
Application of Plackett–Burman Design to Optimize Bioprocess Variables for Decolorization of Reactive Red 195 by a Termite Associated Bacterial Consortium BUTC7

K. Nanthakumar, K. Karthikeyan, S. Suriyanarayanan, and P. Lakshmanaperumalsamy

Abstract

In the present study, an attempt has been made to understand about the dye decolorization capacity of termite based microbial consortia. Initial decolorization experiments were carried out to know about the decolorization ability of the consortia and in continuation, its ability of decolorizing reactive red 195 by applying response surface methodology (RSM) was assessed. Plackett–Burman design was applied for this study to understand the most influencing variables out of all the variables and central composite design was applied in order to find out the most suitable concentration range of the selected variables. The quadratic model having R^2 of 0.8950 was generated to describe the combined effect and the interactions. Statistical analysis indicates that the interactions between the variables are found to be significant. The optimum conditions responsible for maximum decolorization efficiency (97.23 %) were found to be pH 7.0, 0 rpm (static mode), 500 mg/L of dye concentration, and 0.25 % yeast extract. All these factors had a significant cumulative positive effect for maximum decolorization performance in the medium. The experimental and predicted values derived during the present study supported the reliability of this design. Hence from the promising results observed in the present study, it is

K. Nanthakumar
Shriram Institute for Industrial Research,
Bangalore 560048, Karnataka, India

K. Karthikeyan
Gujarat Institute of Desert Ecology,
Bhuj Kachchh 370001, Gujarat, India

S. Suriyanarayanan (✉)
Department of Water and Health, JSS University,
Mysore 570015, Karnataka, India
e-mail: sunsjk@gmail.com

P. Lakshmanaperumalsamy
Karpagam University, Eachanari Post,
Coimbatore 641021, Tamil Nadu, India

confirmed that the microbial consortium isolated from termite can be employed as potential candidate for the treatment of reactive dyes containing effluents.

Keywords

Termite · Microbial consortium · Decolorization · Reactive red 195 · Plackett–Burman design

Introduction

The textile industry is one of the greatest generators of liquid effluent, due to the high quantities of water used in the dyeing processes and more than 800,000 t of dyes are annually produced worldwide, of which 60–70 % are azo dyes (Moutaouakki et al. 2000; Jin et al. 2007). Reactive azo group of dyes is widely used as textile colorants, typically for cotton dyeing, due to their variety of brilliant colors shades, high wet fastness profiles, ease of application, and minimal energy consumption (Carliell et al. 1995; Hao et al. 2000).

Discharge of wastewater from dye manufacturing units and textile processing industries results in pollution of aquatic systems (Keharia and Madamwar 2003). Hence, treatment of such dye containing effluent is essential to prevent deterioration of ecosystem. Bioremediation of textile dyes is gaining significance as it is cost-effective, environment friendly, and produces less sludge (Robinson et al. 2000; Chen et al. 2003). Many microorganisms belonging to different taxonomic groups of bacteria, actinomycetes, fungi, and algae have been reported for their ability to decolorize azo dyes (Chang et al. 2001; Khehra et al. 2005). Microorganisms are capable of utilizing a variety of complex chemicals including dyes as their sole source of carbon but only few researchers have been successful in isolating such culture (Sarnaik and Kanekar 1999). Microorganisms, being highly versatile, have developed enzyme systems for the decolorization and mineralization of dyes under certain environmental conditions. Microorganisms

have the ability not only to decolorize dyes but also to detoxify it (Adedayo et al. 2004; Kumar et al. 2007).

In termites, this study has been selected as source for potent dye degrading bacteria, because they are assumed to have harbor microorganisms responsible for digestion of complex substances as the gut flora consists of bacteria, archaea, and archaezoa (Konig and Varma 2006). When a biological treatment system for dye containing wastewaters is concerned, several considerations have to be made in which environmental conditions of a biotreatment system greatly influence its efficiency.

Keeping the above background information and our preliminary results on decolorization, we made an attempt to assess the efficiency of a novel termite associated bacterial consortium on decolorization of reactive red 195 by Plackett–Burman design and central composite design.

Materials and Methods**Dye Stuff and Chemicals**

Reactive red 195 was procured from a local textile industry situated in Coimbatore, Tamilnadu, India and used for the study. One gram of dye was dissolved in de-ionized water and made up to 1,000 ml to give 1,000 mg/L. From the stock solution, required concentrations of dye solutions were prepared and filter sterilized using 0.45 μ membrane filter and used. Chemicals used in the study were of highest purity and are of analytical grade.

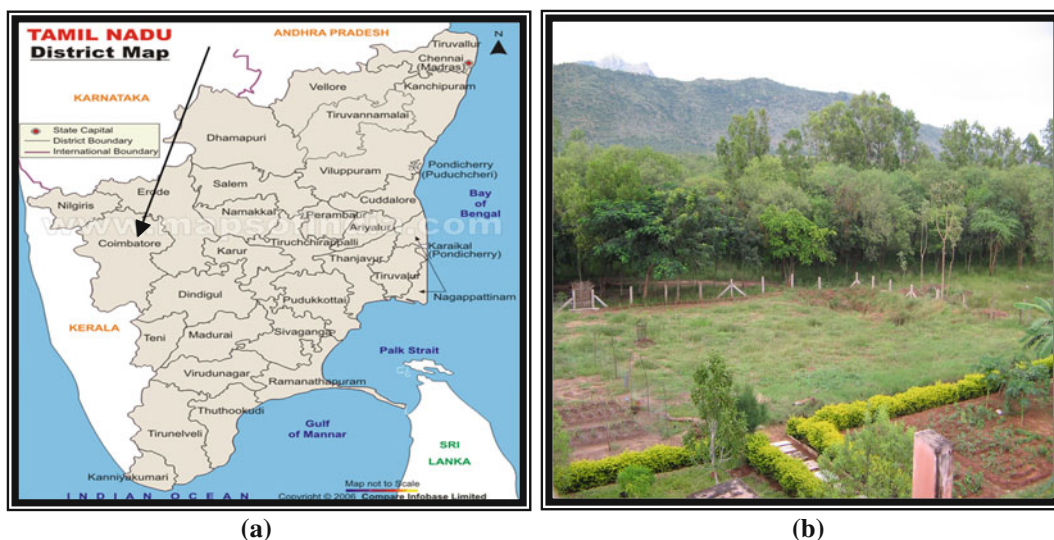


Fig. 1 a The study area (Coimbatore district) and b environment termatorium habitat

Preparation of Termite Homogenate

Termites were collected from a termatorium located in Bharathiar University campus (Fig. 1a, b), which was washed with sterile distilled water and surface sterilized with diluted ethanol (70 %). 10 g of termites were homogenized in a sterile mortar with sterile distilled water and the homogenate was transferred to flasks containing 50 ml of sterile saline blank. The flask was kept in a shaker at 120 rpm for 15 min, for uniform distribution and this homogenate was used for decolorization studies.

Isolation of Axenic Bacterial Strains from Termites by Pour Plate Technique

Individual bacterial strains present in the homogenate were isolated by pour plating technique (Azamujja et al. 2004). After incubation, the bacterial colonies were isolated and further purified for its usage in dye decolorization experiment.

Selection of Dyes and Dye Decolorizers

The dye decolorization capacity of the individual isolates was assessed and the potent strains were grown in the same medium as consortium in nutrient broth for up to 18 h and selected as potent consortia (BUTC7) and this was used for further experiments.

Decolorization Measurement

After incubation, the samples were centrifuged at 10,000 rpm for 15 min and the suspended biomass was separated. The absorption spectra (545 nm) of the clear supernatant were recorded at λ_{\max} of the dyes using a spectrophotometer (UV-Vis 3210, Hitachi, Japan). Medium containing dyes without the inoculum was taken as control. The initial and final absorbance values obtained were then used to calculate percentage decolorization of the dye.

$$\% \text{ decolorization} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

Preparation of Bacterial Consortium Inoculum

One ml of grown culture of potent dye decolorizer was taken from each flask and inoculated in 50 ml sterile nutrient broth and incubated up to 18 h. After 18 h of incubation, 10 ml of the broth was drawn from each flask, mixed thoroughly and adjusted to 1.0 OD at 600 nm using sterile medium in a spectrophotometer (UV-Vis Hitachi, 3210). One ml of the above bacterial consortium was used as inoculum for all the experiments. The potent dye decolorizing bacterial strains were used as consortia to check the efficiency in decolorizing reactive red 195 by statistical analysis by applying response surface methodology and central composite design.

Response Surface Methodology and Central Composite Design for Decolorization of Reactive Red 195

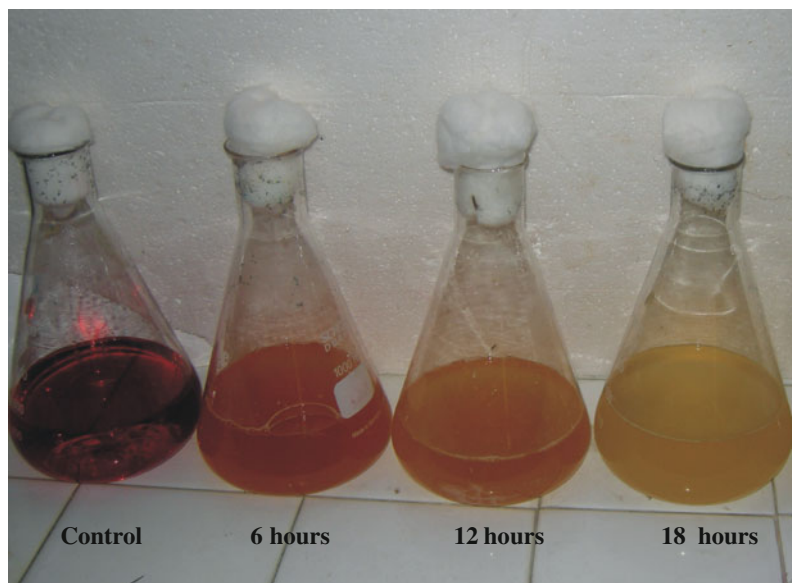
The main goal of response surface is to hunt efficiently for the optimum values of the variables, so that the response is maximized (Dey et al. 2001). Media components used in any bioprocesses such as pH, temperature, and nutrients in general are

considered to be the most important parameters which affect process of any industrial system. This is mainly achieved by optimizing the different variables by changing one parameter at a time and maintaining others at a constant level. This method is found to be time consuming and also often does not yield reliable results. In this regard, statistical method such as RSM is found to be a useful model for studying the effect of copious factors influencing the responses by varying them simultaneously and carrying out a limited number of experiments. This methodology consists of Plackett–Burman design as first optimization step and CCD as a second step to optimize the factors that have significant effects on the process, and hence this methodology was adopted for the study. The influence of pH, temperature, agitation, incubation time, dye concentration, glucose, yeast extract, KH_2PO_4 , chromium, phenol, and sodium salts in the dye decolorization was investigated in 12 runs using Plackett–Burman design.

Plackett–Burman Design for Screening of Nutrients

Plackett–Burman design was applied for screening of nutrients with respect to their main

Fig. 2 Reactive red 195 decolorization by the bacterial consortium BUTC17 at various time intervals



effects and not their interaction effects. The medium components were screened for 11 variables at two levels of maximum (+) and minimum (−). According to this design, the number of positive signs (+) is equal to $(N + 1)/2$ and the number of negative signs (−) is equal to $(N - 1)/2$ in a row (Plackett and Burman 1946).

This design was used to formulate the media components and the flask culture experiments on decolorization were performed and the response was calculated as percentage of dye decolorization. In general, all the experiments were performed in triplicate and the average percentage dye decolorization was considered. The effect of each variable was calculated using the following equation (Plackett and Burman 1946).

$$E = \left(\sum M_+ - \sum M_- / N \right)$$

where, E is the effect of tested variable, M_+ and M_- are responses (dye decolorization) of trials at which the parameter was at its higher and lower levels respectively, and N is the number of experiments was carried out. The standard error (SE) of the variables was calculated by using Student's t .

Optimization of Medium Components Using RSM

RSM is an efficient experimental methodology to determine the ideal conditions for a system containing multiple variables and is found to be useful rather than optimizing by the conventional method where variables are changed in consecutive steps while keeping one factor (Liu and Tzeng 1998). The screened medium components affecting dye decolorization were optimized using CCD (Box and Wilson 1951; Box and Hunter 1957).

According to this design, the treatment of combinations is represented as $2^k + 2k n_0$ where 'k' is the number of independent variables and n_0 being the number of times the experiment was performed. For statistical analysis, the variables X_i have been coded as x_i according to the following transformation (Plackett and Burman 1946):

$$x_i = X_i - X_0 / \delta X$$

where, x_i is dimensionless coded value of the variable X_i , X_0 the value of the X_i at the center point, and δX is the step change. A total number of 30 experiments were employed for optimizing the medium components and this system behavior was explained by the following quadratic equation (Plackett and Burman 1946):

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j$$

where, Y is the predicted response, β_0 is the intercept term, β_i is the linear effect, β_{ii} is the squared effect, and β_{ij} is the interaction effect. The statistical model was validated for decolorization of reactive red 195 under different environmental conditions.

Results

Development of Bacterial Consortium

The decolorization pattern of reactive red 195 by the bacterial consortium at various time intervals.

Optimization of Process Using RSM for Decolorization of Reactive Red 195

The decolorization response in the 12 run experimental set up is represented in Table 1 and Fig. 2. The Pareto chart depicts the order of significance of the variables involved in decolorization of reactive red 195 (Fig. 3). Among the variables screened for this experiment, the most effective factors with high level of significance were in the order as follows: dye concentration, yeast extract, static condition, and pH and consequently these factors were further investigated with CCD to find the optimal range of these variables. Statistical analysis of this design demonstrates that the model F value of 0.89 is significant and values of $p < 0.05$ indicate

Table 1 Plackett–Burman design to evaluate factors influencing reactive red 195 decolorization by bacterial consortium

Run	A	B	C	D	E	F	G	H	J	K	L	Decolorization (%)
1	4	20	0	0	10	0.25	0.25	0.1	5.0	2.0	1.0	9.6
2	4	20	0	72	10	1.0	1.0	0.1	50.0	10.0	5.0	75.7
3	4	60	0	72	1000	0.25	1.0	0.5	50.0	2.0	1.0	54.6
4	11	20	300	72	10	1.0	1.0	0.5	5.0	2.0	1.0	25.6
5	4	60	300	0	1000	1.0	1.0	0.1	5.0	2.0	5.0	19.8
6	4	60	300	72	10	0.25	0.25	0.5	5.0	10.0	5.0	23.4
7	4	20	300	0	1000	1.0	0.25	0.5	50.0	10.0	1.0	5.8
8	11	20	0	0	1000	0.25	1.0	0.5	5.0	10.0	5.0	12
9	11	60	0	72	1000	1.0	0.25	0.1	5.0	10.0	1.0	45.6
10	11	20	300	72	1000	0.25	0.25	0.1	50.0	2.0	5.0	66.8
11	11	60	0	0	10	1.0	0.25	0.5	50.0	2.0	5.0	12.7
12	11	60	300	0	10	0.25	1.0	0.1	50.0	10.0	1.0	12.5

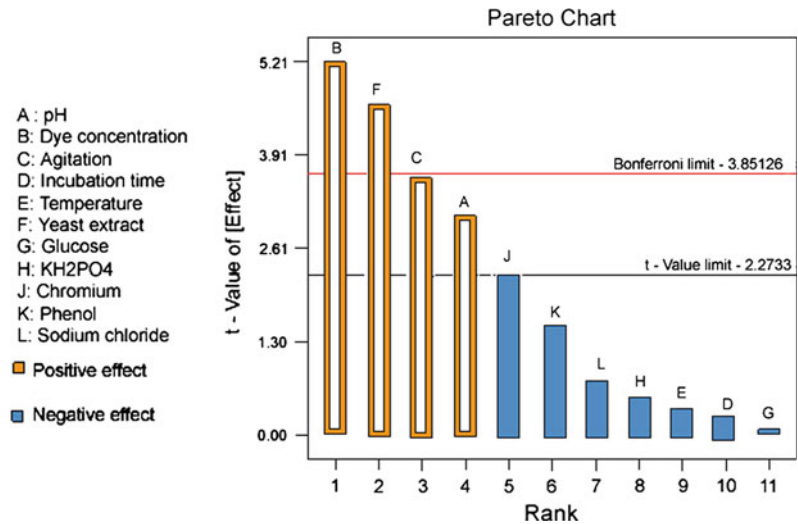
A pH B Temperature ($^{\circ}$ C) C Agitation (rpm)

D Incubation time (hrs) E Dye concentration (mg/L) F Yeast extract (%)

G Glucose (%) H KH_2PO_4 (%) J Chromium (g/L)

K Phenol (mg/L) L Sodium chloride (%)

Fig. 3 Pareto chart for reactive red 195 decolorization



model terms are significant. Regression analysis was also performed on the results and first-order polynomial equation was derived in order to notify the independent variable which showed the decolorization of reactive red 195 and the equation is as follows:

$$\text{Reactive red 195 (\% decolorization)} \\ = + 34.09 + 14.53A + 7.51C + 11.43E \\ + 3.01F$$

Central Composite Design

The result of 30 run CCD of four variables with + effects such as pH, agitation, dye concentration, and yeast extract were chosen for optimization of decolorization of reactive red 195 by the bacterial consortium BUCT17 is shown in Table 2, which is presented as decolorization corresponding to combined effect of four components in their specified ranges.

Table 2 Experimental plan for optimization of variables involved in reactive red 195 decolorization using CCD

Run	pH	Agitation (rpm)	Dye concentration (mg/L)	Yeast extract (%)	Decolorization (%)	
					Experimental	Predicted
1	0	2	0	0	39.42	33.00
2	0	0	0	2	32.06	36.17
3	0	0	0	-2	22.95	22.17
4	1	1	-1	-1	21.91	22.17
5	1	1	-1	1	46.09	39.17
6	-1	1	1	-1	46.23	42.50
7	0	0	2	0	64.17	58.67
8	1	1	1	1	21.9	27.67
9	0	0	0	0	87.07	94.17
10	0	0	0	0	97.23	94.17
11	-1	1	1	1	24.54	31.50
12	0	0	0	0	96.83	94.17
13	0	0	0	0	94.15	94.17
14	1	-1	1	-1	40.04	42.83
15	1	-1	1	1	41.12	33.83
16	-1	-1	-1	-1	31.63	31.67
17	-1	1	-1	-1	31.79	31.50
18	0	0	0	0	96.03	94.17
19	-1	-1	-1	1	25.22	22.67
20	2	0	0	0	11.01	5.33
21	1	-1	-1	1	15.01	19.83
22	1	-1	-1	-1	11.34	14.83
23	1	1	1	-1	22.22	28.67
24	-1	-1	1	-1	23.09	24.17
25	0	0	-2	0	54.99	53.67
26	0	0	0	0	95.01	94.17
27	-2	0	0	0	2.01	2.00
28	-1	-1	1	1	21.65	21.17
29	0	-2	0	0	15.43	15.33
30	-1	1	-1	1	54.55	54.50

Decolorizations varied markedly with the condition were tested, in the range of 2.01–97.23 %. Lowest decolorization was observed at run 27, where lower pH with optimum concentrations of yeast extract, dye concentration, and static condition, whereas maximum decolorization of 97.23 % was observed at pH 7, 0 rpm, 500 mg/L of the dye with 0.25 % yeast extract (run 10), which strongly suggests that these variables had played a significant role in decolorization pattern of reactive red 195.

The results obtained were subjected to the analysis of variance with the regression model given as; $Y = + 94.17 + 0.83A + 4.42 B + 1.25 C + 3.50 D - 3.13 A B + 3.88 A C - 1.50 A D - 5.37 BC + 3.00 BD - 3.50 C D - 22.62 A^2 - 17.50 B^2 - 9.50 C^2 - 16.25 D^2$.

Where Y is the response value (% decolorization of reactive red 195) with coded levels of pH, agitation, dye concentration, and yeast extract as A,B,C, and D respectively. Next to pH, another most influencing variable for

Table 3 Analysis of variance for reactive red 195 decolorization

Source	Sum of square	Degree of freedom	Mean square	F-value	p-value	
Model	4,774.58	3	1,591.53	0.89	0.0043	Significant
A-pH	2,531.71	1	2,531.71	16.0607	0.0039	–
C-agitation	676.501	1	676.501	4.29159	0.0720	–
E-dye concentration	1,566.37	1	1,566.37	9.93673	0.0136	–
F-yeast extract	22.1408	1	22.1408	1.40243	0.3019	–
Residual	1,261.07	8	157.634	–	–	–
Cor total	6,035.65	11	–	–	–	–

Table 4 ANOVA for CCD (quadratic model) for reactive red 195 decolorization

Source	Sum of square	Degree of freedom	Mean square	F-value	p-value	
Model	25,683.1	14	1834.51	9.12893	<0.0001	Significant
A-pH	16.6667	1	16.6667	0.08294	0.7773	–
B-agitation	468.167	1	468.167	2.3297	0.1477	–
C-dye concentration	37.5	1	37.5	0.18661	0.6719	–
D-yeast extract	294	1	294	1.46301	0.2452	–
AB	156.25	1	156.25	0.77754	0.3918	–
AC	240.25	1	240.25	1.19554	0.2915	–
AD	36	1	36	0.17914	0.6781	–
BC	462.25	1	462.25	2.30026	0.1501	–
BD	144	1	144	0.71658	0.4106	–
CD	196	1	196	0.97534	0.3390	–
A ²	14,040.4	1	14,040.4	69.8683	<0.0001	–
B ²	8,400	1	8,400	41.8003	<0.0001	–
C ²	2,475.43	1	2,475.43	12.3183	0.0032	–
D ²	7,242.86	1	7,242.86	36.0421	<0.0001	–
Residual	3,014.33	15	200.956	–	–	–
Lack of fit	2,947.5	10	294.75	22.0511	0.0016	Significant
Pure error	66.8333	5	13.3667	–	–	–
Cor Total	28,697.5	29	–	–	–	–

CV 4.19; R^2 0.8950

effective decolorization of reactive red 195 was found to be yeast extract at a concentration of 0.25 %. In this study, dye concentration has an influence on decolorization of reactive red 195 and an optimum dye concentration of 500 mg/L was observed, whereas 1,000 mg/L was the maximum concentration used in the present study. The next influencing variable for decolorization of reactive red 195 is static condition (no agitation was employed), which influences decolorization effectively, whereas aeration

(increased dissolved O₂ level) strongly inhibited decolorization of reactive red 195.

The ANOVA results are presented in Tables 3 and 4, the quadratic regression model suggested that it is very significant with a model F value of 9.12 and R^2 value (multiple correlation coefficient) of 0.89 (near 1) denotes better correlation between the experimental and predicted values. In the present case, a low value (4.19) denotes that the experiments performed are highly reliable. The p value denotes the

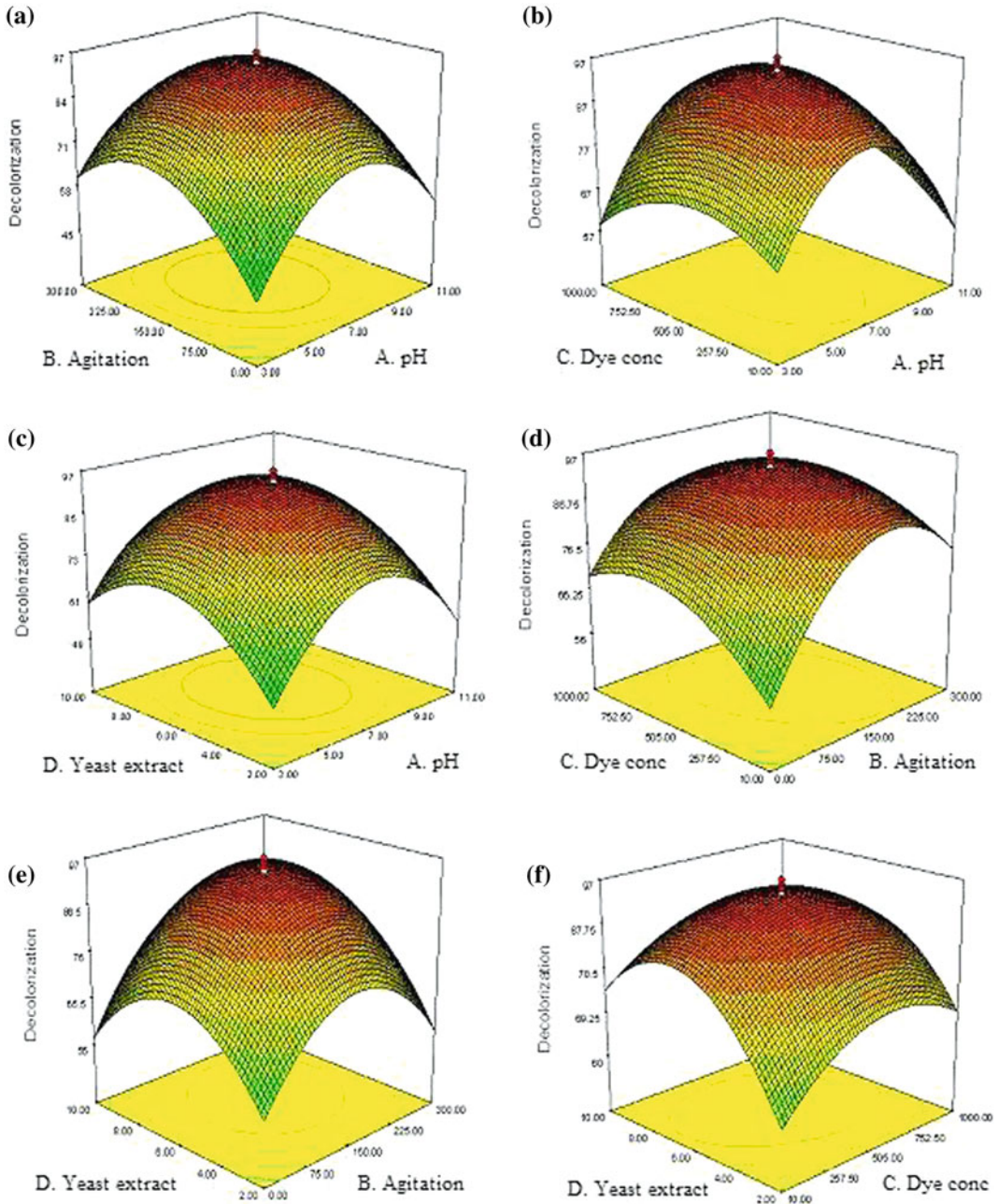


Fig. 4 The effect of various factors on reactive red 195 decolorization by bacterial consortium BUTC17

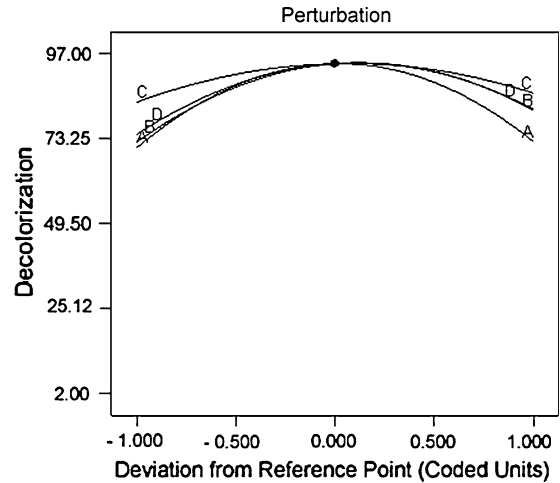
significance of coefficients and also important in understanding the pattern of mutual interactions between the variables.

The interaction effects and optimal levels of the variables affecting decolorization were

determined by plotting the response surface curves (Fig. 4), which is showcase the behavior of response (percentage decolorization) with respect to simultaneous change in two variables.

Fig. 5 Optimum values of the tested variables for reactive red 195 decolorization

Decolorization
Actual Factors
A: pH= 7.00
B: Agitation = 0 rpm
C: Dye conc = 500 mg/L
D: Yeast Extract = 0.25 %



Model Validation

The maximum experimental decolorization of reactive red 195 was 97.23 %, whereas the predicted value was 94.17 %, which established a strong agreement between them. The optimum values of the tested bioprocess variables are pH 7.0, agitation 0 rpm, dye concentration 500 mg/L, and yeast extract of 0.25 % as shown in perturbation graph (Fig 5). Confirmation of the decolorization pattern was done to validate the optimum combination of the process variables. The results were obtained from these experiments employed RSM was found to be encouraging for optimizing similar dye decolorization studies under pilot or industrial scale conditions.

Discussion

Development of Bacterial Consortium

Based on the initial decolorization experimental results (data not shown), the study was planned to optimize the operational variables by applying RSM and CCD. The main objective of RSM is to determine the optimum operational conditions for the system or to determine a region that satisfies operating specifications (Ravikumar et al. 2006).

Optimization of Process Using RSM for Decolorization of Reactive Red 195

Microbial decolorization process which uses several variables (carbon and nitrogen source, dye concentration, inoculum size, pH, and temperature) is considered to be the most important parameters which affect the process (Wong and Yuen 1996; Chen et al. 2003; Nachiyar and Rajkumar 2003; Khehra et al. 2005), and hence it is required to investigate the influencing nature of these variables on the decolorization process.

In line with our studies, decolorization (80 %) of a textile azo dye, Disperse Yellow 211 (DY 211) in simulated aqueous solution by *Bacillus subtilis* involving Box-Behnken design matrix and found temperature, pH, and initial dye concentration as the most influencing variables (Sharma et al. 2009). Similar report on decolorization of 91.0 % of Direct Black 22, a textile dye, by a novel microbial consortium by RSM (Mohana et al. 2008).

Central Composite Design

Bacterial culture generally exhibits maximum decolorization at a neutral pH value or a slightly alkaline pH value and the rate of color removal tends to decrease rapidly at highly acidic or

alkaline pH conditions (Bhatt et al. 2005; Handayani et al. 2007). Bacterial consortium employed in the present study also exhibited a similar better performance at pH 7. Studies reported glucose were utilized during decolorization of dye acid red 27 and reactive red 2 by *Enterococcus faecalis* under a batch system and concluded that it might be due to the role of glucose as a cosubstrate (Handayani et al. 2007) i.e. the source of electron donors, which are needed for azo bond cleavage (Sponza and Isik 2004; Mendez et al. 2005) and it is also established that glucose acts as an effective electron donors for dye reduction (Tan 2001).

Decolorization percentage of the dye decreases with increase in the initial dye concentrations, which might be due to inhibitory effects caused due to toxicity of dye (Korbahti and Rauf 2008). Decolorization and degradation of Navy blue HER by *Trichosporon beigelii* NCIM-3326 within 24 h of incubation under static condition and concluded that it may be due to enzyme activity by the bacterial consortium got induced under static conditions with maximum decolorization (Saratale et al. 2009).

Similarly, R^2 values of 0.993, 0.987 and 0.968 had been derived for decolorization and degradation of Ponceau 2R, malachite green and anthraquinone blue and suggests that the fitted linear plus interactions models could explain 99.3, 98.7, and 96.8 % respectively of the total variation (Levin et al. 2005). In general, the larger magnitude of t and smaller the value of p , more significant is the corresponding coefficient term (Montgomery 1991).

Conclusion

It can be concluded that the present study, that the bacterial consortium obtained from termites possess higher color removal efficiency and interestingly the experimental values were very much concurrent with the predicted values, which reflected the suitability of RSM. By applying these statistical methods for optimization of experiments, we could observe the process variables completely achieving

decolorization values up to 97.23 %. From this study, the bacterial consortium has the ability to decolorize dyes which indicates its potential application for decolorizing textile dyeing effluents which are reactive in nature, it was strong evidence. Further it is planned to design a bioreactor for treating similar dyes in large-scale system with the aid of this novel consortia and application of this design as well for future large-scale applications. To our knowledge, decolorization of reactive red 195 by termite associated bacterial consortium is reported for the first time.

Acknowledgments The author K. Nanthakumar acknowledges Council of Scientific and Industrial Research, New Delhi, India for providing SRF and the author K.Karthikeyan acknowledges UGC, New Delhi, India for providing JRF and they also thank Department of Environmental Sciences, Bharathiar University for providing facilities for this work.

References

- Adedayo O, Javadpour S, Taylor C, Anderson WA, Moo-Young M (2004) Decolorization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant. *World J Microbiol Biotechnol* 20:545–550
- Azambuja PD, Feder E, Garcia S (2004) Isolation of *Serratia marcescens* in the midgut of *Ghodnius prolixus*: impact on the establishment of the parasite *Trypanosoma cruzi* in the vector. *Exp Parasitol* 107:89–96
- Bhatt N, Patel KC, Keharia H, Madamwar D (2005) Decolorization of diazo dye Reactive Blue 172 by *Pseudomonas aeruginosa* NBAR12. *J Basic Microbiol* 45:407–418
- Box GEP, Hunter JS (1957) Multifactor experimental design for exploring the response surfaces. *Ann Math Stat* 28:195–242
- Box GEP, Wilson KB (1951) On the experimental attainment of optimum conditions. *J Roy Stat Soc B* 13:1–45
- Carliell CM, Barclay SJ, Naidoo N, Buckley CA, Mulholland DA, Senior E (1995) Microbial decolorization of a reactive azo dye under anaerobic conditions. *Water SA* 21:61–69
- Chang JS, Chou C, Chen SY (2001) Decolorization of azo dyes with immobilized *Pseudomonas luteola*. *Process Biochem* 36:757–763
- Chen KC, Wu JY, Liou DJ, Hwang SCJ (2003) Decolorization of the textile azo dyes by newly isolated bacterial strains. *J Biotechnol* 101:57–68

- Dey G, Mitra A, Banerjee R, Maiti BR (2001) Enhanced production of alpha amylase by optimization of nutritional constituents using response surface methodology. *Biochem Eng J* 7:227–231
- Handayani W, Meitiniarti VI, Timotius KH (2007) Decolorization of acid red 27 and reactive red 2 by *Enterococcus faecalis* under a batch system. *World J Microbiol Biotechnol* 23:1239–1244
- Hao OJ, Kim H, Chiang PC (2000) Decolorization of wastewater. *Crit Rev Environ Sci Technol* 30:449–505
- Jin X, Liu G, Xu Z, Yao W (2007) Decolorization of a dye industry effluent by *Aspergillus fumigatus* XC6. *Appl Microbiol Biotechnol* 74:239–243
- Keharia H, Madamwar D (2003) Bioremediation concepts for treatment of dye containing waste water: a review. *Ind J Exp Biol* 41:1068–1075
- Khehra MS, Saini HS, Sharma DK, Chadha BS, Chimmi SS (2005) Decolorization of various azo dyes by bacterial consortium. *Dyes Pigments* 67:55–61
- Konig H, Varma A (2006) *Intestinal microorganisms of termites and other invertebrates*, vol 24. Springer, Heidelberg, pp 483–493
- Korbahti BK, Rauf MA (2008) Application of response surface analysis to the photolytic degradation of basic red 2 dye. *Chem Eng J* 138:166–171
- Kumar K, Devi SS, Krishnamurthi K, Dutta D, Chakrabarti T (2007) Decolorization and detoxification of Direct Blue-15 by a bacterial consortium. *Bioresour Technol* 98:3168–3171
- Levin L, Forchiassin F, Viale A (2005) Ligninolytic enzyme production and dye decolorization by *Trametes trogii*: application of the Plackett–Burman experimental design to evaluate nutritional requirements. *Process Biochem* 40:1381–1387
- Liu BL, Tzeng YM (1998) Optimization of growth medium for production of spores from *Bacillus thuringiensis* using response surface methodology. *Bioproc Eng* 18:413–418
- Mendez Paz D, Omil F, Lema JM (2005) Anaerobic treatment of azo dye Acid Orange 7 under batch conditions. *Enzyme Microb Technol* 36:264–272
- Mohana S, Shrivastava S, Divecha J, Madamwar D (2008) Response surface methodology for optimization of medium for decolorization of textile dye Direct Black 22 by a novel bacterial consortium. *Bioresour Technol* 99:562–569
- Montgomery DC (1991) *Design and analysis of experiments*. Wiley, New York
- Moutaouakki A, Zeroula Y, Dzayri FZ, Talbi M, Lee K, Belgani M (2000) Purification and partial characterization of azo reductases from *Enterococcus agglomerans*. *Arch Biochem Biophys* 413:139–146
- Nachiyar C, Rajkumar GS (2003) Degradation of a tannary and textile dye, Navitian Fast Blue S5R by *Pseudomonas aeruginosa*. *World J Microbiol Biotechnol* 19:609–614
- Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. *Biometrika* 33:305–325
- Ravikumar K, Ramaligam S, Krishnan S, Balu K (2006) Application of response surface methodology to optimize the process variables for reactive red and acid brown dye removal using a novel biosorbent. *Dyes Pigments* 70:18–26
- Robinson T, Mc Mullan G, Marchant R, Nigam P (2000) Remediation of dyes in textile effluents: a typical review on current treatment technologies with a proposed alternative. *Bioresour Technol* 77:247–255
- Saratale RG, Saratale GD, Chang JS, Govindwar SP (2009) Decolorization and biodegradation of textile dye navy blue HER by *Trichosporon beigelii* NCIM-3326. *J Hazard Mater* 166:1421–1428
- Sarnaik S, Kanekar P (1999) Biodegradation of methyl violet by *Pseudomonas mendocina* MCM B-402. *Appl Microbiol Biotechnol* 52:251–254
- Sharma P, Singh L, Dilbaghi N (2009) Optimization of process variables for decolorization of disperse yellow 211 by *Bacillus subtilis* using Box–Behnken design. *J Hazard Mater* 164:1024–1029
- Sponza DT, Isik M (2004) Decolorization and inhibition kinetic of direct black 38 azo dye with granulated anaerobic sludge. *Enzyme Microb Technol* 34:147–158
- Tan NCG (2001) *Integrated and sequential anaerobic/aerobic biodegradation of azo dyes*. Ph.D. Thesis, Wageningen University, Wageningen
- Wong PK, Yuen PY (1996) Decolorization and degradation of methyl red by *Klebsiella pneumoniae* RS 13. *Water Res* 30:1736–1744