Research Paper Environment

Optimization Of Medium Components For Reactive Orange M2r Dye Degradation By Consortium Vss Using Response Surface Methodology

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ABSTRACT

Discharge of wastewater from textile dyeing industries has been a problem in terms of pollution and treatment of these waters is a great task. Keeping this in mind, the aim of this current research is to study the effect of various bioprocess variables on decolorization of an azo dye, Reactive orange M2R (ROM2R) by a bacterial consortium VSS.

Response surface methodology was used to optimize the important physical parameters screened by Placket-Burman design. 12 Physical parameters such as Glucose(%), Starch(%), Sucrose(%), Yeast extract(%), Peptone(%), Ammonium nitrate(%), pH, temperature(0C), dye concentration(ppm), inoculum size (v/v) and time (h) were tested by using Placket-Burman design criterion and 4 parameters out of 12 (Yeast extract, pH, Temperature, Inoculum size) showed significant effect (P<0.05)) on decolorization of ROM2R using consortium VSS. The values of parameters were optimized by applying central composite design (CCD) and the most suitable values for ROM2R decolorization by consortium VSS, as predicted by the statistical tool, was Yeast Extract 0.3%, pH 6; temperature 35 0C, inoculum size (v/v) (%) 12.5%. At these optimum levels of parameters, bacterial decolorization of ROM2R by 95% was obtained under static conditions.

Keywords : Azo dye, biodegradation, ROM2R, Placket-Burman design, Response Surface Methodology.

Introduction

Rapid urbanization and industrialization has lead to a vast increase of waste to the environment adding to the pollution load. Majority of colored effluents contain dyes released from textile, dye stuff and dyeing industries (Senan & Abraham, 2004). ROM2R dye has been used in the textile industries for dyeing of cotton, woolen and nylon fabrics worldwide. It is reported to be toxic and cause allergic reactions of respiratory tract.

A number of microorganisms have been found to be able to decolorize textile dyes including bacteria, fungi, and yeasts (Olukanni *et al*.,2006; Wesenberg *et al*., 2003). Keeping in view the importance of biological treatment over conventional modes of treatment of azo dyes, an attempt has been made to study the decolorization abilities of the newly isolated bacterial consortium VSS for Reactive orange M2R (ROM2R), selected as model azo dye. This article describes optimization of parameters for ROM2R decolorization by consortium VSS. Process optimization by one-factor-at-a-time method involves changing one variable (pH, temperature, dye concentration, inoculum size, etc.) while fixing the others at a certain arbitrary levels. The conventional "one-factor-at-a-time" approach is laborious and time consuming, especially for large number of variables. Moreover, it seldom gurantees the determination of optimal conditions (Choudhari and Singhal, 2007). These limitations of a single factor optimization process can be overcome by using statistical methods. In statistical based approaches, response surface methodology (RSM) has been extensively used in media optimization (Fu et al., 2009; Shih et al., 2008). RSM is a collection of statistical based techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions (Kalil *et al*., 2000). It is a statistically designed experimental protocol in which several factors are simultaneously varied. In this work, we have screened out eleven most effective parameters such as pH, temperature, dye concentration (mg/l), time (h) and inoculum size % (v/v) for decolorization of ROM2R by Consortium VSS using response surface methodology (RSM). Placket-Burman design was used to select the factors having significant effect on decolorization of ROM2R by Consortium VSS and optimization of the selected parameters for the decolorization of ROM2R was done by central composite design (CCD).

Materials and Methods

Dyes and chemicals

The azo dye Reactive Orange M2R was obtained from Space Industries, Vatva GIDC, Ahmedabad.

Organisms and culture conditions

The bacterial consortium VSS was isolated from the textile dye contaminated soil collected from Vatva GIDC, Ahmedabad by enrichment culture technique. The six bacterial cultures in the consortium VSS (1 to 6) were identified by 16SrDNA sequencing and deposited in NCBI gene bank with the accession numbers KF282710 to KF282715 respectively.

Decolorization Studies

The decolorization studies were carried out in 250 ml Erlenmeyer flasks containing 100 ml of Bushnell Hass Broth (BHB). The medium was inoculated with 18 hrs old consortium VSS. Dye solutions of ROM2R were filter sterilized as stock solution (1.0% w/v) and added aseptically to the BHB to the desired concentration. The incubation was done at 37 ^{0}C for 24 hours at static conditions. The decolorized medium was then centrifuged at 10000 rpm at 4 ^{0}C for 10 minutes and the cell free supernatant was used for determination of percentage decolorization of ROM2R.

Response Surface Methodology

Response Surface Methodology (RSM) was divided into 2 stages, first to identify the significant process parameters for decolorization of ROM2R by consortium VSS using Plackett-Burman design criterion and later significant parameters resulted from Plackett-Burman design were optimized by using a central composite design (CCD). The experimental design and statistical analysis of the data were done using

statistical software Design Expert 8.

Screening of important nutrient components using Plackett-Burman design

The medium components were screened for eleven variables at two levels, maximum (+) and minimum (-) (Plackett and Burman, 1946). The experimental design and levels of each variable are shown in Table-1. The medium was formulated as per the design and the flask culture experiments for dye decolorization were assayed as described earlier. Response was calculated as dye decolorization and expressed as % decolorization. All experiments were performed in triplicates. The effect of each variable was calculated using the following equation:

$Y=\beta 0+\beta ixi$……..(1)

Y is the predicted response, β0 and βi are constant coefficients, and xi is the coded independent factors.

Table-1: The Placket-Burman design variables with % decolorization as response

Run	A	B	C	D	E	F	G	H	J	K	L	% Decolorization
	%	%	%	%	%	%	PPM	%	pH	^{0}C	hrs	%
1	-1	1	1	1	-1	-1	-1	1	-1	1	1	95
2	1	1	1	-1	-1	-1	1	-1	1	1	-1	73
3	1	-1	1	1	1	-1	-1	-1	1	-1	1	89.95
4	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	30.87
5	1	1	-1	-1	-1	1	-1	1	1	-1	1	88
6	-1	-1	1	-1	1	1	-1	1	1	1	-1	75.52
7	1	1	-1	1	1	1	-1	-1	-1	1	-1	84.35
8	1	-1	-1	-1	1	-1	1	1	-1	1	1	75
9	-1	1	-1	1	1	-1	1	1	1	-1	-1	90
10	-1	-1	-1	1	-1	1	1	-1	1	1	1	95
11	-1	1	1	-1	1	1	1	-1	-1	-1	1	35.34
12	1	-1	1	1	-1	1	1	1	-1	-1	-1	72

A: Glucose; B: Starch; C: Sucrose; D: Yeast extract; E: Peptone; F: Ammonium nitrate; G: Dye concentration; H: Inoculum size; J: pH; K: Temperature; L: Time

Optimization of the screened medium components using response surface methodology

The screened medium components affecting the dye decolorization were optimized using central composite design (CCD) (Box & Wilson, 1951; Box and Hunter, 1957). Four important parameters, i.e., Concentration of Yeast Extract(A), pH (B), Temperature (C) and Inoculum size (D), were screened from Plackett-Burman design as the independent variables and percentage of decolorization was the dependent response variable. Each of the four independent variables was studied at five different levels as per CCD in a total of 30 experiments. The percentage of dye decolorization corresponding to combined effects of four variables was studied in their specified ranges: Yeast extract: 0.3-0.5%; pH: 4-8; Temperature: 30-40 ^{0}C; Inoculum size: 5- 20%. The other process variables were kept constant throughout the 30 experiments. The plan of CCD in coded levels of the four independent variables is as shown in Table-2.

Table-2: Experimental design and results of the central composite design

Std	Run	A:YEAST EXTRACT	B:pH	C: TEMP.	D: INOCULUM SIZE	%Decolorization	
						Experimental	Predicted
		%	pH	0C	%	%	
1	16	-1	-1	-1	-1	28.02	25.19
2	13	1	-1	-1	-1	31	26.04
3	23	-1	1	-1	-1	60.79	49.88
4	7	1	1	-1	-1	30.37	31.95
5	19	-1	-1	1	-1	25.23	25.85
6	10	1	-1	1	-1	55.08	44.07
7	14	-1	1	1	-1	57.03	55.53
8	1	1	1	1	-1	60.3	54.97
9	4	-1	-1	-1	1	60	57.7
10	12	1	-1	-1	1	75	68.35
11	6	-1	1	-1	1	72	74.87
12	21	1	1	-1	1	75	66.75
13	11	-1	-1	1	1	65	55.27
14	15	1	-1	1	1	80	83.29
15	8	-1	1	1	1	80.09	77.42
16	28	1	1	1	1	92	86.68
17	17	-2	0	0	0	55	60.34
18	27	2	0	0	0	60	70.44
19	5	0	-2	0	0	15	23.9
20	26	0	2	0	0	45.09	51.97
21	22	0	0	-2	0	30.12	37.95
22	9	0	0	2	0	50.6	58.54
23	3	0	0	0	-2	28	37.28
24	29	0	0	0	2	95	101.49
25	20	0	0	0	0	67	68.87
26	18	0	0	0	0	65.03	68.87
27	24	0	0	0	0	65	68.87
28	2	0	0	0	0	76.13	68.87
29	30	0	0	0	0	70	68.87
30	25	0	0	0	0	70.03	68.87

Statistical Analysis

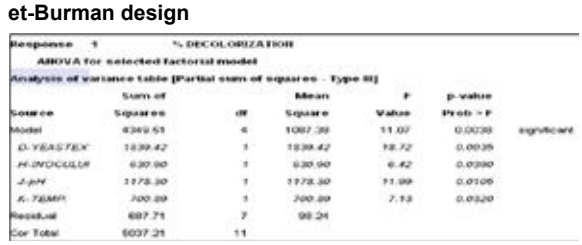
Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). The quality of the polynomial model equation was judged statistically by the coefficient of determination R^2, and its statistical significance was determined by an F-test.

Results and Discussion

The bacterial consortium VSS was able to decolorize the dye ROM2R using it as sole source of carbon and energy. The interaction of eleven culture conditions namely Glucose, Starch, Sucrose, Yeast Extract, Peptone, Ammonium nitrate, Dye conc., Inoculum conc., pH, Temperature and Incubation Time in dye decolorization investigated in 12 runs using Placket-Burman design is presented in Table-1. The data indicated a wide variation in the dye decorization, ranging from 30.87% to 95%. This variation reflected the effect of the interaction among the variables in the dye decolorization. Among the variables screened, the most effective factors with high significance level were in the order of Yeast extract, Inoculum size, pH and Temperature. They were selected for further optimization.

The statistical analysis of the Plackett-Burman design (Table-3) demonstrated that the model F-value of 11.07 was significant. The p-value < 0.05 indicated that the model terms were significant.

Table-3: ANOVA for selected factorial model for Plackett-Burman design


Response 1 % DECOLORIZATION

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4349.53	4	1087.38	11.07	0.0038	significant
D-YEASTEX	1839.42	1	1839.42	18.72	0.0035	
H-INOCULUM	630.90	1	630.90	6.42	0.0390	
J-pH	1178.30	1	1178.30	11.99	0.0105	
K-TEMP	700.89	1	700.89	7.13	0.0320	
Residual	687.71	7	98.24			
Cor Total	5037.21	11				

The model's goodness of fit was checked by determination coefficient (R^2). In this case, the value of R^2 (0.86) closer to 1 denoted better correlation between the observed and predicted responses. The coefficient of variation (CV) indicated the degree of precision with which the experiments were compared. The reliability of the experiment is usually indicated by low value of CV. In the present case, a low CV (13.16) denoted that the experiments performed were highly reliable.

From the analysis, it was inferred that the dye decolorization was supported by yeast extract, inoculums size, pH and temperature as shown by their F-values and p-values (Kalavati *et al*, 2012). Therefore these variables were considered as

highly significant for dye decolorization by consortium VSS and were further investigated with central composite design to find the optimal range of these variables.

Central composite Design

The result of 30 run CCD for four variables, Yeast extract concentration, Inoculum size, pH and Temperature chosen for optimization of dye decolorization process are shown in Table-2. The decolorization varied markedly ranging from 30.12 - 95 % in the conditions tested.

The results obtained from the central composite design were fitted to a second order polynomial equation to explain the dependence of decolorization on the medium components.

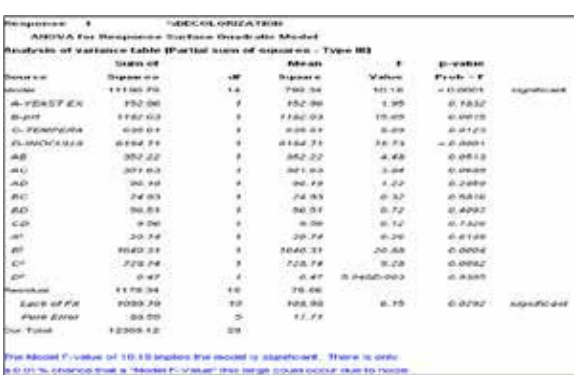
$$Y= +68.87 +2.52 \times A +7.02 \times B +5.15 \times C +16.05 \times D -4.69 \times A \times B +4.34 \times A \times C +2.45 \times A \times D +1.25 \times B \times C -1.88 \times B \times D -0.77 \times C \times D -0.87 \times A^2 -7.73 \times B^2 -5.15 \times C^2 +0.13 \times D^2$$

Where Y is the predicted response (% decolorization), A, B, C and D are the coded values of Yeast extract, Inoculum size, pH and temperature respectively.

The analysis of variance of the quadratic regression model suggested that the model was significant (Table-4). The model's goodness of fit was checked by determination coefficient (R^2). In this case, the value of R^2 (0.904) closer to 1 (Table-5) denoted better correlation between the observed and predicted responses. The low CV (15.29) denoted that the experiments performed were reliable. The p-values denoted the significance of the coefficients and also the pattern of the mutual interactions between the variables.

The response surface curves are plotted to explain the interaction of the variables and to determine the optimum level of each variable for maximum response (Fig.1 to 6). The model predicted the optimal values of the 4 most significant variables. The maximum dye decolorization obtained was 95% with Yeast extract-0.3%, pH-6, and Temperature and Inoculum size

Table-4: Analysis of Variance (ANOVA) for the fitted quadratic polynomial model for optimization of Dye decolorization



Effect of carbon and nitrogen sources on degradation efficiencies of microorganisms have been worked out by few investigators. Nosheen *et al.* and Wang *et al* have reported increased efficiencies of bacterial cultures with addition of carbon and nitrogen sources to the degradation medium. Many different co-substrates have been found to suit as electron donor, like yeast extract (carliell *et al*, 1995; Nigam *et al* 1996). Bhatt *et al* (2005) observed decrease in dye decolorization efficiency at high concentration of yeast extract. The nutrient requirement for optimum decolorization depends on the nature of the microbial species employed. Rengadurai *et al*. (2012) reported the significant effects of pH, Temperature and inoculum size on degradation of reactive textile dyes.

Table-5: Various Analysed values for response surface model

Std. Dev.	8.86	R-Squared	0.9047
Mean	57.96	Adj R-Squared	0.8158
C.V. %	15.29	Pred R-Square	0.4822
PRESS	6404.72	Adeq Precision	12.381

Figure 1: Response surface curve for % decolorization by Consortium VSS showing interaction between pH and Yeast extract

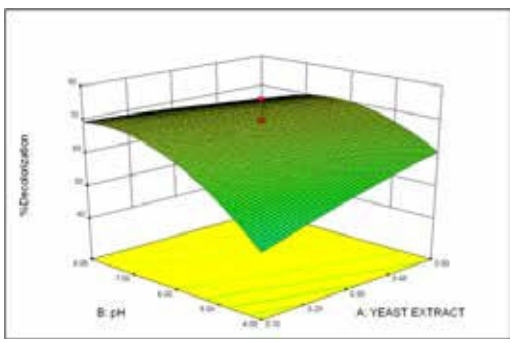


Figure 2: Response surface curve for % decolorization by Consortium VSS showing interaction between Temperature and Yeast extract

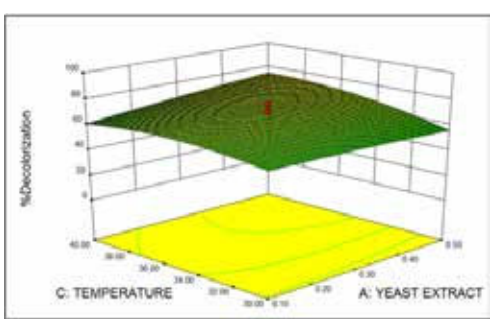


Figure 3: Response surface curve for % decolorization by Consortium VSS showing interaction between Inoculum size and Yeast extract

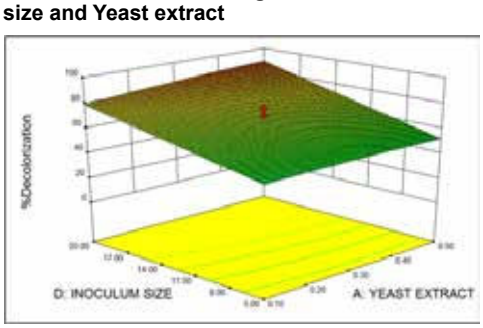


Figure 4: Response surface curve for % decolorization by Consortium VSS showing interaction between pH and Temperature

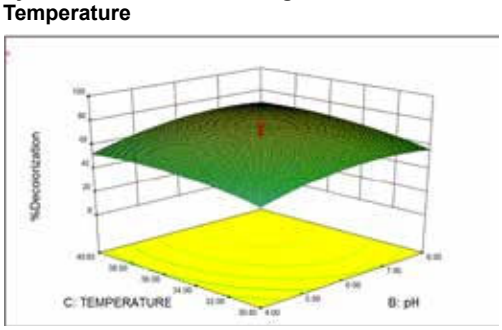


Figure 5: Response surface curve for % decolorization by Consortium VSS showing interaction between pH and Inoculum size

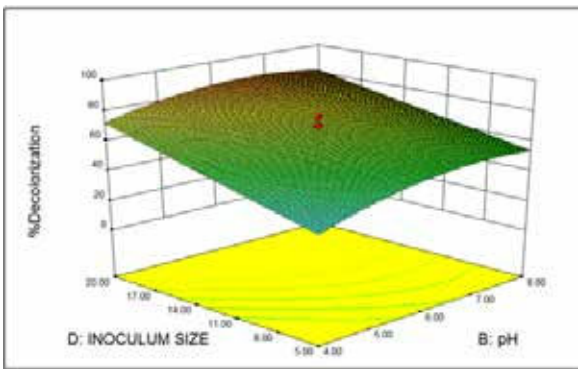
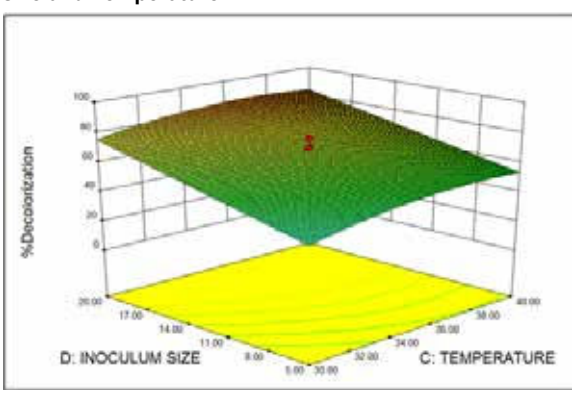


Figure 6: Response surface curve for % decolorization by Consortium VSS showing interaction between Inoculum size and Temperature



Conclusion

The application of bacterial consortium VSS to decolorize the azo dye ROM2R seemed to be one of a pragmatic approach. This study showed that the response surface methodology was an appropriate method to optimize the culture conditions for obtaining the maximum decolorization of the dye. By applying central composite design and RSM to the optimization experiments, the process variables were investigated to achieve the maximum decolorization of 95%. The experimental and predicted values were very close, which reflected the accuracy and the applicability of RSM. Moreover the ability of Consortium VSS to decolorize ROM2R of 95 % indicated its potential for decolorizing the dyeing effluents.

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