Bioremoval of Textile Effluent Dye by Aspergillus fumigates

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Abstract: This study dealing with optimization of the conditions affecting the formation of extracellular lignindegrading enzymes; to achieve maximal decolorization activity of Direct Violet dye by one fungal strain; as technology for remediation wastewater of textile dyes for the possibility of reusing of this water. In this study Aspergillus fumigates fungal strain used for production extracellular ligninolytic enzymes for removing Direct Violet dye under different conditions: culture medium, incubation period, pH and temperatures. The results indicated that the removal efficiency of A. fumigatus was enhanced by addition glucose and peptone to the culture medium. The addition of peptone and glucose was found to increase the decolorization activity of the fungal isolate from 51.38% to 93.74% after 4 days of incubation. The highest production of extracellular lignin degrading enzymes also recorded in Direct Violet dye medium supplemented with peptone and glucose. It was also found the decolorization activity of A. fumigatus was decreased gradually by increasing the incubation period up to 4 days. Also it was found that the fungal strain can grow and produce extracellular ligninolytic enzymes which accompanied by efficient removal of Direct Violet dye in a wide pH range of 4-8. The results also found that the maximal biosynthesis of ligninolytic enzymes which accompanied with maximal removal of Direct Violet dye was obtained at a temperature of 28°C. This indicates that the different conditions of culture medium, incubation period, pH and temperatures are effective on dve decolorization on the fungal biomass and played a role in Direct Violet dye removal along with enzymatic activity of A. fumigatus.

Key words: Aspergillus fumigates · Extracellular lignin · Degrading enzymes · Textile dye · Dye removing

INTRODUCTION

Several investigators worked on the fungal strain used for production extracellular ligninolytic enzymes for removing textile dyes under different conditions: culture medium, incubation period, pH and temperatures. Many authors are reported the maximal fungal growth, enzymes production as well as dyes bioremoval were obtained on medium supplemented with glucose and peptone. Maria et al. [1] and Keck et al. [2] reported that using precursor materials enhanced the bioremediation process for recalcitrant compounds such as dyes. Rodriguez et al. [3] reported that the decolorization of dye by the strain of Trametes hispida is mainly ascribed to exracellular activity. On the other hand, Wafaa et al. [4] reported that the possible mechanism for dyes removal of the isolated fungi could be based on a biosorption of such chemicals on the intact fungal biomass as shown by the capacity of removing dyes by the fungal isolates in a relatively short

period of time and the slowing degrading power with the same fungi by increasing the incubation time. Bumpus et al. [5] found that the presence of another carbon source was necessary as supplement of the growth substrate. Parshetti et al. [6] also found that the rate of decolorization of Reactive Blue-25 by Aspergillus ochraceus was slower in the medium containing only peptone than medium containing glucose and peptone. Hu [7] found minimal increase in removal of color reactive azo dyes when Phanerochaete luteola was cultured in a medium with low nitrogen content. Chao and Lee [8] also found that the fungal strains of Phanerochaete chrysosporium, pre-cultured in high nitrogen medium, were able to enhance the decolorization of two azo dyes. Generally, medium containing dye supplemented with peptone and glucose was the best medium to support high fungal growth, enzymes production and consequently decolorization percentage. Parshetti et al. [6] reported that the complete

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decolorization of reactive blue-25 (100 ppm) by A. ochraceus NCIM-1146 occurred at pH 5.0. However, 87% 81% and 70% decolorization was obtained at pH 3, 7 and 9, respectively. Tavares et al. [9] reported that laccase losses stability at pH of 3.0 whilst for pH of 5.0 no loss of enzyme activity is observed. The decrease of pH at the end of experiment might also be referred to the excretion of organic acids by the fungus itself (Abde-Aal et al., [10] and Naima et al. [11]. Wafaa et al. [12] reported the decrease in the initial pH values of the media at the end of experiments for Direct Violet dye bioremoval by A. niger at different pH values. Jadhav et al. [13] found that Comamonas sp. UVS has the ability to decolorize Direct Red 5B dye within the pH range of 6-12 with maximal decolorization at pH 6.5. Concerning the effect of different degrees of temperature on the decolorization activity of fungal strains, the decrease in decolorization activity at higher temperature could be attributed to the loss of cell viability [15] or might be due to the denaturation of ligninolytic enzymes. Ambrosio and Campos-Takaki [15] found that the optimum temperature for decolorization of Orange II dye by Cunnighamella elegans was 28°C. Revankar and Lele [16] also found that the temperature of 28°C was the optimum for bioremoval of Amaranth dye (100 mg/L) by Ganoderma Sp. WR-1. This work aimed study of Aspergillus fumigates fungal strain for production extracellular ligninolytic enzymes for removing Direct Violet dye under different conditions: culture medium, incubation period, pH and temperatures.

MATERIAL AND METHODS

Decolorization Assay of Dyes: Dye decolorizing activity was expressed in terms of decolorization percentage and was determined by measuring the absorbance at 542nm, 396nm, 632 nm, 555nm and 691 for Direct Violet, Direct Green, Reactive Blue, Reactive Red and mix respectively, against the original color of the medium. Decolorization activity (%) was calculated according to the equation:

$$Decolourization\% = \frac{O.D\ of\ control - O.\ D\ of\ sample.}{O.D\ of\ control} x 100$$

Control = Original color of medium, Sample = Color of medium after microbial growth

Biochemical Tests

Enzymatic Assay: Activities of lignin peroxidase, laccase and tyrosinase were assayed spectrophotometrically in both culture supernatant

and cell free extract. The methods for determination of the activities of these enzymes are described below:

Assay of Laccase Enzyme: Laccase activity was determined according to a method of Paszczynski *et al.* [17]. This method based on the oxidation of a phenolic compound Dimethoxy Phenol (DMP) by laccase enzyme.

Assay of Tyrosinase Enzyme: Tyrosinase activity was determined according to a method of Kalme *et al.* [18]. This method based on the oxidation of a phenolic compound (catechol) by tyrosinase enzyme.

Assay of Lignin Peroxidase Enzyme: Lignin peroxidase was determined according to a method of (Jadhav and Govindwar, [19]. This method based on oxidation of n-propanol in the presence of H₂O₂ by lignin peroxidase enzyme with the formation of propanaldehyde.

Statistical Analysis: Statistical analysis was carried out using one way Analysis of Variance (ANOVA) with post test if P < 0.05 and using software GraphPadInStat 3.06 Guide.

RESULTS

Influence of Different Types of Media on Production of Ligninolytic Enzymes, Decolorization of Direct Violet Dye and Growth of Aspergillus Fumigates: The most efficient fungal isolate Aspergillus fumigatus in dye removal was tested for enhancing fungal growth, enzyme production and the subsequent Direct Violet dye (100 ppm) decolorization using different media. Three different media were used in order to establish the most suitable condition for decolorization of solution containing Direct Violet dye (100 ppm). Table (1) indicates that the highest fungal growth and the dye bioremoval have been enhanced greatly in dye containing medium amended with glucose and peptone after 4 days of incubation. The percent of decolorization was 93.97. On the other hand, the rate of fungal growth as well as decolorization percent of Direct Violet dye was slower in the medium containing only peptone as compared with the medium containing glucose and peptone (Fig.1b). The decolorization percent was 60.81, while the mineral basal medium recorded the lowest percent of decolonization being 51.38 and the lowest mycelia dry weight at the end of the experiment (Table 1).

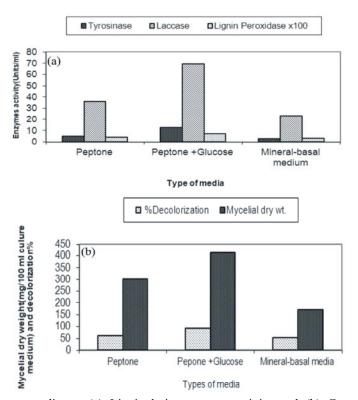


Fig. 1: Influence of different media on (a) Ligninolytic enzymes activity and (b) Growth of *A. fumigatus* and decolorization percentage of Direct Violet dye.

Table 1: Influence of different media on growth of A. fumigates, production of ligninolytic enzymes and decolorization of Direct Violet dye.

	Lignin peroxidase activity	Laccase activity	Tyrosinase activity	Total soluble proteins	Decolorization	Mean Dry Wt.(mg/100ml
Media	(Units/ml) (mean \pm SE)	(Units/ml) (mean \pm SE)	(Units/ml) (mean \pm SE)	(mg/ml) (mean \pm SE)	percentage (mean $\pm SE$)	culture medium) ±SE
Peptone	0.04067± 0.001780***	35.882± 3.113*	4.844±0.6815***	12.74±0.1970**	60.813± 3.943***	299±13.868***
Peptone+Glucose	0.07280 ± 0.003774	69.412± 9.245	12.325 ± 0.6815	21.98±0. 7165	93.743±0.4099	412±5.132
Mineral basal medium	0.03280±0.003064***	22.941±0.8985**	2.674±0.03554***	9.47±1.194**	$51.387 \pm 2.349***$	172± 8.021***

^{**-}Values were given as mean ± SE and the data are significant to P<0.01 (**) and if or P<0.01 (***) as compared with that of pepton+glucose.

Data in Table (1) and Figure (1a) also indicate that the highest production of enzymes (laccase, lignin peroxidase and tyrosinase) was obtained with dye containing medium amended with peptone and glucose. On the other hand, enzymes biosynthesis decreased with dye containing medium amended with peptone only. While, a significant decrease in enzymes biosynthesis was recorded with mineral basal medium containing dye.

Generally, medium containing dye amended with glucose and peptone as carbon and nitrogen sources was the best medium for fungal growth, enzymes production and Direct Violet dye decolorization.

The growth of *A. fumigatus*, enzymes production and decolorization percent were statistically significant for all tested media (Table 1). This significance is observed between dye containing medium amended with glucose and peptone versus the two other tested media.

Influence of Incubation Period on Growth of Aspergillus Fumigatus, Decolorization Percent of Direct Violet Dye and Production of Ligninolytic (Laccase, Lignin Peroxidase and Tyrosinase) Enzymes: The present experiment was carried out to determine the time at which maximum growth of A. fumigatus, decolorization of Direct Violet dye as well as synthesis of Laccase, tyrosinase and lignin peroxide enzymes takes place. Table (2) indicates that the growth of A. fumigatus, grown on media containing dye supplemented with peptone and glucose and the decolorization percentage of Direct Violet dye were increased by increasing incubation days till the fourth day of growth. After the fourth day of incubation period, no significant increase in both growth of A. fumigatus and decolorization percent of Direct Violet dye was occurred till the sixth day of incubation. At the seventh day of growth, there was significant decrease in

Table 2: Influence of incubation periods on growth of A. fumigatus, production of ligninolytic enzymes and decolorization percentage of Direct Violet dye.

Incubation	Lignin peroxidase activity	Laccase activity	Tyrosinase activity	Total soluble proteins	Mean Dry Wt.(mg/100ml	Decolorization
periods (Days)	(Units/ml) (mean ±SE)	(Units/ml) (mean ±SE)	(Units/ml) (mean ±SE)	(mg/ml) (mean \pm SE)	culture medium) ±SE	percentage (mean ±SE)
2	0.03920±0.0005995***	57.647± 2.696**	5.465± 0.2685***	18.78±0.2695***	216.70± 4.493***	86.553±0.7374***
3	$0.06330 \pm 0.0005033 ***$	73.529±3.056	7.906±0.2926***	1990±0.2037***	384.00+9.609***	90.917±0.3769**
4	0.07800 ± 0.0004509	64.706±5.793	10.930±0.4084	25.14±0.1764	446.80±6.102	94.450± 0.2442
5	0.07527±0.0004910	52.941± 3.673***	10.465±0.5496	24.10±0.2037	469.00±5.292	93.863±0.2466
6	0.07113±0.0002603***	48.235±2.696***	9.767±0.3075	23.52± 00.2695**	439.60±5.930	92.920±0.1386
7	0.06350±0.0005686***	46.666±3.240***	8.488± 0.3554**	21.06±00.1764***	386.00±6.658***	90.913±0.2954**
8	0.05767±0.001255***	41.176±2.696***	7.325± 0.3554***	20.82±00.2341***	351.00±6.429***	88.790± 0.3800***
9	0.05113±0.0006173***	33.529±5.970***	6.162± 0.2928***	20.40±00.1006***	338.00±5.196***	84.430±1.145***

⁻Values were given as mean ± SE and the data are significant to P<0.01 (**) and if or P<0.001 (***) as compared with that of 4 days.

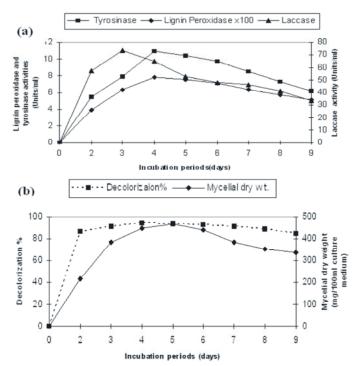


Fig. 2: Influence of incubation period on (a) Ligninolytic enzymes activity and (b) Growth of *A. fumigatus* and decolorization percentage of Direct Violet dye.

the fungal growth as well as the decolorization percent compared with the growth and the decolorization percent at the fourth day of incubation. However, the decrease in the fungal growth as well as the decolorization percent was nearly constant at the eighth and ninth days of incubation (Fig. 2 b). While Figure (2a) demonstrates that the highest production of enzymes (laccase, lignin peroxidase and tyrosinase) was obtained at the fourth day of growth then the level decreased. It is clear from the results that laccase, lignin peroxidase and tyrosinase synthesis in the grown culture of A. fumigatus takes place during the logarithmic phase of growth. In all the subsequent experiments the mycelia were harvested at the fourth day of growth at which the enzyme formation as well as the decolorization percent of Direct Violet dye were maximal.

Influence of Different Hydrogen Ion Concentrations on Growth of Aspergillus Fumigatus, Production of Ligninolytic Enzymes (Laccase, Tyrosinase and Lignin Peroxidase) and Decolorization Percent of Direct Violet Dye: The experiment aims at studying the effect of the original pH value of the culture medium on the growth of A. fumigatus, the production of laccase, tyrosinase and lignin peroxidase enzymes as well as the decolorization percent of Direct Violet dye. Nine pH values ranging from 3 to 8 were chosen for this study. Results in Table & Fig. (3) show that the fungal growth, bioremoval of Direct Violet dye as well as enzymes (laccase, lignin peroxidase and tyrosinase) production were increased gradually with increasing the pH value within the acidic range from 3 to 5. However, the maximal growth, dye bioremoval and enzymes (laccase, tyrosinase and lignin peroxidase)

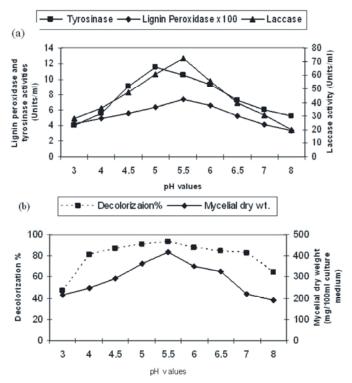


Fig. 3a,b: Effect of different hydrogen ion concentrations on a) Ligninolytic enzymes biosynthesis and b) Growth of *A. fumigatus* and decolorization percentage of Direct Violet dye.

Table 3: Influence of hydrogen ion concentration on growth of A. fumigatus, production of ligninolytic enzymes and decolorization percentage of Direct Violet dye.

Initial	Final	Lignin peroxidase activity	Laccase activity	Tyrosinase activity	Total soluble proteins	Mean Dry Wt.(mg/100ml	Decolorization
pH	pН	(Units/ml) (mean \pm SE)	(Units/ml) (mean ±SE)	(Units/ml) (mean ±SE)	(mg/ml) (mean ±SE)	culture medium) ±SE	percentage (mean ±SE)
3	2.5	0.04257±0.0005783***	28.235± 1.480***	4.070± 0.3738***	17.63±0.1556***	216±2.646***	47.343±2.340***
4	3	0.04900±0.001050***	35.882±1.225***	5.581±0.1777***	18.32±0.1176***	250±7.810***	80.710±0.1039***
4.5	3.2	0.05553±0.001906***	47.059±0.8985***	9.069±0.4700 *	21.20±0.3402***	294.20±6.437***	86.870±1.421**
5	4	0.06350±0.0007371***	60.588±1.556**	11.511 ± 0.4403	20.21±0.2354***	363±15.133*	90.800±0.6653
5.5	4.2	0.07403 ± 0.001841	72.352±2.449	10.581±0.3075	24.75±0.5893	417±11.358	93.637± 0.2378
6	5	0.06557±0.001490**	55.882±1.556***	9.302±0.5331*	22.86±0.1556***	352± 4.726**	87.397± 0.1299**
6.5	5.3	0.05297±0.0009939***	39.411±2.038***	7.325±0.2926***	21.44±0.3205***	327±15.308***	84.243±0.4971***
7	6	0.04183±0.001157***	30.588±2.227***	6.046±0.5331***	20.79±0.2120***	217±4.163***	82.723± 0.2999***
8	6.8	0.03323±0.001245***	20±1.891***	5.232±0.4841***	20.75±0.1819***	192±3.606***	64.190±0.5041***

-Values were given as mean \pm SE and the data are significant to P<0.01 (**) and if or P<0.001 (***) as compared with that of pH 5.5.

production were obtained at pH 5.5. The results also indicate that the fungal growth, decolorization percent of Direct Violet dye and the enzymes formation were markedly reduced when the fungus grew at pH range from 6 to 8 compared with pH 5.5. The final pH values of the culture media were measured at the end of incubation period. From the data given in Table (3), it is clear that there was a significant decrease in the original pH values of the culture media after 4 days of incubation. The obtained results showed the importance of initial media pH on bioremoval process. In all the subsequent experiments the initial media pH was adjusted at pH 5.5 at which the enzymes formation and the bioremoval of dye were maximal.

Influence of Different Temperature Degrees on Growth of Aspergillus fumigatus, Production of Ligninolytic Enzymes (Laccase, Lignin Peroxidase and Tyrosinase) and Decolorization Percent of Direct Violet Dye: The present experiment was carried out to determine the degree of temperature at which optimum fungal growth, color removal and enzymes (laccase, tyrosinase and lignin peroxidase) production could be achieved using A. fumigatus. The experimental temperature ranged from 20 to 40°C. Data in Table (4) indicate that the fungal growth was increased significantly by increasing temperature from 20°C to 28°C, reaching its maximum at 30°C. Further increase in temperature above 30°C resulted in a marginal significant decrease in fungal growth. Similarly, dye

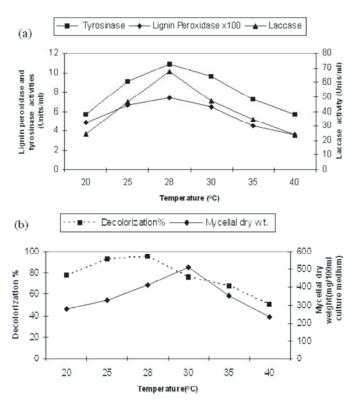


Fig. 4a,b: Effect of different temperature degrees on a) Ligninolytic enzymes biosynthesis and b) Growth of *A. fumigatus* and decolorization percentage of Direct Violet dye.

 $Table \ 4: Influence \ of temperature \ degrees \ on \ growth \ of \ \textit{A. fumigatus}, \ production \ of \ ligninolytic \ enzymes, \ decolorization \ percentage \ of \ Direct \ Violet \ dye$

Temperature	Lignin peroxidase activity	Laccase activity	Tyrosinase activity	Total soluble proteins	Mean Dry Wt.(mg/100ml	Decolorization
(°C)	(Units/ml) (mean ±SE)	(Units/ml) (mean ±SE)	(Units/ml) (mean ±SE	(mg/ml) (mean ±SE)	culture medium) ±SE	percentage (mean ±SE)
20	0.04840±0.001266***	24.176±10.236***	5.697±0.5329***	19.25± 0.3951***	277.00±13.421**	77.273±0.1410***
25	0.06627±0.0008876**	46.470±0.08987**	9.069±0.4194	21.44±03.135**	326.67±16.915**	92.957±0.2194
28	0.07437±0.001488	67.646±2.449	10.930±0.4194	23.76±0.2990	414.00±4.041	95.160±0.06928
30	0.06430±0.0005508***	47.058± 2.652**	9.612±0.3172	22.25±0.3577	509.33±37.966*	75.867±2.672***
35	0.04550±0.0002082***	34.705±1.556**	7.209±0.4194***	15.90±0.3312***	354.00±13.650**	68.157±0.2466***
40	0.03573±0.001214***	23.920±3.082***	5.698±0.3552***	12.70±0.3712***	234.00±20.744***	50.820±1.417***

-Values were given as mean \pm SE and the data are significant to P<0.01 (**) and if or P<0.001 (***) as compared with that 28°C.

decolorization activity of A. fumigatus was found to increase with increasing incubation temperature from 20°C to 28°C and maximal decolorization percent of Direct Violet dye was obtained at 28°C. While, every increasing in temperature above 28°C resulted in a highly significant decrease in decolorization activity. Figure (4a) shows that increasing the temperature from 20 to 28°C resulted in a significant increase in enzymes (laccase, lignin peroxidase and tyrosinase) production. The figure also shows that the maximal enzymes activity was occurred at 28°C. Furthermore, increasing the temperature above 28°C resulted in significant lowering in the enzymes activity. Therefore, in all the subsequent experiments, the incubation temperature were adjusted at 28°C at which the enzymes formation as well as the decolorization activity of A. fumigates were maximal.

DISCUSSION

Growth of the fungal isolates in nitrogen and carbon limited medium indicted that the fungus utilized the dyes as the sole source of carbon and nitrogen by production of extracellular lignin-degrading enzymes to degrade the dyes. The increasing in the decolorization percent of Direct Violet dye by time with the fungal isolates confirmed the previous observation. This result is in agreement with Rodriguez *et al.* [16] who reported that the decolorization of dye by the strain of *Trametes hispida* is mainly ascribed to exracellular activity. On the other hand, such finding is in contrary to those obtained by Wafaa *et al.* [12] who reported that the possible mechanism for dyes removal of the isolated fungi could be based on a biosorption of such chemicals on the intact fungal

biomass as shown by the capacity of removing dyes by the fungal isolates in a relatively short period of time and the slowing degrading power with the same fungi by increasing the incubation time. The growth of the most efficient strain A. fumigatus, previously selected, was tried in several media in the presence of 100 ppm Direct Violet dves to select the most suitable medium for rapid fungal growth, enzymes productions and consequently fast decolorization. Data obtained during this investigation indicate that Direct Violet dye medium supplemented with carbon and nitrogen source enhanced the fungal growth, enzymes production and consequently the dye removal. This result is in agreement with those of Maria et al. [1] and Keck et al. [2] who reported that using precursor materials enhanced the bioremediation process for recalcitrant compounds such as dyes. The maximal fungal growth, enzymes production as well as Direct Violet dye bioremoval were obtained on Direct Violet dye medium supplemented with glucose and peptone whereas the medium containing only peptone showed slower rates in fungal growth, enzymes production as well as decolorization process. Similar results were obtained by Bumpus et al. [5] who found that the presence of another carbon source was necessary as supplement of the growth substrate. Parshetti et al. [6] also found that the rate of decolorization of Reactive Blue-25 by Aspergillus ochraceus was slower in the medium containing only peptone than medium containing glucose and peptone. The lowest rate of fungal growth, enzymes biosynthesis as well as the dye removal was obtained in mineral basal medium without glucose and peptone. Hu [7] found minimal increase in removal of color – reactive azo dves when Phanerochaete luteola was cultured in a medium with low nitrogen content. Chao and Lee [8] also found that the fungal strains of *Phanerochaete chrysosporium*, pre-cultured in high nitrogen medium, were able to enhance the decolorization of two azo dyes. Generally, medium containing dye supplemented with peptone and glucose was the best medium to support high fungal growth. enzymes production and consequently decolorization percentage. Data also showed that the ligninolytic enzymes production which accompanied with the decolorization activity was increased gradually with increasing peptone concentration from 2.0 to 8.0 g/L. Whereas, maximal stimulation of enzymes as well as decolorization activity were obtained at a concentration of 10g/l. On the other hand, increasing peptone concentration above 10 g/L had an inhibitory effect. Mineralization studies with several dyes have revealed that most of the dves investigated were degraded extensively only in a certain range of nitrogen concentrations [28-31]. With regard to the enzymes production as well as decolorization percent at different stages of A. fumigatus, it was indicted that maximal fungal growth, enzymes production as well as dye bioremoval were obtained at the fourth day of incubation. After four days of incubation, the level of enzymes production as well as the decolorization activity was markedly decreased. The decreasing in the decolorization after the fourth day supports that the decolorization of Direct Violet dye in a medium supplemented with peptone and glucose might be due to enzymatic biodegradation activity along with physical binding (biosorption) of dye on the fungal biomass. These results are in harmony with those of Pazarlioglu et al. [32] who reported that the bioremoval of Direct blue by Phanerochaete chrysosporium is due to enzymatic degradation while biosorption mechanism played a minor role. Yesilada et al. [33] also found that the mechanism of Astrazon red dye decolorization by Funaliatrogii is based on biosorption and enzymatic biodegradation. Parshetti et al. [6] also found that the removal of Reactive blue 25 by Aspergillus ochraceusNCIM-1146 is attempt by both biosorption and biodegradation mechanisms. With respect to the effect of hydrogen ion concentration on the decolorization process, it was found that the maximal fungal growth as well as the ligninolytic enzymes production was obtained at pH 5.5. The highest decolorization efficiency (93.63%) was also obtained at pH 5.5. This indicates that the optimum pH for Direct Violet dve decolorization by the fungal strain lies in the slightly acidic range. These results are in agreement with Parshetti et al. [6] who reported that the complete decolorization of reactive blue-25 (100 ppm) by A. ochraceusNCIM-1146 occurred at pH 5.0. However, 87% 81% and 70% decolorization was obtained at pH 3, 7 and 9, respectively. On the other hand, the lowest ligninolytic enzymes production was obtained at pH 3 which was accompanied by the decreasing in the decolorization activity (47.34%). Similar results were observed in other studies [25 and 26]. These results suggest that acidic pH values may influence the stability of the enzymes causing denaturation. According to Tavares et al. [9] laccase losses stability at pH of 3.0 whilst for pH of 5.0 no loss of enzyme activity is observed. The results also showed that the originally adjusted pH values of the culture medium were decreased after four days of incubation. These results might be attributed to the production of organic acid due to dve

removal, reaction of the fungal biomass with dve and possible bioremediation of dye by ligninolytic enzymes. The decrease of pH at the end of experiment might also be referred to the excretion of organic acids by the fungus itself [10 and 11]. Such finding is similar with those obtained by Wafaa et al. [12] who reported the decrease in the initial pH values of the media at the end of experiments for Direct Violet dye bioremoval by A. niger at different pH values. The results also indicated that A. fumigatus can grow and produce extracellular ligninolytic enzymes which accompanied by efficient removal of Direct Violet dye in a wide pH range of 4-8 with different degrees. Since the low pH is not suitable for the wastewater treatment, fungal strains capable of decolorizing dye efficiently at wide pH ranges are desirable for industrial applications. However, to achieve best decolorization by A. fumigatus, it is suggested that the pH of textile effluent should be adjusted to around 5.5. These results are in harmony with those of Jadhav et al. [13] who found that *Comamonas* sp. UVS has the ability to decolorize Direct Red 5B dye within the pH range of 6-12 with maximal decolorization at pH 6.5. Concerning the effect of different degrees of temperature on the decolorization activity of A. fumigatus, it was shown that the dye decolorization activity of the fungal strain was found to increases with increasing incubation temperature from 20 to 28°C. Further increase in temperature above 30°C resulted in a marginal decrease in decolorization activity. The decrease in decolorization activity at higher temperature could be attributed to the loss of cell viability [27] or might be due to the denaturation of ligninolytic enzymes. The data also indicate that the maximal extracellular ligninolytic enzymes production which accompanied with the highest percent of Direct Violet dye decolorization was obtained at a temperature of 28°C. These results are in agreement with those of Ambrosio and Campos-Takaki [15] who found that the optimum temperature for decolorization of Orange II dye by Cunnighamell aelegans was 28°C. Revankar and Lele [16] also found that the temperature of 28°C was the optimum for bioremoval of Amaranth dye (100 mg/L) by Ganoderma Sp. WR-1.

CONCLUSION

The obtained results throw the light on the optimal conditions of textile effluent depolarization before discharge to the sewer system or reuse it in other industrial purposes.

REFERENCES

- Maria, B.P.G., N.S. Buurke, S. Goszczynski and D.L. Crawford, 1996. Transformation of azo dye isomers by *Streptomyces chromofuscus* A11. Appl. Environment. Microbiol., 62: 1814-1817.
- Keck, A., J. Klein, M. Kudlich, A. Stolz, H.J. Knackmuss and R. Mattes, 1997. Reduction of azo dyes by redox mediators originating in the naphthalenesulfonic acid degradation pathway of *Sphingomonas sp.* Strain BN6. Appl. Environ. Microbiol., 63(9): 3684-3690.
- Rodriguez, E., M.A. Pickard and Vazquez-Duhalt 1999. Industrial dye decolorization by laccases from ligninolytic fungi. Curr. Microbial., 38: 27-32.
- Wafaa, M.A., H. Moawad and M. Khalafallah, 2003b. Microflora involved in textile dye waste removal. Journal of Basic Microbiology, 43: 167-174.
- Bumpus, J.A., T. Fernando, M.A. Jurek and S.D. Aust, (1989). Factors affecting biodegradation of xenobiotices by the white rot fungus *Phanerochaete chrysosporium*. Biotechnology Applications in Hazardous Waste Treatment, 1989: 363-381.
- Parshetti, G.K., S.D. Kalme, S.S. Gomare and S.P. Govindwar, 2007. Biodegradation of Reactive blue-25 by *Aspergillus ochraceus* NCIM-1146. J. Bioresour. Technol., 98: 3638-3642.
- Hu, T.L., 1994. Decolorization of reactive azo dyes by transformation with *Pseudomonas luteola*. Biroresource Technology, 49(1): 47-51.
- 8. Chao, W.L. and S.L. Lee, 1994. Decolorization of azo by three white fungi: influence of carbon source. World J. Microbiol. Biotechnol., 10: 556-559.
- Tavares, A.P.M., M.A.Z. Coelho, M.S.M. Agapito, J.A.P. Coutinho and A.M.R.B. Xavier, 2006. Optimization and modeling of laccase production by *Trametes versicolor* in a bioreactor using statistical experimental design. Appl. Biochem. Biotechnol., 134: 233-248.
- Abdel-Aal, S.E., A.M. Dessouki and Y.H. Gad, 2001.
 Removal of some dyes from industrial effluents by polymeric materials and gamma-irradiation, J. Radioanal. Nucl. Chem., 247(2): 399-405.
- Naima, C., P. Delia-Laura, A.M. Douglas, C. Arani,
 L. Dieter, D.R. Alexander, S. Karl- Werner and
 J.C. Terrence, 2007. Fe (III)-TAML-catalyzed green oxidative degradation of the azo dye Orange II by
 H₂O₂ and organic peroxides: products, toxicity, kinetics and mechanisms, Green Chem., 9: 49-57.

- 12. Wafaa, M.A., A.M.E. Ola and H.A.M. Fatma, 2009. The effect of pH on bioremediation potential for the removal of direct violet textile dye by Aspergillus niger. Desalination, 249: 1206-1211.
- 13. Jadhav, J.P. and S.P. Govindwar, 2006. Biotransformation of malachite green by Saccharomyces cerevisiae MTCC 463. Yeast, 23: 315-323.
- Jadhav, U.U., V.V. Dawkar, G.S. Ghodake and S.P. Govindwar, (2008). Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas sp.* UVS. Journal of Hazardous Materials, 158: 507-516.
- Ambrosio, S.T. and G.M. Campos-Takaki, 2004.
 Decolorization of reactive azo dyes by Cunninghamella elegans UCP 542 under cometabolic conditions. Bioresour Technol., 91: 69-75.
- Revankar, M.S. and S.S. Lele, 2007. Synthetic dye decolourization by white rot fungus, Ganoderma sp. WR-1. Bioresour. Technol., 98: 775-780.
- 17. Paszczynski, A., R.L. Crawford and V.B. Huynh, 1988. Purification Method. Enzymol., 161: 264-270.
- Kalme, S.D., G.K. Parshetti, S.U. Jadhav and S.P. Govindwar, 2007. Biodegradation of benzidine based dye direct blue-6 by *Pseudomonasdesmolyticum* NCIM 2112. Bioresource Technol., 98: 1405-1410.
- Swamy, J. and J.A. Ramsay, 1999. Effects of glucose and NH₄⁺ concentrations on sequential dye decolouration by Trametes versicolour. Enzyme Microb. Technol., 25: 278-284.
- Sumathi, S. and B. Manju, 2000. Uptake of reactive textile dyes by Aspergillus foetidus. Enzyme Microb. Technol., 27: 347-355.

- Galhaup, C., H. Wagner, B. Hinterstoisser and D. Haltrich, 2002. Increased production of laccase by the wood-degrading basidiomycete *Trametes pubescens*. Enzyme Microb. Technol., 30: 529-536.
- Hatvani, N. and I. Mecs, 2002. Effect of the nutrient composition on dye decolorisation and extracellular enzyme production by Lentinus edodes on solid medium. Enzyme Microb. Technol., 30: 381-386.
- Pazarlioglu, N.K., R.O. Urek and F. Ergun, 2005. Biodecolourization of Direct Blue 15 by immobilized *Phanerochaete chrysosporium*. Process Biochem., 40: 1923-1929.
- 24. Yesilada, O., S. Sing and D. Asma, 2002. Decolourization of textile dye Astrazon Red FBL by *Funalia trogii* pellets. Bioresour Technol., 81: 155-157.
- 25. Eggert, C., U. Temp and K.L. Eriksson, 1996. The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus*: purification and characterization of the laccase. Appl. Environ. Microbiol., 62: 1151-1158.
- Nyanhongo, G.S., J. Gomesa, G.M. Gubitz, R. Zvauya, J. Readd and W. Steiner, 2002. Decolorization of textile dyes by laccases from a newly isolated strain of *Trametes modesta*. Water Res., 36: 1449-1456.
- 27. Pearce, C.I., J.R. Lioyd and J.T. Gutherie, 2003. The removal of color from textile wastewater using whole bacterial cells: a review. Dyes Pigments., 58: 179-186.