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Enhanced Decolourization of Solar Orange RSN by White-rot Fungus *Ganoderma lucidum* IBL-05

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This study deals with the process optimization of biological decolourization of dye solar orange RSN, a synthetic textile by *Ganoderma lucidum* IBL-05. In initial time course study of 10 d, a maximum decolourization (55 ± 3 %) of solar orange RSN was caused by *Ganoderma lucidum* IBL-05 after 7 d. The effect of media composition, initial pH of the solution, incubation temperature, carbon and nitrogen additives and initial dye concentration was investigated. Maximum dye decolourization (92 ± 4 %) was achieved in basal nutrient medium II containing 0.01 % solar orange RSN and supplemented with 1 % glucose and maize seed meal after 3 d incuba-tion at pH 4.5 and 30 ºC. The ligninolytic profile of *G. lucidum* IBL-05 was also investigated and manganese peroxidase was the dominant enzyme activity involved in biodegradation of solar orange RSN with minor activities of lignin peroxidase and laccase.

Key Words: White-rot fungi, *Ligninases*, Solar orange RSN, *Ganoderma lucidum*, Decolourization.

INTRODUCTION

Large amounts of synthetic dyes are used for various industrial applications such as textile dyeing, printing and significant proportion of these dyes enters the environment in wastewater[1]. The presence of very low concentrations of dyes in effluents can be highly visible and undesirable[2-4]. The dyestuffs disposed to the surface water can prevent its recreational and economic use and affect its aesthetic and sentimental value. As a consequence, sunlight transmittance is reduced, photosynthetic processes are negatively influenced and the water's self-purification potential is also affected. Dyes may also be problematic if they are broken down anaerobically in the sediments, as toxic amines are often produced due to incomplete degradation by bacteria. Some dyes are reported to cause allergy, dermatitis, skin irritation, cancer and mutations in humans. Hence removal of hazardous industrial effluents is one of the growing needs of the present time[5]. But removing dyes from

textile wastewater with conventional methods is a difficult and expensive process[6]. One approach is the use of enzyme-based methods, which have a minimal impact on ecosystems and low energy requirements[7]. The enzyme has different functions in the individual organism[8] and among others, it is thought to be involved in the synthesis of lignin by plants and the biodegradation of lignin by white-rot fungi[9]. Several combined anaerobic and aerobic microbial treatments have been suggested to enhance the degradation of textile dyes. Biological processes have received increasing interest as a viable alternative owing to their cost effectiveness, ability to produce less sludge and environmental friendliness[10,11].

In recent years, the utilization of biodegradative abilities of some white-rot fungi seems to be promising. They don't require preconditioning to particular pollutants and owing to their extracellular nonspecific free radical-based enzymatic system; they can degrade to a non-detectable level or even completely eliminate a variety of xenobiotics including synthetic dyes. White-rot fungi variously secrete one or more of three extra cellular enzymes that are essential for lignin degradation and which combine with other processes to effect lignin mineralization. They are often referred to as lignin-modifying enzymes.

Solar orange RSN is a direct dye, commonly used in textile industry of Pakistan for dyeing cellulose fibers and cellulosic component in fiber blends and viscous rayon. The main objective of this study was to investigate the biodegradation potential of *Ganoderma lucidem*-IBL-05 for solar orange RSN. This investigation may form the basis of using this white-rot fungus for the development of an effective decolourization process for textile industry effluents containing solar orange RSN and other structurally related dyes.

EXPERIMENTAL

The direct dye solar orange RSN was procured locally. All the chemicals used in this study were of analytical grade and mainly purchased from Merck (Darmstad, Germany) and Fluka (Sigma-Aldrich Chemie, Inc. Germany). Pure culture of white-rot fungus *Ganoderma lucidum* IBL-05 was obtained from the culture collection of Industrial Biotechnology Laboratory, Department of Chemistry, University of Agriculture, Faisalabad, Pakistan. The culture was grown on PDA (OXOIDE, Hampshire, UK) slants for 5-6 d and preserved at 4 ºC.

Basal nutrient media: The four basal nutrient media of different composition[12,13] were used to select the most suitable medium. Glucose (5 g/L) and dyestuff (0.01 %) were added into each medium. The pH of all media was adjusted to 4.5 by using 2,2-dimethyl succinic acid.

Medium I: It was Kirk's basal salts medium[14].

Medium II: It was of the same composition as that of the Kirk's basal salts medium I, excluding veratryl alcohol and Tween-80 solution.

Medium III: This medium had the following composition (g/L): Urea, 0.036; KH_2PO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.5 and $CaCl_2 \cdot 2H_2O$, 0.099. No Tween-80 and veratryl alcohol was added.

Medium IV: 0.036 g/L Urea, 0.09 g/L KH_2PO_4, 0.1 g/L K_2HPO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.05 g/L $CaCl_2 \cdot 2H_2O$, 0.1 mg/L thiamine and 10 mL/L trace elements solution[13].

Inoculum development: Aqueous suspension of conidia was prepared in 1 % sterile glucose solution from maintenance medium. The suspension was passed through sterile glass wool columns to remove hyphal fragments. The concentration of the conidial suspension[15] was adjusted to get 1×10^8 conidia/mL.

Initial time course study and decolourization experiments: Initial time course study for the decolourization of solar orange RSN by *Ganoderma lucidum* IBL-05 was carried out in 250 mL conical flasks containing 100 mL of sterilized Kirk's basal medium with 0.01 % of the direct dye. All the decolourization media were maintained at pH 4.5 (except the pH optimization experiment) with 1 M NaOH/1 N HCl. The entire test flasks were sterilized (121 ºC) in autoclave for 15 min and inoculated with 5 mL of the fungal conidial suspension and incubated for 10 d at 30 °C (120 rpm) unless otherwise stated in case of optimization studies. The samples were withdrawn from each flask after every 24 h and centrifuged at 10,000 rpm for 10 min to determine the percent decolourization.

Dyestuff analysis: The supernatants recovered after centrifugation of the fermented samples were subjected to dyestuff analyses using spectrophotometer[13]. Absorbance was measured by using a UV/Visible spectrophotometer (U-2001, Hitachi, Japan). Wavelength resulting in maximum absorbance (λ_{max} 425 nm) by 0.01 % solar orange RSN was used. The amount of decolourized dyestuff was calculated by subtracting the dyestuff concentration in the liquid phase from the initial value.

Optimization of process parameters: Optimization of various process parameters was done using the traditional step wise strategy of varying one parameter at a time by maintaining the previously optimized conditions[16]. Shake flasks were incubated for 7 d at different initial pH values for selection of best pH for dye decolourization. For investigating the effect of temperature, the shake flasks were incubated at different temperatures (30-45 ºC) at optimum pH for 7 d. In the next experiment different carbon sources (1 %) like glucose, fructose, sucrose, maltose, starch and molasses, were used in decolourization flasks to study their effect on dye decolourization under pre-optimized conditions. Subsequently, the decolourization medium was supplemented with different nitrogen sources such as peptone, yeast extract, urea and maize seed meal each at 1 % level.

Effect of varying dyestuff concentrations: In order to determine the maximum dyestuff concentration tolerated by *G. lucidem* IBL-05, the optimum decolourization medium was developed by varying the dyestuff concentration (0.01-0.1 %). Decolourization flasks containing varying concentrations of solar orange RSN, basal nutrient medium II and glucose (1 %) were processed for 3 d (final optimized incubation time) at optimum pH (4.5) and temperature (30 ºC).

Lignin peroxidase, manganese peroxidase and laccase activity: Lignin peroxidase (LiP) activity was determined by monitoring the oxidation of 4 mM veratryl alcohol to veratraldehyde in 100 mM sodium acetate buffer pH 3.0 at 310 nm in the presence of 0.2 mM of H_2O_2[17]. Laccase was assayed by following the oxidation of 2,2'-azino*bis*(3-ethylbenzothiazoline)-6-sulfonate (ABTS)[18] in sodium acetate buffer (50 mM) pH 4.0 at 420 nm. Manganese peroxidase (MnP) activity was determined[19] by the oxidation of 1 mM $MnSO_4$ in 50 mM sodium malonate buffer (pH 4.5) in the presence of 0.1 mM H_2O_2 at 270 nm.

RESULTS AND DISCUSSION

In the present study, biodegradation of direct dye solar orange RSN by white-rot fungus *G. lucidum* IBL-05 was investigated. The initial time course study for the biodegradation of the direct dye was carried out for 10 d using Kirk's basal medium. The results showed that there was negligible colour removal during initial days. *G. lucidum* IBL-05 reduced the colour from 6 to 16 % within first 3 d. Then % decolourization increased gradually and maximum decolourization (55 ± 3 %) was observed on 7th day (Fig. 1). A slight decline in percent decolourization was noted on 8th-10th day.

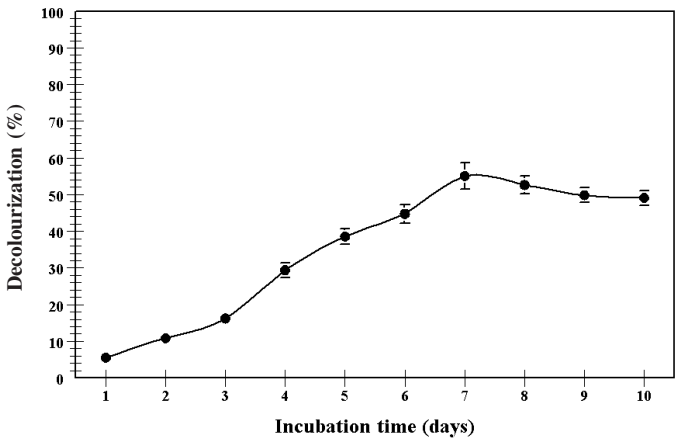

Fig. 1. Initial time course study of decolourization of solar orange RSN by *Ganoderma lucidum* IBL-05

This decrease in the dye decolourization is probably due to the formation of metabolites and this decreasing trend continued till the 10th day. White-rot fungi have different decolourization potential on chemically different substances and screening for ligninolytic fungi for dye decolourization must be conducted under conditions as similar as possible[20-22]. Maximum decolourization (73 %) of solar golden yellow R by *Schizophyllum commune* was observed on the 6th day of incubation[16]. The first sign of fungal mycelia growth was seen 24 h after inoculation and that medium was completely decolourized by *Lentinula edodes*[23] within 6 d.

Four different basal media were used for decolourization of solar orange RSN by *G. lucidum* IBL-05. The results regarding the effect of media are shown in Fig. 2. The modified Kirk's nutrient medium M-II showed better dye removal (58 ± 3 %) as compared to other media. Minimum decolourization (41 ± 2 %) was observed in medium M-IV after 7 d. Medium I contained the components of Kirk's basal medium. The medium II differed from medium I in composition to some extent. Medium II did not contain costly chemicals like veratryl alcohol and Tween-80. Media III and IV contained additional unique source of urea that created a difference in the composition of these media. However, the decolourization was low in medium III and 1V indicating that these media were deficient in some essential components for growth of *G. lucidum* IBL-05. It has also been reported that biodegradation of different dyes by different white-rot fungi is variable in different basal media[12].

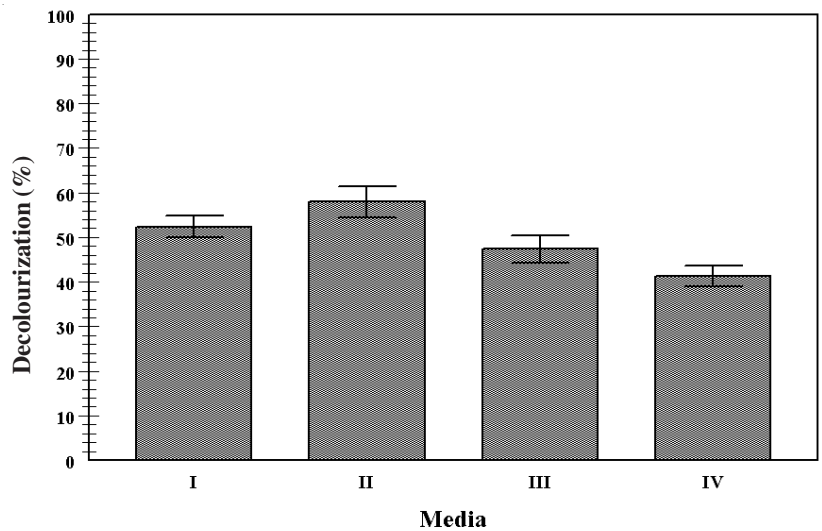

Fig. 2. Effect of media on decolourization of solar orange RSN by *Ganoderma lucidum* IBL-05

Results regarding the effect of varying pH on dye decolourization by *G. lucidum* IBL-05 are shown in Fig. 3. With an initial increase in pH the decolourization efficiency of the fungus gradually increased. Maximum colour removal (63 ± 3 %) was recorded at pH 4.5. A further increase in pH resulted in low dye decolourization. The pH value of the medium plays a significant role in decolourization of dyes by white-rot fungi. White-rot fungi have been reported to show maximum growth and dye decolourization in acidic pH range[12,16]. In a recent study[24], the growth and decolourization properties of *Schizophyllum commune* on acid orange 7, acid red 18 and reactive black 5 were studied in pH varying from 1 to 6 and optimum was found to be pH 2 for both growth and colour removal of these azo dyes. Maximum decolourization of solar golden yellow R by *Schizophyllum commune* was also noted[16] at pH 4.5. At higher pH values, reactive dye solutions were more negatively charged and dye removal efficiency of white-rot fungi is decreased[25].

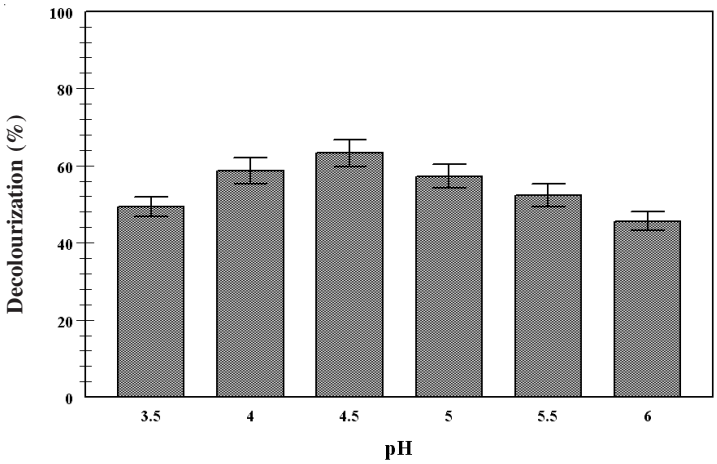

Fig. 3. Effect of pH on decolourization of solar orange RSN by *Ganoderma lucidum* IBL-05

The variation in the solar orange RSN decolourization by *G. lucidum* IBL-05 as a function of incubation temperature is shown in Fig. 4. The fungus exhibited better dye decolourization ability in the temperature range 30-35 ºC. Maximum decolourization (65 ± 4 %) was recorded in the flasks incubated at 30 ºC for 7 d under optimum conditions. A decreasing trend in dye decolourization was observed at higher temperature (40-50 ºC). White-rot fungi show better growth under medium temperature as compared to those at higher temperatures[26]. Temperature optima of 30-37 ºC have also previously been reported[13,16,23] for different white-rot fungi for decolourization of different dyestuffs.

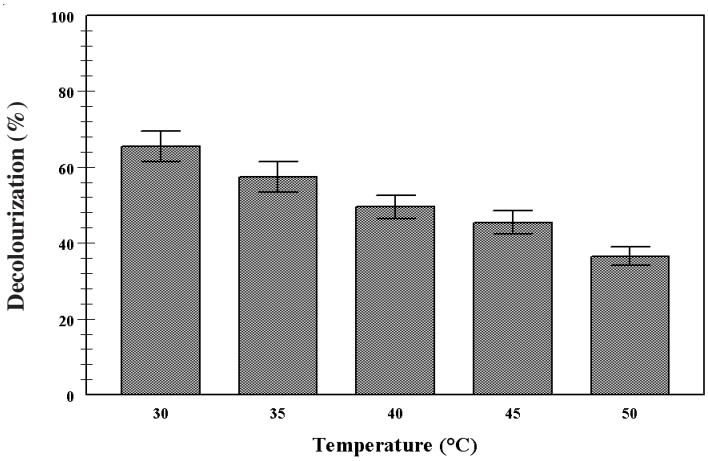

Fig. 4. Effect of temperature on decolourization of solar orange RSN by *Ganoderma lucidum* IBL-05

Different carbon sources were added to observe their stimulatory/ inhibitory effect on dye decolourization. Decolourization experiments were performed using glucose, maltose, sucrose, starch and molasses (1 %) as carbon additives and the flasks were incubated at 30 °C for 7 d. It was observed that all the carbon additives stimulated the dye decolourization (Fig. 5). However, the results were very interesting in case of glucose and maximum colour removal (81 ± 4 %) was observed on 3rd day in the medium supplemented with glucose followed by molasses (78 ± 3 %). While the medium supplemented with maltose decreased only 71 ± 3 % colour of the dye as compared to control. The fungus utilized glucose as readily consumable carbon source and it caused a significant shortening of lag phase. Addition of glucose to the dye decolourization medium provides easily metabolizable energy source to the fungus and creates an environment to enhance decolourization rate of dyes. Decolourization of Poly R 478 dye by 10 white-rot fungi was also reported to vary in response to different carbon regimes and fastest decolourization rates were achieved with monomers (glucose, xylose) as carbon source[27]. Stimulatory effect of glucose on decolourization of dyes by white-rot fungi have been reported by different researchers[12,16]. Everzol turquoise blue G was optimally (77 %) decolourized by *Coriolus versicolor* in presence of 0.5 % glucose supplement as compared to only 65 % colour removal with 0.2 % glucose[21].

Various nitrogen sources were also tested to study their effects on the decolourization of solar orange RSN by *G. lucidum* IBL-05 under optimum conditions. All nitrogen sources (urea, peptone, maize seed meal and yeast extract at 1 %) were found to have stimulatory effect on fungal growth and hence dye decolourization (Fig. 6). Maximum colour removal (83 ± 5 %) was

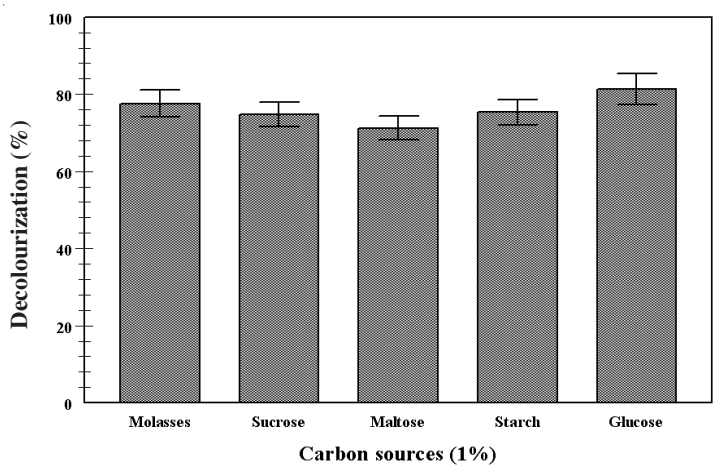

Fig. 5. Effect of carbon additives on decolourization of Solar Orange RSN by *Ganoderma lucidum* IBL-05

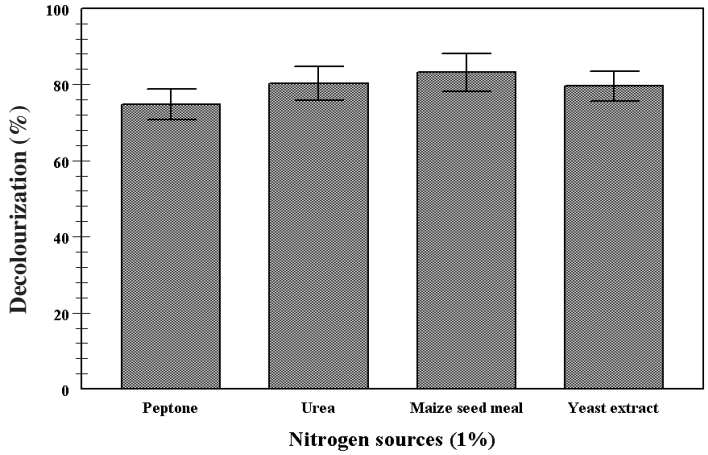

Fig. 6. Effect of nitrogen additives on decolourization of solar orange RSN by *Ganoderma lucidum* IBL-05

recorded with maize seed meal followed by urea (80 ± 4 %). Higher growth rate and ligninolytic activities of *Ganoderma lucidum* have been reported in nitrogen enriched media used for decolourization of different types of dyestuffs[28-30]. It has also been reported[31] that the highest dye removal efficiency by *Ganoderma australe* was in N-rich medium.

The effect of different concentrations (0.01-0.1 %) of solar orange RSN on the dye removal by *G. lucidum* IBL-05 was investigated under pre-optimized conditions. A maximum of 92 ± 4 % dye decolourization was observed with 0.01 % dye solution after 3 d. Higher concentrations were

not tolerated by the fungus and a significant lower ligninolytic enzyme formation and consequently decreased dye decolourization was noted in the culture flasks having concentrations higher than 0.01 %. Synthetic dyes are toxic chemicals and are well tolerated and best decolourized by the microbial systems at lower concentrations[32]. Dye concentrations in industrial effluents vary within a range of 0.01-0.02 per cent[33]. *G. lucidum* IBL-05 decolourized solar orange RSN higher than the reported dye concentrations in textile wastewaters.

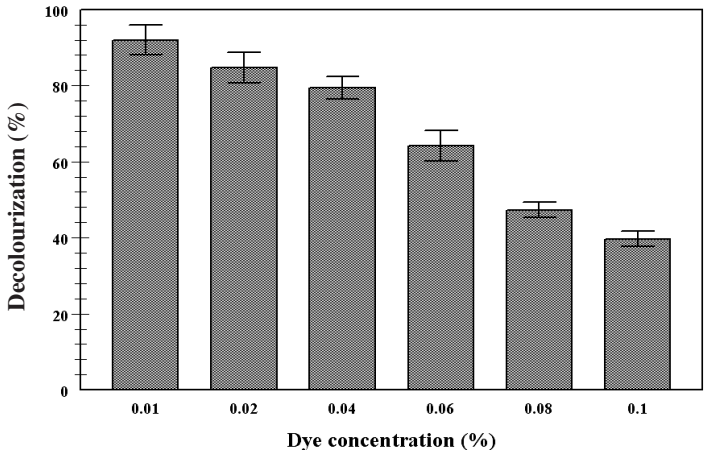

Fig. 7. Effect of dye concentration on decolourization of solar orange RSN by *Ganoderma lucidum* IBL-05

The ligninolytic enzyme profile was also investigated under optimized conditions. Manganese peroxidase (1156 ± 38 U/mL) was found to be the major enzyme involved in degradation of solar orange RSN by *G. lucidum* IBL-05. Lower activities of lignin peroxidase (345 ± 24 U/mL) and laccase (213 ± 18 U/mL) were also detected in the culture supernatant of optimal decolourized medium. Lignolytic enzymes profile showed that MnP was the major lignolytic activity involved in dye degradation with minor LiP and laccase activities. This is consistent with present initial finding on selection of basal nutrient medium. The selected medium II did not contain varatryl alcohol but it gave better decolourization results as compared to medium one containing varatryl alcohol. Varatryl alcohol is a fungal secondary metabolite that plays an important role in stimulating LiP oxidation of a wide range of recalcitrant substrates[34]. Ligninase profiles and pattern of their expression vary among different white-rot fungi cultures and this appears to be of most interest for practical applications for dye degradation[23,35,36]. MnP has also previously been reported as major enzyme involved in degradation of different dyes by white-rot fungi[21,37].

Conclusion

Ganoderma lucidum IBL-05 isolated from rotting wood samples, exhibited high ability to decolourize a synthetic dye, solar orange RSN commonly used in textile industry of Pakistan. The fungal growth was markedly influenced by this dye. However, its ability can be improved by further careful process optimization using some additives. The results can be translated in terms of developing an indigenous bioprocess for bioremediation of textile effluents.

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