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ORIGINAL ARTICLE

Enhanced decolorization of Solar brilliant red 80 textile dye by an indigenous white rot fungus *Schizophyllum commune* IBL-06

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KEYWORDS

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Ligninolytic enzymes

Abstract An indigenously isolated white rot fungus, *Schizophyllum commune* IBL-06 was used to decolorize Solar brilliant red 80 direct dye in Kirk's basal salts medium. In initial screening study, the maximum decolorization (84.8%) of Solar brilliant red 80 was achieved in 7 days shaking incubation period at pH 4.5 and 30 °C. Different physical and nutritional factors including pH, temperature and fungal inoculum density were statistically optimized through Completely Randomized Design (CRD), to enhance the efficiency of *S. commune* IBL-06 for maximum decolorization of Solar brilliant red 80 dye. The effects of inexpensive carbon and nitrogen sources were also investigated. Percent dye decolorization was determined by a reduction in optical density at the wavelength of maximum absorbance (λ_{max}, 590 nm). Under optimum conditions, the *S. commune* IBL-06 completely decolorized (100%) the Solar brilliant red 80 dye using maltose and ammonium sulfate as inexpensive carbon and nitrogen sources, respectively in 3 days. *S. commune* IBL-06 produced the three major ligninolytic enzymes lignin peroxidase (LiP), manganase peroxidase (MnP) and lacaase (Lac) during the decolorization of Solar brilliant red 80. LiP was the major enzyme (944 U/mL) secreted by *S. commune* IBL-06 along with comparatively lower activities of MnP and Laccase.

1. Introduction

Synthetic dyes are one among the major chemical pollutants that originate mainly from textile and plastic industries and pose serious health hazards to the entire ecosystem, especially the animals and human beings. These dyes are unusually resistant to degradation and decolorization by physical or chemical methods including adsorption, precipitation, chemical degradation or photo degradation. The physio-chemical remediation techniques also have financial and methodological disadvantages (Ali and El-Mohamedy, 2012; Asgher and

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Iqbal, 2013). There is a pressing need for development of eco-friendly biological treatment techniques for such dyes and textile dye containing effluents. A number of reports are available in literature on bioremediation of dyes by micro-organisms that have dye degrading capabilities (Chen, 2006; Asgher et al., 2008, 2009, 2012a,b,c; Oves et al., 2013). Biological processes provide an alternative to existing expensive and commercially or environmentally unattractive, physio-chemical technologies. The biological treatment is cost effective and eco-friendly, and can be applied to wide range of dyes or dye containing industrial effluents (Senthilkumar et al., in press; Iqbal and Asgher, 2013).

A wide spectrum of microorganisms including bacteria, filamentous white rot fungi, yeasts and algae are capable of decolorizing a wide range of dyes via anaerobic, aerobic and sequential anaerobic–aerobic treatment processes (Martins et al., 1999; Asgher et al., 2012d). Anaerobic systems could reduce the color intensity more satisfactorily than the aerobic processes. However, the carcinogenic aromatic amines formed with reductive cleavage of azo bonds by bacterial azoreductase need to be further decomposed by an aerobic treatment (Pearce et al., 2003). WRF have the potential capability to aerobically degrade such contaminants by virtue of its extracellular ligninolytic enzymes (Asgher et al., 2008; Iqbal and Asgher, 2013). Individual azo-, triphenylmethane-, phthalocyanine and heterocyclic dyes (Tavčar et al., 2006), as well as complex industrial effluents are efficiently decolorized by the action of nonspecific ligninolytic enzymes of WRF (Ünyayar et al., 2005; Asgher et al., 2008, 2012c; Senthilkumar et al., in press).

In recent years many efforts have been made for the development of bioremediation processes using white rot fungi. The current study was focused on the development and optimization of bioremediation process for Solar brilliant red 80 direct textile dye by *S. commune* IBL-06.

2. Materials and methods

2.1. Chemicals and textile dyestuff

The direct textile dye Solar brilliant red 80 used in this study was obtained from Clariant Pakistan Limited, Faisalabad. The chemical structure of Solar brilliant red 80 is shown in Fig. 1. All other chemicals were of analytical grade and were purchased from Sigma–Aldrich Chemicals (USA) and Merck (Germany).

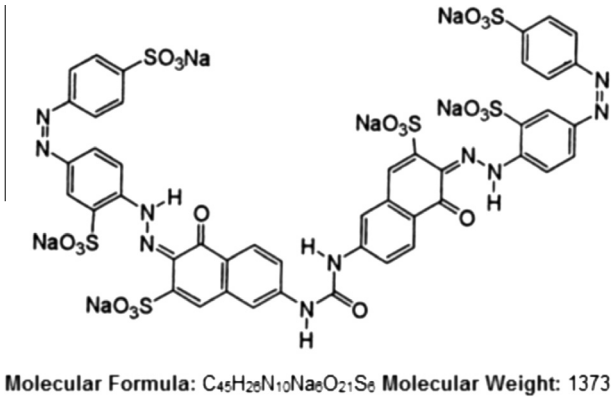

Figure 1 Chemical Structure of Solar brilliant red 80 direct textile dye.

2.2. Fungal culture and spore inoculum preparation

Pure culture of locally isolated indigenous white rot fungus *S. commune* IBL-06 was obtained from Industrial Biotechnology Laboratory, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan. The culture was initially grown on potato dextrose agar (PDA) medium slants at pH 4.5 and 30 °C. After having sufficient population of spores, the PDA slants were refrigerated for subsequent use in further experimental studies. Inoculum medium was prepared by adding 1% (w/v) sterile glucose solution to the Kirk's basal salts medium (Tien and Kirk, 1988). Fresh culture of *S. commune* IBL-06 was added to the inoculum medium and the flask was incubated (120 rpm) at 30 °C for 5 days to get a homogenous inoculum (1×10^6–10^8 spores/mL).

2.3. Fermentation protocol for decolorization

Decolorization flasks (500 mL) were prepared in triplicate each containing 100 mL of 0.01% (w/v) Solar brilliant red 80 dye solution in Kirk's nutrient medium (pH 4.5).The flasks were sterilized in autoclave (121 °C) for 15 min. and on cooling to room temperature, 5 mL inoculum was aseptically added to each flask in laminar air flow under sterilized environment. In the initial time course study, experimental samples were incubated for 10 days at 120 rpm in a temperature controlled shaking incubator. The triplicate flasks were removed after every 24 h and the contents were filtered through Watman No. 1 filter paper. After centrifugation, the supernatants were collected and analyzed for residual dyestuff concentration.

2.4. Decolorization process optimization

The decolorization process was optimized by studying the effect of different physical and nutritional factors on decolorization of Solar brilliant red 80 by *S. commune* IBL-06. The classical method under Completely Randomized Design (CRD) for medium optimization was followed where one parameter was varied keeping the previously optimized at optimum level.

2.4.1. Effect of pH

pH is an important physical factor that needs proper consideration during microbial process optimization studies. The pH of the decolorization media was adjusted at different levels (3, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0) using M HCl/M NaOH. After sterilization each flask was inoculated and incubated for stipulated time period.

2.4.2. Effect of temperature

To study the effect of varying temperature on dye decolorization efficiency of the fungus, the media were adjusted to initial optimum pH 4 and incubated at 25, 30, 35, 40, and 45 °C for 10 days. Samples harvested after every 24 h were spectrophotometricaly analyzed for residual dyestuff concentration.

2.4.3. Effect of carbon and nitrogen sources

The effects of inexpensive carbon supplements (glucose, maltose, sucrose, fructose, and starch), and different nitrogen

additives (0.02% ammonium nitrate, ammonium sulfate, ammonium di-hydrogen phosphate, peptone, and urea) was also investigated to select the best combination of carbon and nitrogen source for optimum dye decolorization at optimum pH and temperature.

2.4.4. Effect of inoculum size

For optimization of inoculum size, Kirk's basal medium containing Solar brilliant red 80 was inoculated with varying volumes (1–7 mL) of freshly prepared fungal spore inoculum. After inoculation, all the flasks were incubated under continuous shaking conditions (120 rpm) for 10 days at optimum pH and temperature.

2.5. Decolorization assay

Solar brilliant red 80 solution was scanned in the wavelength range from 200–800 nm using UV/Vis spectrophotometer (T60, UV/Visible, PG Instruments, UK) to determine the wavelength of maximum absorbance (λ_{max} 588 nm). The collected filtrates from decolorization flasks were carefully centrifuged at 5000*g* for 15 min at 4 °C and clear supernatants were analyzed spectrophotometrically at 588 nm to determine the percent decolorization of Solar brilliant red 80 using the following formula:

$$\% \text{ Decolorization} = 100 \times \frac{A_{ini} - A_{fin}}{A_{ini}}$$

where, A_{ini} = Initial absorbance of dye before incubation, A_{fin} = Final absorbance of dye after incubation

2.6. Ligninolytic enzymes assays

Supernatants from optimally decolorized dye samples were analyzed for LiP, MnP and Lac activities to study the enzymes secreted by the *S. commune* IBL-06 during decolorization of Solar brilliant red 80. LiP was assayed by the method of Tien and Kirk (1988). To determine the activity of MnP, the method of Wariishi et al. (1992) was followed. Laccase activity of culture supernatants was measured by monitoring the oxidation of 2, 2 azinobis (3-ethylbenzthiazoline)-6 sulphonate (ABTS) at 436 nm as described eariler (Iqbal et al., 2011). Blanks contained 100 μL of distilled water instead of culture supernatants.

2.7. Statistical analysis

All the data on dye decolorization and enzyme assays was statistically analyzed using the statistical software Minitab, Windows version 15. The means and standard errors of means (S.E) were calculated for each treatment. The SE values have been displayed as Y-error bars in figures.

3. Results and discussion

In the time course study on decolorization of Solar brilliant red 80, *S. commune* IBL-06 showed maximum decolorization (84.83%) on 7th day of incubation at pH 4.5 and 30 °C (Fig. 2). The rate of color removal kept on increasing within first 7 days and no further decolorization occurred from 7 to 10 days. All the three ligninolytic enzymes including LiP, MnP, and Lac were secreted by *S. commune* IBL-06 that are mainly involved in the decolorization process. LiP (507 U/

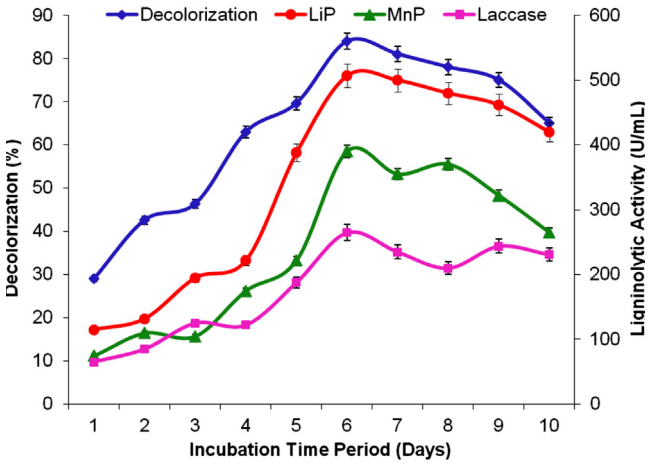

Figure 2 Decolorization of Solar brilliant red 80 by *S. commune* IBL-06 in time course study.

mL) was the major enzyme recorded in the culture, followed by MnP and laccase (Fig. 2). The capability of WRF to completely/partially degrade or decolorize different textile dyes is directly correlated to their ability to produce ligninolytic enzyme when cultured in the dye containing medium. Similar findings on co-relationship between ligninolytic enzymes and textile dyes decolorization has also been observed by Zille et al. (2004). It has also been reported that dye concentration also affects dye degradation by WRF and their ligninolytic enzymes (Levin et al., 2012). The results demonstrate that *S. commune* belong to several known white rot fungal species, i.e. *Trametes versicolor*, *Phanerochaete chrysosporium*, *Irpex lacteus*, *Pleurotus ostreatus* and *Bjerkandera* sp., that are capable of efficient decolorization/degradation of a broad spectrum of chemically different textile dyes (Novotný et al., 2001).

3.1. Effect of pH on dye decolorization

UV–Visible spectra and pH versus dye decolorization profile (Figs. 3 and 4) showed maximum dye decolorization efficiency

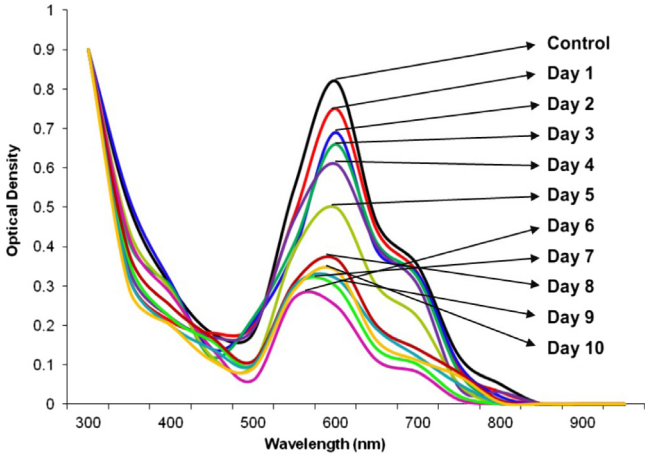

Figure 3 UV–Vis absorption spectra of Solar brilliant red 80 processed at optimum pH for ten days time period with *S. commune* IBL-06.

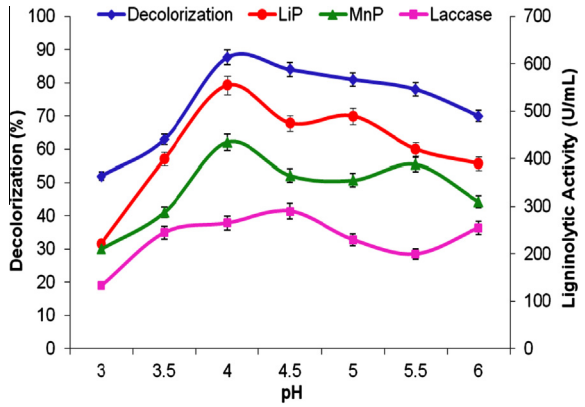

Figure 4 Effect of varying pH on percent decolorization of Solar brilliant red 80 and ligninolytic enzymes activities produced during decolorization by *S. commune* IBL-06.

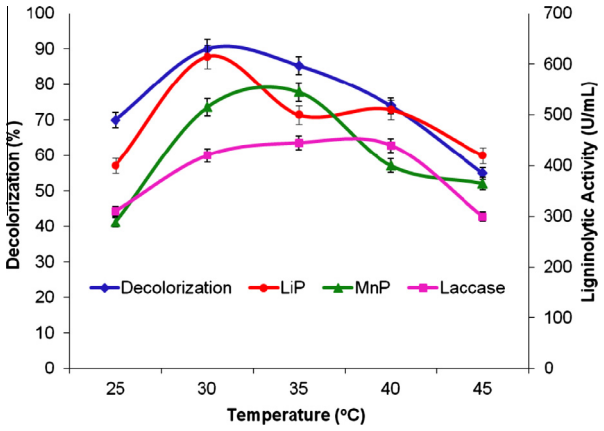

Figure 6 Effect of different temperatures on percent decolorization of Solar brilliant red 80 and ligninolytic enzymes activities produced during decolorization by *S. commune* IBL-06.

(87.70%) after 6 days of incubation in the medium adjusted at pH 4. The culture supernatant of the media processed at varying pH showed that LiP was the major enzyme secreted by *S. commune* IBL-06 for decolorization of Solar brilliant red 80. The effect of variation on decolorization of different textile dyes by a variety of micro-organisms has been reported (Sawhney and Kumar, 2011; Asgher et al., 2012c; Kumar et al., 2012). Most of the WRF secrete different sets of ligninolytic enzymes during the decolorization of different dyes that is favored in pH range of 3–6 (Asgher et al., 2008).

3.2. Effect of incubation temperature

The UV–Visible spectra of dye (Fig. 5), enzyme activity profiles and dye decolorization pattern (Fig. 6) showed maximum decolorization (89.2%) of Solar brilliant red 80 in the optimum pH flasks incubated at 30 °C after 4 days that decreased at further increase in temperatures. Maximum LiP (625 U/mL) activity was also noted in culture filtrates shaken at 30 °C.

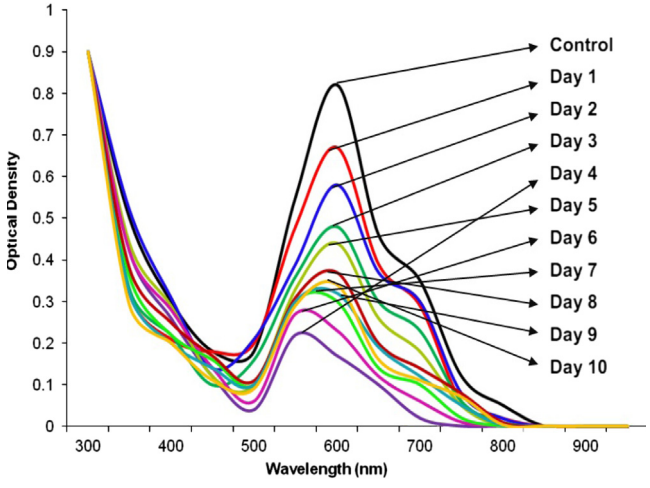

Figure 5 UV–Vis absorption spectra of Solar brilliant red 80 processed at optimum temperature for ten days time period with *S. commune* IBL-06.

Swamy and Ramsay (1999) and Asgher et al., (2008) also observed increase in fungal dye decolorization efficiency with initial rise in temperature to up to certain optimum levels and inhibition of the organism growth and enzyme formation at higher temperatures. For mostly white rot fungi the optimum temperatures for decolorization of chemically different dyestuffs have been reported in the range from 25 to 37 °C (Asgher et al., 2009). It has also been reported that WRF like *P. chrysosporium* and *Coriolus versicolor* give maximum dye decolorization around 35 °C; temperatures below 25 °C and above 40 °C inhibit fungal growth and ligninolytic enzymes synthesis WRF (Assadi et al., 2001; Asgher et al., 2008).

3.3. Effect of additional carbon and nitrogen sources

Different combination of carbon and nitrogen had different influences on enzyme synthesis and decolorization efficiency of the fungus for Solar brilliant red 80. However, the combination of maltose and ammonium sulfate caused maximum dye color removal (91.5%) in 3 days, followed by glucose and ammonium di-hydrogen phosphate (86.6%), (Table 1). Maximum LiP synthesis (775 U/mL) was also noted in the flasks receiving maltose and ammonium sulfate. Similarly, the decolorization efficiency of *T. versicolor* was also enhanced with increasing concentrations of ammonium nitrate (Asgher et al., 2009). Additional carbon and nitrogen sources have also previously been reported to enhance the formation of ligninolytic enzymes involved in dye removal (Selvam et al., 2006; Asgher et al., 2012a).

3.4. Effect of inoculam size

Varying volumes of fresh fungal spore inoculum (1–7 mL) were used to inoculate the triplicate decolorization flasks and the flasks were incubated for stipulated time period at optimum pH and temperature. The change in UV–Visible spectra, enzymes synthesis and dye decoloriztion in response to varying inculum size under optimum conditions has been displayed in Figs. 7 and 8. Complete decolorization (100%) of the direct dye Solar brilliant red 80 after 3 days of incubation time period was observed in the flasks receiving 5 mL inoculum of

Table 1 Activities of ligninolytic enzymes produced during decolorization of Solar brilliant red 80 by *S. commune* IBL-06 with different carbon and nitrogen sources.

Nitrogen sources			Carbon sources				
			Glucose	Maltose	Sucrose	Fructose	Starch
Ammonium nitrate	Enzyme Activities (U/mL)	LiP	715 ± 4.2	555 ± 5.2	402 ± 4.6	688 ± 6.6	475 ± 6.9
		MnP	505 ± 2.8	345 ± 4.6	288 ± 5.2	474 ± 5.8	345 ± 5.4
		Laccase	488 ± 2.9	265 ± 3.8	220 ± 3.4	465 ± 4.2	340 ± 6.8
	Decolorization (%)		77.9 ± 2.3	62.4 ± 1.36	45.5 ± 3.2	72.8 ± 3.5	55.2 ± 1.8
Ammonium sulfate	Enzyme Activities (U/mL)	LiP	745 ± 7.2	775 ± 6.8	588 ± 4.6	760 ± 6.8	590 ± 6.5
		MnP	519 ± 6.2	595 ± 8.6	375 ± 5.3	590 ± 7.5	388 ± 4.8
		Laccase	502 ± 5.8	532 ± 5.8	288 ± 4.3	555 ± 6.5	302 ± 5.5
	Decolorization (%)		79.9 ± 2.9	91.5 ± 3.6	65.8 ± 2.9	82.5 ± 3.9	66.7 ± 2.5
Ammonium dihydrogen phosphate	Enzyme Activities (U/mL)	LiP	745 ± 4.6	705 ± 3.9	425 ± 2.3	392 ± 2.4	570 ± 5.2
		MnP	520 ± 4.3	499 ± 0.45	310 ± 3.8	265 ± 2.9	399 ± 5.3
		Laccase	499 ± 6.9	462 ± 2.95	245 ± 2.9	262 ± 2.6	313 ± 4.5
	Decolorization (%)		86.6 ± 3.3	82.9 ± 3.6	46.5 ± 2.6	44.6 ± 2.4	65.8 ± 2.7
Peptone	Enzyme Activities (U/mL)	LiP	492 ± 5.4	625 ± 6.8	375 ± 4.6	222 ± 1.6	420 ± 2.3
		MnP	372 ± 4.8	415 ± 5.8	264 ± 3.9	188 ± 3.7	298 ± 3.8
		Laccase	362 ± 4.6	352 ± 8.5	240 ± 4.7	115 ± 2.6	252 ± 2.8
	Decolorization (%)		57.5 ± 2.3	69.5 ± 2.8	41.2 ± 2.6	32.3 ± 3.2	49.2 ± 2.4
Urea	Enzyme Activities (U/mL)	LiP	445 ± 3.5	505 ± 2.5	210 ± 2.6	202 ± 3.4	209 ± 3.8
		MnP	310 ± 4.6	399 ± 2.4	162 ± 2.5	150 ± 2.4	159 ± 2.8
		Laccase	272 ± 3.6	402 ± 3.6	110 ± 3.9	101 ± 2.6	105 ± 2.5
	Decolorization (%)		51.3 ± 1.8	58.3 ± 2.6	31.3 ± 1.5	26.5 ± 1.6	28.6 ± 1.2

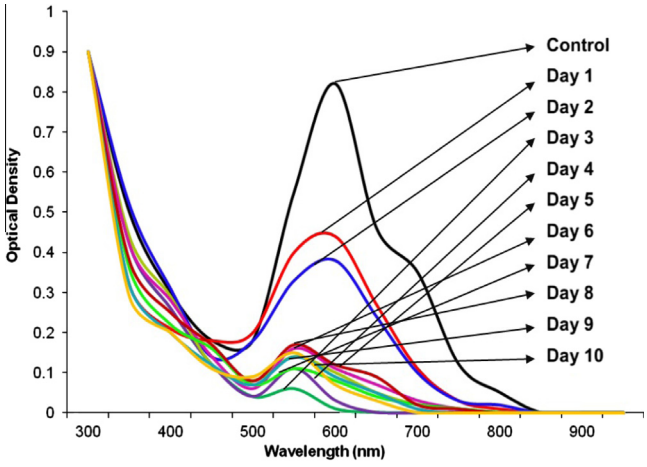

Figure 7 UV–Vis absorption spectra of Solar brilliant red 80 processed for ten days time period with *S. commune* IBL-06 with optimum inoculums size.

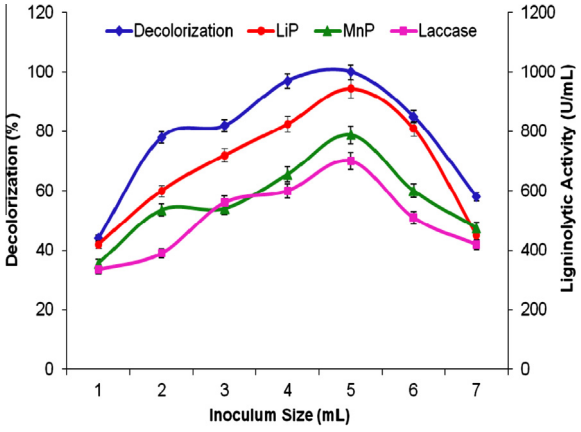

Figure 8 Effect of different inoculum levels on percent decolorization of Solar brilliant red 80 and ligninolytic enzymes activities produced during decolorization by *S. commune* IBL-06.

S. commune IBL-06.The production of enzymes and dye decolorization rate increased with an increase in inoculum size from 1–5 mL but a further increase in inoculum density caused reduction in the decolorization efficiency of the fungus that may be due to early depletion of nutrients. For best enzyme production and dye decolorization the bio-technological processes involving microorganisms require an optimum amount of the microbial spores/cells (Radha et al., 2005; Asgher et al., 2009). Similar to our findings, Sivaraj et al., 2011 and Kumar et al., 2012, who also reported 4.0% (v/v) optimum inoculum density for textile wastewater decolorization.

4. Conclusions

The decolorization process for Solar brilliant red 80 using fresh fungal culture was optimized and complete decolorization (100%) of the dye was achieved in 3 days at pH 4 and 30 °C using 5 mL spore inoculums which was correlated with the maximum formation of LiP (944 U/mL) followed by MnP and Lac activities. The indigenous WRF strain *S. commune* IBL-06 with its efficient ligninolytic enzyme system has an excellent scope for use in the treatment of industrial effluents that contain unused residual textile dyes.

Acknowledgments

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