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Response Surface Technique for optimization of parameters for decolorization of Reactive red BS using *Trametes hiruta*

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Abstract: The aim of our research was to study the initial dye concentration, pH, temperature and inoculums size on Reactive red B.S dye decolorization using *Trametes hirsuta* have been investigated. The central composite design matrix and response surface methodology (RSM) have been applied to design the experiments to evaluate the interactive effects of four most important operating variables the initial dye concentration (100mg/lit – 300mg/lit), pH (3 – 7), temperature (25°C -37°C) and inoculum size (1- 5 disc) of 4 milli meter fungal mycelia on the biodegradation of Reactive red B.S. The total 31 experiments were conducted in the present study towards the construction of a quadratic model. Very high regression coefficient between the variables and response R^2= 0.9754 indicated excellent evaluation of experimental data by second order polynomial regression model. The RSM indicated that the maximum decolorization of 88% was observed at optimum growth conditions.
Keywords: Decolorization, Degradation, Design of Experiments, Surface surface methodology, *Trametes hirsute.*

1.Introduction

More than 100,000 commercial dyes are available to textile industries worldwide with over 700,000 tons of commercial dyes a year being produced. An estimated 10 – 15% of synthetic dyes are discharged into water ways as effluent which carries the potential to cause environmental damage. Up to 50% of the synthetic dyes are degraded by hydrolysis in process and their environmental fate is unknown [1,2]. Textile and dye stuff industries discharge large volumes of effluent into the environment has become a major concern in waste water treatment since some azo dyes or their metabolities may be mutagens or carcinogens [3]. Removal of color seems to be critical problem for textile industries because it is difficult to treat [4].

Different treatment methods are available for decolorization of dye containing effluents such as, adsorption, oxidation, coagulation, ion exchange, flocculation, electrochemical, membrane technology, chemical degradation and photo degradation, this are under the category of physical or chemical methods [5-7], but these methods have lot disadvantages when compare with the biological methods. In biological methods are give lot advantages such as inexpensive,ecofriendly and do not proudce sludge during the treatment [8,9]. Many microorganisms have been reported for their ability to decolorize the dyes [10,11]. Among the micro organisms, white rot fungi are the most intensively studied, dye decolorizing microorganisms [12], with their lignolytic enzyme systems are involved for the decolorization of textile dyes in to CO_2 annd water [13].

Most optimization studies during the development of a process involve variation of one factor at a time, keeping all other factors constant. But the experiments conducted using the factorial designs, enable all factors to vary simultaneously. This helps in quantifying linear, square and interactive effects of the test variables. Another important advantage is that, the experimental designs could be changed progressively until a fitted model is found to describe the studied phenomenon [14,15]. Response surface methodology (RSM) is an empirical statistical technique employed for multiple regression analysis of quantitative data obtained from statistically designed experiments by solving the multivariate equations simultaneously. The graphical representation of these equations are called as response surfaces, could be used to describe the individual and cumulative effect of the test variables on the response and to determine the mutual interaction between the test variables and their subsequent effect on the response [16,17].

The objective of the present study was to investigate the decolorization of Reactive red BS by *Trametes hirsuta* grown in Laccase production medium by employing Central Composite Design using Response Surface Methodology.

2.Materials and Methods

2.1 Materials

Reactive red B.S, a commercial textile dye was procured from a local company. The purity of dye was 90%. A stock solution of 1000 mg/l of reactive red bs dye was prepared by mixing 1g of dye in one liter of double distilled water and used for further studies by diluting as required.

2.2 Microorganisms and culture conditions

Trametes hirsuta (MTCC-136) a white-rot fungus procured from Institute of Microbial Technology, Chandigarh, India was used in this study. The culture was maintained on YEA medium containing yeast extract – 5 g/l; glucose – 10 g/l and agar-agar – 5 g/l. The pH of the medium was adjusted to 5.8 with dilute sulphuric acid before sterilization. After ten days of incubation at 25°C, the agar slants were stored at 4°C.

2.3 Batch experimental studies

Batch decolorization experiments were carried out using Laccase production medium containing wheat bran flakes 4.5%, yeast extract 1.5%, glucose 1%, NH_4Cl 0.25%, thiamine dichloride 0.05%, KH_2PO_4 0.2%, $MgSO_4.7H_2O$ 0.05%, $CaCl_2$ 0.01% and KCl 0.05% and maintained at 25°C and pH 5.0. The factors affecting the growth and dye decolorizing rate of growing *Trametes hirsuta* were examined in 250ml Erlenmeyer flask with 25 ml accumulation medium containing varying concentrations of aqueous reactive red bs dye solution. The pH of the solution was adjusted to the desired value by using dilute sulphuric acid and autoclaved at 121°C for 15 min. The sterilized accumulation medium was mixed with 25 ml of sterilized enrichment media namely Laccase production medium with desired pH. 4 mm fungal disc was added to the above medium and the culture was grown at 25°C and aeration was maintained by shaking at 120 rpm for 10 days. This shaking frequency supplied the culture with enough oxygen to attain logarithmic growth. For each dye concentration a non-inoculated media was served as blank .The samples were drawn at predetermined time intervals and analyzed for residual dye concentration and biomass concentration. The residual dye concentration in the medium was determined by measuring the absorbance in UV-Spectrophotometer. The dry weight of *Trametes hirsuta* was determined after the organism had been dried at 40°C for 2hrs.

2.4 Experimental design and statistical analysis

Response surface methodology (RSM) was used to optimize the parameters for the decolorization of reactive red bs dye using *Trametes hirsuta*. A central composite experimental design with eight star points ($F = 8$), six axial points and six replicates at the center point ($n_0 = 6$), resulting in a total of 31 experiments ($\alpha = 2$) which covers the entire range of spectrum of combinations of variables, was used for fitting a second order response surface. The experiments were conducted in a randomized fashion. The dependent variable selected for this study was percentage decolorization of dye. The independent variables chosen were initial dye concentration (100-300 mg/l)X_1, initial pH (4-8) X_2, temperature (25°C-37°C)X_3 and inoculum size (1 to 5 disc of 4mm mycelia) X_4 (Table 1).

A mathematical model, describing the relationships among the process dependent variable and the independent variables in a second-order equation, was developed. Design-based experimental data were matched according to the following second-order polynomial equation (1).

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad \text{……... (1)}$$

The quality of fit of the second order equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by *F*-test. The significance of each coefficient was determined using Student’s *t*-test. The coefficients of the equation and Analysis of Variance (ANOVA) for the final predictive equation was done using MINITAB version 15. The response surface equation was optimized for maximum Percentage decolorization of dye and the response surfaces were made by the fitted quadratic polynomial equation obtained from the software, holding independent variables with one parameter at a constant value, and changing the other two variables.

3.Results and discussion

The levels of the chosen independent variables used in the experiments are given in **Table 1.** Thirty one experimental runs were carried out according to central composite four variable designs using Laccase production medium for a period of ten days as per the design, various combination of the four parameters, the results of percentage decolorization of dye in each case are summarized in **Table 2.** A quadratic equation was fitted to the above data, using multiple linear regressions available in the same software as given in Eq. (2).

$$Y=88.000-3.5917X_1+0.8583X_2-1.9917X_3+2.9250X_4-2.9542X_1^2-7.9292X_2^2-12.1792X_3^2-8.9917X_4^2+4.7625X_1X_2-0.0125X_1X_3-2.1500X_1X_4-7.8125X_2X_3+2.5750X_2X_4+5.150X_3X_4 \quad \text{.................. (2)}$$

The fit of the model was checked by the coefficient of determination R^2 which was calculated to be 0.9754, indicating that 97.54% of variability in the response could be explained by the model. The significance of each co-efficient was determined by student's t-test and p-values which are listed in **Table 3.** The larger the magnitude of the t-value and the smaller the P value, the more significant is the corresponding coefficient. In this case, the coefficients X_1, X_2, X_3, X_4, X_1^2, X_2^2, X_3^2, X_4^2 and X_1X_4 were found to be highly significant model terms. The coefficient of interaction terms (X_2X_3, X_2X_4, X_3X_4 & X_1X_4) of temperature and inoculum size was found to be highly significant. The results of analysis of variance for the models used for the decolorization of reactive red bs are given in Table 4. The ANOVA demonstrates that the quadratic model was highly significant, as is evident from the calculated F value (45.32) and a very low probability value (p model > F = 0.0001). It was observed from **Table 4**, the coefficient for the square effects (p = 0.0001) and interaction effects (p = 0.0001) were highly significant when compared with linear effects.

Table 1: Range and level used in central composite design for the decolorization of Reactive red BS by *Trametes hirsuta.*

Independent Variable	Range and Level				
	-α	-1	0	+1	+α
Initial dye concentration (mg/l) (X_1)	100	150	200	250	300
Initial pH (X_2)	4	5	6	7	8
Temperature(°C) (X_3)	25	28	31	34	37
Inoculum size (4mm disc) (X_4)	1	2	3	4	5

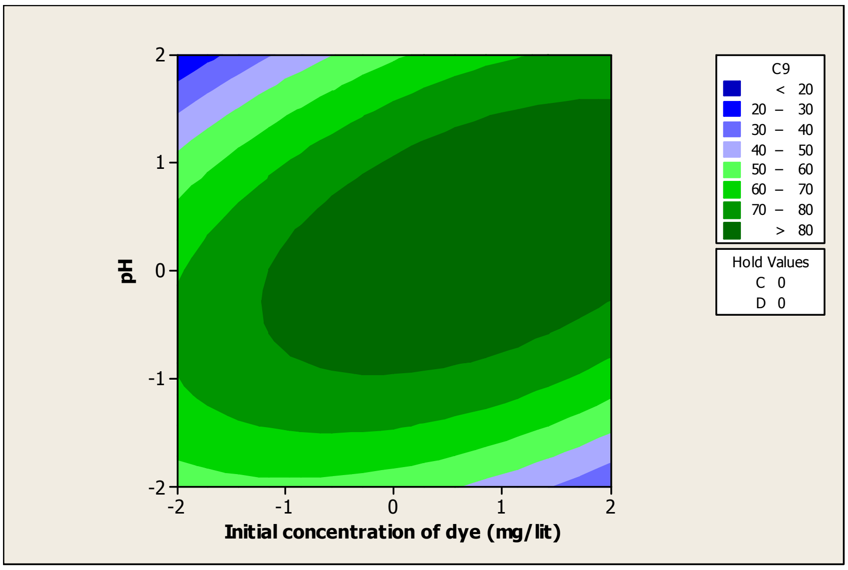


Fig.1 Response surface contour plot showing interactive effect of initial dye concentration and pH on percentage decolorization of dye.

Table 2: Central Composite design matrix of orthogonal and real values along with observed responses for the decolorization Reactive red BS.

Run no.	Independent Variables								Response (% decolorization)	
	Orthogonal Values				Real Values				Exp	Pre
1	0	-2	0	0	200	4	31	1.5	57.6	54.56
2	0	0	0	-2	200	6	31	0.5	45.8	46.183
3	0	0	0	0	200	6	31	1.5	88.0	88.0
4	0	0	0	0	200	6	31	1.5	88.0	88.0
5	1	-1	1	-1	250	5	34	1.0	55.0	56.37
6	-1	1	1	1	150	7	34	2.0	48.0	51.45
7	-1	1	-1	-1	150	7	28	1.0	54.0	55.742
8	1	1	1	1	250	7	34	2.0	66.8	63.84
9	2	0	0	0	300	6	31	1.5	79.7	83.367
10	0	0	0	0	200	6	31	1.5	88.0	88.0
11	-1	1	1	-1	150	7	34	1.0	30.1	25.858
12	-1	-1	1	1	150	5	34	2.0	65.2	69.742
13	1	1	-1	1	250	7	28	2.0	76	73.17
14	0	0	-2	0	200	6	25	1.5	39.4	43.267
15	1	-1	-1	-1	250	5	28	1.0	58.9	55.058
16	0	0	0	0	200	6	31	1.5	88.0	88.0
17	-1	-1	-1	-1	150	5	28	1.0	50.4	53.075
18	0	0	0	0	200	6	31	1.5	88.0	88.0
19	1	1	1	-1	250	7	34	1.0	43.6	46.842
20	1	-1	-1	1	250	5	28	2.0	37.2	41.158
21	0	0	0	2	200	6	31	2.5	57.6	57.883
22	0	0	2	0	200	6	37	1.5	38.5	35.3
23	0	2	0	0	200	8	31	1.5	54.3	58.0
24	1	1	-1	-1	250	7	28	1.0	81.6	76.775
25	-1	1	-1	1	150	7	28	2.0	62.4	60.74
26	-1	-1	1	-1	150	6	34	1.0	52.0	54.442
27	-2	0	0	0	100	6	31	1.5	72.0	69.0
28	0	0	0	0	200	6	31	1.5	88.0	88.0
29	1	-1	1	1	250	5	34	2.0	65.2	63.075
30	0	0	0	0	200	6	31	1.5	88.0	88.0
31	-1	-1	-1	1	150	5	28	1.0	51.4	47.77

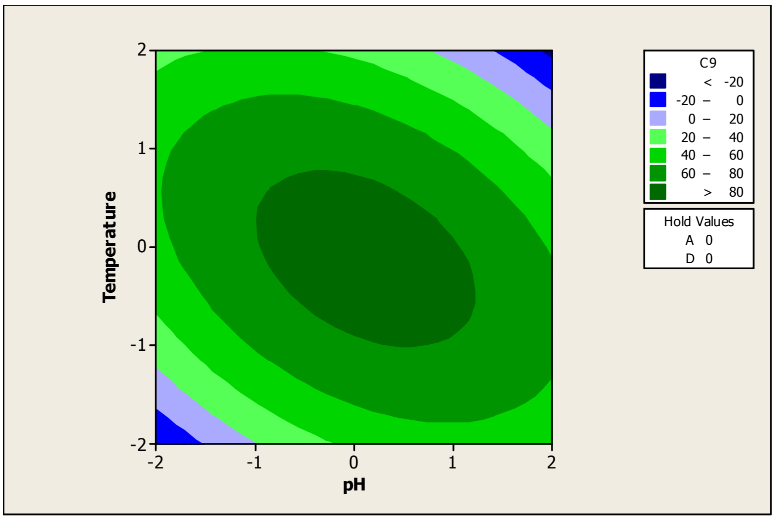


Fig. 2. Response Surface Contour plot showing interactive effect of pH and Temperature on percentage decolorization of dye.

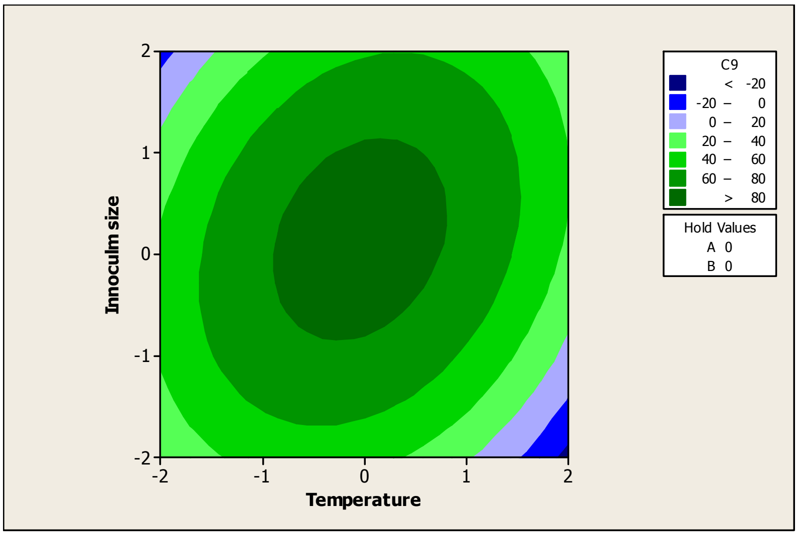


Fig. 3. Response Surface Contour plot showing interactive effect of temperature and inoculum size on percentage decolorization of dye.

Table 3: Significance of regression coefficients obtained for the decolorization of Reactive red BS.

Model Term	Parameter estimate (Coefficients)	T	P
Constant	88.00	60.024	0.000
X_1	3.5917	4.536	0.000
X_2	0.8583	1.084	0.294
X_3	-1.9917	-2.515	0.023
X_4	2.9250	3.694	0.002
$X_1 *X_1$	-2.9542	-4.073	0.001
$X_2 *X_2$	-7.9292	-10.931	0.000
$X_3 *X_3$	-12.1792	-16.791	0.000
$X_4 *X_4$	-8.9917	-12.396	0.000
$X_1* X_2$	4.7625	4.911	0.000
$X_1 *X_3$	-0.0125	-0.013	0.990
$X_1 *X_4$	-2.1500	-2.217	0.041
$X_2 *X_3$	-7.8125	-8.056	0.000
$X_2 *X_4$	2.5750	2.655	0.017
$X_3 *X_4$	5.150	5.311	0.000

X_1, X_2, X_3, X_4= Linear effects
X_1^2, X_2^2, X_3^2, X_4^2= Squared effects
X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4 = Interaction effects

Table 4 : Analysis of Variance (ANOVA) for the selected quadratic model for the decolorization of Reactive red BS.

Sources of variation	Sum of squares	Degrees of Freedom	Mean square	F	P
Regression	9545.15	14	681.80	45.32	0.000
Linear	627.82	4	156.96	10.43	0.000
Square	6973.46	4	1743.36	115.87	0.000
Interaction	1943.88	6	323.98	21.53	0.000
Residual error	240.73	16	15.05		
Total	9785.89	30			

Linear effect = Significant; Squared effect = Significant; Interaction effect = Significant

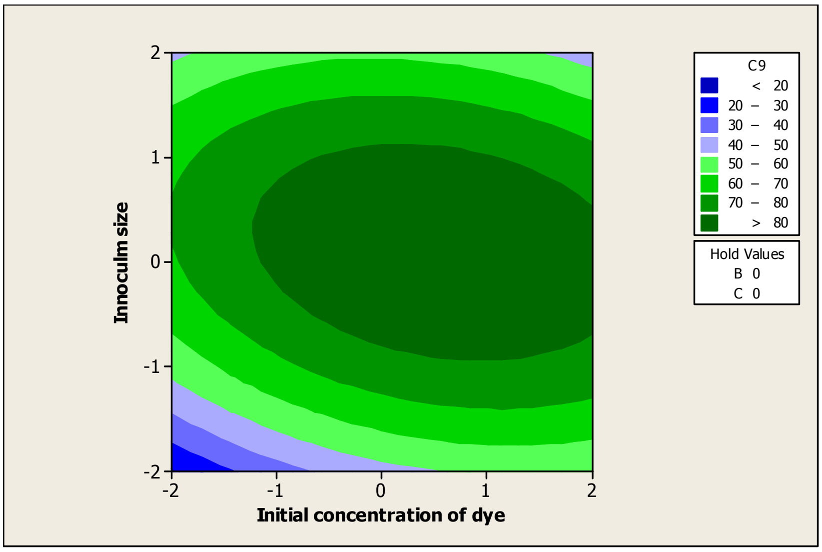


Fig. 4. Response Surface Contour plot showing interactive effect of initial dye concentration and inoculum size on percentage decolorization of dye.

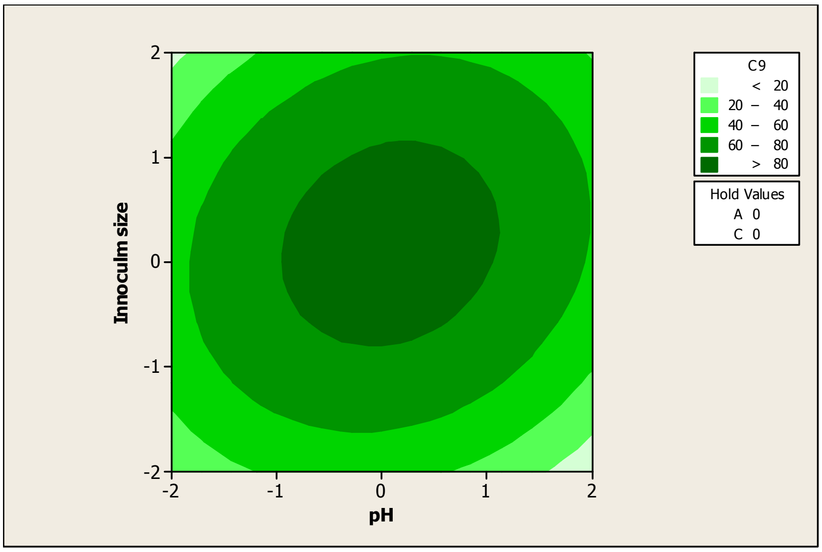
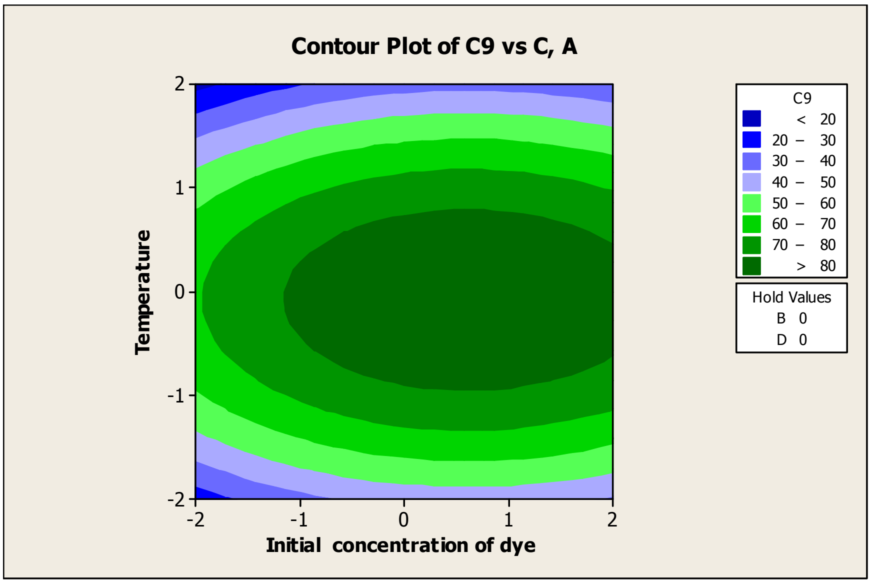
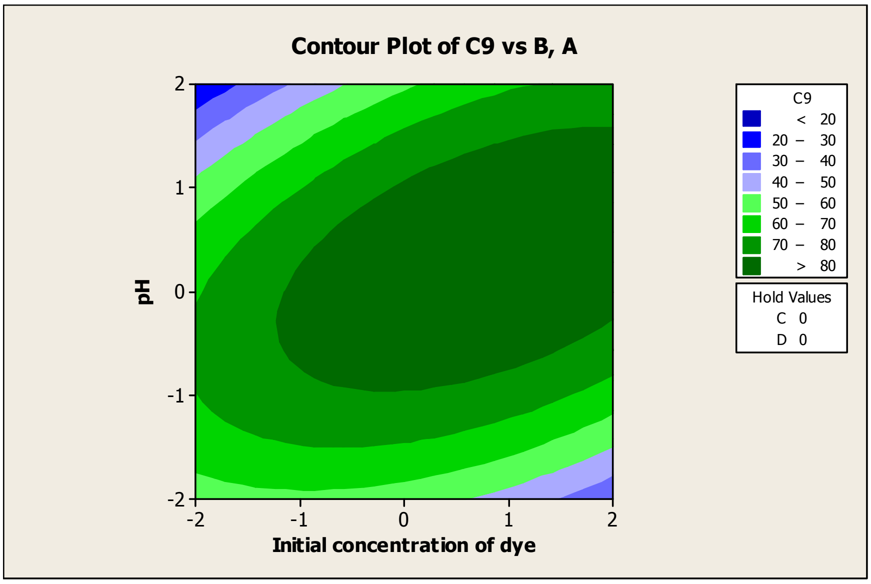


Fig. 5. Response Surface Contour plot showing interactive effect of initial pH and inoculum size on percentage decolorization of dye.

Figs.1, 2 and 3 shows the contour plot for the interactive effects of initial dye concentration, pH, temperature and inoculum size on percentage decolorization of dye. From all the figures, it was observed that the lower and higher levels of all the variables did not result in higher percentage decolorization. The interactions between initial dye concentration and inoculum size were apparent not only from the low probability value ($P < 0.041$, Table 3), but also from the elliptical contour plot (Fig. 4). Since a circular contour plot indicates that the interactions between the corresponding variables are negligible, while an elliptical contour plot indicates that the interactions between them are significant. The other pair of the independent variables pH and inoculum size showed similar effects (Fig. 5). The percentage decolorization of dye was significantly affected by pH, temperature and inoculum size where initial pH and inoculum size producing greater effects. Response surface analysis revealed the maximum percentage decolorization of dye using Laccase production medium by *Trametes hirsuta* could be achieved at the optimum conditions and the optimum values of the parameters X_1, X_2. X_3 and X_4 were found to be 246 mg/l, 6.4, 30.5°C and 3disc of 4mm mycelium. Under these optimum process conditions a maximum percentage dye decolorization of 92% was obtained.





4.Conclusion

The use of an experimental design permitted the rapid screening of a large experimental domain for optimization of percentage decolorization ability of fungal strain *T.hirsuta*. The fit of the model is checked by determination coefficient R^2.In this case, the value of determination coefficient R^2 is 0.9754 indicates the mean about negligible of total variations were not explained by the model. The optimized conditions for highest decolorization 88% of Reactive red B.S in simulated dye solution are at initial dye concentration of 245 mg/lit, pH 6.45, Temperature 30.75^{o}C and inoculums size 3.01 disc of 4 milli meter mycelia.

References

[1] Robinson, and T, Mcmullan, G, 2001, “Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative”, Bioresource Technol, 77(3), 247- 255.

[2] Park, C, and Lee, M, 2007 “Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*”, Bio chem.Eng. J., 36(1),59 - 65.

[3] Harazono, K, and Nakamura, K, 2005, “Decolorization of mixtures of different reactive

textile dyes by the white – rot basidiomycete *phanerochaete ordid* and inhibitory effect of polyvinyl alcohol", Chemosphere, 59(1), 63-68.

[4] Pratum, C, Wongthanate, J, Arunlertaree, C, Prapagdee, B, 2011, "Decolorization of reactive dyes and textile dyeing effluent by *Pleurotus sajor-caju",* Int.J.of integrative Biology, 11(1), 52-57.

[5] Banat, I. M, Nigem, P, Singh, D, Marchant R, 1996, " Microbial Decolorization of textile-Dye-containg effluents: A Review", Bioresourse, 217-227.

[6] Aksu, Z, and Tezer, S, 2000 **"**Equilibrium and kinetic modelling of biosorption of Remazol Black B by *Rhizopus arrhizus* in a batch system: effect of temperature", Process Biochemistry, 36, 431-439.

[7] Aksu, Z, 2003, **"**Reactive dye bioaccumulation by *Saccharomyces cerevisiae"***,** Process Biochemistry, 38, 1437-1444.

[8] Akar, S. T, Akar, T, Cabuk A, 2009, "Decolorization of a textile dye, reactive red 198 (rr198), by *Aspergillus parasiticus* fungal biosorbent"**,** Brazilian Journal of Chemical Engineering, 26, 399-405.

[9] Akar, T, Tunali, S, 2005, "Biosorption performance of Botrytis cinerea fungal by-products for removal of Cd(II) and Cu(II) ions from aqueous solutions", Minerals Engineering, 18, 1099-1109.

[10] Chang, J. S, Chou, C, Chen, S. Y, 2001, "Decolorization of azo dyes with immobilized *Pseudomonas luteola".* Process Biochem, 36, 757–763.

[11] Khehra, M. S, Saini, H. S, Sharma, D. K, Chadha, B. S, Chimmi, S. S, 2005, "Decolorization of various azo dyes by bacterial consortium", Dyes Pig, 67, 55–61.

[12] Coughlin, M. F, Kinkle, B. K, Bishop, P. L, 1999, "Degradation of azo dyes containing aminonaphthol by *Sphingomonas* sp. strain 1CX". J. Ind. Microbiol.Biotechnol, 23, 341–346.

[13] Wasenberg, D, Kyriakides, I, Agathos, S. N, 2003, " White rot fungi and their enzymes for the treatment of industrial dye effluents", Biotechnol.Adv. 22, 161-187.

[14] Box, G. E, and Draper, N. R, 1987, "Empirical Model Building and Response Surfaces", Wiley, New York.

[15] Mason, R. I, Gunst, R. F, Hess, J. L, 1989, " Statistical Design and Analysis of Experiments", Wiley, New York.

[16] Khuri, A. I, and Cornell, J. A, 1987, "Response Surfaces: Design and Analysis", Marcel Dekker, New York.

[17] Montgomery, D.C, 1991," Design and Analysis of Experiments", 3rd ed., Wiley, New York.

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