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Process Optimization of Experimental Variables Using Plackett-Burman Design for Decolourisation of Reactive Blue 222 by a Novel Bacterial Consortium Isolated from the Gut of Termites

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Abstract. Bacterial consortium derived from termite was tested for its efficiency to decolourise Reactive Blue 222 aerobically. The central composite design matrix and response surface methodology (RSM) were applied to design experiments for the assessment of interactive effects of four most important operating variables viz., pH (3.0-11.0), agitation (300 rpm), temperature (20-60 °C) and glucose (0.1-0.5 g/litre) on the biodegradation of Reactive Blue 222 out of eleven different variables. Optimisation was achieved using the Plackett-Burman statistical design. A regression coefficient between variables and the response indicated excellent evaluation of experimental data by the Stat-Ease package. The experimental values were in good agreement with the predicted ones and the model was highly significant, correlation coefficient being 0.89. RSM indicated that pH 7.0 at static condition; temperature at 20 °C and a glucose concentration of 0.50 g/litre resulted in 99.21% decolourisation.

Keywords: decolourisation, Reactive Blue 222, bacterial consortium, termite gut, central composite design

Introduction

Recently, many of the South Asian countries are experiencing severe environmental problems due to their rapid industrialisation. This phenomenon is very common where the polluting industries like textile dyeing, leather tanning, paper and pulp processing, sugar manufacturing, etc. thrive as clusters.

Textile dyeing industry consumes large quantities of water and produces large volumes of wastewater at different steps of printing, dyeing and finishing processes, which is often rich in colour, containing residues of reactive dyes and chemicals, and requires proper treatment before being released into the environment. India produces dyestuff and pigments close to 80,000 tonnes and is the second largest exporter of dyestuffs and intermediates among developing countries, after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80% (Mathur *et al*., 2005). During textile production, potentially hazardous compounds at various stages of operations are released with a negative impact on the ecosystem (Asamudo *et al*., 2005).

Treatment and disposal of effluents from the textile and dyeing industries is quite difficult by common physical and chemical methods, mainly because of the high BOD, COD, heat, colour, pH and presence of metal ions. Besides, they are highly expensive, emit toxic substances and form large amounts of sludge (Johnson *et al*., 1978), posing disposal problem (Banat *et al*., 1996). Several physicochemical decolourisation techniques have been reported, a few of which were accepted by the textile industries (DaSilva and Faria, 2003; Okazaki *et al*., 2002).

Microbial degradation and decolourisation is an environment friendly and cost-competitive alternative to chemical decomposition processes. Many microorganisms belonging to different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolourise azo dyes (Khehra *et al*., 2005). Pure fungal cultures have been used to develop bioprocesses for the mineralisation of azo dyes (Zhang *et al*., 1999). However, the long growth cycle and moderate decolourisation rate limit the performance of fungal decolourisation system (Banat *et al*., 1996). In contrast, bacterial decolourisation is normally faster, but it requires a mixed community of bacteria to mineralise azo dyes through a combined metabolic mode of anaerobic-aerobic sequence (Chang *et al*., 2004). In previous studies, mixed bacterial cultures having the highest decolourisation activity under anaerobic conditions were grown on rich media supplemented with yeast extract or glucose (Chen *et al*., 2003; Oxspring *et al*., 1996; Nigam *et al*., 1996a).

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Mixed culture studies may be more appropriate for decolourisation of azo dyes. About 80% of colour removal in an effluent sample containing mixture of azo and diazo reactive dyes was observed by He *et al*. (2004) using mixed bacterial culture. As the catabolic activities of microorganisms in mixed consortium complement each other, obviously syntrophic interactions present in the mixed communities can lead to complete mineralisation of azo dyes (Chang *et al*., 2004). Recently, different kinds of dye decolourising bacterial cultures under anaerobic conditions were isolated from textile effluents (Kapdan *et al*., 2000; Nigam *et al*., 1996b). In general, a single parameter method or single dimensional search which involves changing one variable while others are kept constant at a certain level, is laborious and time consuming especially when the number of variables is large. An alternative and effective approach is, therefore, increasingly being involved in the use of statistical methods (McDaniel *et al*., 1976). The Plackett-Burman (PB) experimental design (Plackett and Burman, 1946) was applied to maximise enzyme production and decolourisation efficiency.

Termites, in this study were selected as the source of potent dye-degrading bacteria because they are assumed to harbour microorganisms responsible for digestion of lignocellulosic substances; a complex symbiotic community of prokaryotic and eukaryotic microorganisms has been found in the intestinal tract of termites. The gut flora consists of bacteria, archaea and archaezoa (Konig and Varma, 2006). In this respect, the use of a bacterial consortium has several advantages. Generally, such bacteria do not require sterile conditions, thus greatly reducing costs. Besides, they are usually more resistant to changes in pH, temperature and feed composition as compared to individual species (Forgacs *et al*., 2004). Finally, there is a higher possibility of complete mineralisation of the dye since few strains have been found which can metabolise such types of compounds (Pearce *et al*., 2003). Hence the present study is aimed at employing the termite-derived bacterial consortium for decolourisation of Reactive Dye. Sani and Banerjee (1999) found 92, 96 and 96% decolourisation rates of Magenta, Crystal Violet and Malachite Green dyes, respectively, using Kurthia sp. They found higher COD removal efficiency (56-85%) and decolourisation efficiency (19-33 mg/g).

Reactive Blue 222 is one of the reactive azo dyes and has large consumption in the textile dyeing processes. It is a well-known surrogate for non-biodegradable Reactive Azo dyes. In the present study, the decolourisation of Reactive Blue 222 was accomplished by a bacterial consortium isolated from the termite gut. The optimisation conditions were also assessed by the response surface methodology using Plackett-Burman design.

Materials and Methods

Chemicals and media. The dye powder used in this study was obtained from Hi Media, Mumbai. All the chemicals used were of the highest purity or analytical grade, obtained from recognised chemical suppliers. Decolourising medium (DM) was used for decolourisation and the composition (g/litre) was as follows: glucose, 2.0; peptone, 2.0; NaCl, 5.0; $CaCl_2$, 0.04; $MgSO_4$, 0.02; distilled water 1000 ml; pH of the medium was adjusted to 7.0. For solid media, agar was added at concentration of 20 g/litre.

Preparation of dye solution. The dyes were used in dissolved form throughout the experiment. One gram of dye was dissolved in deionised water and made up to 1000 ml to give 1000 mg/litre. From the stock solution, required concentrations were prepared. Whenever needed, dye solutions were filter sterilised in 0.45µ membrane filter and aseptically added to the medium.

Development of consortium from termites. Wood-eating termites (*Macrotermes* sp.), were collected from a soil mound of Western Ghats region, Coimbatore, Tamilnadu, India. Termites were collected in a sterile container and separated from soil and used for bacterial isolation. Collected termites were washed with sterile de-ionised water and surface sterilised with diluted ethanol under aseptic conditions. Ten grams of termites were homogenised in a sterile glass mortar with distilled water and made up to 100 ml. The entire process was carried out under ascetic conditions.

Preparation of inoculum of consortium. Individual bacterial strains (six in all) were grown in nutrient broth for 16-18 hs; one ml of culture was taken from each flask and mixed well. Optical density was adjusted to 1 OD containing the cell concentration of 32.0×10^4 CFU/ml.

Dye concentration measurement (decolourisation assay). A standard solution of dye was taken and the absorbance was determined at its wavelength to obtain a plot of absorbance *vs.* wavelength. The wavelength corresponding to maximum absorbance (λ_{max}) was determined from this plot for the dyes (612 nm for Reactive Blue 222). These wavelengths were used for preparing the calibration curves between absorbance and the concentration of dye solution. During estimation, samples were pelletised by brief centrifugation at 10,000 rpm for 15 mins to prevent absorbance interferences from the cellular or other suspended debris. The absorption spectrum of the clear supernatant was recorded using a spectrophotometer (UV-Vis 3210, Hitachi, Japan). Dye solution incubated without

the inoculum was taken as positive control and uninoculated culture without dye was used as negative control (blank) for the dye and the rate of decolourisation was calculated. Samples with higher concentration of dyes were diluted, when necessary, for accurate determination of the dye concentration in the solution. The absorbance value obtained in each case was then used to calculate percentage decolourisation of the dye. Decolourisation of culture supernatant was measured at the absorbance maxima of dye, as follows:

$$\text{Decolourisation (\%)} = [\{A_i - A_f\} / A_i] \times 100$$

where, A_i and A_f are the initial and the final absorbance values, respectively. Results were corrected according to the blank (dye free) samples.

Optimisation of the operational variables for decolourisation of the dyes. In order to optimize the conditions required for decolourisation, various operational variables were employed such as pH, agitation condition (shaking), static condition, temperature, incubation time, and dye concentration. Influence of sodium salts and phenols on colour removal was also monitored.

Medium optimization for decolourisation of Congo Red by the bacterial consortium. Process optimization could be carried out by empirical or statistical methods. The empirical method is time consuming and does not necessarily enable an effective optimization. A statistics-based procedure called the Response Surface Method (RSM) is a powerful experimental design tool to recognise the performance of composite systems (Kiran *et al*., 2007; Linder *et al*., 2005; Ravikumar *et al*., 2005). The RSM represents an assemblage of experimental design and multiple regression-based methods that can be applied to evaluate tribulations where several factors might influence a response (Gardiner and Gettinby, 1998).

For any microbial decolourisation process, media components such as carbon source, nitrogen source, dye concentration, inoculum size, pH and temperature are the most important parameters which affect the process. The main conventional strategy used is media engineering for which the optimal operating conditions of a process are optimized by changing one parameter at a time and keeping the others at a constant level. This method often does not yield reliable results, and is also laborious, time consuming and impractical. In this regard, the recently developed response surface methodology is a useful model for studying the effect of several 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 central composite design as a second step to optimize the factors that have significant effects on response surface analysis. This methodology was adopted for the study.

Screening of important nutrient components using Plackett-Burman design. ***Experimental design and statistical analysis.*** The Plackett-Burman experimental design was used to evaluate the relative importance of various nutrients for dye decolourisation by the bacterial consortium in static condition. This design presumes that there are no interactions between different media constituents, in the range of variables under consideration. A linear approach is considered to be sufficient for screening.

This study was carried out using Plackett-Burman design for screening medium components with respect to their main effects and not to their interaction effect (Plackett and Burman, 1946) on dye decolourisation by the bacterial consortium. The medium components were screened for eleven (11) variables at two levels, maximum (+) and minimum (-). According to the Plackett-Burman 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. A column should contain equal number of positive and negative signs. The first row contains (N+1)/2 positive signs and (N-1)/2 negative signs; the choice of placing the signs is arbitrary. The next (N-1) rows are generated by shifting cyclically one place (N-1) and the last row contains all negative signs. The experimental design and levels of each variable are shown in Table 1.

The medium was formulated as per design and the flask culture experiments; dye decolourisation was assayed as described earlier. Response was calculated as percentage rate of the dye decolourisation. All experiments were performed in triplicate.

The effect of each variable was calculated using the following equation:

$$E = (\Sigma M^{+} - \Sigma M^{-}) / N$$

where E is the effect of tested variable, M^{+} and M^{-} are responses (dye decolourisation) of trials at which the parameter was at its higher and lower levels, respectively, and N is the number of experiments carried out.

The standard error (SE) of the variables was the square root of variance and the significance level (p - value) of each variable calculated by using Student's t - test.

$$t = E_{xi} / SE$$

where E_{xi} is the effect of the tested variable. The variables with higher confidence levels were considered to influence the response or the output variable.

Table 1. Plackett-Burman experimental design for screening significant process variables affecting decolorisation of Reactive Blue 222

Run	pH	Temp (°C)	Agitation (rpm)	Incubation time (h)	Dye conc. (ppm)	Glucose (g/litre)	Amm. nitrate (g/litre)	Mag. sulphate (g/litre)	Chromium (g/litre)	Phenols (µg/litre)	Trace salts (g/litre)
1	3	20	0	0	10	0.1	0.1	0.05	0.05	0.05	0.05
2	11	20	300	72	1000	0.1	0.1	0.05	0.5	0.05	0.5
3	11	60	0	0	10	0.5	0.1	0.5	0.5	0.05	0.5
4	11	20	300	72	10	0.5	0.5	0.5	0.05	0.05	0.05
5	3	20	0	72	10	0.5	0.5	0.05	0.5	0.5	0.5
6	11	20	0	0	1000	0.1	0.5	0.5	0.05	0.5	0.5
7	3	60	300	0	1000	0.5	0.5	0.05	0.05	0.05	0.5
8	11	60	0	72	1000	0.5	0.1	0.05	0.05	0.5	0.05
9	3	20	300	0	1000	0.5	0.1	0.5	0.5	0.5	0.05
10	11	60	300	0	10	0.1	0.5	0.05	0.5	0.5	0.05
11	3	60	0	72	1000	0.1	0.5	0.5	0.5	0.05	0.05
12	3	60	300	72	10	0.1	0.1	0.5	0.05	0.5	0.5

Optimisation of concentrations of the selected medium components using response surface methodology. Response surface methodology is an emprical statistical modeling technique employed for multiple regression analysis using quanitative data obtained from factorial design to solve multivariable equations simultaneously. The screened medium components affecting dye decolourisation were optimized using cenral composite design (CCD) (Box and Hunter, 1957; Box and Wilson, 1951).

According to this design, the total number of treatment combinations is $2^k + 2k + n0$ where 'k' is the number of independent variables and $n0$ the number of repetitions of the experiments at the center point. For statistical calculation, the variables X_i have been coded as x_i according to the following transformation:

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

where x_i is dimensionless coded value of the variables X_i, X_0 the value of the X_i at the center point, and δX is the step change. A 2^k-factorial design with eight axial points and six replicates at the center point with a total number of 30 experiments was employed for optimizing the medium components.

The behaviour of the system was explained by the following quadratic equation:

$$Y = \beta_o + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j$$

where Y is the predicted response, β_0 the intercept term, β_i the linear effect, β_{ii} the squared effect, and β_{ij} is the interaction effect. The regression equation was optimized for maximum value to obtain the optimum conditions using Design Expert Version 7.1.5 (State Ease, Minneapolis, MN).

Validation of the experimental model. The statistical model was validated with respect to dye decolourisation under the conditions predicted by the model in flask conditions. Samples were withdrawn at the desired intervals and the dye decolourisation was determined as described above.

Extraction and analysis of the degraded products. After 48 hs of decolourisation, the entire decolourised medium was centrifuged at 5000 rpm for 20 mins. Supernatant of decolourised components of the dyes were extracted using equal volume of ethyl acetate, dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary vacuum evaporator. The biodegradation was analysed by FTIR spectroscopy. Biodegradation of Reactive Blue 222 was characterised by Fourier Transform Infrared Spectroscopy (Shimadzu), compared with the control dye and changes in transmission (%) at different wavelengths were observed. FTIR analysis was performed in the mid IR region of 400-4000/cm with scan speed of 16. The pellets prepared using spectroscopic pure KBr (5:95) were fixed in sample holder and analyses were carried out.

Results and Discussion

Optimisation of bioprocess variables using factorial design and response surface methodology for decolourisation of the dye Reactive Blue by bacterial consortium. *Plackett–Burman design*. In the present study, the influence of eleven factors (A - L) namely pH, temperature, agitation, incubation time, dye concentration, glucose, yeast extract, KH_2PO_4, chromium, para nitrophenol and trace salts on the dye decolourisation was investigated in 12 runs using Plackett-Burman design. Table 2 represents the Plackett-Burman design for the eleven (11) selected variables and the corresponding

Table 2. Plackett-Burman experimental design for evaluating factors influencing dye degradation by the bacterial consortium

Run	A	B	C	D	E	F	G	H	J	K	L	Decolourisation (%)
1	3	20	0	0	10	0.1	0.1	0.05	0.05	0.05	0.05	9.05
2	3	20	0	72	10	0.5	0.5	0.05	0.5	0.5	0.5	64.8
3	3	60	0	72	1000	0.1	0.5	0.5	0.5	0.05	0.05	33.9
4	11	20	300	72	10	0.5	0.5	0.5	0.05	0.05	0.05	24.8
5	3	60	300	0	1000	0.5	0.5	0.05	0.05	0.05	0.5	18.9
6	3	60	300	72	10	0.1	0.1	0.5	0.05	0.5	0.5	22.9
7	3	20	300	0	1000	0.5	0.1	0.5	0.5	0.5	0.05	5.5
8	11	20	0	0	1000	0.1	0.5	0.5	0.05	0.5	0.5	11.9
9	11	60	0	72	1000	0.5	0.1	0.05	0.05	0.5	0.05	44.8
10	11	20	300	72	1000	0.1	0.1	0.05	0.5	0.05	0.5	79.4
11	11	60	0	0	10	0.5	0.1	0.5	0.5	0.05	0.5	13.1
12	11	60	300	0	10	0.1	0.5	0.05	0.5	0.5	0.05	13.1

A: pH; B: temperature (°C); C: agitation (rpm); D: incubation time (h); E: dye concentration (ppm); F: glucose (g/litre); G: yeast extract (g/litre); H: KH_2PO_4 (g/litre); J: chromium (g/litre); K: paranitro phenol (µg/litre); L: trace salts (g/litre)

response for the dye decolourisation. Variations ranging from 5.5 to 79.4% in the dye decolourization were observed by Plackett-Burman design. Our reports concur with the results observed by Prouty (1990), Bumpus and Aust (1986) and Reid (1979) that dye decolourisation was up to 98.0% within 1-2 days which is similar to our report on Reactive Blue 222 decolourisation. They also reported that dye degradation decreased with the increase in the dye concentration.

The Pareto chart (Fig. 1) illustrates the order of significance of the variables affecting dye decolourisation. Among the

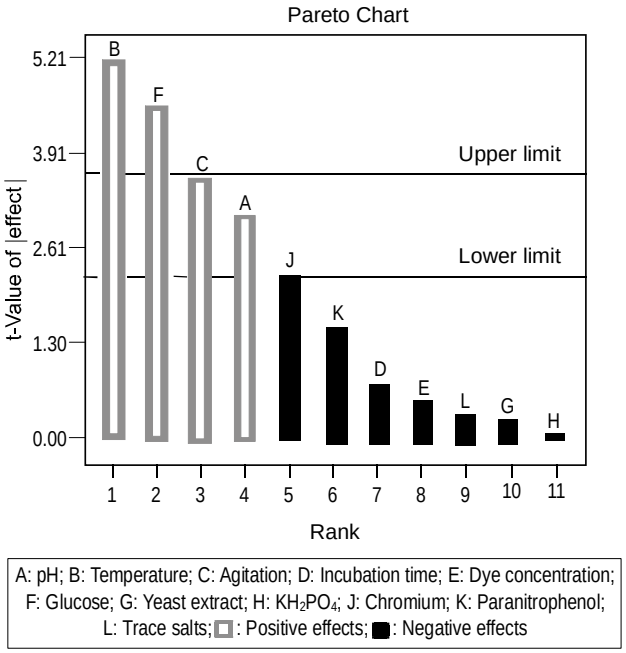

Fig. 1. Pareto chart for Plackett-Burman design for 11 factors on decolourisation of Reactive Blue 222 by bacterial consortium.

variables screened, the most effective factors with high significance level indicated by Pareto chart were in the order of pH, temperature, agitation and glucose. They were identified as the most significant variables in dye decolourisation and selected for further optimization. A report of Sani *et al*. (1998) suggested that the agitated cultures have often been found more efficient in decolourisation of various dyes, compared to the static ones, presumably, because of an increased mass and oxygen transfer.

Statistical analysis of the Plackett-Burman design demonstrates that the model F value of 0.81 is significant. The values of $p < 0.05$ indicate that the model terms are significant (Table 3).

Regression analysis was performed on the results and the first order polynomial equation was derived representing dye decolourisation as a function of the independent variables.

Reactive Blue decolourisation (%) = + 48.93 + 23.73 A + 9.84 B + 17.83 C + 6.93 F

The magnitude of the effect indicates the level of significance of the variable on the decolourisation of Reactive Blue. Consequently, based on the results from this experiment, statistically significant variables i.e. pH, agitation, temperature and the glucose with positive effect were further investigated with central composite design to find the optimal range of these variables.

Central composite design. Results of 30 runs of Central Composite Design (CCD) in four variables, pH, agitation, temperature and glucose chosen for the optimisation of decolourisation of Reactive Blue dye by bacterial consortium are shown in Table 4. It shows decolourisation (%) corresponding to the combined effect of four components in their

Table 3. Analysis of variance (ANOVA) for dye decolourisation

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value	Remarks
Model	2466.5	7	352.357	0.8187	0.0047	significant
A-pH	22.1408	1	22.1408	1.40243	0.3019	
B-Temperature	1857.54	1	1857.54	117.659	0.0004	
C-Agitation	102.668	1	102.668	6.50309	0.0633	
F-Glucose	22.1408	1	22.1408	1.40243	0.3019	
Residual	63.15	4	15.7875			
Total	2529.65	11				

specified ranges. Decolourisation varied markedly with the conditions tested, in the range of 12.69-99.21%. Decolourisation values of 99.21% was observed at pH 7, agitation 0 rpm, temperature 20 °C and the glucose 6 g/litre (run 30). The experimental results suggested that these variables strongly affect the decolourisation of Reactive Blue. The results obtained were subjected to analysis of variance on Stat-Ease package, with the regression model given as:

$$Y = + 97.17 - 3.13\,A - 3.96\,B - 4.13\,C + 2.96\,D + 1.94\,AB + 2.94\,AC + 0.063\,AD - 5.56\,BC - 2.94\,BD - 1.44\,CD - 22.18\,A^2 - 12.68\,B^2 - 17.68\,C^2 - 7.43\,D^2$$

where Y is the response value (percent decolourisation of Reactive Blue) and A,B,C and D are the coded levels of pH, agitation, temperature and glucose, respectively. The adequacy of the model was checked using analysis of variance (ANOVA) and the results are presented in Table 5. The analysis of variance of the quadratic regression model suggested that the model is very significant as was evident from the Fisher's F-test. The model F value of 9.30 implies that the model is significant. The R^2 value (multiple correlation coefficient) closer to 1 denotes better correlation between the experimental and predicted responses. In this case, the value of R^2 (0.89) indicates good correlation between the experimental and predicted values. It was also reported that in a study of the stability and kinetics of *b*-1,3-glucanase from *Trichoderma harzianum*, a very low value of R^2 was obtained. A central composite design with two independent variables, pH and temperature, was applied and the estimated responses were compared with the experimental values. R^2 has been reported as 0.6899 and AAD value was calculated as 20.63% (Rana *et al*., 2003).

The coefficient of variation (CV) indicates degree of precision with which the experiments are compared. The lower reliability of the experiment is usually indicated by high value of CV. In the present case, a low CV (3.14) denotes that the experiments performed are highly reliable. The *p* value denotes the significance of coefficients and is also important in understanding the pattern of mutual interactions between the variables.

The main goal of response surface is to efficiently hunt for the optimum values of the variables so that the response is maximised (Dey *et al*., 2001). The interaction effects and optimal levels of the variables were determined by plotting the response surface curves. The response surface contour plots are presented in Fig. 2. The contour plots showcase the

Table 4. Experimental plan for optimisation of dye decolourisation using central composite design

Run	pH	Agitation (rpm)	Temperature (°C)	Glucose (g/litre)	Decolourisation (%)	
					Experimental	Predicted
1	1	1	1	-1	21.65	26.6667
2	1	1	-1	1	35.39	40.3333
3	-2	0	0	0	22.67	24.3333
4	1	-1	1	-1	36.98	35.9583
5	0	-2	0	0	55.98	54.375
6	-1	-1	1	-1	32.35	40.3333
7	-1	1	1	-1	23.22	23.2917
8	1	-1	-1	1	43.25	39.125
9	-1	1	-1	-1	46.07	45.6667
10	0	0	0	0	94.17	97.1667
11	-1	1	1	1	19.98	20.3333
12	-1	-1	-1	-1	48.24	40.4583
13	-1	-1	1	1	48.22	49.125
14	0	0	0	2	76.94	73.375
15	1	1	-1	-1	34.01	37.2917
16	-1	1	-1	1	43.93	48.4583
17	-1	-1	-1	1	51.29	55
18	0	0	0	-2	68.04	61.5417
19	0	0	2	0	17.93	18.2083
20	0	2	0	0	37.44	38.5417
21	0	0	-2	0	37.01	34.7083
22	0	0	0	0	98.02	97.1667
23	0	0	0	0	97.19	97.1667
24	0	0	0	0	98.31	97.1667
25	2	0	0	0	24.09	22.2083
26	0	0	0	0	97.3	97.1667
27	1	-1	1	1	41.22	45
28	1	1	1	1	22.92	23.9583
29	1	-1	-1	-1	12.69	14.7083
30	0	0	0	0	99.21	97.1667

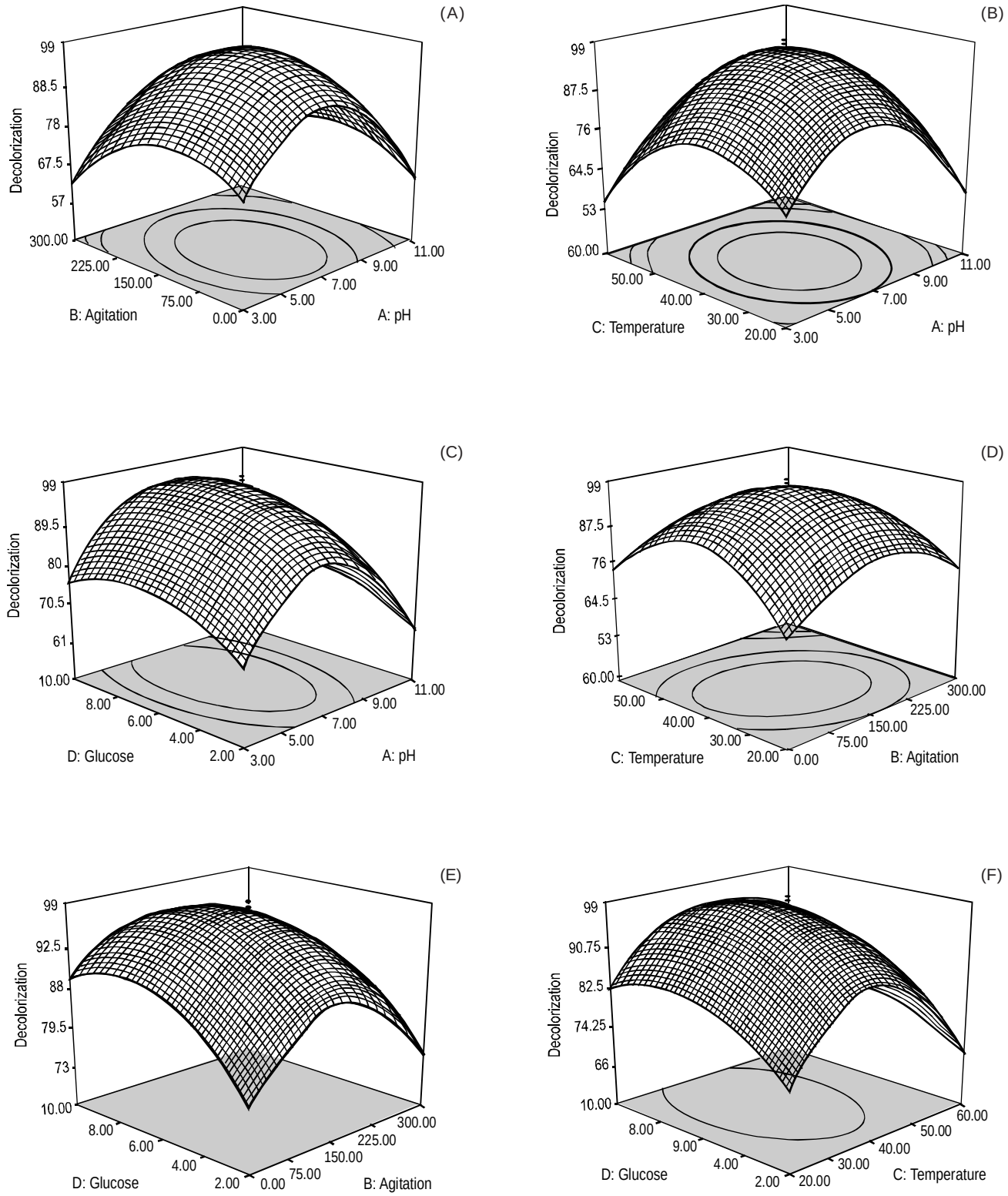

Fig. 2. Three dimensional response surface plot for the effect of (A) pH, agitation; (B) pH, temperature; (C) pH, glucose; (D) agitation, temperature; (E) agitation, glucose; (F) temperature, glucose on decolourisation of Reactive Blue by bacterial consortium.

Table 5. ANOVA for the experimental results of the central composite design (quadratic model)

Source	Sum of squares	Degree of freedom	Mean square	*F* - Value	*p* - Value	Remarks
Model	23234.7	14	1659.62	9.30949	< 0.0001	Significant
A-pH	234.375	1	234.375	1.3147	0.2695	
B-Agitation	376.042	1	376.042	2.10937	0.1670	
C-Temperature	408.375	1	408.375	2.29074	0.1509	
D-Glucose	210.042	1	210.042	1.17821	0.2949	
AB	60.0625	1	60.0625	0.33691	0.5702	
AC	138.063	1	138.063	0.77445	0.3927	
AD	0.0625	1	0.0625	0.00035	0.9853	
BC	495.063	1	495.063	2.777	0.1164	
BD	138.063	1	138.063	0.77445	0.3927	
CD	33.0625	1	33.0625	0.18546	0.6728	
A^2	13490	1	13490	75.6708	< 0.0001	
B^2	4408	1	4408	24.7262	0.0002	
C^2	8570.86	1	8570.86	48.0774	< 0.0001	
D^2	1513	1	1513	8.48704	0.0107	
Residual	2674.08	15	178.272			
Lack of fit	2659.25	10	265.925	89.6376	< 0.0001	Significant
Pure error	14.8333	5	2.96667			
Cor total	25908.8	29				

CV - 3.14; R^2 - 0.89

behaviour of response (decolourisation percentage) with respect to the simultaneous change in two variables. The roles played by various factors affecting the decolourisation percentage are highlighted by 3D graphs. The 3D response surface plots described by the regression model were drawn to illustrate the effects of the independent variables and combined effects of each independent variable on the response variable.

Validation of the model. The maximum experimental response for decolourisation of Reactive Blue was 99.21% whereas the predicted value was 97.16% indicating a strong agreement between them. The optimum values of the tested variables are pH 7.0, agitation 0 rpm, temperature 20 °C and glucose 6 g/litre as shown in perturbation graph (Fig. 3). The results are encouraging for optimization under pilot scale or industrial scale conditions. Similar R^2 values were reported by Levin *et al*. (2005) being 0.993 for Ponceau 2R decolourisation, 0.987 for Malachite Green and 0.968 for Anthraquinone Blue degradation. It suggests that the fitted linear plus interactions models could explain 99.3, 98.7 and 96.8%, respectively, of the total variation.

FTIR analysis. Results of FTIR analysis of the control and the sample obtained after decolourisation showed various peaks. The FTIR spectra of the control displays various significant peaks at 1135.99 cm^{-1}, 1045.35 cm^{-1}, 1018.34 cm^{-1} and 617.18 cm^{-1} which supports the presence of secondary amines CN stretch, alkyl substituted ether C-O stretch, thioethers, CH_3 -S -(C - S stretch), stretched vibrations with O-H as functional groups etc. The FTIR spectra of the treated Reactive Blue 222 showed the specific peaks in finger print region for primary amine which is supporting the peak at 3469.70 cm^{-1}, 3384.84 cm^{-1} and 1458.08 cm^{-1} for stretching vibrations of the functional groups with N-H bonds and primary amines having two bonds. The group frequency region shows specific peaks for functional groups; the peak at 1635.52 cm^{-1} for -N=N- stretching vibrations represents the presence of hydroxyl (-OH) and secondary amino (-NH-)

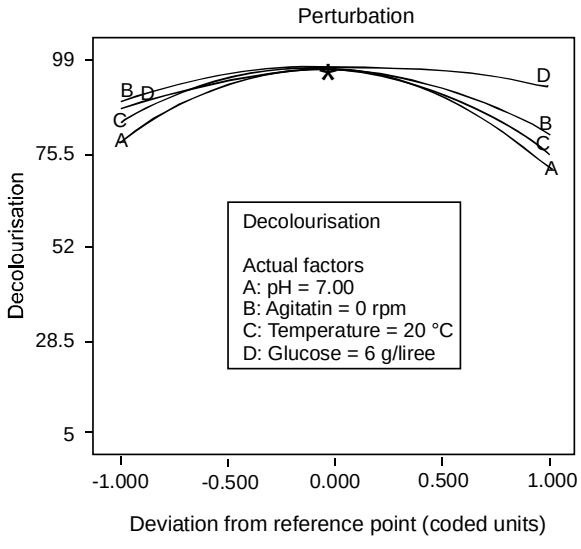

Fig. 3. Perturbation graph showing the optimum values of the tested variables.

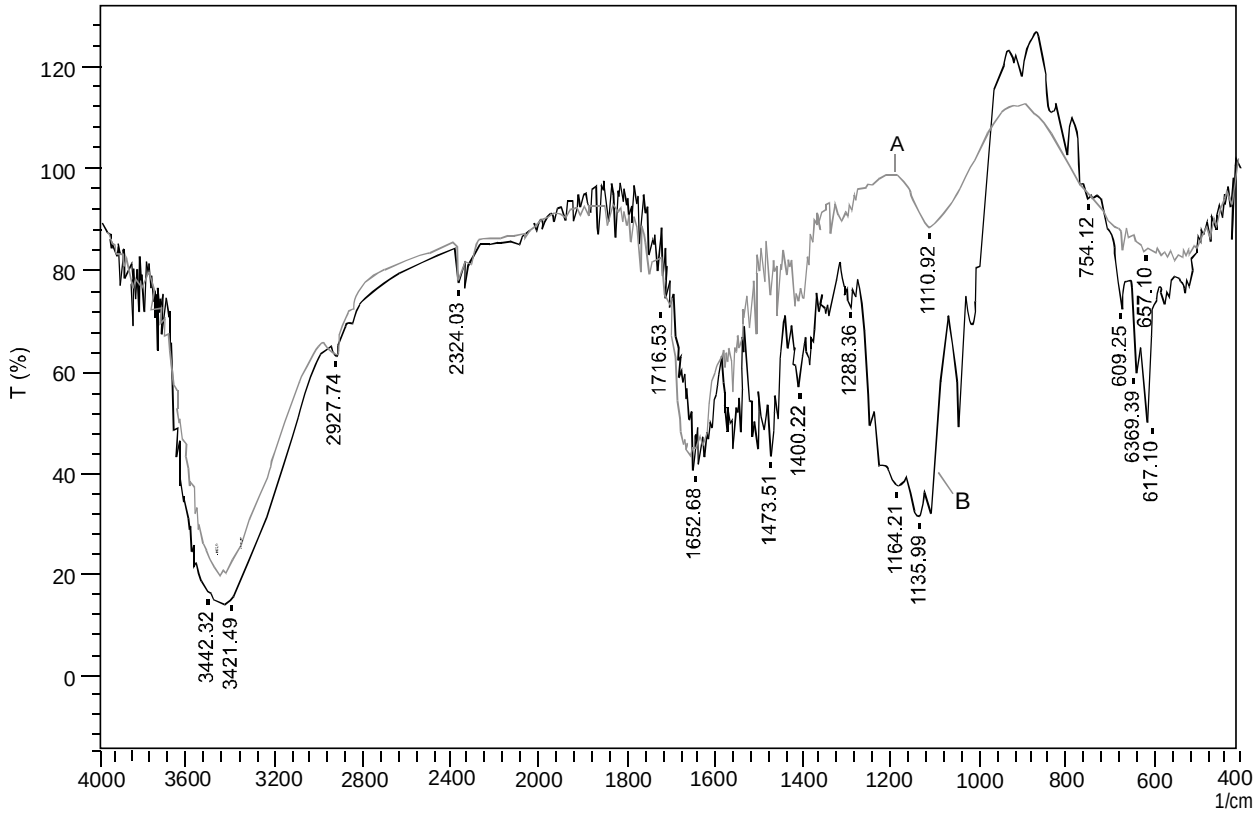

Fig. 4. FTIR spectra of Reactive Blue 222 (A) before and (B) after treatment with the bacterial consortium.

groups in the dye. Considerable difference in the FTIR spectrum of Reactive Blue 222 reveals biodegradation (Fig. 4).

Conclusion

The bacterial consortium exhibited greatest ability in decolorising Reactive Blue 222 which seems to be a practical approach. It can be concluded from the study that the bacterial consortium obtained from termites possesses higher colour removal efficiency due to synergistic activity among the strains. FTIR spectra of raw dye and treated degradation products proved that the dye was completely mineralised by the bacterial consortium. This study shows that the response surface methodology is a suitable system to optimise the excellent culture conditions for achieving the maximum decolourisation of dye. The experimental and the predicted values were very close which reflected the accuracy and the applicability of RSM. By applying central composite design and RSM to the optimization experiments, the process variables could be completely investigated and decolourisation values up to 99.21 could be achieved. The culture not only decolourised the dye but it also degraded it as seen in the FTIR spectra. The efficiency of the bacterial consortium supports the merits of using a compatible mixed strain which could be further exploited for treating similar dye-bearing waste waters. Thus biodecolourisation and biodegradation of textile dye Reactive Blue 222 by the isolated bacterial consortium from termite gut can be claimed as an environment friendly method of dye degradation. Further work on identification of the intermediates formed is in progress.

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