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Exploring Bio sorption potential of dry immobilized biomass of cyanobacteria for removal of triphenylmethane dye under thermal conditions

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Abstract
Dry composite biomass of cyanobacterial consortium collected from thermal springs was used as biosorbent in immobilized form for decolorization of Crystal violet (CV) dye under thermal conditions that generally prevail in textile effluent. Response surface methodology (RSM) was employed to investigate the effect of process variables on the biological treatment of dye containing water. The variables investigated were pH, initial dye concentration and temperature using Box-Behnkhen Design (BBD) model. Predicted values were found to be in good agreement with experimental values (R^2=0.9501), which indicated suitability of the model. Maximum decolorization occurred at pH-6, 150mg/l dye concentration and 45ºC indicating suitability of biosorbent for removal of Crystal violet dye.

Keywords: Cyanobacteria, immobilized, consortium, crystal violet, RSM

1. introduction

Dyes and pigments are used extensively in several industries including textiles, printing, food, paper, plastic and cosmetics and hence, are common contaminants in such industrial wastewaters some of these dyes are known to be toxic and carcinogenic in nature. Triphenylmethane dyes are a group of dyes used extensively in a variety of industries (Gregory, 1993) and which have potential toxicity to animals and humans. Crystal violet is a triphenylmethane dye that is used extensively as a biological stain, as a dermatological agent and as a colorant in various commercial textile operations (Kumar et al., 2006). It is also known as hexamethyl pararosaniline chloride, a basic dye with molecular formula $C_{25}H_{30}N_3Cl$ and chemical structure as shown in fig. 1. The dye is reported to be a mitotic poisoning agent, which is recalcitrant and carcinogenic, and thus regarded as a biohazard (Au et al., 1978). Its removal from wastewaters is therefore, essential for environmental safety. However, conventional wastewater treatment facilities have proved to be rather ineffective in removing commercial dyestuffs including crystal violet dye from wastewaters (Shaul et al., 1991). Biosorption is considered to be more efficient and economical method for the treatment of wastewaters containing dyes, pigments and other colorants (Akbal, 2005).

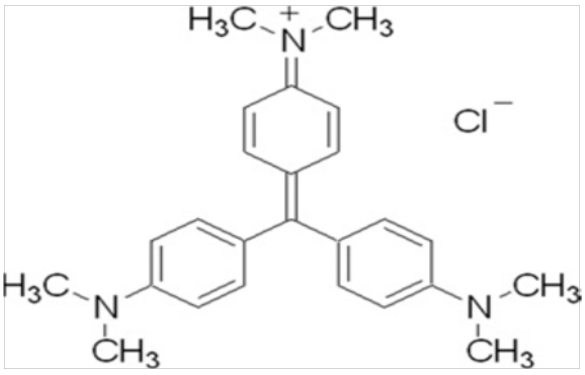

Fig.1: Chemical structure of crystal violet

While there are a number of reports on decolorization of crystal violet dye under normal temperatures, its removal under high temperature has been found to decline (Mona et al., 2011). In the present study we explored therefore, dead immobilized cyanobacterial biomass from hot spring for removal of the dye under thermal conditions. Considering the advantages of immobilized cells over free cell systems (Chu et al., 2009), the dead biomass of thermophilic consortium was immobilized in calcium alginate before using it as a biosorbent. Full range of response surface methodology (RSM) approach was adopted using Box-Behnkhen design (BBD) model to analyze the effectively different process variables and optimize their combinations for maximum response.

2. Materials& Methods

2.1. Preparation of biosorbent from composite cyanobacterial biomass

Cyanobacterial biomass along with spring water was collected in sterilized reagent bottles from two hot springs, at Sohana, Haryana (28º14'47.67"and 77º3'52.06") and Manikaran, Himachal Pradesh (32º02'70.11"and 77º34'49.40"), India. Inoculum of the two consortia (10 ml each) was added separately to sterilized BG-11 medium (Steiner et al., 1971) taken in 250 ml Erlenmeyer flasks under aseptic conditions. The pH of the culture medium was adjusted to 7.5±0.5 using 0.1N HCl or 0.1N NaOH solutions before autoclaving. It was incubated under continuous illumination of 3000lux light intensity at 45ºC in an orbital shaker (Orbitek LT-IL) at 120rpm for two months and sub culturing was done after every 15 days for maintenance of the culture in active form. The consortial biomass was then harvested during the exponential growth phase (7th day of incubation) and concentrated by centrifugation and mixed together (1:1 w/w) to form a composite consortium that was used for further experiments after oven drying at 70ºC. For immobilization, known quantity of dry composite biomass was added into the 0.5g per 25ml of the sodium alginate solution and it was then dropped into 0.025M calcium chloride solution using the 25ml burette (3mm ±1 diameter). The cyanobacterial beads formed were kept overnight at 4ºC in the $CaCl_2$ solution for complete gelation process. The beads were then washed with double distilled water and stored in distilled water for further experimental work.

2.2. Preparation of dye solution and its estimation

Stock solution of the dye (1g/L) for biosorption experiments was prepared by dissolving textile dye Crystal violet obtained from a textile mill in aqueous solution. The desired dye solutions were prepared from this stock by dilutions with water. For each experimental run 100ml of the dye solution was taken with required concentration of the dye and samples from the flasks are withdrawn at required time intervals for measuring dye removal.

Absorbance of the dye solution was measured at 590nm (λ_{max}) using Biomate UV-visible spectrophotometer to determine residual dye concentration. A standard curve was plotted taking different concentrations of crystal violet solution in the medium. Dye biosorption was calculated in different batch experiments as follows:

$$\text{Removal of the dye (\%)} = C_o - C_e / C_o \times 100 \quad (1)$$

Where C_o is the initial dye concentration (mg/L) and C_e is the residual concentration of the dye (mg/L) at different time intervals.

2.3. Optimization of biosorption using response surface methodology (RSM) approach.

The RSM approach was adopted in order to achieve best dye removal response from the biosorbent by experimenting at different combinations of the operating variables viz., pH, initial dye concentration and temperature at the already optimized contact time 3h and pH 7. The quadratic model used is expressed as follow:

$$Y=\beta_o + \sum \beta_i X_i + \sum \beta_{ii} X^2_{ii} + \sum \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

Where β_o is the constant coefficient, β_i the slope or linear effect of the input factor, X_i and β_{ij} the linear by linear interaction effect between the input factor X_i, X_j and β_{ij} and is the quadratic effect of input factor X_i (Benyounis et al., 2005).

Box-Behnken Design (BBD) model of RSM was used in the study with two levels (the minimum and the maximum) for each of the three parameters. For calculation of 10 coefficients of second-order polynomial equation 17 experiments are required to be performed.

In the experimental design model, pH (6–8), initial dye concentration (50-150mg/L) and temperature (40-50ºC) were taken as input variables of live immobilized biomass and percent dye removal was taken as the response of the system. The data were subjected to analysis of variance and the coefficient of regression (R^2) was calculated to find out the goodness of fit of the model.

3. Results and discussion

3.1. Optimization of process variables based on Box-Benkhen Design model

Combination experiments along with predicted and observed responses in Box-Behnkhen Design are represented in Table 1. Observed values of % removal of the dye was very close to the values predicted by the model, indicating that the model used is quite good for the process. The predicted response, Y (% dye removal) was obtained as follows:

$$\text{Dye removal (\%)} = +39.10-18.89*A+4.64*B-0.13*C+4.92*A*B-2.00*A*C-4.70*B*C +2.76*A2 + 4.01*B2-7.01 * C2 \quad (5)$$

Where A, B and C are the coded values of the test variables, pH, initial dye concentration (mg/l) and temperature (ºC), respectively. The equation predicts that first order effect of pH (A) and temperature as negative which indicates that biosorption would decrease as pH increase from 6 to 8 and the decrease in pH is statistically significant ($P<0.05$) as indicated by ANOVA (Table-2). Though a decline in dye removal occurred as temperature increased, but the decline was not statistically significant in the thermal range studied (Table 2). The second order main effects A^2 and B^2 are however, positive. Interactive effects of pH and initial dye concentration are positive. However the interactive effects of pH and temperature, initial dye concentration and temperature are negative indicating antagonistic influence of these parameters. Using the Box-Behnken design the optimum conditions indentified were pH 6, initial dye concentration 150mg/l and temperature of 45ºC when 63.41% decolorization was achieved.

The regression coefficient (R^2) quantitatively evaluates the correlation between the experimental data and the predicted responses as shown in the Fig. 2. The model adequacy check is an important part of the data analysis procedure, as the approximating model would give poor or misleading results if it were an inadequate fit. This is done by looking at residual plots, which are examined for approximating the model (Box *et al.* 1978). The studentized residual and normal %probability

plot of decolorization of crystal violet dye by the composite cyanobacterial biomass consortia is shown in Fig. 2. The studentized residuals measure the number of standard deviations separating the actual and predicted values. Linear relationship between the two variables shows that no response transformation was needed. A high value of R^2 (0.9501) indicates a strong correlation between the experimental and predicted values of response. The lack of fit test was conducted to measure the failure of the model to represent the data in the experimental domain at points which are not included in the regression. A non-significant F-value obtained for the test of significance for lack of fit again confirms the applicability of the quadratic model for the desired response.

Three dimensional response surface plots were drawn to illustrate the effects of the independent variables and effect of interactions of a pair of independent variables on the response (Fig.3, a-c). The shape of the corresponding plots and contour lines indicates whether the mutual interactions between the independent variables are significant or not. The main goal of response surface is to track efficiency for optimum values of variables such that the response is maximized and by analyzing the plots, the best response range can be calculated. Range of pH selected in this present study was 6 to 8, based on our earlier batch studies. Maximum biosorption took place at pH 6 and on increasing the pH the dye decolourization decreased. In the batch studies, maximum biosorption was observed at pH 7, but in the BBD experiments, optimal pH was found to be 6, which may be due to the interactions of the different process variables taken together. An increase in initial dye concentration with respect to pH favoured the dye removal response (Fig.3a). This may be due to the fact that on increasing the initial dye concentration, there is greater probability of contact between dye molecules and the biomass used. The finding was in agreement with literature reports where higher initial concentration of the dyes resulted in higher dye biosorption efficiency (Daneshvar et al., 2007; Khataee *et al.* 2011).

Removal of the dye was maximum in the middle range of 45ºC temperature (Fig.3b). Increase in pH led to decrease in dye removal as already explained. The three dimensional plot between temperature and initial dye concentration, showed that with respect to these variables maximal response is in the middle range, 50 to 150mg/L at 40ºC temperature.

Fig. 3c shows the interaction between temperature and pH. It shows the colour removal efficiency of CV solution as a function of pH and temperature increases when the pH decreases from 8 to 6 and the temperature from 40 to 50ºC. We get the maximum dye removal (63.4%) at150mg/L dye concentration, pH 6 and temperature of 45ºC. In earlier batch mode study maximum dye removal was observed at pH 7, while using the RSM approach, pH 6 was found more favourable, which may be due to the fact that in response surface methodology quadratic effects modify the optimal parameter conditions.

The results indicate that the present biosorbent derived from biomass of cyanobacteria of hot springs is suitable for removal of the carcinogenic crystal violet dye from wastewaters.

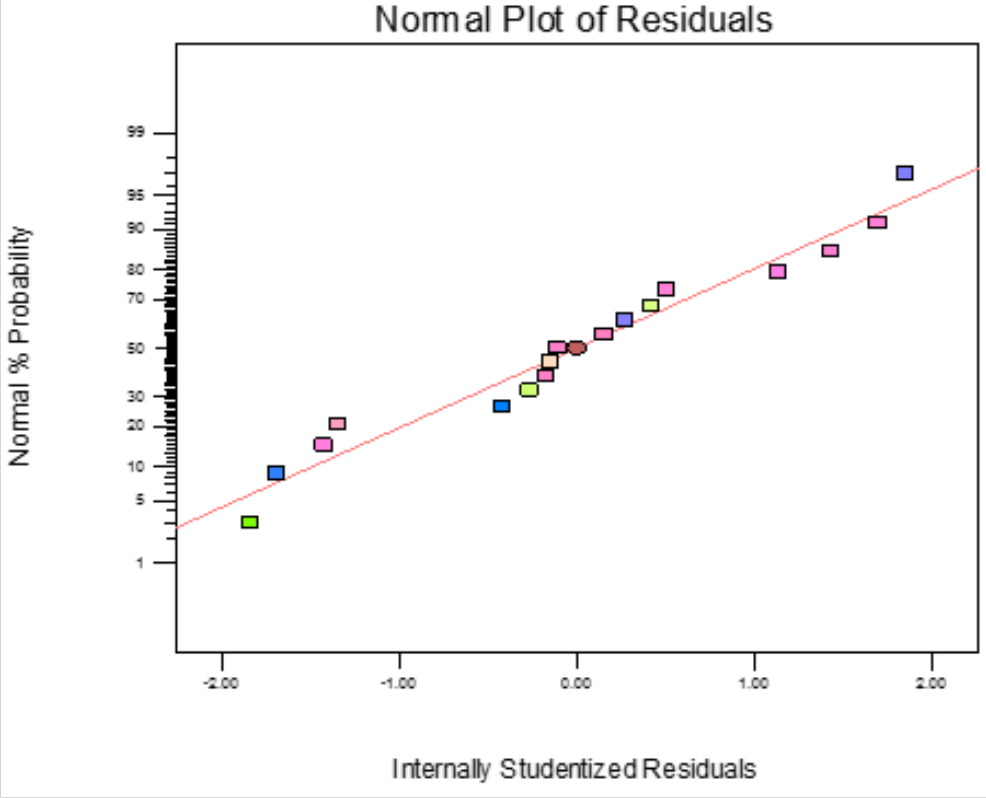

Fig.2: The studentized residual and normal % probability plot of decolorization of CV dye using immobilized biomass.

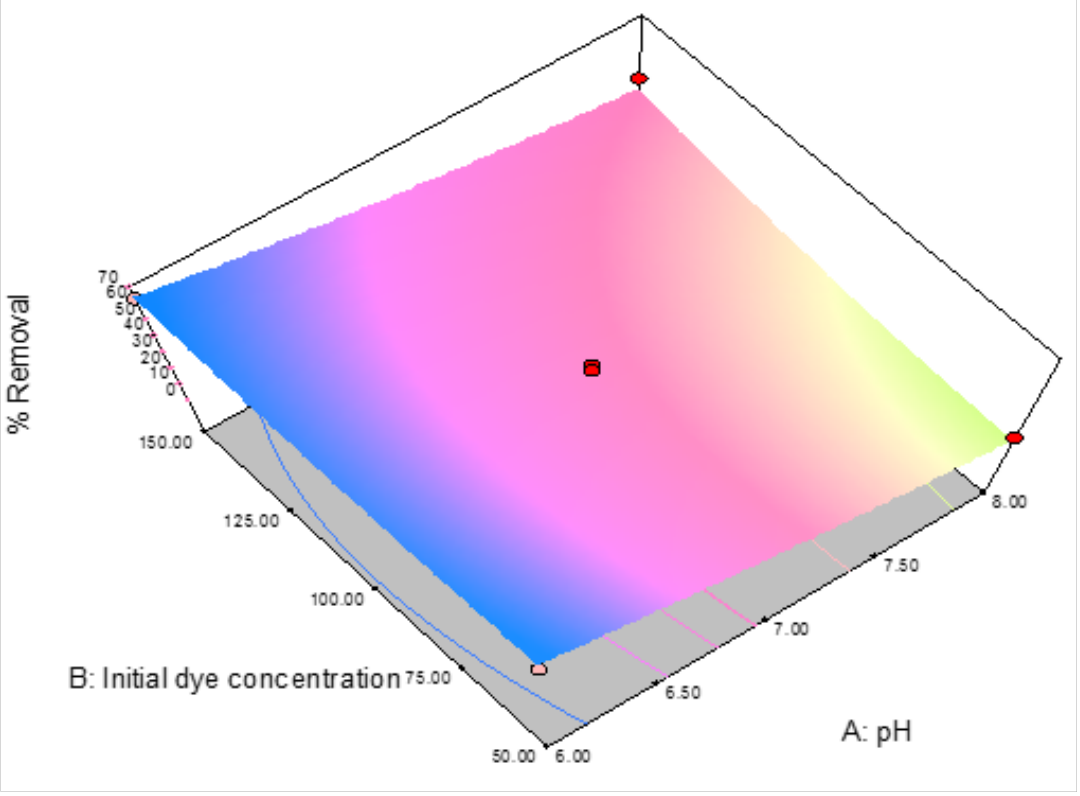
% Removal
70
60
50
40
30
20
10
0
150.00
125.00
100.00
75.00
50.00
B: Initial dye concentration
6.00
6.50
7.00
7.50
8.00
A: pH

Fig.3.a

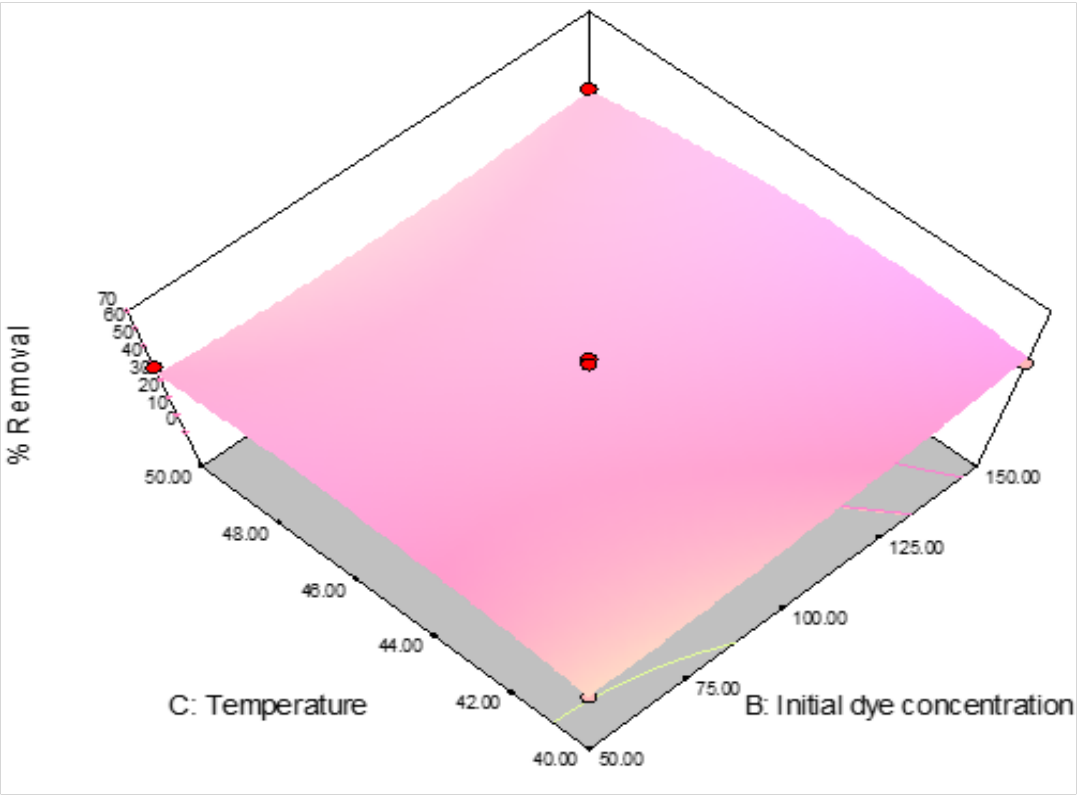
% Removal
70
60
50
40
30
20
10
0
50.00
48.00
46.00
44.00
42.00
40.00
C: Temperature
50.00
75.00
100.00
125.00
150.00
B: Initial dye concentration

Fig.3.b

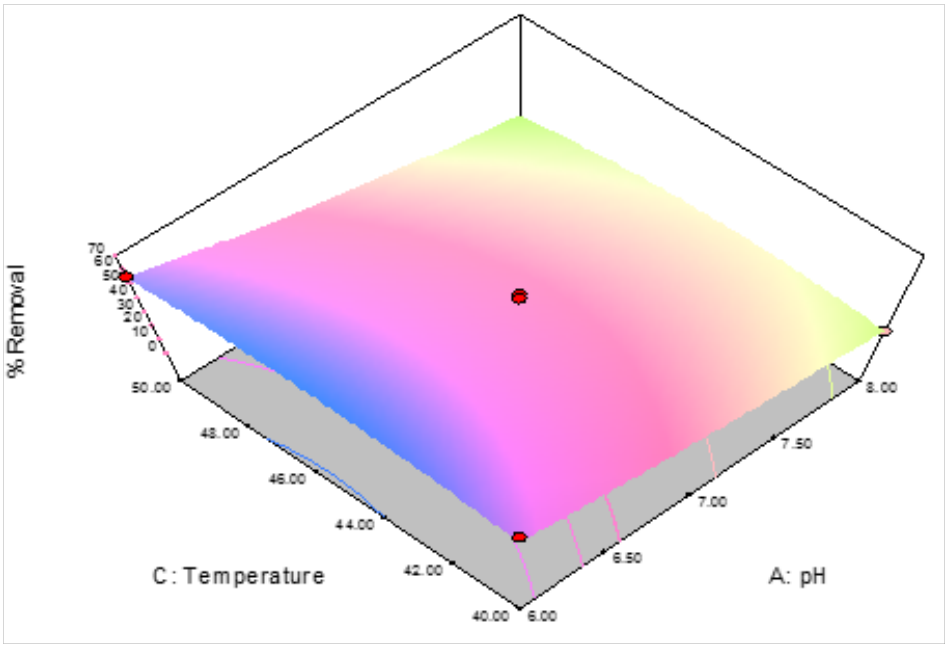

Fig.3.c

Fig.3: Three-dimensional response surface plot showing effect of (a) Initial dye conc. and pH (b) temperature and initial dye conc. (c) temperature and pH by dry immobilized biomass.

Table-1: Box-Behnken matrix for three variables along with observed and predicted values of dye removal using dry immobilized biomass

Std run	A:pH	B:Initial dye concentration (mg/L)	C:Temperature (^{o}C)	Dye Removal (%) Experimental	Dye removal (%) Predicted
1	6	50	45	60.7	65.1
2	8	50	45	18.5	17.4
3	6	150	45	63.4	64.5
4	8	150	45	40.9	36.6
5	6	100	40	56.6	51.9
6	8	100	40	17.4	18.1
7	6	100	50	56.3	55.6
8	8	100	50	9.1	13.8
9	7	50	40	26.5	26.9
10	7	150	40	41.9	45.6
11	7	50	50	39.7	36.0
12	7	150	50	36.3	35.9
13	7	100	45	38.3	39.1
14	7	100	45	38.6	39.1
15	7	100	45	44.3	39.1
16	7	100	45	41.4	39.1
17	7	100	45	32.9	39.1

Table-2: Analysis of variance table ANOVA for RSM parameters fitted to polynomial equation

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	3519.1	9	391.01	14.81	0.0009	Significant
*A-pH	2853.90	1	2853.90	108.12	< 0.0001	
*B-Initial dye concentration	172.05	1	172.05	6.52	0.0379	
C-Temperature	0.13	1	0.13	0.00	0.9471	
AB	97.02	1	97.02	3.68	0.0967	
AC	16	1	16	0.61	0.4617	
BC	88.36	1	88.36	3.35	0.1100	
A^2	32.13	1	32.13	1.22	0.3064	
B^2	67.79	1	67.79	2.57	0.1531	
*C^2	207.05	1	207.05	7.84	0.0265	
Residual	184.78	7	26.40			
Lack of Fit	113.12	3	37.71	2.10	0.2424	not significant
Pure Error	71.66	4	17.92			
Cor Total	3703.88	16				

*showing the significant model terms

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