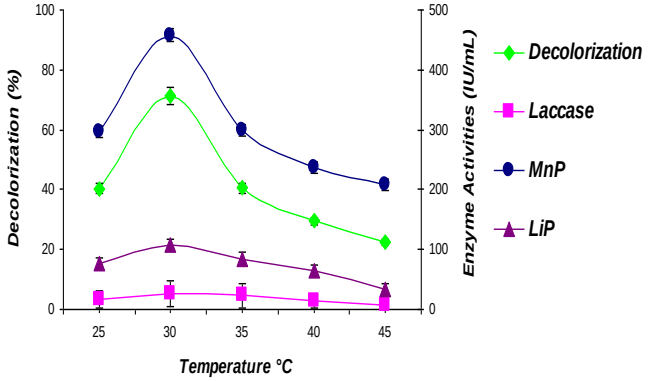

Figure 5. Effect of temperature on the production of (LMEs) and decolorization of NOVACRON Reactive black dye by *Agaricus bisporus* A21

It is obvious from the figure that percentage removal of dye increased with an increase in temperature from 25 to 30 ˚C. Maximum decolorization (71.47 %) was achieved at 30°C. The percentage removal of dye decreased with further increase in temperature up to 45˚C. Similarly maximum activity MnP was recorded at 30 °C. The decolorization efficiency as well as MnP activity of the fungus decreased above 30 °C. The enhanced decolorization of the dye can be correlated to high MnP activity at 30 °C. Decolorization activity of the fungus was significantly suppressed at 45˚C (22.1%), which might be due to the loss of cell viability or deactivation of the enzymes responsible for discoloration at 45 ˚C. Temperature is an important parameter which drastically affects fungal growth, enzyme activities and rate of color removal. Maximum decolourization (83.78±5 %) was observed for Solar Golden Yellow R at temperature 30 °C after 6th day (Bibi *et al.*, 2009). Many WRF fungi have been reported to have 30 °C as the optimum growth temperature (Asgher *et al.*, 2009). Optimum temperature was found to vary between 25-37 °C among various isolates of WRF.

***Effect of carbon additives*:** Various carbon sources, viz. glucose, fructose, maltose, molasses and sucrose (at 1 %) were used as co-substrates to investigate their effects on dye decolorization by *Agaricus bisporus* A21 and the results are shown in Fig. 6. Decolorization of NOVACRON Reactive black dye was found to be dependent on the presence of additional carbon sources. Results clearly show that maximum removal of dye (74.14%) was achieved after 24 h using glucose as a carbon source, with MnP activity of 478 IU mL^{-1}.

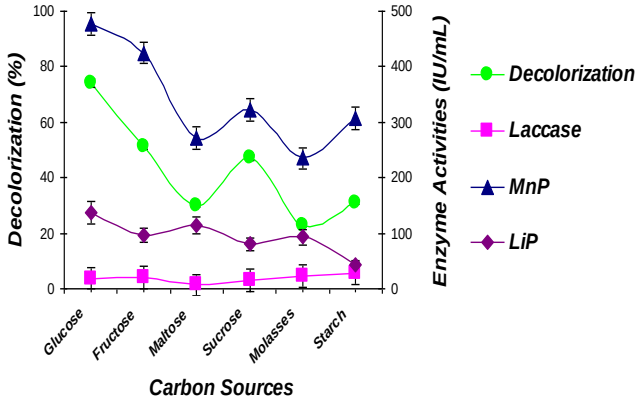

Figure 6. Effect of different carbon additives on production of (LMEs) and decolorization of NOVACRON Reactive black dye by *Agaricus bisporus* A21

This clearly indicated that *Agaricus bisporus* A21 effectively utilized glucose as carbon source. Rapidly metabolizable carbon sources affect the extent of color removal as well as the secretion of extracellular fungal enzymes. Nosheen *et al.* (2010) used glucose and starch as carbon sources for optimizing the maximum decolorization of Reactive Black B and Reactive Orange 16. The results clearly demonstrated that in a medium amended with glucose, the rate of decolorization was more profound than as in a medium supplemented with other tested carbon supplements. Similar results are reported by Hadibarata *et al.* (2011) who revealed that degradation of Reactive Black 5 (RB5) by *Pleurotus* sp. F019 and *Trametes* sp. F054 was extremely correlated with the presence of glucose in culture media. Very low color removal was observed when the medium was amended with molasses. As molasses contain much amount of mineral as well as sugar and many other components which inhibit the growth of fungi. Generally the addition of carbon supplements to basal media have been reported to increase the fungal growth and enzyme biosynthesis of white rot fungi to achieve enhanced decolorization of different dyes (Chander and Arora, 2004; Asgher *et al.*, 2008; Bibi *et al.*, 2010).

***Effect of nitrogen additives on the rate of color removal*:** In order to see the effect of nitrogen additives on the color removal of the dye by *Agaricus bisporus* A21 the growth

medium was supplemented with various nitrogen supplements (1 %) and the results are shown in Fig.7. The data indicated that additional nitrogen sources have slightly stimulatory effect on color removal and maximum decolorization of Reactive black (75.88 %) was observed with ammonium sulphate after 24 h of incubation followed by ammonium tartarate. Maximum MnP activity (506.72 IU/mL) was also observed with ammonium sulphate.

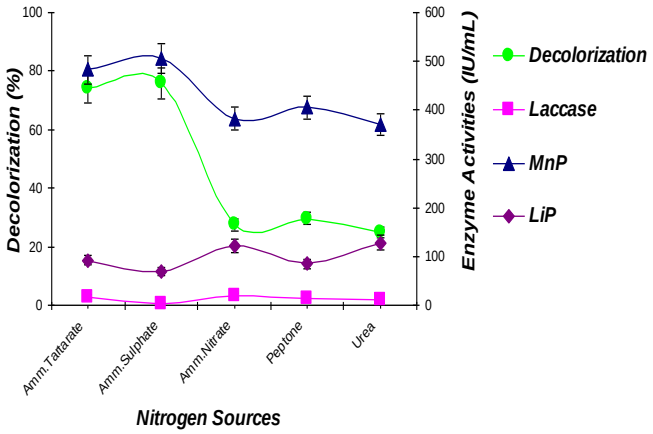

Figure 7. Effect of different nitrogen additives on production of (LMEs) and decolourization of NOVACRON Direct black by *Agaricus bisporus* A21

Different strains of white rot fungi show different behavior when the growth medium was amended with same nitrogen additives. Extent of color removal was slightly enhanced by the addition of nitrogen additive to the growth medium of *P. tremellosa* (Robbinson *et al.*, 2003). Ligninolytic peroxidase production in Bjerkandera sp. Strain BOS55 was greatly stimulated by high nitrogen, depending upon the combination of initial pH and type of nitrogen source (Kaal *et al.*, 1993). High nitrogen in the form of asparagines and phenylalanine stimulated MnP production in wild type *Dichomitus squlenes* (Peric and Gold, 1991). Our findings are in line with the earlier reported results in which higher growth and ligninolytic activities have been reported in nitrogen rich media for decolorization of different types of dyestuff by WRF (D'Souza *et al.*, 1999; Rigas and Dritsa; 2006; Bibi *et al.*, 2010).

***Effect of surfactant on the rate of color removal*:** The effect of surfactant on the decolorization of NOVACRON Direct black by *Agaricus bisporus* A21 was investigated by amending the growth media with different levels of Tween-80 (1-5 mL). Enhanced color removal of Reactive black (78.31%) was observed in medium containing 3 mL of surfactant (Fig. 8). This is directly correlated with high MnP activity (545.73 IU mL^{-1}). Decrease in dye decolorization and MnP biosynthesis was observed below and above the optimum level.

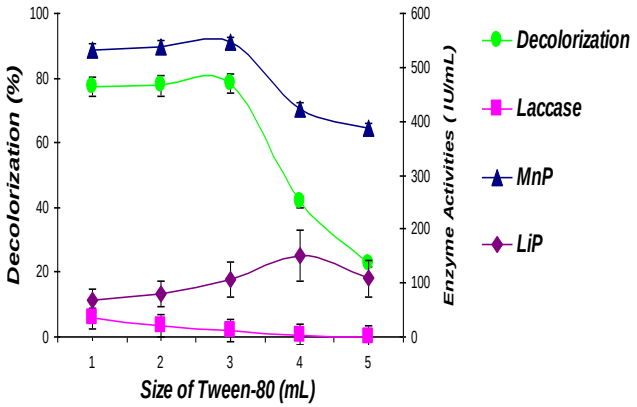

Figure 8. Effect of different size of surfactant on production of (LMEs) and decolorization of NOVACRON Reactive black dye by *Agaricus bisporus* A21

Tween-80 plays a key role in enhancing the extent of color removal by increasing the surface area of the substrate (Asgher *et al.*, 2008).Tween 80 transformed the cell membrane structure and promoted the permeation of MnP from the cell into the medium. However, the mechanism by which surfactants (i.e.,Tween 80) enhance extracellular enzyme production in filamentous fungi has not been established.

***Effect of different redox mediators on the rate of color removal*:** Redox mediators play very important role in the removal of color as the presence of mediators increase the reaction rate to many folds as compare to fungus or enzyme alone. Various redox mediators viz. ABTS, $MnSO_4$, veratryl alcohol, glyoxlate and guaiacol (1 mL of 1 mM solution) were added to optimum growth medium to observe their effect on dye decolorization and MnP biosynthesis. The results reveal that $MnSO_4$ stimulated the maximum decolorization (83.34 %) and MnP activity (820.04 IU mL^{-1}) within 5 h (Fig. 9). The results indicated that the production of enzymes as well as the extent of color removal dramatically effected in the presence of these redox mediators.

Sundramoorthy *et al.* (2005) reported that the presence of manganous ions (Mn^{2+}) in the medium has the stimulating effect on the production of MnP. MnP catalyzes the peroxide dependent oxidation of Mn^{2+} to Mn^{3+} that makes complex with oxalate or with other chelators that enhance the activity of MnP. Similarly Urek *et al.* (2005) studied the effect of adding various concentrations of Mn^{2+} to SSF cultures of *P. chrysosporium*. MnP activity increased steadily with increasing Mn^{2+} concentration. The addition of $MnSO_4$ to the cultures notably improved MnP activities.

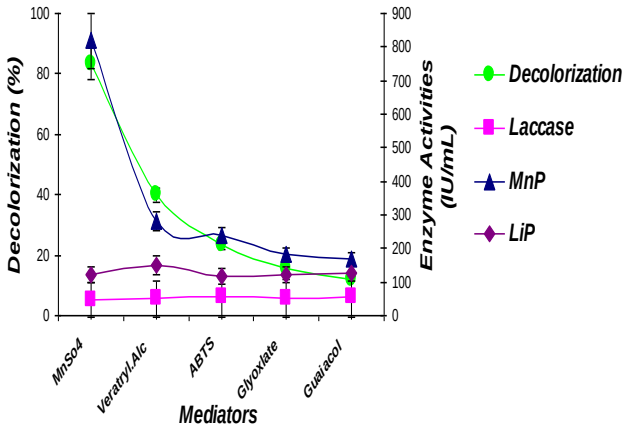

Figure 9. Effect of various redox mediators on production of (LMEs) and decolorization of NOVACRON Reactive black dye by *Agaricus bisporus* A21

***Effect of dye stuff concentration on the rate of color removal*:** The effect of initial dye concentration on the rate of dye decolorization was determined by varying the initial dye concentration from (0.01-0.03 %. The results regarding the effect of initial dye concentration on dye decolorization and MnP biosynthesis are shown in Fig. 10. According to the results, highest color removal (84 %) was observed in flask containg 0.01% dye solution in 5 h only. However, above this concentration, the decolorization efficiency of *Agaricus bisporus* A21 became exhausted, especially when it was more than 0.02 %. Therefore the increase of initial dye concentration caused a steady decrease in decolorization capability of *Agaricus bisporus* A21.

The ligninolytic profile of *Agaricus bisporus* A21 was also noted during dye decolorization. MnP was observed to be the major enzyme (820 IU mL^{-1}) involved in the decolorization of NOVACRON Reactive black followed by LiP with minor activity of laccase. Dyes being toxic compounds are well tolerated and best decolorized only at lower concentration (Hai *et al*., 2006). WRF tolerate and decolorize dyes to variable extent. Earlier studies on dye biodegradation showed that low concentrations (50-500 mg L^{-1}) of the dyes were well decolorized and tolerated by fungi (Kapdan *et al.*, 2000; Nilsson *et al.*, 2005; Regas and Drista, 2006; Hai *et al*., 2006; Bhatti *et al*., 2008).

In all above mentioned screening as well as optimization experiments, visually it was observed that the dyes initially adsorbed on fungal mycelium but were ultimately degraded by the WRF ligninases ending up with no net adsorption.

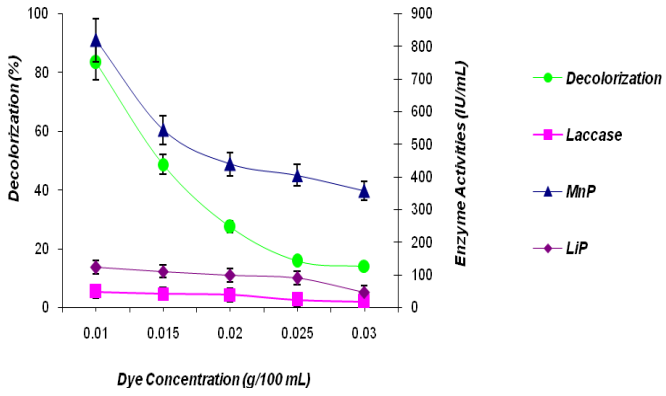

Figure 10. Effect of various concentration of NOVACRON Reactive black on production of (LMEs) and decolorization by *Agaricus bisporus* A21

Conclusions: In conclusion, a look at the present studies clearly reveals that *Agaricus bisporus* A21, is the best white rot fungi for the efficient removal of NOVACRON Reactive black and MnP production. The rate of color removal and enzyme production ability of this organism can be enhanced on supplementing the basal media with various operational parameters upto 84 % and 820.04 IU mL^{-1} respectively. Moreover, its enzyme systems seemed to be much responsive to the inductive effect of various supplements, of which $MnSO_4$, Tween-80, ammonium sulphate and glucose were excellent MnP inducers which ultimately enhanced the rate of color removal. The biological treatment for synthetic dyes removal is a very perspective, environmentally protective and low cost approach for solution of such problems. The studies discussed in this paper indicated that fungal decolourization has a great potential to be developed further as a decentralized wastewater treatment technology for small textile or dyeing units.

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