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Response surface methodology mediated optimization of textile azo dye, Eriochrome Black T decolorization by *Bacillus cereus* RC1

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ABSTRACT

Decolorization of azo dye, Eriochrome Black T was studied using the bacterium, *Bacillus cereus* RC1, isolated from dye contaminated site. At an initial dye concentration of 100 mg/L, the decolorization efficacy of 78.15% was observed in 24 h at pH 7. The effect of process and medium parameters such as pH, temperature, dye concentration, glucose, yeast extract and $(NH_4)_2SO_4$ on the decolorization of dye by the bacterium was evaluated using response surface methodology (RSM) involving a central composite design. The optimum conditions determined for the decolorization by RSM were pH 7.01, temperature 35.39°C, initial dye concentration 106.25 mg/L, glucose 3.06 g/L, yeast extract 0.75 g/L and $(NH_4)_2SO_4$ 1.11 g/L. Under the optimized conditions, the decolorization of dye was found to be 92.34% which was in good agreement with the predicted value of 93.47% in 24 h of incubation. The nontoxic nature of the decolorized end product was determined by phytotoxicity study using tomato seeds. Hence, the bacterial isolate can be employed in dye decolorization process in industrial wastewater treatment as an eco-friendly and nontoxic alternative.

Keywords: Decolorization; Eriochrome Black T; *Bacillus cereus* RC1; Optimization; Response surface methodology

1. Introduction

With the estimated worldwide production of over 1×10^6 ton, more than 10,000 different commercially available dyes are indiscriminately being used in textile industry for dyeing and printing purposes. The complex aromatic structure of these textile dyes makes them highly stable, recalcitrant and toxic in nature. Azo dyes account for about 70% of all dyes produced and are the most widely used synthetic dyes in textile dyeing purposes. About 10%–15% of these chemically diverse xenobiotic dyes are discharged as effluent during the dyeing processes without proper effluent treatment, making them the most problematic group of synthetic colorants released into the environment [1,2]. Azo dyes are characterized by the coupling of diazotized amine with either associated amine or a phenol and the presence of an azo group (–N=N–). They are recalcitrant pollutants and hence, negatively affect industrial wastewater treatment. Therefore, accelerating azo-reduction by cleavage of azo bonds is crucial for a promising bioremediation process [3].

The high color content of these dyes deteriorates the aesthetic property of the water and also interferes in light penetration in the aquatic environment thereby, inhibiting the photosynthetic activity of aquatic plants and algae. Hence, dye decolorization is an important goal in wastewater treatment processes. Moreover, the discharge of these dyes in natural ecosystem poses serious environmental and health hazards due to the transformation of azo group into aromatic amines. The amines formed are toxic to aquatic life and carcinogenic and mutagenic to the humans [4]. In addition, the discharge of untreated effluent into water bodies like rivers and lakes increases the biological oxygen demand and chemical oxygen demand [1].

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A variety of conventional physicochemical strategies such as coagulation, flocculation, membrane filtration, precipitation, irradiation, activated carbon adsorption and reverse osmosis technique are being practicing for the removal of dyes from textile effluent. Although these methods are effective in the removal of nonionic dyes, they possess limitations such as complex process and generate large amounts of dye contaminated sludge which requires high cost for further processing [5]. Due to the inherent disadvantages associated with these physicochemical methods, the biological methods have gained much interest as an eco-friendly and cost-effective alternative in the effluent treatment. Many microorganisms, including bacteria, fungi and algae have been reported for their ability in biodegradation and bioaccumulation of azo dyes [3]. In anaerobic conditions microorganisms cleave azo bond (–N=N–) by producing azo reductase. The effectiveness of microbial degradation/decolorization of dyes depends on the adaptability and the activity of selected microorganisms [5]. The rapid growth rate, metabolic versatility and genetic diversity of bacteria make them a promising biological candidate to remediate the pollution caused by dyes [1]. Microbial decolorization of azo dyes serves as a cost-effective and comparatively less or nontoxic alternative for the treatment of these dyes [6,7]. Different bacterial genera have been reported for the biodegradation of azo dyes which include *Clostridium*, *Eubacterium*, *Enterobacter*, *Escherichia*, *Bacillus* and *Pseudomonas* [8].

Eriochrome Black T (EBT) is an azo dye commonly used for dyeing silk, wool and nylon after pre-treatment with chromium salts. EBT is hazardous as such and its degradation also leads to carcinogenic by-products like Naphthoquinone [9]. The exploitation of microorganisms as promising strategy to decolorize azo dyes proves to be environmentally friendly. Recently, *Bacillus cereus, Bacillus subtilis*, *Micrococcus* sp., *Pseudomonas* sp. and *Klebsiella* sp. were reported for the decolorization of EBT [8,10]. In the present study, an eco-friendly approach was employed to decolorize azo dye, EBT using *B. cereus* RC1 isolated from textile dye effluent. Further, the medium and culture conditions were optimized by response surface methodology (RSM) using central composite design (CCD) method.

2. Materials and methods

2.1. Chemicals

The textile azo dye, EBT and media components were purchased from HiMedia Laboratories Pvt. Ltd., India.

2.2. Isolation and identification of the bacteria

Enrichment culture method was used to isolate the bacterium RC1 from the textile effluent discharge collected from Tirupur, Tamil Nadu, India, according to Singh et al. [11] with modifications. Briefly, 10 mL of textile effluent discharge samples were mixed with 100 mL of mineral salt medium amended with 100 mg/L of dye. The flasks were incubated at 37°C for 24 h. Further, the samples were serially diluted (10^7) and the obtained solution was spread on mineral salt agar medium containing 100 mg/L of EBT. The isolated colonies showing clear zones as an indication of decolorization were further purified. The pure bacterial isolates were individually grown on mineral salt medium containing 100 mg/L of dye and the percentage dye decolorization was calculated. Among the isolates, the bacterial stain showing the maximum decolorization was selected for characterization and further decolorization experiments.

The bacterial isolate was identified by 16S rRNA gene sequencing. Briefly, the bacterial DNA was isolated by phenol–chloroform method and the 16S rDNA region was amplified using the primers, 785F (GGATTAGATACCCTGGTA) and 907R (CCGTCAATTCMTTTRAGTTT). The obtained polymerase chain reaction product was sequenced (Macrogen Inc., Seoul, Korea) and the sequence obtained was entered in National Centre of Biological Information (NCBI) database and BLAST search was performed. Further, the phylogenetic tree was constructed using the highly similar sequences obtained from the BLAST search. The Bootstrap method in Mega 5.05 software was employed to construct the phylogenetic tree [10].

2.3. Culture medium and culture conditions

The isolated bacterium was cultured in mineral salts medium containing glucose (3.0 in g/L), yeast extract (2.0 g/L), $(NH_4)_2SO_4$ (2.0 g/L), K_2HPO_4 (6.0 g/L), KH_2PO_4 (1.0 g/L), $MgSO_4.7H_2O$ (0.1 g/L), NaCl (5.0 g/L), $FeSO_4.5H_2O$ (0.001 g/L) and $MnSO_4.H_2O$ (0.01 g/L) [12,13]. The medium was autoclaved at 121°C for 20 min and 100 mg/L concentration of dye (EBT) was added to it. The flasks were incubated for 24 h at 37°C in a shaker incubator at 160 rpm and the percentage dye decolorization was calculated.

2.4. Dye decolorization experiment

The dye decolorization ability of RC1 was studied according to Hailei et al. [14] with few modifications. Briefly, the bacterium (10^7 CFU/mL) was inoculated in 20 mL conical flasks contain mineral salt medium amended with 100 mg/L of EBT. The conical flasks were incubated at 37°C in a shaker (rpm) for 24 h. After incubation, the bacterial suspension was centrifuged at 5,000 rpm and the supernatant was collected. The concentration of dye present in the supernatant was measured at a wavelength of 523 nm using a UV–visible spectrophotometer [15]. The percentage decolorization of dye (%D) was determined using the formula [16]:

$$\%D = 100 \times (C_i - C_t/C_i) \quad (1)$$

where C_i is the initial dye concentration and C_t is the dye concentration after incubation time.

2.5. Response surface methodology

Influence of various physical and chemical parameters, that is, pH, temperature (°C), dye concentration (mg/L), glucose concentration (g/L), yeast extract (g/L) and $(NH_4)_2SO_4$ concentration (g/L) on the dye decolorization efficacy of bacterial isolate, RC1 was determined. To further optimize the parameters a standard RSM design, CCD was employed. A CCD consist of 52 experimental runs was employed by

Table 1
The levels of selected parameters

Parameters	Levels					Step change (ΔX)
	–2	–1	0	1	2	
pH (*X*1)	5	6	7	8	9	1
Temperature (°C) (*X*2)	33	34	35	36	37	1
Dye concentration (mg/L) (*X*3)	50	75	100	125	150	25
Glucose (g/L) (*X*4)	1	2	3	4	5	1
Yeast extract (g/L) (*X*5)	0.5	0.75	1	1.25	1.5	0.25
$(NH_4)_2SO_4$ (g/L) (*X*6)	0.5	0.75	1	1.25	1.5	0.25

varying the six selected independent variables. The levels and ranges of the selected parameters were depicted in Table 1. The parameters were coded in the range of –2 to +2, according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad i = 1,2,3.....k \tag{2}$$

where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the central point and ΔX_i is the step change. Table 2 shows the CCD experimental plan and levels of independent variables. A second-order polynomial model was employed to correlate the response variable to the independent variables. The general form of the second-degree polynomial equation is:

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i}^{i<} \sum_{j}^{j} \beta_{ij} x_i x_j + e \tag{3}$$

where Y_i is the predicted dye decolorization, β_0 is the offset term, β_i is the *i*th linear coefficient, β_{ij} is the *i*th quadratic coefficient, x_i and x_j are input variables that influence the response variable and *e* is the error. The design was analyzed and the coefficients of the model were tested for their significance by linear regression analysis. The R^2 value was used to determine the percentage of variability of the optimization parameter explained by the model. The statistical analysis of the model was performed in the form of an analysis of variance. The interactive effect of the selected parameters on dye decolorization was evaluated by surface (3D) and contour (2D) plots. All experiments were conducted in triplicates and mean values were used for analysis.

2.6. Toxicity assay

Phytotoxic studies were conducted to evaluate the toxicity of EBT and its degraded product using germination assay with tomato seeds. The culture broth was centrifuged at 7,000 rpm for 15 min and the supernatant was collected. Equal volume of *n*-butanol was added to the supernatant to extract the decolorized end product [17]. The dye and the degraded product was weighed and dissolved in water to obtain a final concentration of 1,000 ppm. These were evaluated for phytotoxic on tomato seeds at room temperature (35°C ± 2°C). Ten seeds were irrigated separately with 5 mL of 100 mg/L of decolorized product and EBT per day. A control was maintained using distilled water. All the experiments were performed in triplicate. The seed germination percentage was recorded after 7 d of incubation [18].

3. Results and discussion

3.1. Characterization of bacterium and dye decolorization

The 16S rRNA gene sequence analysis identified the bacterial isolate as *B. cereus* RC1. The sequence was submitted to the NCBI GenBank database with an accession number KU308402. The decolorization efficiency of *B. cereus* RC1 in the mineral salt medium was determined as 78.15%.

3.2. Response surface methodology

RSM is the most suitable multivariable system for media and process optimization analysis for dye decolorization [19]. In general, bacteria can able to utilize dyes as sole source of carbon. As *B. cereus* RC1 unable to utilize dye as sole carbon source; in the present study, medium components were optimized to attain maximum decolorization of dye [20]. Mohana et al. [21] reported glucose to be the best carbon source in the bacterial decolorization medium. Among the organic nitrogen sources, yeast extract is widely used as organic nitrogen source in the bacterial dye decolorization process [22–25]. Garg and Tripathi [26] reported the importance of ammonium sulfate ($(NH_4)_2SO_4$) in decolorization and biodegradation of orange II (Acid Orange 7) dye using *B. cereus*. The most important influencing factors for dye decolorization are pH, temperature and initial dye concentration [27]. Saratale et al. [28] showed the importance of pH in the decolorization of textile dye, Navy blue HER by *Trichosporon beigelii* NCIM-3326. Initial dye concentration and temperature are the significant parameters in dye decolorization efficacy of bacteria [29,30]. Based on these observations medium and culture conditions such as glucose (g/L), yeast extract (g/L), $(NH_4)_2SO_4$ (g/L), pH, temperature (°C) and dye concentration (mg/L) were optimized by RSM using CCD method.

RSM is an empirical modeling technique used to evaluate the relationship between a set of manageable experimental variables and results obtained. The RSM involves three major steps: (i) designing and conducting the experiments, (ii) developing the mathematical model and determination of the coefficients and (iii) predicting the response and testing the suitability of the model [17]. In this study, CCD was

Table 2
CCD design along with experimental and predicted dye decolorization values

S. no.	pH	Temperature (°C)	Dye concentration (mg/L)	Glucose (g/L)	Yeast extract (g/L)	$(NH_4)_2SO_4$ (g/L)	Decolorization (%)		
							Experimental	Predicted	Error
1	–1	–1	–1	–1	–1	–1	58.36	58.43	–0.07
2	1	–1	–1	–1	–1	1	55.33	54.02	1.31
3	–1	1	–1	–1	–1	1	61.96	66.77	–4.81
4	1	1	–1	–1	–1	–1	67.37	65.31	2.06
5	–1	–1	1	–1	–1	1	66.65	66.68	–0.03
6	1	–1	1	–1	–1	–1	59.44	59.88	–0.44
7	–1	1	1	–1	–1	–1	73.14	71.96	1.18
8	1	1	1	–1	–1	1	83.23	81.13	2.10
9	–1	–1	–1	1	–1	1	69.17	69.95	–0.78
10	1	–1	–1	1	–1	–1	40.34	41.53	–1.19
11	–1	1	–1	1	–1	–1	71.34	70.90	0.44
12	1	1	–1	1	–1	1	76.01	74.67	1.34
13	–1	–1	1	1	–1	–1	52.24	54.49	–2.25
14	1	–1	1	1	–1	1	75.29	76.62	–1.33
15	–1	1	1	1	–1	1	76.38	75.91	0.47
16	1	1	1	1	–1	–1	54.04	53.98	0.06
17	–1	–1	–1	–1	1	1	58.36	59.82	–1.46
18	1	–1	–1	–1	1	–1	64.85	66.72	–1.87
19	–1	1	–1	–1	1	–1	74.93	75.00	–0.07
20	1	1	–1	–1	1	1	68.10	67.25	0.85
21	–1	–1	1	–1	1	–1	58.01	60.76	–2.75
22	1	–1	1	–1	1	1	69.52	71.36	–1.84
23	–1	1	1	–1	1	1	49.35	49.56	–0.21
24	1	1	1	–1	1	–1	62.33	62.95	–0.62
25	–1	–1	–1	1	1	–1	53.69	57.19	–3.50
26	1	–1	–1	1	1	1	59.81	62.39	–2.58
27	–1	1	–1	1	1	1	56.91	57.87	–0.96
28	1	1	–1	1	1	–1	48.27	49.65	–1.38
29	–1	–1	1	1	1	1	47.55	51.01	–3.46
30	1	–1	1	1	1	–1	40.87	37.46	3.41
31	–1	1	1	1	1	–1	29.55	32.26	–2.71
32	1	1	1	1	1	1	49.72	51.05	–1.33
33	–2	0	0	0	0	0	78.53	70.85	7.68
34	2	0	0	0	0	0	68.45	70.53	–2.08
35	0	–2	0	0	0	0	72.13	65.52	6.61
36	0	2	0	0	0	0	71.75	72.76	–1.01
37	0	0	–2	0	0	0	76.01	72.49	3.52
38	0	0	2	0	0	0	69.52	67.44	2.08
39	0	0	0	–2	0	0	79.89	79.36	0.53
40	0	0	0	2	0	0	69.35	64.28	5.07
41	0	0	0	0	–2	0	85.42	87.25	–1.83

(Continued)

Table 2 *(Continued)*

S. no.	pH	Temperature (°C)	Dye concentration (mg/L)	Glucose (g/L)	Yeast extract (g/L)	$(NH_4)_2SO_4$ (g/L)	Decolorization (%)		
							Experimental	Predicted	Error
42	0	0	0	0	2	0	78.45	71.01	7.44
43	0	0	0	0	0	–2	57.20	55.15	2.05
44	0	0	0	0	0	2	73.41	69.85	3.56
45	0	0	0	0	0	0	88.26	89.46	–1.20
46	0	0	0	0	0	0	85.74	89.46	–3.72
47	0	0	0	0	0	0	84.30	89.46	–5.16
48	0	0	0	0	0	0	86.47	89.46	–2.99
49	0	0	0	0	0	0	91.35	89.46	1.89
50	0	0	0	0	0	0	86.10	89.46	–3.36
51	0	0	0	0	0	0	90.42	89.46	0.96
52	0	0	0	0	0	0	91.85	89.46	2.39

employed with six variables at five levels. The observed dye decolorization at respective experimental runs was used as a response variable in the analysis. The varied dye decolorization from 29.55% to 91.85% indicates that the selected variables and their levels have significant effect on the process of dye decolorization.

By taking dye decolorization as a response, a multiple regression analysis was performed and the accuracy of the data was analyzed by determination of the regression coefficient (R^2) and percentage of error. The R^2 value of 0.9606 suggested that only 3.94% of the variability in the response could not explain by the constructed mathematical model. The observed differences between the experimental and predicted % dye decolorization values suggested the enhanced prediction capability of the constructed model. Further, the observed adjusted R^2 value of 0.9163 suggested a high significance of the model. The correlation between the observed and predicted values was graphically presented in Fig. 1. In this figure, all the values near to the line specify the best correlation between the experimental and predicted value.

The low model p value 0.0001 and high F value is 21.69 (model F value > p value), suggesting the significance of the model and it could be promising in design space. The adequate precision value of 18.696 (>4 is desirable) indicating an adequate signal. The coefficient of variance (CV) value of 6.17% indicates good precision and reliability of the experiments conducted. The model p and F values, R^2 value, adequate precision value and CV values suggest that the developed model could be useful to optimize and to enhance the % dye decolorization by 92.34%.

The application of CCD yielded the following regression equation, which is an experimental relationship between the selected parameters and % dye decolorization.

$$\begin{aligned}\text{Dye decolorization (\%)} = {} & 89.46242 - 0.08075\,X_1 + 1.80975 \times X_2 - 1.26175 \times X_3 \\ & - 3.77075 \times X_4 - 4.06025 \times X_5 + 3.67475 \times X_6 - 4.69369 \\ & \times X_1 \times X_1 - 5.08119 \times X_2 \times X_2 - 4.87494 \times X_3 \times X_3 \\ & - 4.41119 \times X_4 \times X_4 - 2.58244 \times X_5 \times X_5 - 6.73994 \times X_6 \\ & \times X_6 + 0.44031 \times X_1 \times X_2 + 2.06906 \times X_1 \times X_3 - 1.30906 \\ & \times X_1 \times X_4 + 1.66594 \times X_1 \times X_5 + 2.63844 \times X_1 \times X_6 \\ & - 1.77531 \times X_2 \times X_3 - 0.83219 \times X_2 \times X_4 - 3.12969 \times X_2 \\ & \times X_5 - 1.03719 \times X_2 \times X_6 - 1.94719 \times X_3 \times X_4 - 3.70469 \\ & \times X_3 \times X_5 + 1.92406 \times X_3 \times X_5 - 3.38781 \times X_4 \times X_5 \\ & + 3.95094 \times X_4 \times X_6 - 1.90406 \times X_5 \times X_6 \end{aligned} \quad (4)$$

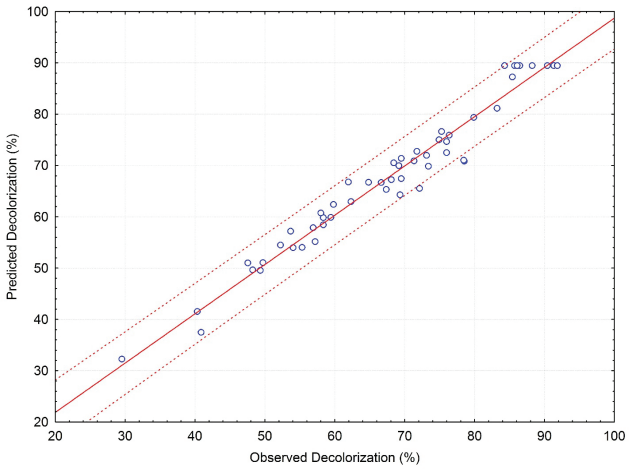

Fig. 1. Correlation between the observed and predicted dye decolorization values.

The constant (coefficients) terms were carefully chosen based on their t, p and F values (Table 3). Coefficients having low p value and high F value are considered to be significant. Based on this criterion, linear terms of pH and dye decolorization and interaction terms of pH with temperature and glucose concentration, temperature with glucose concentration and $(NH_4)_2SO_4$ concentration were observed to be insignificant. Among all the studied parameters, the squire term of $(NH_4)_2SO_4$ has the highest effect value of –13.4798 which corresponds to its immense impact on dye decolorization. In addition, the negative sign signifies the low concentration is more suitable than higher concentration. The parameters such as pH and dye decolorization were significant at square term than their linear terms

Table 3
Estimated regression coefficient and corresponding *t* and *p* value for Eriochrome Black T decolorization

	Effect	Coefficients	*t* Value	*p* Value
Intercept	89.4624	89.46242	64.88058	0.000000
*X*1	–0.1615	–0.08075	–0.12249	0.903530
*X*2	3.6195	1.80975	2.74525	0.011271
*X*3	–2.5235	–1.26175	–1.91398	0.067621
*X*4	–7.5415	–3.77075	–5.71993	0.000007
*X*5	–8.1205	–4.06025	–6.15908	0.000002
*X*6	7.3495	3.67475	5.57431	0.000010
*X*1 × *X*1	–9.3874	–4.69369	–6.47019	0.000001
*X*2 × *X*2	–10.1624	–5.08119	–7.00435	0.000000
*X*3 × *X*3	–9.7499	–4.87494	–6.72004	0.000001
*X*4 × *X*4	–8.8224	–4.41119	–6.08076	0.000003
*X*5 × *X*5	–5.1649	–2.58244	–3.55986	0.001588
*X*6 × *X*6	–13.4799	–6.73994	–9.29091	0.000000
*X*1 × *X*2	0.8806	0.44031	0.59741	0.555832
*X*1 × *X*3	4.1381	2.06906	2.80725	0.009763
*X*1 × *X*4	–2.6181	–1.30906	–1.77610	0.088397
*X*1 × *X*5	3.3319	1.66594	2.26030	0.033152
*X*1 × *X*6	5.2769	2.63844	3.57977	0.001512
*X*2 × *X*3	–3.5506	–1.77531	–2.40870	0.024047
*X*2 × *X*4	–1.6644	–0.83219	–1.12909	0.270019
*X*2 × *X*5	–6.2594	–3.12969	–4.24628	0.000282
*X*2 × *X*6	–2.0744	–1.03719	–1.40723	0.172178
*X*3 × *X*4	–3.8944	–1.94719	–2.64190	0.014281
*X*3 × *X*5	–7.4094	–3.70469	–5.02643	0.000039
*X*3 × *X*5	3.8481	1.92406	2.61052	0.015335
*X*4 × *X*5	–6.7756	–3.38781	–4.59650	0.000116
*X*4 × *X*6	7.9019	3.95094	5.36054	0.000017
*X*5 × *X*6	–3.8081	–1.90406	–2.58339	0.016305

suggesting the importance of these parameters in the dye decolorization process. Hence, a small alteration of these conditions has significant effect on the dye decolorization (Table 4).

The surface and contour plots were plotted based on the regression Eq. (3). Each plot represents the inestimable number of combinations of two test variables in the selected range and all other variables kept at their respective central level. Figs. 2–5 show the surface plots of selected parameters and their interactions with another. Figs. 2 and 3(a) depict the interaction of pH with other selected parameters. The contours in Fig. 3 are slightly inclined toward the $(NH_4)_2SO_4$ concentration suggesting the pH dependency on it. The interactions of temperature with other variables are depicted in Fig. 3. Fig. 3(d) indicates that yeast extract concentration has slightly depended on temperature concentration. Though, glucose and $(NH_4)_2SO_4$ concentrations have no influence on the dye concentration (Fig. 4); yeast concentration has influence on the dye concentration (Fig. 4(c)). Fig. 5(a) represents that glucose concentration has influenced the yeast extract concentration; however, it has no influence on $(NH_4)_2SO_4$ concentration (Fig. 5(b)). Fig. 5(c) represents the interaction of yeast extract with $(NH_4)_2SO_4$. This study also signifies that the selected organic and inorganic nitrogen sources concentrations were depended on each other.

The optimum conditions, pH 7.01, temperature 35.39°C, dye concentration 106.25 mg/L, glucose 3.06 g/L, yeast extract 0.75 g/L and $(NH_4)_2SO_4$ 1.11 g/L were determined by solving Eq. (3). At these conditions the predicted dye decolorization was 93.47%, however, by conducting the experiments 92.34% decolorization was observed.

3.3. Phytotoxic studies

In the phytotoxic studies of decolorized product, tomato seeds were treated with 100 mg/L of EBT and degraded product solution. The dye showed significant toxicity on the tomato seed germination and seedling root and shoot length as compared with the control and the decolorized product. The result showed significant reduction in seed germination (61.38%) compared with the control (94.85%), whereas the decolorized product showed 89.30% germination. The results showed significant reduction in the toxicity of the decolorized product compared with the dye. Similar results were observed with root and shoot lengths. The dye treated seeds showed reduced root and shoot length.

Table 4
Analysis of variance results of Eriochrome Black T decolorization by *B. cereus* RC1

	SS	df	MS	*F* Value	*p* Value
*X*1	0.26	1	0.261	0.01500	0.903530
*X*2	131.01	1	131.008	7.53639	0.011271
*X*3	63.68	1	63.681	3.66330	0.067621
*X*4	568.74	1	568.742	32.71762	0.000007
*X*5	659.43	1	659.425	37.93427	0.000002
*X*6	540.15	1	540.152	31.07290	0.000010
*X*1 × *X*1	727.73	1	727.725	41.86331	0.000001
*X*2 × *X*2	852.84	1	852.844	49.06091	0.000000
*X*3 × *X*3	785.01	1	785.013	45.15889	0.000001
*X*4 × *X*4	642.76	1	642.762	36.97569	0.000003
*X*5 × *X*5	220.29	1	220.292	12.67259	0.001588
*X*6 × *X*6	1,500.55	1	1,500.550	86.32105	0.000000
*X*1 × *X*2	6.20	1	6.204	0.35689	0.555832
*X*1 × *X*3	136.99	1	136.993	7.88067	0.009763
*X*1 × *X*4	54.84	1	54.837	3.15455	0.088397
*X*1 × *X*5	88.81	1	88.811	5.10897	0.033152
*X*1 × *X*6	222.76	1	222.763	12.81474	0.001512
*X*2 × *X*3	100.86	1	100.856	5.80184	0.024047
*X*2 × *X*4	22.16	1	22.161	1.27485	0.270019
*X*2 × *X*5	313.44	1	313.438	18.03093	0.000282
*X*2 × *X*6	34.42	1	34.424	1.98030	0.172178
*X*3 × *X*4	121.33	1	121.329	6.97962	0.014281
*X*3 × *X*5	439.19	1	439.191	25.26500	0.000039
*X*3 × *X*5	118.46	1	118.465	6.81482	0.015335
*X*4 × *X*5	367.27	1	367.273	21.12783	0.000116
*X*4 × *X*6	499.52	1	499.517	28.73535	0.000017
*X*5 × *X*6	116.01	1	116.015	6.67388	0.016305
Error	417.20	24	17.383		
Total SS	10,595.67	51			

Note: SS, sum of squire; df, degrees of freedom; MS, mean square.

In EBT suspension treated seeds, root length was 3.91 cm whereas in the seeds treated with decolorized product was 4.31 cm. Shoot length of the control, EBT and decolorized product treated seeds was 7.05, 5.18 and 6.81 cm, respectively. The results emphasized the reduced toxicity of the degraded product to that of EBT treated seeds.

Nezamzadeh-Ejhieh and Khorsandi [31] used Ni^{2+}-exchanged zeolite P as a heterogeneous catalyst in the photodecolorization of aqueous solution of EBT under UV irradiation. The maximum decolorization efficiency of 63.2% was achieved over Ni–P zeolite catalyst after 110 min reaction time at pH 9. The biodecolorization capability of *Pseudomonas luteola* was investigated by Hsueh and Chen [32]. The maximum decolorization observed was 86% for 110 ppm of dye concentration when incubated for 10 h. Zhu et al. [33] reported the EBT decolorizing ability of laccase isolated from an edible mushroom *Russula virescens* with a decolorization percentage of 61.4 ± 5.5 after 24 h of incubation at a concentration of 250.0 mg/L. Very few reports are available on the decolorization of EBT using bacteria. EBT, a naphthol containing azo dye is difficult to decolorize/degrade by the bacteria [32]. However, in the present study, *B. cereus* RC1 isolated from textile effluents significantly decolorized the azo dye EBT by 78.15% in mineral salt medium and 92.34% in optimized conditions.

4. Conclusions

The bacterium *B. cereus* RC1 isolated from the textile effluents has the ability to decolorize the textile azo dye EBT. RSM involving a CCD in six factors was employed for the optimization of decolorization process. Using the RSM 92%

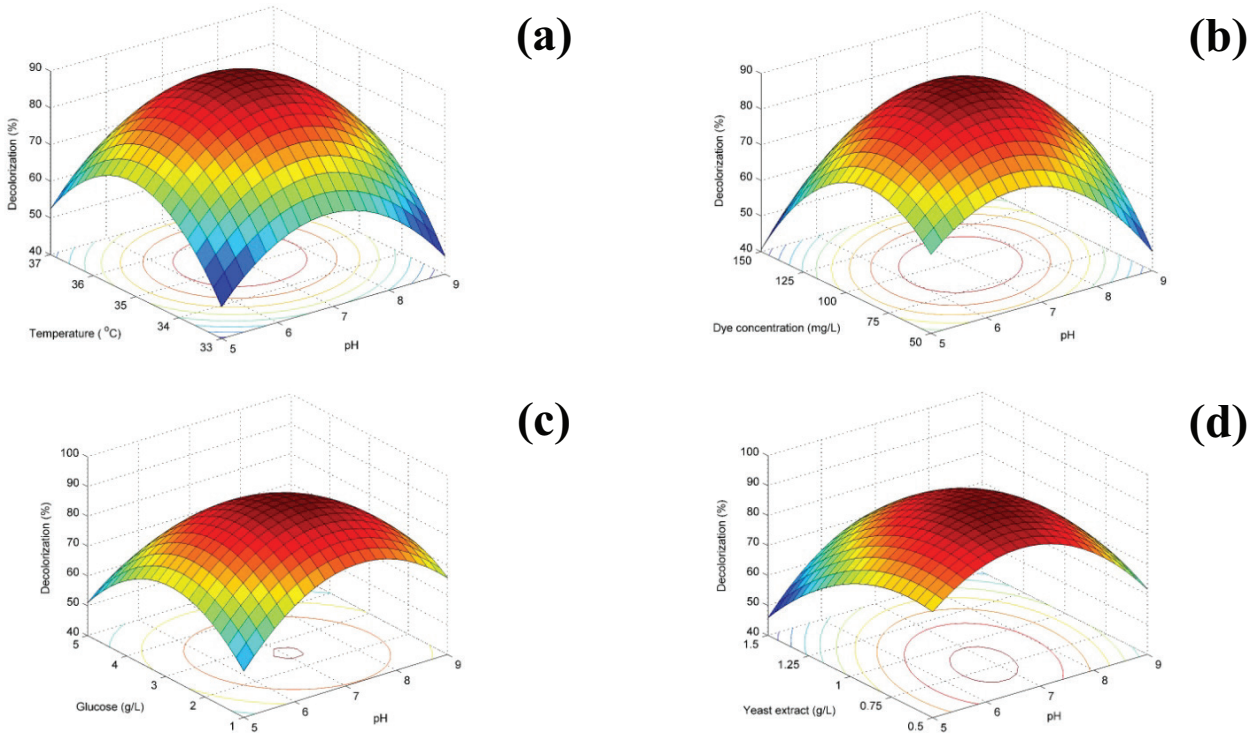

Fig. 2. Response surface plots of EBT decolorization: (a) interaction influence of pH with temperature, (b) interaction influence of pH with dye concentration, (c) interaction influence of pH with glucose and (d) interaction influence of pH with yeast extract.

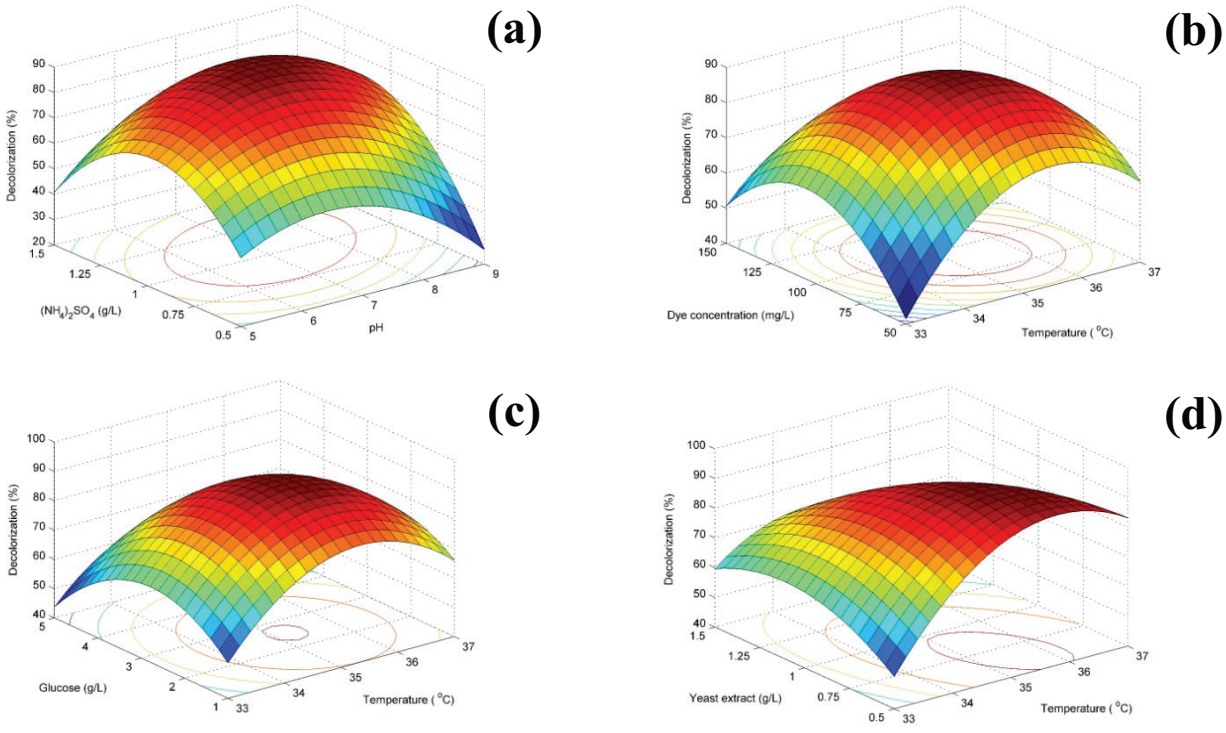

Fig. 3. Response surface plots of EBT decolorization: (a) interaction influence of pH with $(NH_4)_2SO_4$, (b) interaction of temperature with dye concentration, (c) interaction of temperature with glucose and (d) interaction of temperature with yeast extract.

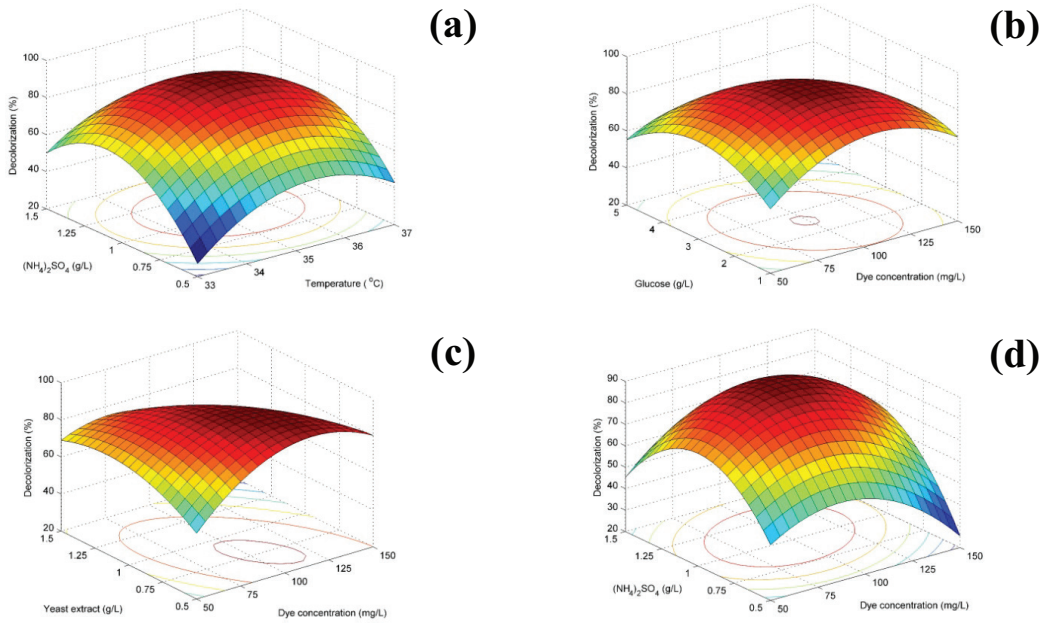

Fig. 4. Response surface plots of EBT decolorization: (a) interaction of temperature with $(NH_4)_2SO_4$, (b) interaction of dye concentration with glucose, (c) interaction of dye concentration with yeast extract and (d) interaction of dye concentration with $(NH_4)_2SO_4$.

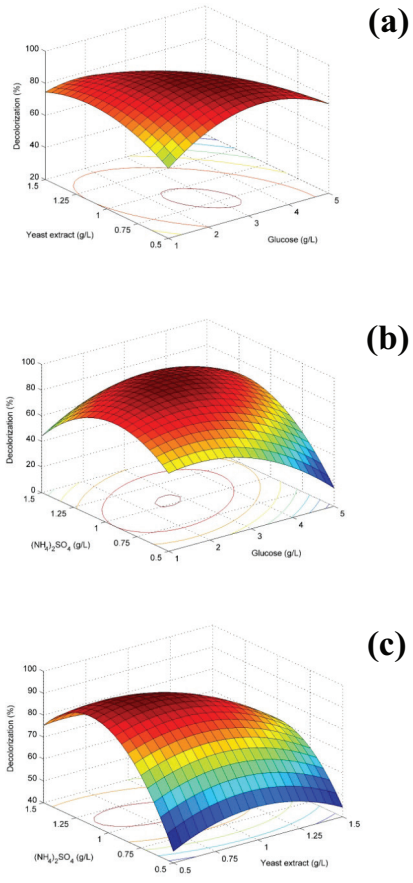

Fig. 5. Response surface plots of EBT decolorization: (a) interaction of glucose with yeast extract, (b) interaction of glucose with $(NH_4)_2SO_4$ and (c) interaction of yeast extract with $(NH_4)_2SO_4$.

of decolorization was achieved in 24 h under the conditions, pH 7.01, temperature 35.39°C, dye concentration 106.25 mg/L, glucose 3.06 g/L, yeast extract 0.75 g/L and $(NH_4)_2SO_4$ 1.11 g/L. Moreover the ability of *B. cereus* RC1 to decolorize EBT with 92% decolorization efficiency warrants its potential application in large scale wastewater treatment plants.

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