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# Optimization of parameters for decolorization of Coomassie Brilliant Blue using Trametes hirsute

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## ABSTRACT

The widely used textile dye Coomassie Brilliant Blue effluent was treated using Laccase producing Trametes hirsuta, by employing statistical optimization using Response Surface Methodology (RSM). A $2^4$ Central Composite Design (CCD) was chosen to evaluate the interactive effects of four variables in different ranges namely the initial dye concentration, pH, temperature and inoculum size respectively. Experiments were conducted in the present study towards the construction of a quadratic model. Model validation showed a good agreement between experimental results and the predicted responses. Maximum decolorization of dye 94% was observed at optimum process conditions at initial dye concentration of 180.5 mg/l, initial pH of 5.5, temperature of 31$^{o}$C and inoculum size of 4mm of 3 mycelium disc.

**Keywords:** Decolorization, Degradation, Design of Experiments, Response Surface Methodology, Trametes hirsuta

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## INTRODUCTION

Colour removal of industrial effluents has been a major concern in wastewater treatment, especially for wastewater that originates from textile and dyestuff plants with a continuous discharge of a great quantity of remaining dyes to the environment [1]. Normally colors are noticeable at a dye concentration of more than 1 mg/l and an average concentration of 300 mg/l have been reported in effluents from textile manufacturing processes [2].

Dye production estimated in 1978 was that 2%, (or 9000 tones) of the 4,50,000 tones of dye produced worldwide is discharged as effluent from manufacturing operations. While 40,000 tones, or 9% is from the coloration industries, making the total to 50,000 tons of colored effluents [3]. Today nearly one million metric tons of dye is annually produced in the world of which azo dyes ($R_1$-N=N-$R_2$), represent about 70% on weight basis [4]. In India dyestuff industry produces around 60,000 metric tons of dyes, which is approximately 6.6% of total world output [5]. The largest consumer of these dyes is the textile industry accounting for two third of its market. Recent estimates indicate that 12% of the synthetic textile dyes used yearly are lost to wastewater streams. Approximately 20% of these enter the environment through effluents from wastewater treatment plant [6].

Dyes and their degradation products are carcinogenic in nature [7]. A review of the mutagenicity of effluents showed that textile and other dye-related industries produce consistently more potent wastewaters when compared to other industrial discharges [8]. Recent studies shown that azo dyes contribute to mutagenic activity of ground and surface waters polluted by textile effluents [9,10]. Further, their discharge into surface water leads to aesthetic problems and obstructs light penetration and oxygen transfer into water bodies, hence affecting aquatic life. Thus, the removal of color from textile effluents has been a major concern. So the dye containing industrial effluents must be treated before discharge into the environment.

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 [11], these methods have its own demerits when compared with biological methods. In biological methods major advantages are inexpensive, ecofriendly and do not produce sludge during the treatment [12]. Many microorganisms have been reported for their ability to decolorize the dyes [13] Among the micro organisms, white rot fungi are the most intensively studied, dye decolorizing microorganisms [14], with their lignolytic enzyme systems are involved for the decolorization of textile dyes in to $CO_2$ and water [15].

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 [16]. Another important advantage is that, the experimental designs could be changed progressively until a fitted model is found to describe the studied phenomenon [17]. Response surface methodology is an empirical statistical technique employed for multiple regression analysis of quantitative data obtained from statistically designed experiments by solving the multivariable equations simultaneously [18]. 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 [19].

The objective of the present study was to investigate the decolorization of Coomassie Brilliant Blue by Trametes hirsuta grown in laccase production medium by employing Central Composite Design using Response Surface Methodology.

## MATERIALS AND METHODS

Trametes hirsuta (MTCC-136), a white-rot fungus procured from Institute of Microbial Technology, Chandigarh, and 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 – 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$^{o}$C, the agar slants were stored at 4$^{o}$C.

Coomassie Brilliant blue (CBB), a commercial textile dye was procured from a local company. The purity of dye was 90%. A stock solution of 1000 mg/l of dye was prepared by mixing 1g of coomassie brilliant blue dye in one liter of double distilled water and used for further studies by diluting as required.

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$^{o}$C. 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 dye solution. The pH of the solution was adjusted to the desired value by using dilute sulphuric acid and autoclaved at 121$^{o}$C for 15 min. The sterilized accumulation medium was mixed with 25 ml sterilized enrichment media namely Laccase production medium with desired pH. 4mm mycelium disc was added to the above medium and the culture was grown at 25$^{o}$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 at 523 nm using UV-Spectrophotometer. The dry weight of Trametes hirsuta was determined after the organism had been dried at 40$^{o}$C for 2hrs.

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**Experimental design and statistical analysis:**

Response surface methodology was used to optimize the parameters for the decolorization of 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 (α = 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 (1-5 Disc) of 4mm mycelia $X_4$ (Table 1).

**Table 1: Range and level used in central composite design for the decolorization of dye 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 (Number of 4mm disc) ($X_4$) | 1 | 2 | 3 | 4 | 5 |

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).

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

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.

## 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,

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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).

$$Y=93.3871-5.0464X_1-8.0105X_2+3.7382X_3+2.4572X_4-101678X_1^2-11.5153X_2^2-71515X_3^2-11.5628X_4^2+0.5492X_1X_2+91961X_1X_3-7.6442X_1X_4-4.8252X_2X_3-7.8854X_2X_4-4.7823X_3X_4 \quad (2)$$

**Nomenclature:**

$x_i$ = Coded value of the $i^{th}$ variable
$X_i$ = Uncoded value of the $i^{th}$ test variable
$X_o$ = Uncoded value of the $i^{th}$ test variable at the center point
Y = Predicted response
$\beta_o$ = Offset term
$\beta_i$ = Coefficient of linear effect
$\beta_{ii}$ = Coefficient of square effect
$\beta_{ij}$ = Coefficient of interaction effect

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

| Run no. | Independent Variables | | | | | | | | Response (% decolorization) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Orthogonal Values | | | | Real Values | | | | Exp | Pre |
| | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_1$ | $X_2$ | $X_3$ | $X_4$ | | |
| 1 | 0 | -2 | 0 | 0 | 200 | 4 | 31 | 1.5 | 71.34 | 64.95 |
| 2 | 0 | 0 | 0 | -2 | 200 | 6 | 31 | 0.5 | 25.86 | 43.82 |
| 3 | 0 | 0 | 0 | 0 | 200 | 6 | 31 | 1.5 | 94.85 | 93.39 |
| 4 | 0 | 0 | 0 | 0 | 200 | 6 | 31 | 1.5 | 95.02 | 92.90 |
| 5 | 1 | -1 | 1 | -1 | 250 | 5 | 34 | 1.0 | 70.69 | 75.95 |
| 6 | -1 | 1 | 1 | 1 | 150 | 7 | 34 | 2.0 | 21.95 | 37.53 |
| 7 | -1 | 1 | -1 | -1 | 150 | 7 | 28 | 1.0 | 50.78 | 53.46 |
| 8 | 1 | 1 | 1 | 1 | 250 | 7 | 34 | 2.0 | 30.52 | 31.44 |
| 9 | 2 | 0 | 0 | 0 | 300 | 6 | 31 | 1.5 | 37.55 | 42.12 |
| 10 | 0 | 0 | 0 | 0 | 200 | 6 | 31 | 1.5 | 90.41 | 92.15 |
| 11 | -1 | 1 | 1 | -1 | 150 | 7 | 34 | 1.0 | 56.85 | 4216 |
| 12 | -1 | -1 | 1 | 1 | 150 | 5 | 34 | 2.0 | 71.79 | 79.67 |
| 13 | 1 | 1 | -1 | 1 | 250 | 7 | 28 | 2.0 | 24.52 | 24.58 |
| 14 | 0 | 0 | -2 | 0 | 200 | 6 | 25 | 1.5 | 66.05 | 56.91 |
| 15 | 1 | -1 | -1 | -1 | 250 | 5 | 28 | 1.0 | 50.58 | 30.67 |
| 16 | 0 | 0 | 0 | 0 | 200 | 6 | 31 | 1.5 | 95.81 | 94.05 |
| 17 | -1 | -1 | -1 | -1 | 150 | 5 | 28 | 1.0 | 37.67 | 45.56 |
| 18 | 0 | 0 | 0 | 0 | 200 | 6 | 31 | 1.5 | 92.03 | 93.00 |
| 19 | 1 | 1 | 1 | -1 | 250 | 7 | 34 | 1.0 | 76.05 | 67.55 |
| 20 | 1 | -1 | -1 | 1 | 250 | 5 | 28 | 2.0 | 22.63 | 45.63 |
| 21 | 0 | 0 | 0 | 2 | 200 | 6 | 31 | 2.5 | 75.68 | 53.65 |
| 22 | 0 | 0 | 2 | 0 | 200 | 6 | 37 | 1.5 | 66.78 | 71.86 |
| 23 | 0 | 2 | 0 | 0 | 200 | 8 | 31 | 1.5 | 30.58 | 32.91 |
| 24 | 1 | 1 | -1 | -1 | 250 | 7 | 28 | 1.0 | 40.63 | 41.56 |

ISSN: 0975-8585

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 25 | -1 | 1 | -1 | 1 | 150 | 7 | 28 | 2.0 | 64.31 | 67.46 |
| 26 | -1 | -1 | 1 | -1 | 150 | 6 | 34 | 1.0 | 58.06 | 5316 |
| 27 | -2 | 0 | 0 | 0 | 100 | 6 | 31 | 1.5 | 71.55 | 62.41 |
| 28 | 0 | 0 | 0 | 0 | 200 | 6 | 31 | 1.5 | 93.19 | 93.25 |
| 29 | 1 | -1 | 1 | 1 | 250 | 5 | 34 | 2.0 | 78.8 | 71.78 |
| 30 | 0 | 0 | 0 | 0 | 200 | 6 | 31 | 1.5 | 92.3 | 93.85 |
| 31 | -1 | -1 | -1 | 1 | 150 | 5 | 28 | 1.0 | 86.53 | 90.69 |

The fit of the model was checked by the coefficient of determination $R^2$ which was calculated to be 0.8490, indicating that 84.90% 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 $X_2$, $X_1^2$, $X_2^2$, $X_3^2$, $X_1X_3$, $X_1X_4$ and $X_2X_4$ were found to be highly significant model terms. It was found from the coefficient $X_1$, the percentage decolorization was higher at low initial dye concentration. Because higher dye concentrations inhibit the microbial growth. The effect of pH was found to be highly significant (p = 0.009). The coefficient of interaction terms ($X_1*X_3$; $X_1*X_4$ and $X_2*X_4$) of concentration, pH, temperature and inoculums size was found to be highly significant. The results of analysis of variance for the models used for the decolorization of dye are given in Table 4. The ANOVA demonstrates that the quadratic model was highly significant, as is evident from the calculated F value (6.43) 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 = 0.014) were highly significant when compared with linear effects.

**Table 3: Significance of regression coefficients obtained for the decolorization of dye.**

| Model Term | Parameter estimate ( Coefficients) | T | P |
|---|---|---|---|
| Constant | 93.3871 | 18.765 | 0.000 |
| $X_1$ | -5.0464 | -1.878 | 0.079 |
| $X_2$ | -8.0105 | -2.980 | 0.009 |
| $X_3$ | 3.7382 | 1.391 | 0.583 |
| $X_4$ | 2.4573 | 0.914 | 0.374 |
| $X_1*X_1$ | -101678 | -4.570 | 0.001 |
| $X_2*X_2$ | -11.5153 | -4.514 | 0.000 |
| $X_3*X_3$ | -71515 | -2.945 | 0.010 |
| $X_4*X_4$ | -11.5628 | -4.533 | 0.000 |
| $X_1*X_2$ | 0.5492 | 0.567 | 0.870 |
| $X_1*X_3$ | 91961 | 2.824 | 0.012 |
| $X_1*X_4$ | -7.6442 | -2.322 | 0.034 |
| $X_2*X_3$ | -4.8252 | -1.466 | 0.562 |
| $X_2*X_4$ | -7.8854 | -2.395 | 0.029 |
| $X_3*X_4$ | -4.7823 | -1.453 | 0.566 |

$X_1$, $X_2$, $X_3$, $X_4$= 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
$X_2$ = Significant; $X_1^2$, $X_2^2$, $X_3^2$= Significant; $X_1*X_3$; $X_1*X_4$, $X_2*X_4$= Significant; $X_2*X_4$= Significant

ISSN: 0975-8585

**Table 4: Analysis of Variance (ANOVA) for the selected quadratic model for the decolorization of dye.**

| Sources of variation | Sum of squares | Degrees of Freedom | Mean square | F | P |
|---|---|---|---|---|---|
| Regression | 15597.3 | 14 | 1114.50 | 6.43 | 0.000 |
| Linear | 2631.5 | 4 | 657.88 | 3.79 | 0.024 |
| Square | 8910.5 | 4 | 2227.52 | 12.85 | 0.000 |
| Interaction | 4055.8 | 6 | 675.96 | 3.90 | 0.014 |
| Residual error | 2774.5 | 16 | 173.38 | | |
| Total | 18371.4 | 30 | | | |

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

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 ($P < 0.034$, 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 temperature showed similar effects (Fig. 5). 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 180.5 mg/l, 5.5, 31.2°C and 4mm of 3 myclium disc. Under these optimum process conditions a maximum percentage dye decolorization of 94% was obtained.

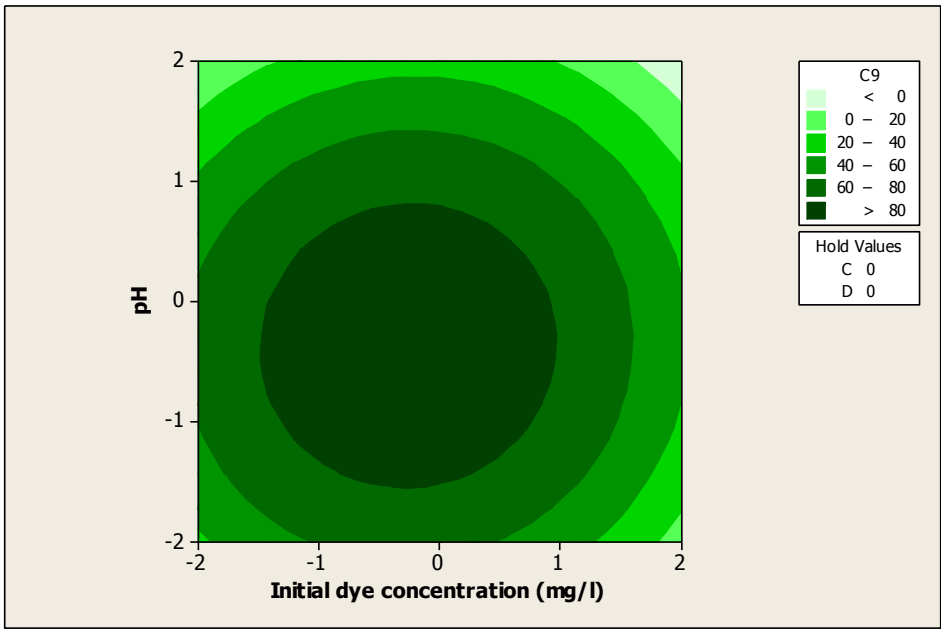

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Fig. 1: Response surface contour plot showing interactive effect of initial dye concentration and pH on percentage decolorization of dye.

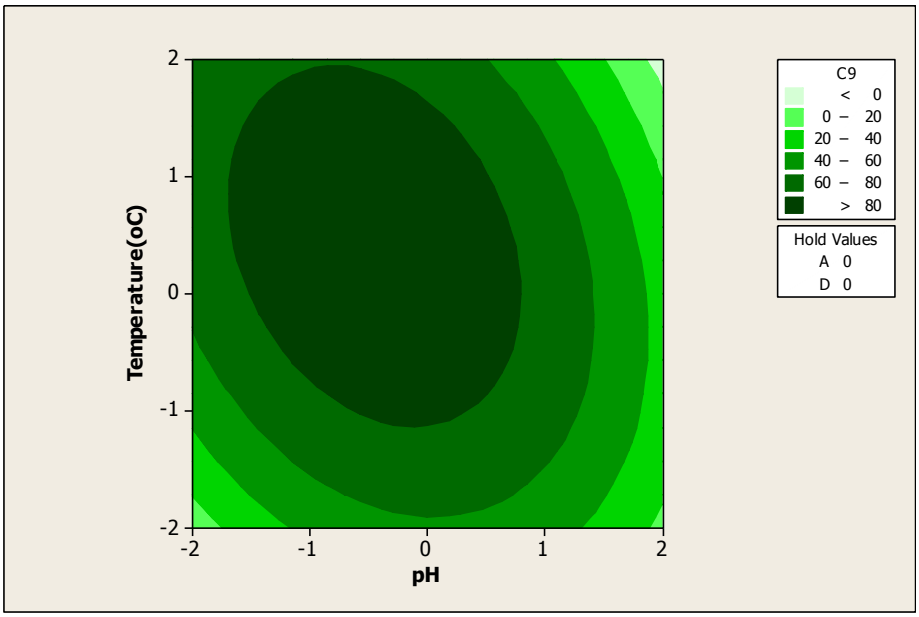


Fig. 2: Response Surface Contour plot showing interactive effect of pH and Temperature on percentage decolorization of dye.

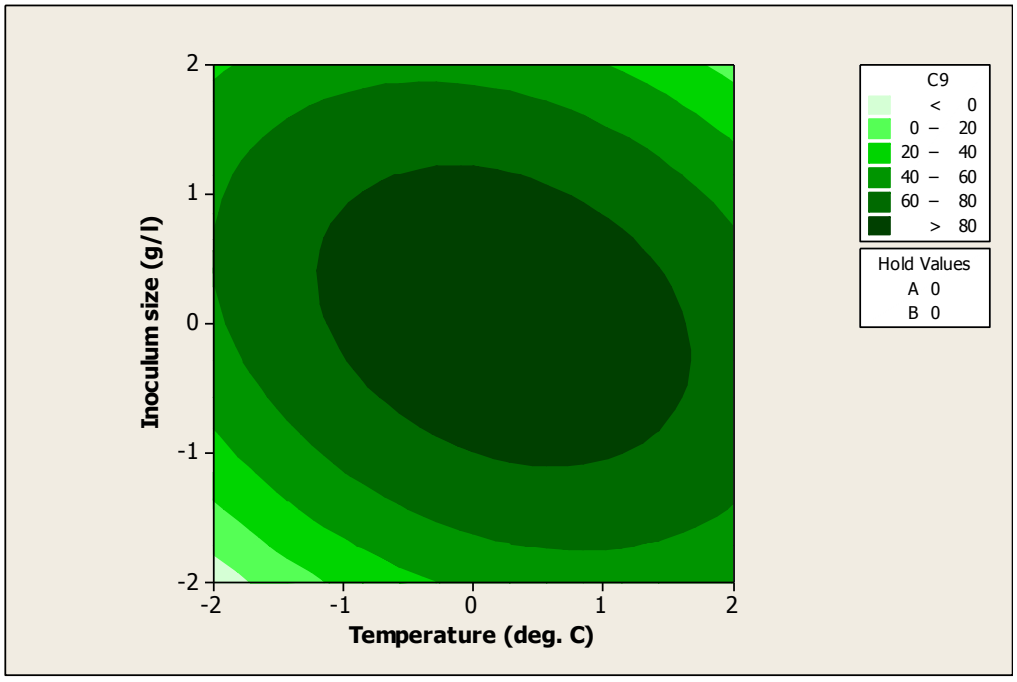


Fig. 3: Response Surface Contour plot showing interactive effect of temperature and inoculum size on percentage decolorization of dye.

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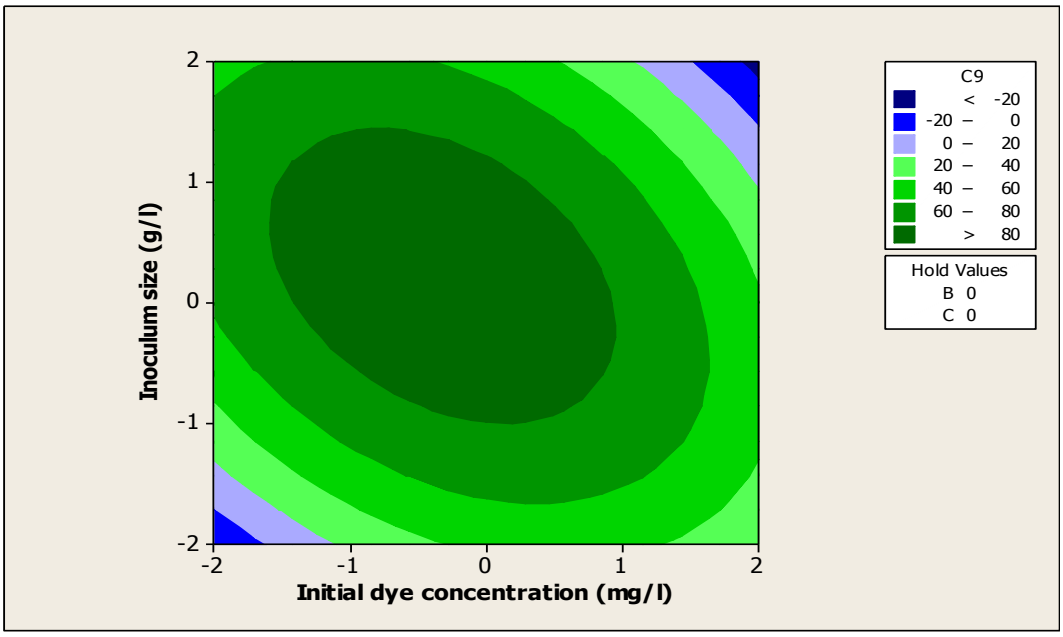


**Fig. 4: Response Surface Contour plot showing interactive effect of initial dye concentration and inoculum size on percentage decolorization of dye.**

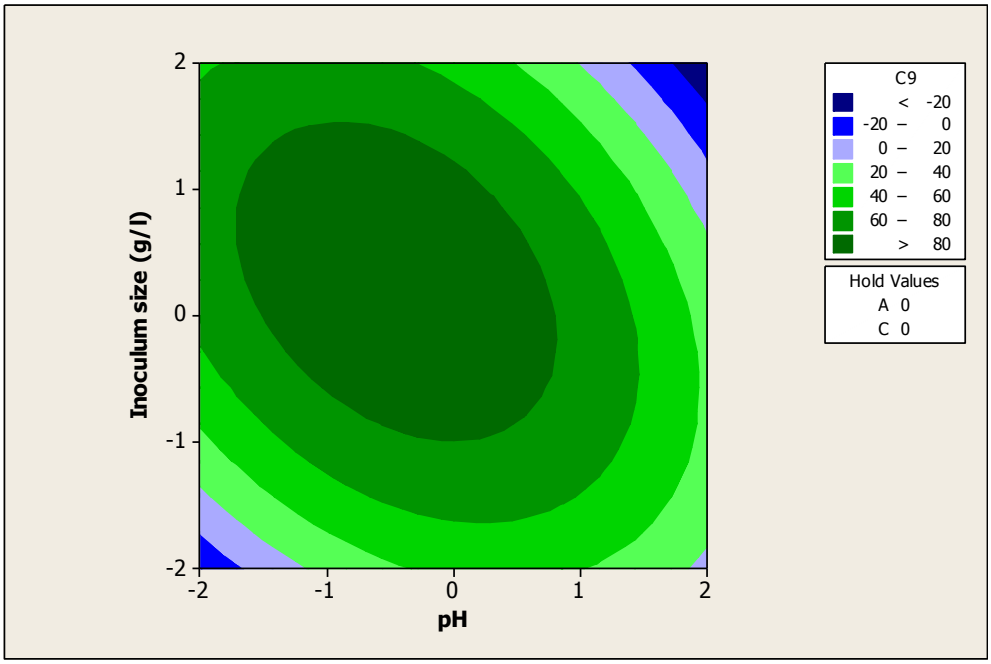


**Fig. 5: Response Surface Contour plot showing interactive effect of initial pH and inoculum size on percentage decolorization of dye.**

## CONCLUSION

The application of Trametes hirsuta grown in Laccase production medium to decolorize the synthetic dye seemed to be one of a pragmatic approach with maximum decolorization of

94% using central composite design. The optimum process conditions namely initial dye concentration, pH, temperature and inoculum size was found to be 180.5 mg/l, 5.5, 31.2°C and 4mm of 3 mycelium disc. Hence Trametes hirsuta could be successfully employed to decolorize commassie brilliant blue present in the industrial effluent.

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