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Development of stripping process for cotton fabric dyed with sandalfix black BR 150%

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Abstract

White-rot fungal strain *Trametes versicolor* IBL-04 was investigated for its potential for decolorization of cotton fabric dyed with sandal fix black BR 150% and stripping process was optimized using response surface methodology (RSM) under central composite design (CCD). Statistically, the significance of the discoloration process was analyzed and presented through analysis of variance (ANOVA). Biological stripping is an alternative method to chemical stripping that remove dyes fixed on the cotton fabric with eco-friendly approach i.e. reduced pollution in the environment. The fungal lignin modifying enzymes (laccase, manganese-peroxidases, lignin-peroxidases) possess the potential of degrading a vast variety of synthetic dyes. Several physical factors like temperature, inoculum size, pH and incubation time were statistically optimized. Biological color stripping resulted in 56.80% discoloration from cotton fabric. Results showed that the color strength have no influence on fabric strength, rather biological or chemical stripping does affect the quality of cotton fabric regarding durability/bursting strength. *Trametes versicolor* IBL-04 present good dye decolorization and can be used as alternative to chemical stripping. Fungal stripping is better than chemical stripping in terms of quality and stripping percentage of fabric.

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Introduction

In textile industries, 100% utilization of the dyes from dye baths is not possible, resulting in 10-15% of the dye is left unused and is discharged as effluents into the water reservoirs (Saratale *et al.,* 2011). The discharged effluents containing toxic synthetic dyes are a serious threat to the aquatic life as these dyes are resistant to be degraded by aquatic organisms. Dyes are being separated by other physicochemical techniques e.g. coagulation, flocculation, oxidation, adsorption, ion-exchange and other electrochemical methods (Khlifi *et al.,* 2010). The dyeing unit of the textile industry consume large volumes of fresh water for wet processing of fabric. Almost annual dye production has crossed 7×10^5 metric tons, from which 5-10% is cleared and lost in the water as effluents (McMullan *et al.,* 2001; Toh *et al.,* 2003). These effluents are highly complex in their composition because of the production of derivative compounds of the main chemical colorants discharged in the water. These effluents contain a wide range of chemicals including acids, bases, some toxic chemicals and colored compounds which are always undesirable.

This has become a serious issue in the current scientific research. All over the world, scientists working on reducing the water pollution are keen on addressing and evaluating the possible solutions to remove chemical contaminants and colorants from waste water so that it may not pose any threat to the aquatic life as well as terrestrial crops (Anjaneyulu *et al.,* 2005). One of the most important problem posed by the effluents is their genotoxicity and cytotoxicity, also they seep down in the underground fresh water reservoirs affecting all life forms. Soil fertility may increase in response to accumulating effluents and may result in eutrophication. The ultimate consequences of eutrophication in aquatic environment are algal bloom and oxygen deficit which causes large scale death of organism. These contaminants indirectly enter into the human food chain and cause liver inflammation and cancer (Fernando *et al.,* 2014).

Keeping all these facts in view, researchers are encouraged to develop biological or enzymatic methods for the degradation of hazardous dyes (Saratale *et al.,* 2011; Murugesan *et al.,* 2007). The removal of such chemicals by enzymes is much more environment friendly and quite economical with a minor impact on the ecosystem (Telke *et al.,* 2011; Forootanfar *et al.,* 2011). Immobilization technique is more effective in the removal of these synthetic dyes as the immobilized enzymes being attached to a matrix do not get washed away and can be reused again and again (Cristovao *et al.,* 2011).

The conventional physicochemical techniques e.g. adsorption, precipitation and flocculation have different potential in removing dyes from fabric and they vary in their treatment time, economy, decolorization level, amount of sludge/toxins produced and reusability (Spadaro *et al.,* 1992). These methods work well on small-scale, but they become ineffective on industrial scale where large volumes of water have to be treated (Robinson *et al.,* 2008). Instead of using these methods, biotechnological and biochemical methods have been evaluated and proven to be far more effective on industrial scale. In addition, biological methods are more economical and do not produce toxins, as the aerobic organisms degrade the chemical colorants to relatively simpler compounds (Khalaf, 2008; Vijayaraghavan *et al.,* 2008).

White-rot basidiomycetes can produce a unique set of hydrolytic and oxidative extracellular enzymes; laccase (Lac), lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and some minor enzymes e.g. cellobiose dehydrogenase, and pyranose 2-oxidase, which can degrade an array of structurally similar natural chemical polymers such as lignin, cellobioses and lignocelluloses. Among all these enzymes, laccase is the major contributor in degrading such natural polymers and laccase also present wide range of industrial biotechnological applications (Nyanhongo *et al.,* 2007). This study was designed to optimize the biological stripping process including major lignolytic enzymes involved in

stripping of textile dyes, using *Trametes versicolor* IBL-04 a white-rot fungus.

Materials and methods

Place of work

All the research and analytical work was performed in Industrial Biotechnology Lab (IBL), Biochemistry Department of University of Agriculture, Faisalabad, Pakistan.

Collection of materials

Grey cotton fabric was obtained from regional hosiery shop located at Jinnah Colony, Faisalabad. All of the reagents and chemicals utilized in this study were of analytical grade, purchased from local distributor of sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Sandalfix black BR 150% dye was obtained from a globally popular dyes industry "Sandal Dyestuff (Pvt.) Limited, located in Faisalabad, Pakistan".

Dyeing of cotton fabric

Fabric was bleached prior to dyeing using a solution of 50% H_2O_2, that was diluted to 20g/L concentration and solution with 50% concentration of sodium hydroxide (15g/L), at 50°C for 30 minutes. Cotton fabric was dyed with a Sandalfix black BR 150%, with 2% (by weight of fabric) overall shade strength using the slight modified method from the one described by Pearce *et al.,* (2003). Liquid solution with 1:15 ratio was used for shade strength 2%. Solution was made by taking sodium carbonate (Na_2CO_3), and sodium sulphate (Na_2SO_4) each with a concentration of 30g/L. Exhaust dying method was used for dyeing of cotton fabric for 15 minutes at 45°C followed by 60 minutes at 65°C. The dyed cotton was rinsed with tape water thoroughly.

Fungal culture

Pure fungal culture of *Trametes versicolor* IBL-04 was obtained from the Industrial Biotechnology Lab., University of Agriculture, Faisalabad. Culture was refreshed and maintained on PDA slants. Kirk's basal salt medium was used with the composition (g/L) glucose 10, $MgSO_4.7H_2O$, 0.05, ammonium tartrate 0.22, $CaCl_2.2H_2O$, 0.01, KH_2PO_4, 0.21, 10% Tween-80, 10, thiamine 0.001, 100mM veratryl alcohol 10, trace element solution 10, chloramphenicol, 1. Trace element solution was used with the following composition $CuSO_4$, 0.08, $Na_2MoO_4.H_2O$, 0.05, $MnSO_4.4H_2O$, 0.07, $ZnSO_4.7H_2O$, 0.043, $Fe_2(SO_4)$, 0.05. The media was adjusted to pH 4.5 and sterilized by autoclaving at 121°C for 15 minutes. Media was inoculated with a loopful of fungal culture and was incubated for 7-10 days at 37°C and 150rpm.

Procedure for biological stripping

The stripping mixture was treated in a set of triplicates in 500mL flasks, each comprising of 100mL Kirk's basal media and a 2 × 2-inches small square chunks of fabric dyed with Sandalfix black BR 150%, with a shade strength of 2%. After adjusting pH to 4.5, erlenmeyer flasks containing media, were sterilized by autoclaving. Flasks were inoculated with fungal inoculums of respective fungi. The flasks were inoculated at different range of temperature in a shaker at 120rpm for a different time period. Controls were added with fabric piece and media, which was not inoculated with fungal culture and flasks were provided same set of condition. Once the incubation period was completed, the samples were harvested. Fabric pieces were carefully removed from the flasks and washed using tape water to remove fungal spores present on the surface of fabric. Pieces were dried and labeled properly. The fungal strain, *T. versicolor* IBL-04 was estimated to provide maximum decolorization in 15 days of incubation period.

Process optimization of physical factors

The physical parameters including temperature (25–45°C), pH (3–7), inoculum size (1–5mL), and incubation period (3–15 days) were optimized through RSM under CCD requiring 30 runs. Incubation temperature, pH, inoculum size and fermentation time, for increased lignolytic enzyme production were optimized and were applied for final color stripping of fabric stuffs.

Color strength determination

The color strength of fabric (both unstripped and stripped) was estimated in terms of K/S (relative ratio of absorption and radiation refraction), λmax= 600 nm,

using UV/Vis spectrophotometer. Spectrophotometer Spectraflash analysis were performed in Quality Control Laboratory, Sandal Dyestuffs, Faisalabad. The samples were folded in tetra layers, measured with repositioning clockwise at 90° rotation during measurement (Butts and Charlotte, 2004). The percentage of the stripping of cotton fabric was calculated as:

$$\%age\ stripping = \frac{\left[\frac{K}{S}\text{value of control sample} - \frac{K}{S}\text{value of stripped sample}\right] \times 100}{\frac{K}{S}\text{value of unstripped fabric}}$$

Ligninolytic enzyme assays

Each sample was analyzed for enzyme activity assay after the stripping under optimized parameters using UV/Vis spectrophotometer. Samples were obtained right after the harvesting by centrifugation for 10 minutes at 10,000 *rev*/min. Laccase assay was performed using supernatant by the method of Shin and Lee, (2000). The MnP assay was performed by the method of Wariishi *et al.,* (1992). LiP activity assay was performed according to Ollikka *et al.,* (1993). Enzymatic activity assay was performed by the following equation:

$A = l \times c \times \varepsilon$

$c = \frac{A}{\varepsilon \times l} \times 10^7\ (U/mL)$

Here, 'A' is absorbance, 'l' cell path length, 'c' concentration and ε is coefficient of molar absorption.

Determination of stripping percentage

Stripping percentage was calculated using the method given by Chatha *et al.,* (2012).

Statistical data analysis

For stripping process optimization, Stat-Ease Design Expert 11.0 version was used.

Results and discussion

Biological stripping process optimization

RSM statistical technique is a more precious method to design the excellent growth and enzyme production medium for *Trametes versicolor* IBL-04, Employing RSM for submerged fermentation, the interaction effect of the independent variables, the main effects were inoculum size, temperature, pH and incubation time and the effects of interaction among these variables were studied on dyed fabric for color removal. Design Expert version 11.0 software was employed for data analysis and formation of 3D response surface charts. Table 3.1 represents the profile of lignolytic enzymes by WRF *Trametes versicolor*.

Table 3.1. Optimization of physical factors using RSM under CCD.

Runs	pH	Temperature (°C)	Inoculum size (mL)	Incubation period (Days)	Lac's Activity 1U/ml	MnP's Activity 1U/ml	LiP's Activity 1U/ml	Stripping Percentage
1	7	25	5	15	79.1667	322.692	484.946	50.1656
2	5	35	7	9	81.3889	314.064	492.473	50.0597
3	5	35	3	9	67.521	275.237	413.978	52.4445
4	7	45	5	15	76.9444	313.201	470.968	43.6009
5	5	35	3	9	68.0556	272.649	425.806	50.891
6	7	25	5	3	65.2778	265.746	398.925	47.4737
7	5	35	3	9	62.4567	250.325	440.125	55.0926
8	3	25	5	3	51.1111	208.801	313.978	44.7158
9	5	35	3	9	66.3889	269.198	404.301	46.2966
10	5	35	3	9	66.9444	280.414	408.602	51.3796
11	3	45	5	15	62.7778	256.255	384.946	56.8041
12	7	45	1	3	53.6111	218.292	327.957	38.5518
13	5	55	3	9	57.2222	232.097	348.387	39.7315
14	7	25	1	3	55.8333	227.783	341.935	42.2757
15	5	35	3	9	66.6667	276.963	418.281	47.4445
16	3	45	5	3	48.8889	199.31	332.369	45.943
17	5	35	1	9	58.3783	257.118	395.699	44.602
18	3	25	1	15	54.1667	222.606	324.731	43.2055
19	5	35	3	3	58.3333	237.274	356.989	44.2463
20	7	25	1	15	69.7222	284.728	427.957	40.3726
21	3	45	1	15	56.7705	218.292	341.935	51.2015

Runs	pH	Temperature (°C)	Inoculum size (mL)	Incubation period (Days)	Lac's Activity 1U/ml	MnP's Activity 1U/ml	LiP's Activity 1U/ml	Stripping Percentage
22	1	35	3	9	48.3213	199.311	322.457	56.0266
23	3	25	1	3	41.9444	170.837	301.236	54.1099
24	7	45	5	3	61.6667	252.804	375.269	35.8099
25	9	35	3	9	69.4444	242.45	344.086	42.2591
26	7	45	1	15	65.5556	264.021	403.226	41.7196
27	3	45	1	3	39.7222	161.346	270.011	36.3146
28	3	25	5	15	64.1667	265.746	407.527	53.3167
29	5	15	3	9	62.5897	220.88	347.312	44.293
30	5	35	3	21	70.2222	294.219	441.935	42.6289

In this experiment the highest color stripping observed was 56.80% at the ligninases activities of Lac, MnP and LiP as 62.7778, 256.255 and 384.946 U/mL respectively and lowest stripping observed was 35.80% at the activities of Lac, MnP and LiP as 61.6667, 252.804 and 375.269 U/mL respectively. Simply, production of ligninases by strain *Trametes versicolor* IBL-04 improved by the rise in the temperature of incubation from 25-35°C and then reduced by further increase in temperature from 35-45°C. Any variation in incubation period and pH value was significantly ($p \leq 0.0001$) corelated with stripping percentage and their interaction with MnP, LiP and laccase activities.

Table 3.2. Analysis of variance (ANOVA) for quadratic model for laccase.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2767.98	14	197.71	32.70	< 0.0001	significant
A-pH	889.79	1	889.79	147.19	< 0.0001	
B-Temperature	32.49	1	32.49	5.37	0.0350	
C-Inoculum size	387.23	1	387.23	64.05	< 0.0001	
D-Incubation time	869.49	1	869.49	143.83	< 0.0001	
AB	7.35	1	7.35	1.22	0.2876	
AC	0.0375	1	0.0375	0.0062	0.9382	
AD	0.2040	1	0.2040	0.0337	0.8567	
BC	0.1231	1	0.1231	0.0204	0.8884	
BD	0.5797	1	0.5797	0.0959	0.7611	
CD	0.2499	1	0.2499	0.0413	0.8416	
A^2	119.10	1	119.10	19.70	0.0005	
B^2	93.16	1	93.16	15.41	0.0013	
C^2	5.71	1	5.71	0.9442	0.3466	
D^2	140.55	1	140.55	23.25	0.0002	
Residual	90.68	15	6.05			
Lack of Fit	70.79	10	7.08	1.78	0.2725	not significant
Pure Error	19.89	5	3.98			
Cor Total	2858.66	29				

The F-value of 32.70 denotes the significance of model. The chance for large F-value are only 0.01%, which may be due to noise. The P-value less than 0.05 denotes the significance of model terms. In above model A, B, C, D, A^2, B^2, D^2 are terms which are significant. More than 0.100 indicates the insignificance of model terms.

The reduction in number of insignificant terms may prove improved model. F-value (the lack of fit) of 1.78 present non-significance related to pure error. The chances of large F-value are 27.25%, which may exist due to noise. Response surface 3D graphs (Fig. 3.1 A-F) represents the model response (laccase activity U/mL) from results of CCD.

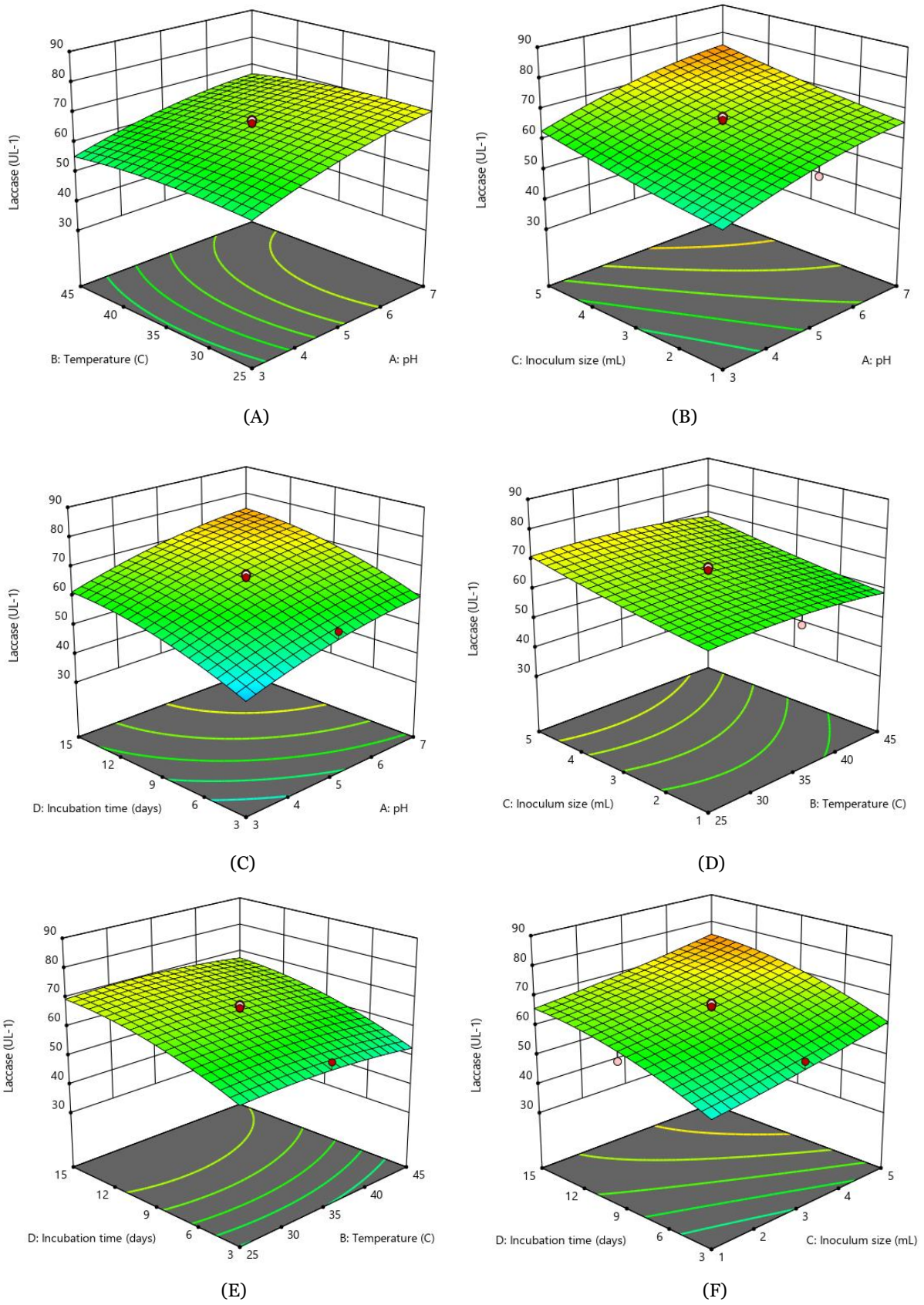


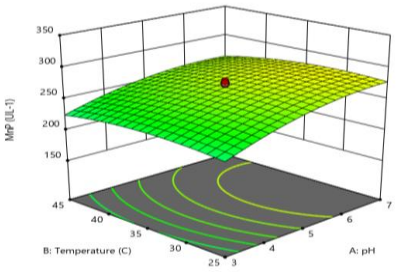
Fig. 3.1. Response surface graphs representing interaction between (A) pH vs temperature (B) pH vs inoculum size (C) pH vs incubation time (D) temperature vs. inoculum size (E) temperature vs incubation time (F) inoculum size vs incubation time.

Laccase gave maximum activity of 81.3889 IU/mL at pH 5, temperature 35°C, inoculum size 7mL and incubation period of 9 days. In our previous study it was observed that maximum decolorization was achieved at acidic pH. The increase in pH from 9 results in decrease in percentage stripping (Asgher *et al.*, 2006).

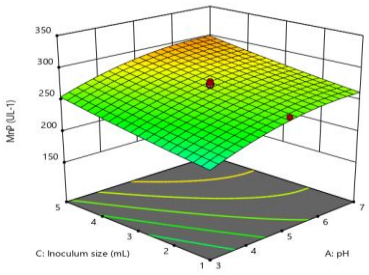
The F-value of 14.74 denotes the significance of quadratic model. The chances (0.01%) for F-value to be larger could occur because of noise. P-values 0.05 or less also denotes the significance of model. In above quadratic model the A, C, D, A^2, B^2, D^2 are significant. Greater value than 0.100 denotes the insignificance of model. F-value of 2.21 denotes the insignificance of lack of fit relative to pure error. There is a 19.68% chance that a Lack of Fit F-value this large could occur due to noise. It's good to have non-significant lack of fit. Response surface 3D graphs (Fig. 3.2 A-F) represents the model response MnP (U/mL).

Table 3.3. Analysis of variance (ANOVA) for quadratic model for MnP.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	42965.49	14	3068.96	14.74	< 0.0001	significant
A-pH	11091.44	1	11091.44	53.28	< 0.0001	
B-Temperature	361.86	1	361.86	1.74	0.2071	
C-Inoculum size	5663.53	1	5663.53	27.20	0.0001	
D-Incubation time	13252.66	1	13252.66	63.66	< 0.0001	
AB	157.69	1	157.69	0.7575	0.3978	
AC	3.02	1	3.02	0.0145	0.9057	
AD	3.02	1	3.02	0.0145	0.9057	
BC	69.00	1	69.00	0.3314	0.5734	
BD	69.00	1	69.00	0.3314	0.5734	
CD	5.16	1	5.16	0.0248	0.8770	
A^2	4275.50	1	4275.50	20.54	0.0004	
B^2	3073.91	1	3073.91	14.77	0.0016	
C^2	42.86	1	42.86	0.2059	0.6565	
D^2	1023.12	1	1023.12	4.91	0.0425	
Residual	3122.78	15	208.19			
Lack of Fit	2547.45	10	254.75	2.21	0.1968	not significant
Pure Error	575.33	5	115.07			
Cor Total	46088.27	29				



(a)



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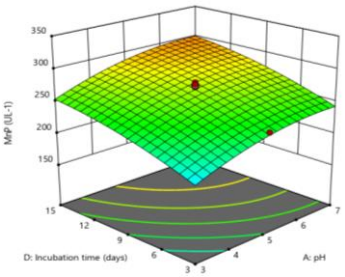

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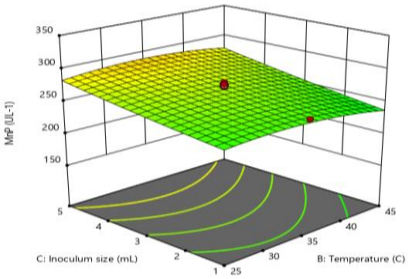

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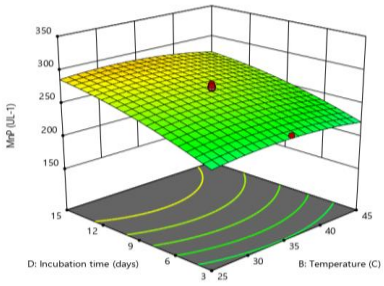

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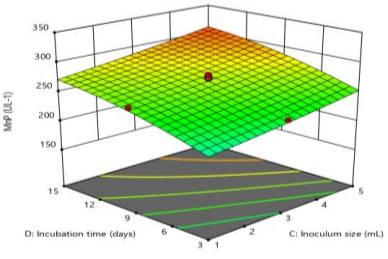

(F)

Fig. 3.2. Response surface graphs representing interaction between (A) pH vs temperature (B) pH vs inoculum size (C) pH vs incubation time (D) temperature vs. inoculum size (E) temperature vs incubation time (F) inoculum size vs incubation time.

Concentration of MnP greatly influence the dye decolorization process. pH and temperature have influence on MnP production by WRFs. These results are similar to a previous study which also represented MnP plays significant role in synthetic dye decolorization by *Schyzophyllum commune* (Pazarlıoglu *et al.,* 2005). *Ganoderma lucidum* a white rote fungus also presented good stripping percentage due to lignolytic enzymes production up to 89.64% to a fabric dyed with 2% shade strength Chatha *et al.,* (2012).

Table 3.4. Analysis of variance (ANOVA) for quadratic model for LiP.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	82240.89	14	5874.35	10.80	< 0.0001	significant
A-pH	13272.86	1	13272.86	24.41	0.0002	
B-Temperature	1065.35	1	1065.35	1.96	0.1820	
C-Inoculum size	11946.49	1	11946.49	21.97	0.0003	
D-Incubation time	23605.99	1	23605.99	43.41	< 0.0001	
AB	982.11	1	982.11	1.81	0.1990	
AC	56.87	1	56.87	0.1046	0.7509	
AD	509.22	1	509.22	0.9364	0.3485	
BC	388.28	1	388.28	0.7140	0.4114	
BD	238.58	1	238.58	0.4387	0.5178	
CD	307.44	1	307.44	0.5653	0.4637	
A^2	12344.56	1	12344.56	22.70	0.0003	
B^2	7731.74	1	7731.74	14.22	0.0019	
C^2	347.47	1	347.47	0.6390	0.4366	
D^2	2963.60	1	2963.60	5.45	0.0339	
Residual	8157.02	15	543.80			
Lack of Fit	7315.92	10	731.59	4.35	0.0592	not significant
Pure Error	841.09	5	168.22			
Cor Total	90397.90	29				

The F-value of 10.80 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, C, D, A^2, B^2, D^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy). The Lack of Fit F-value of 4.35 implies there is a 5.92% chance that a Lack of Fit F-value this large could occur due to noise. This relatively low probability (<10%) is troubling. Response surface 3D graphs (Fig. 3.3 A-F) represents the model response LiP (U/mL).

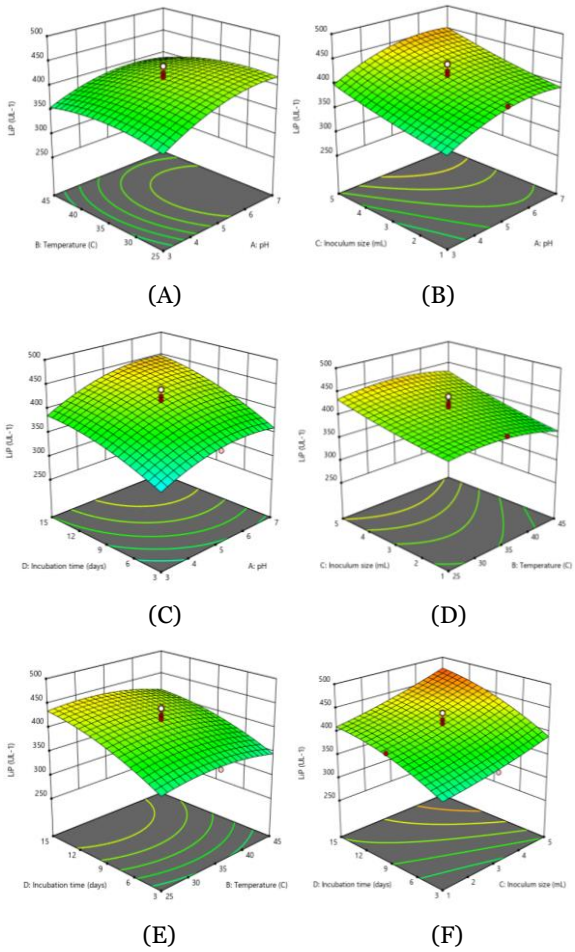

Fig. 3.3. Response surface graphs representing interaction between (A) pH vs temperature (B) pH vs inoculum size (C) pH vs incubation time (D) temperature vs. inoculum size (E) temperature vs incubation time (F) inoculum size vs incubation time.

The optimum pH for various WRFs lies between 4.0-6.0, that was dependent upon the composition of media in discoloration medium (Kapdan *et al.,* 2000; Hai *et al.,* 2006; Jolivalt *et al.,* 2006). The dye decolorization ability of WRF rises by rising the pH at the start from 3-5 and then decrease at pH exceeding from 5-7 (Asgher *et al.,* 2006). Kapdan and Kargi, (2002) studied the decolorization of textile dyes and determined optimum pH as 4.5-6 for various fungal strains. The optimum temperature for WRFs growth and lignolytic enzymatic activities were observed at 30°C temperature (Hai *et al.,* 2006). The optimum range of temperature for dye decolorization has been reported to vary between 25-37°C for various WRF cultures (Toh *et al.,* 2003; Tekere *et al.,* 2001; Chander and Arora, 2007).

Several scientific studies have described that the pH range below neutral i.e. acidic pH is more favorable for most of the WRF to show maximum decolorization efficiency (Asgher *et al.,* 2008; Asgher *et al.,* 2006; Kapdan *et al.,* 2000). Lignolytic enzymes work efficiently for stripping of fabric for reactive dyes within acidic pH and upon elevating pH range above 7, the percent color loss or decolorization clearly suggest the inefficient work of lignolytic enzymes, that may stop working in alkaline range of pH (Alam *et al.,* 2009). Iqbal *et al.*, (2011) has described maximum discoloration of Solar Golden Yellow (a direct dye), up to 83.78% at 30°C temperature and pH 4 with an incubation period of 6 days using white-rot *Trametes versicolor* IBL-04.

Conclusion

In the present research work the potential of indigenous strain of white rot fungi (*Trametes versicolor* IBL-04) was investigated for color stripping of cotton fabric dyed with Sandalfix Black BR 150% dye to develop an inexpensive and environmentally acceptable biochemical stripping process. By optimizing the physical parameters, the fabric color stripping efficiency was significantly improved, and 56.8% color stripping has been achieved by *T. versicolor* under optimum conditions, in biological process for 2% shade strength. The biological stripping proved superior to traditional chemical processing regarding the color removal/stripping of dyed cotton fabric. After initial production of enzymes for textile fabric stripping *T. versicolor* was selected for further optimization so that the production the lignin degrading enzymes may be further enhanced. Optimization for enhanced production of ligninolytic enzymes (laccase, MnP, LiP) was carried out by RSM under CCD. The effect of independent variables such as temperature, pH, inoculum size and incubation time were investigated on stripping percentage and ligninases activities in LSF. Textile industries are the major users of dyes in the world. Bioremediation based technologies has been proved to be the most desirable and cost-effective method to counter textile dye pollution. The ability of the microorganisms to decolorize and metabolize dyes can be employed to treat the environment polluted by textile dyes.

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