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Optimization of Reactive Black 5 dye and Reactive Red 120 dye degradation

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ABSTRACT

The present study was undertaken to study the statistical optimization of medium components for improved Reactive Red 120 dye and Reactive Black 5 dye degradation by P. aeruginosa and A. punctata. Yeast extract, aeration and temperature were identified as significant components influencing Reactive Red 120 dye degradation whereas dye and pH were highly significant on Reactive Black 5 dye degradation. The main factors that had significant positive effects on both the dye degradation were glucose, yeast extract, aeration, inoculum, dye concentration, pH. This statistical optimization approach has led to 100% and 95% degradation of Reactive Red 120 dye and Reactive Black 5 dye respectively within 24 hours of incubation. Statistical approach was found to be very effective in optimizing the medium components in manageable number of experimental runs.

Key words: Reactive Red 120 dye, Reactive Black 5 dye, degradation, optimization, Plackett-Burman Design.

INTRODUCTION

Synthetic dyes are extensively used in textile, paper and printing industries as well as in dye houses [1]. The coloring process discharge huge quantities of dye effluents, which pollute local terrestrial habitat, aquatic bodies as well as rivers. Synthetic dyes are not easily amenable for microbial attack as they contain substitution such as azo, nitro and sulpho groups [2, 3].

Reactive Black 5 dye has been used in the textile industries for the dying of cotton, woolen and nylon fabrics worldwide. It is reported to be toxic and cause allergic reactions of respiratory tract [4, 5]. Reactive Red 120 dye is most suitable for dyeing cellulose fibers because of the effect of the two sulfonic acid groups. It may cause sensitization by inhalation and skin contact.

There are reports on degradation of Reactive Black 5 dye and Reactive Red 120 dye [6-10]. However reports on effect of various parameters on degradation of Reactive Black 5 dye and Reactive Red 120 dye are limited. Hence the present investigation has been selected to study optimization of Reactive Black 5 dye and Reactive Red 120 dye degradation.

EXPERIMENTAL SECTION

Dyes and other chemicals: Reactive Black 5 dye, Reactive Red 120 dye and other chemicals were procured from Hi-media Pvt. Ltd., Mumbai, India.

Culture: *Aeromonas punctata* and *Pseudomonas aeruginosa* isolated from textile industrial effluent, identified by nucleotide sequencing and deposited in gene bank with the accession numbers JN561149 and JN561148 respectively were used for degradation studies.

Degradation studies: One ml of cultures were inoculated into separate flasks containing 100 ml of mineral salts medium with pH 7 and the flasks were incubated at 37°C in static condition [11]. Degradations of dyes were analysed using UV-Vis spectrophotometer reading of samples at 597 nm for Reactive Black 5 dye and 520 nm for Reactive Red 120 dye.

Optimization of media components by Placket-Burman design: Plackett-Burman design, an efficient way to identify the important factors among a large number of variables was used in the present study to screen the important variables that significantly influenced reactive dye degradation [12]. A total of nine variables (table1) were selected for the study with each variable being represented at two levels, high (+) and low (-) and two dummy variables in 12 trials. The number of positive and negative signs per trial was $(k+1)/2$ and $(k-2)/2$, respectively. Each row represents a trial and each column represents an independent (assigned) variable. The main effect of each variable was determined by the following equation:

$$E(X_i) = 2\left(\sum M_i^{+} - M_i^{-}\right) / N \qquad (1)$$

Where, $E(X_i)$ is the concentration effect of the tested variables. M_i^{+} and M_i^{-} represent dye degradation from the trials, where the independent variable (X_i) measured was present at high and low concentrations respectively. N, total number of the trials equals to 12. Experimental error was estimated by calculating the variance among the dummy variables as follows:

$$V_{eff} = \Sigma (E_d)^2 / n \qquad (2)$$

Where, V_{eff} is the variance of concentration effect, E_d is the concentration effect for dummy variable and n is the total number of dummy variables. The standard error (SE) of concentration effect was the square root of variance of an effect, and the significance level (P-value) of each concentration effect was determined using the student's t-test:

$$t(X_i) = E(X_i) / SE \qquad (3)$$

where, $E(X_i)$ is the effect of variable X_i. The statistical analysis of the results was performed with the aid of statistical software package design expert 8.0 (State-Ease, Minneapolis, MN, USA).

Table 1. Variables representing medium components used in placket- Burman design

Variables	Medium components	- level	+level
X_1	Glucose (g/ml)	0.001	0.01
X_2	Maltose (g/ml)	0.001	0.01
X_3	Yeast extract (g/ ml)	0.001	0.01
X_4	Ammonium nitrate (g/ ml)	0.01	0.1
X_5	Aeration (rpm)	0	120
X_6	Inoculum (%)	1	5
X_7	Dye concentration (mg/ ml)	.5	5
X_8	Temperature (°C)	25	42
X_9	pH	7	11

X_1- X_7 represent different assigned variables; the sign '+' is for high concentration of variables and '-' is for low concentration of variables.

LC-MS analysis: LC-MS was carried out using LCMS-2010SA, Shimadzu, Japan. The column used for LC-MS analysis was C18 (4.6 × 250 mm). Mobile phase was methanol:water (50:50 v/v). The flow rate was 0.6 ml/min and UV detector at 254 nm was used. The injection volume of the dye and its degraded product was 10 µl. Mass spectra was obtained using an ion – trap mass spectrometer fitted with an electron spray (ESI,Thermo Finnigan LCQ-DUO, USA) interface operated in negative ionization mode with a spray voltage of 4.5 kV, at a capillary temperature of 275°C, sheath gas at 40 AU (arbitrary unit) and auxillary gas at 26 AU.

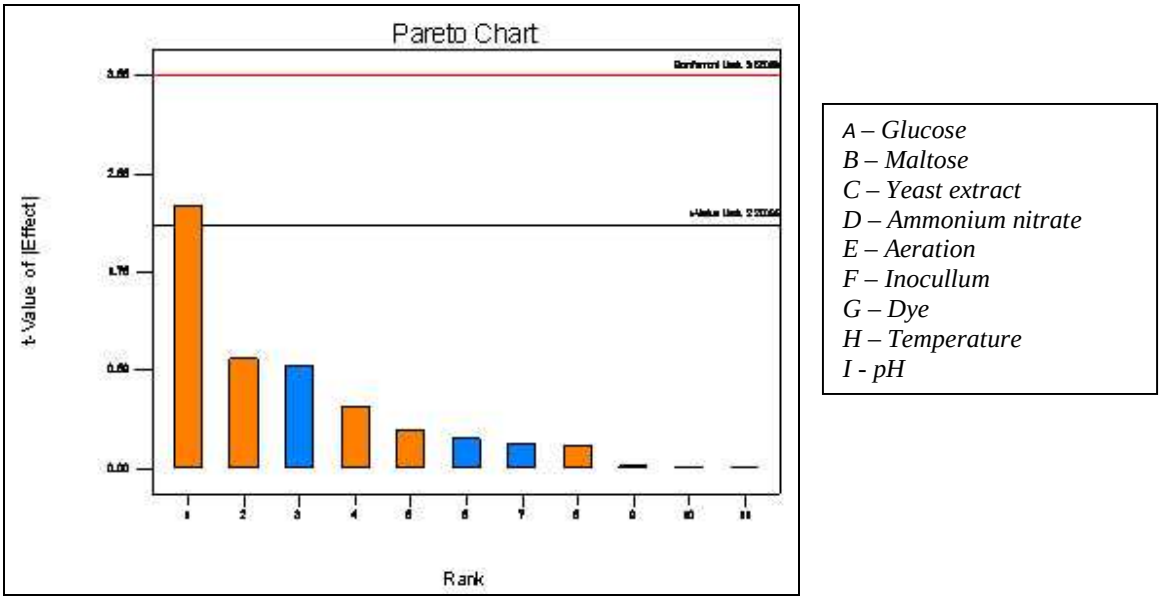

Figure 1: Pareto chart of nine -factor standard effects on Reactive Black 5 dye degradation.

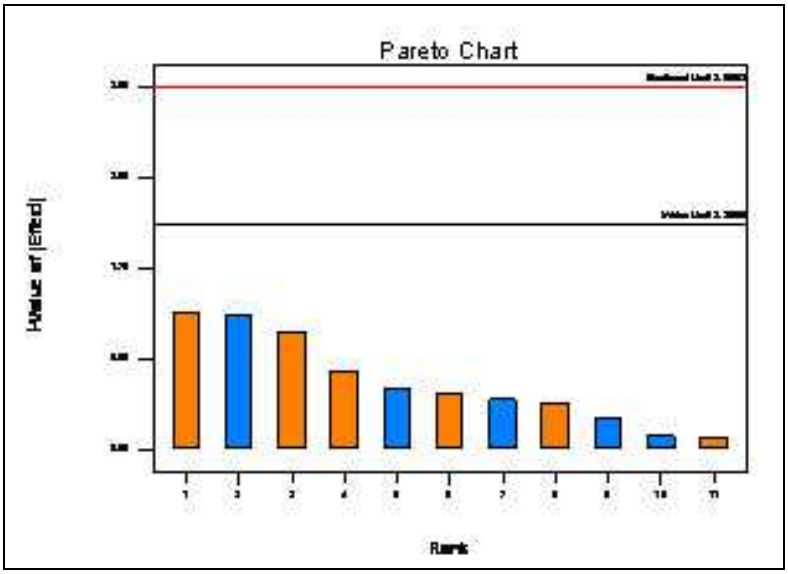

Figure 2: Pareto chart of nine -factor standard effects on Reactive Red 120 dye degradation.

RESULTS AND DISCUSSION

Optimization of media components by Placket-Burman design:

Statistical methods for medium optimization have proved to be a powerful and useful tool. Screening of important media components by placket Burmann design showed a wide variation. This variation reflected the importance of medium optimization to attain higher yields. Within the range of the tested levels of variables, glucose, maltose, yeast extract, aeration, inoculum, dye concentration, pH had positive effects whereas, high levels of the other variables showed negative effects for reactive red dye degradation. In case of reactive black degradation glucose, yeast extract, ammonium nitrate, aeration, inoculum, dye concentration, pH had positive effects whereas, maltose and temperature showed negative effect. Fig. 1 & 2 (Pareto chart) shows, the positive and negative influence of the screened variables on both the dye degradation.

Table 2: Degree of positive or negative effect independent variables on reactive red degradation by *P. aeruginosa*

variables	Medium components	Effect	SE	t (X_i)	Significance Level
X_1	Glucose (g/ml)	0.04	0.051	**0.78**	N.S
X_2	Maltose (g/ml)	0.09	0.051	**1.76**	N.S
X_3	Yeast extract (g/ ml)	0.27	0.051	**5.29**	S
X_4	Ammonium nitrate (g/ ml)	-0.03	0.051	-0.58	N.S
X_5	Aeration (rpm)	0.43	0.051	**8.43**	S
X_6	Inoculum (%)	0.09	0.051	**1.76**	N.S
X_7	Dye concentration (mg/ ml)	0.01	0.051	**0.19**	N.S
X_8	Temperature (°C)	-0.26	0.051	-5.09	S
X_9	pH	0.22	0.051	**4.31**	N.S

Bold type indicates positive effect, italic type indicates negative effect
S-Significant; N.S- Non-Significant

Statistical analyses of the data (*t*-test) showed that variations of yeast extract, aeration and temperature in the tested range of variables had the most considerable effects on reactive red whereas dye concentration and pH were highly significant on reactive black dye degradation. The main effect of each variable upon reactive red and reactive black dye degradation has been given in table 2 and 3. The percentage contribution of each factor is shown in Fig. 3.

Table 3: Degree of positive or negative effect independent variables on reactive black degradation by *Aeromonas punctata*

variables	Medium components	Effect	SE	t (X_i)	Significance Level
X_1	Glucose (g/ml)	0	0.068	**0**	N.S
X_2	Maltose (g/ml)	-0.06	0.068	-0.88	N.S
X_3	Yeast extract (g/ ml)	0.073	0.068	**1.07**	N.S
X_4	Ammonium nitrate (g/ ml)	0.16	0.068	**2.35**	N.S
X_5	Aeration (rpm)	0.18	0.068	**2.64**	N.S
X_6	Inoculum (%)	0.056	0.068	**0.82**	N.S
X_7	Dye concentration (mg/ ml)	0.73	0.068	**10.73**	S
X_8	Temperature (°C)	-0.30	0.068	-4.41	N.S
X_9	p_H	0.28	0.068	**4.11**	S

Bold type indicates positive effect, italic type indicates negative effect
S-Significant; N.S- Non-Significant

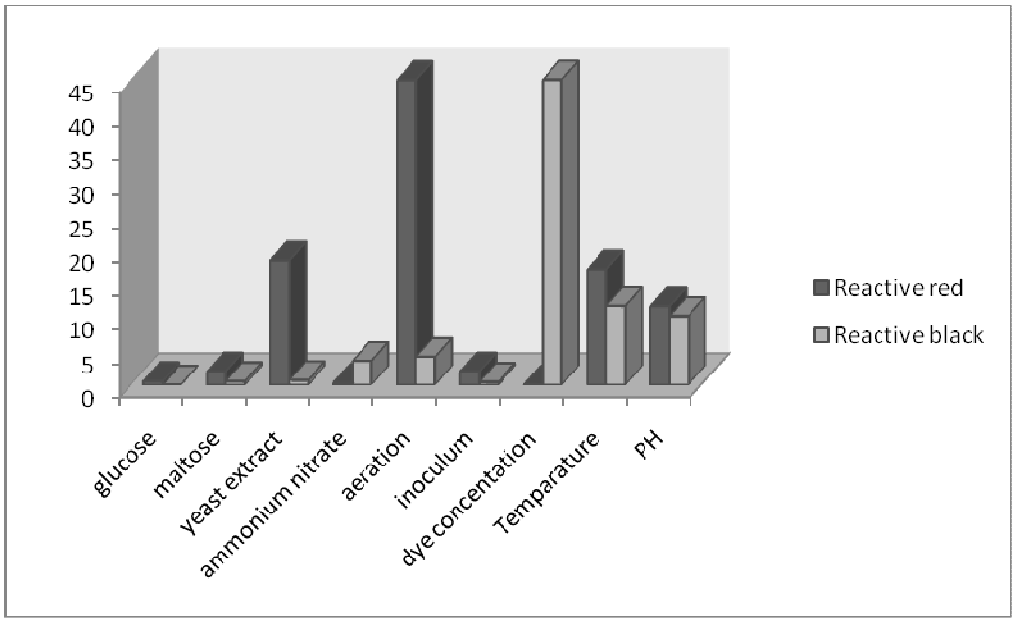

Figure 3: Percentage contribution of each factor for Reactive Red 120 dye and Reactive Black 5 dye degradation

The analysis of variance (ANOVA) table for reactive red and reactive black has been presented in Tables 4 and 5, respectively. The model F-value of 10.88 for reactive red and 16.63 for reactive black implies that the model is significant. The p-value < 0.05 indicated that the model terms were significant. The model's goodness of fit was checked by determination coefficient (R^2). In this study, the value of R^2 (0.80 & 0.78) was closer to 1 which 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 lower reliability of the

experiment is usually indicated by high value of CV. In the present case, a low CV (30.81 & 18.54) denoted that the experiments performed were highly reliable.

Table 4: Analysis of variance for reactive red dye degradation by *P. aeruginosa*

Source	Sum of square	Degree of freedom	Mean square	F- value	P- value	
Model	1.01	3	0.34	10.88	0.0034	Significant
C-yeast extract	*0.23*	1	*0.23*	*7.45*	*0.0259*	
E-aeration	*0.56*	1	*0.56*	*18.27*	*0.0027*	
H-temperature	*0.21*	1	*0.21*	*6.92*	*0.0302*	
Residual	0.25	8	0.031			
Cor Total	1.25	11				

CV - 30.81; R^2 - 0.80

Table 5: Analysis of variance for reactive black dye degradation by *A. punctata*

Source	Sum of square	Degree of freedom	Mean square	F- value	P- value	
Model	1.87	2	0.93	16.63	0.0009	Significant
G-dye	*1.63*	1	*1.63*	*28.97*	*0.0004*	
J-ph	*0.24*	1	*0.24*	*4.29*	*0.0683*	
Residual	0.51	9	0.056			
Cor Total	2.37	11				

CV -18.54; R^2 - 0.78

According to the data obtained from the Plackett-Burman experimental results the predicted optimum medium for reactive red was as follows (g/ml): glucose, 0.01; maltose, 0.01; yeast extract, 0.01; ammonium nitrate, 0.01; dye concentration, 5; temperature, 25°C incubation at 120 rpm shaking condition. Optimum media composition for reactive black dye degradation is (g/ml): glucose, 0.01; maltose, 0.001; yeast extract, 0.01; ammonium nitrate, 0.1; dye concentration, 5; temperature, 25°C incubation at 120 rpm shaking condition. Under optimized conditions, maximum degradation was achieved at one and two days for reactive red and reactive black.

Effect of carbon and nitrogen sources on degradation efficiencies of microorganisms have been worked out 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 [9, 13]. Many different co-substrates have been found to suit as electron donor, like glucose and yeast extract [14, 15].

LC-MS analysis: LC-MS analysis of the dyes and their degraded product confirmed degradation of Reactive Black 5 by *Aeromonas punctata* and Reactive Red 120 dye by *Pseudomonas aeruginosa*. The peak present in the spectra of the dyes were absent in the degraded products, indicating that the entire dye has been decomposed to colourless low molecular fragments by respective cultures. LC-MS analysis of the Reactive Black 5 dye degraded sample demonstrated the presence of a compound with molecular weight of 173 (retention time 3.12 min) which could be interpreted as 2-nitroso-1-napthol. Whereas LC-MS analysis of Reactive Red dye degraded product showed a peak of unidentified compound with a molecular weight of 747 (retention time of 2.83 min) (Fig. 4 and 5).

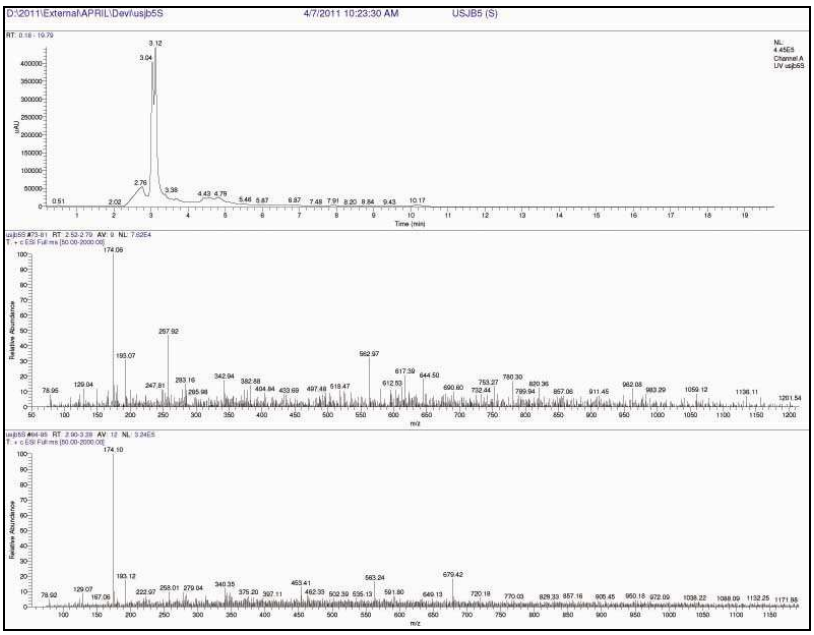

Figure 4: LC-MS spectra of Reactive Black 5 dye degradation product.

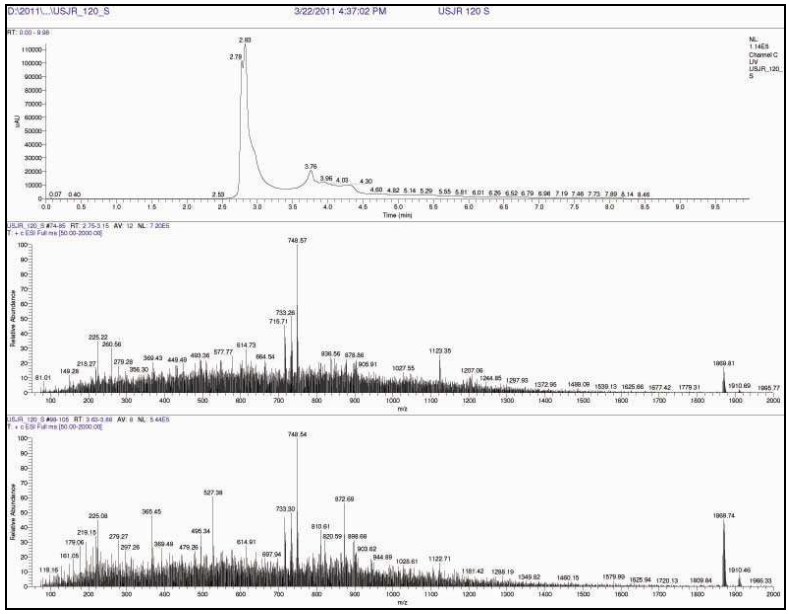

Figure 5: LC-MS spectra of Reactive Red 120 dye degradation product.

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