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Statistical Design for Optimization of Process Parameters for Biodecolorization of Reactive Orange 4 Azo Dye by *Bacillus cereus* Isolate

Satyendra Kumar Garg, M. Tripathi and N. Lal
Centre of Excellence, DST-FIST Supported, Department of Microbiology, Dr. Ram Manohar Lohia Avadh University, Faizabad, 224001, India

Corresponding Author: Satyendra Kumar Garg, Centre of Excellence, DST-FIST Supported, Department of Microbiology, Dr. Ram Manohar Lohia Avadh University, Faizabad, 224001, India Tel: +91-5278-247350 Fax: +91-5278-246330

ABSTRACT

In the present study, different approaches are being compared for the decolorization of reactive orange 4 monoazo dye by *Bacillus cereus* isolate under varied cultural and nutritional conditions. By employing conventional one-factor-at-a-time approach, the bacterial strain exhibited decolorization activity over a wide range of pH (7.0-9.0), temperature (30-38°C), dye concentration (50-200 mg L^{-1} MSM) and inoculum size (1.0-6.0%, v/v), with peak activity (68.2% color removal) at pH 8.0, 35°C, 50 mg dye L^{-1} MSM and 4.0% (v/v) inoculum in the presence of 1.0% (w/v) glucose as carbon/energy source and 0.2% (w/v) ammonium nitrate within 72 h incubation. Under response surface methodology (RSM using Box-Behnken design) approach, the dye decolorization enhanced to 100% at optimized 40 mg reactive orange L^{-1} MSM, glucose 1.0% (w/v) and ammonium nitrate 0.2% (w/v) during 72 h of incubation. The dye decolorization time was advanced by 12 h in bioreactor trial and 100% color removal was achieved within only 60 h incubation. In future experimentation, we envisage to test the potential of our isolate for the decolorization of other variety of azo dyes, mixture of dyes as well as the real textile effluent.

Key words: Azo dyes, *Bacillus cereus*, decolorization, RSM, textile effluent

INTRODUCTION

Dyes make the world more beautiful through colored substances; on the other hand, they create a serious pollution problem for the environment. Azo dyes (R1-N = N-R2) represent about 70% of the total one million tons of dyes annually produced in the world (Hao *et al*., 2000). They are the most common synthetic colorants released to the environment via textile, food, pharmaceutical and chemical industries. The discharge of azo dyes during production and textile processing is problematic not only for aesthetic reasons but also because azo dyes and their anaerobic degradation products (aromatic amines) are carcinogenic (Hao *et al*., 2000).

Azo dyes are generally recalcitrant to biodegradation due to their xenobiotic nature. Dyes can be removed from the wastewater by physicochemical treatment methods. The conventional biological methods employed in the industry include the use of aerated lagoons and activated sludge processes. However, application of these techniques has several shortcomings such as higher energy costs, excessive sludge generation and formation of toxic by-products (Sarioglu and Bisgin, 2007). Lately, dye decolorization through modern biological means has gained momentum because bioprocessing is cheap, eco-friendly and applicable to wide range of dyes. Therefore, more advanced

alternative biological wastewater treatment strategies will be required which are not only economical but also environmentally conducive with no toxic effects on any life forms, including humans. Certain microorganisms, being highly versatile, have developed enzyme systems for decolorization and mineralization of azo dyes under specific environmental conditions (Pandey *et al*., 2007). The anoxic degradation of various azo dyes by mixed aerobic and facultative anaerobic microbial consortia was reported by several researchers (Khehra *et al*., 2006).

Keeping the above facts in view, the present study was envisaged with an aim to optimize various cultural (pH, temperature, dye concentration, inoculums size, incubation time) and nutritional (carbon and nitrogen sources) parameters for maximization of reactive orange 4 mono azo dye decolorization is simulated synthetic Minimal Salt Medium (MSM) using conventional one-factor-at-a-time approach, subsequently followed by response surface (statistical) methodology. The dye decolorization by *Bacillus cereus* isolate was also attempted at bench-scale bioreactor level under RSM optimized conditions.

MATERIALS AND METHODS

Bacterial culture: *Bacillus cereus* isolate (NCBI GenBank number FJ959366), previously isolated in our laboratory from treated tannery effluent was employed in the present study (Tripathi *et al*., 2011). The pure culture was preserved and maintained at 4°C on Glucose Yeast Extract (GYE) agar slants containing (g L^{-1}): Glucose 5.0, yeast extract 5.0, peptone 5.0 and agar 20.0.

Inoculum preparation: The bacterial inoculum was prepared in GYE broth (pH 8.0). The sterilized medium (100 mL) was inoculated with a loopful of *B. cereus* culture and incubated at 33°C in an incubator shaker (150 rpm) for 24 h.

Culture conditions: The dye decolorization trials were performed in sterilized MSM (pH 8.0) containing (g L^{-1}): K_2HPO_4, 1.0; $CaCl_2$, 0.02, $FeCl_3$, 0.05, $MgSO_4$, 0.02, carbon/nitrogen sources as per the treatment and amended with reactive dye orange 4 at 50 mg L^{-1} MSM. The medium was inoculated with *B. cereus* [Absorbance (A_{600}) 1.09] at 3.0% (v/v) containing 2.9×10^6 colony forming units (cfu) mL^{-1} and incubated at 33°C for 120 h. The samples were drawn periodically at 24 h intervals and analyzed employing UV-Vis spectrophotometer (Systronics) for bacterial growth and dye decolorization.

Effect of dye concentration: To determine the effect of varied initial dye concentrations on decolorization and growth response of *B. cereus*, a wide range (50-200 mg L^{-1}) of reactive dye orange 4 was taken in MSM and incubated for 120 h under static culture condition.

Effect of inoculum size: The MSM (pH 8.0) containing optimized (as above) dye concentration was inoculated with 1.0-6.0% (v/v) exponentially growing culture (A_{600} 1.09) and incubated for 96 h under static culture condition.

Effect of carbon and nitrogen sources: The effect of nutritional parameters such as carbon (viz., glucose, sucrose, starch) at 1.0% (w/v) and nitrogen sources (viz., ammonium sulfate, ammonium chloride, ammonium nitrate, peptone, urea) at 0.2% (w/v) were evaluated under above optimized conditions so as to find the most suitable carbon and nitrogen source. Thereafter, the

optimum concentration (out of 0.5, 0.7, 1.0, 1.2, 1.5%, w/v) of best carbon and nitrogen (out of 0.1, 0.15, 0.2, 0.25, 0.3%, w/v) source for bacterial growth response and dye decolorization was determined.

Combined effect of temperature and initial pH: The pH of MSM was adjusted in the range of 7.0-9.0 using 0.1 M HCl or 0.1 M NaOH, prior to sterilization. The sterilized screw capped tubes were then inoculated with the above optimized dose of inoculum (4.0%, v/v) and incubated at 30-38°C for 72 h in an incubator under static condition.

Optimization of dye decolorization using statistical design: Box-Behnken design was applied (using Design-Expert software) which was operated for three variables, viz., glucose [(A), % w/v], ammonium nitrate [(B), % w/v] and dye concentration [(C), mg L^{-1}]. These variables were selected as the important factors to optimize the dye decolorization based on the findings from preliminary experiments employing one-factor-at-a-time approach. Optimal values were obtained by solving the regression equation and three dimensional response surface plots were used for analyzing the interactive effect of each variable.

The effect of each factor on reactive orange 4 dye decolorization was studied at three different levels (-1, 0 and +1) with minimum, central and maximum values (Table 1) and 17 experimental setups were obtained (Table 2). A second order polynomial equation was used for the analysis of decolorization and the data were fitted in the equation by multiple regression procedure. This resulted in an empirical model. The predicted values for dye decolorization were obtained by applying quadratic model using Design Expert software. The model equation for analysis is as under:

Table 1: Experimental range and the levels of three independent variables employed in RSM in terms of actual and coded factors

	Levels		
Variables	-1	0	+1
Dye concentration (mg L^{-1})	40	45	50
Glucose (%, w/v)	0.8	0.9	1.0
Ammonium nitrate (%, w/v)	0.22	0.25	0.28

Table 2: Experimental designs used in RSM studies by using three independent variables showing observed and predicted values of reactive orange 4 dye decolorization

				Dye decolorization (%)	
Standard order	Factor A (glucose, %, w/v)	Factor B (ammonium nitrate, %, w/v)	Factor C (dye concentration, mg L^{-1})	Actual response	Predicted response
1	0.80	0.17	45.00	84.7	83.69
2	1.00	0.17	45.00	85.9	86.81
3	0.80	0.23	45.00	86.5	85.59
4	1.00	0.23	45.00	87.2	87.94
5	0.80	0.20	40.00	92.8	93.44
6	1.00	0.20	40.00	97.8	98.12
7	0.80	0.20	50.00	69.5	70.51
8	1.00	0.20	50.00	70.8	70.16
9	0.90	0.17	40.00	95.5	95.60
10	0.90	0.23	40.00	97.2	97.48
11	0.90	0.17	50.00	70.5	70.23
12	0.90	0.23	50.00	71.2	71.10
13	0.90	0.20	45.00	87.2	87.02
14	0.90	0.20	45.00	87.0	87.02
15	0.90	0.20	45.00	86.9	87.02
16	0.90	0.20	45.00	86.7	87.02
17	0.90	0.20	45.00	87.3	87.02

$$Y = \beta_o + \sum \beta_n X_n + \sum \beta_{nn} X_n^2 + \sum \beta_{nm} X_n X_m \quad (1)$$

where, Y is the predicted response, β_o offset term, β_n liner coefficient, β_{nn} squared coefficient, β_{nm} interaction coefficient, X_n nth independent variable, X_n^2 squared effect and X_nX_m interaction effects. The predicted values for dye decolorization were obtained by applying quadratic model (Design Expert software).

Bench-scale bioreactor level dye decolorization: Under RSM optimized cultural conditions [w/v, reactive orange 4 concentration (40 mg L^{-1}), glucose (1.0%), ammonium nitrate (0.2%)], the dye decolorization was performed in a stirred tank bioreactor (New Brunswick Scientific Co. Inc. Edison, NJ, USA) of 3.0 L capacity by *B. cereus* isolate under static culture conditions. The fermentor was equipped with direct drive dual Rushton style impeller, PID temperature, agitation control, probes and controller of pH and Dissolved Oxygen (DO). The MSM (3.0 L) was inoculated (at optimized 4.0%, v/v) with *B. cereus* culture and fermentor operated without aeration and agitation. The samples (5.0 mL each) were drawn periodically at 12 h intervals up to 72 h. The bacterial growth and dye decolorization were assessed as per the analytical determinations.

Analytical determinations

Bacterial growth: The growth of *B. cereus* isolate was determined at every 24 h interval in dye containing synthetic medium. For bacterial growth measurement, the control and experimental samples (1.0 mL each) were centrifuged at 10 000 rpm (4°C) for 10 min and supernatant decanted. The bacterial pellets were washed with sterilized deionized water to remove the color present on bacterial cell surface and centrifuged as above. The washed pellets were resuspended in deionized water (1.0 mL each) and used for bacterial growth determination spectrophotometrically at 600 nm.

Dye decolorization assay: In order to determine the extent of dye decolorization, the samples drawn at different time intervals were centrifuged at 10 000 rpm for 10 min in refrigerated centrifuge (4°C). The supernatant was analyzed spectrophotometrically at 482 nm (Zimmermann *et al*., 1982) against uninoculated dye medium for decolorization. The extent of dye decolorization was calculated from the difference between initial (at 0 h) and final (at different incubation times) absorbance and represented as under:

$$\text{Decolorization extent (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Statistical analyses: All experiments were performed in triplicate. The standard deviation was calculated using Microsoft Excel program and results are presented as Mean±SD values.

RESULTS AND DISCUSSION

Effect of dye concentration: The dye concentration exerted a profound effect on bacterial growth as well as reactive orange 4 dye decolorization (Fig. 1). In general, the maximum growth (OD 0.137-0.635) was noted in the samples containing 50 mg dye L^{-1} MSM during 24-96 h incubation, which was directly correlated with the extent of dye decolorization. The results further reveal that the increasing dye concentration (50-200 mg L^{-1}) was inhibitory not only for the bacterial growth but also for the extent of dye decolorization, irrespective of incubation time. The

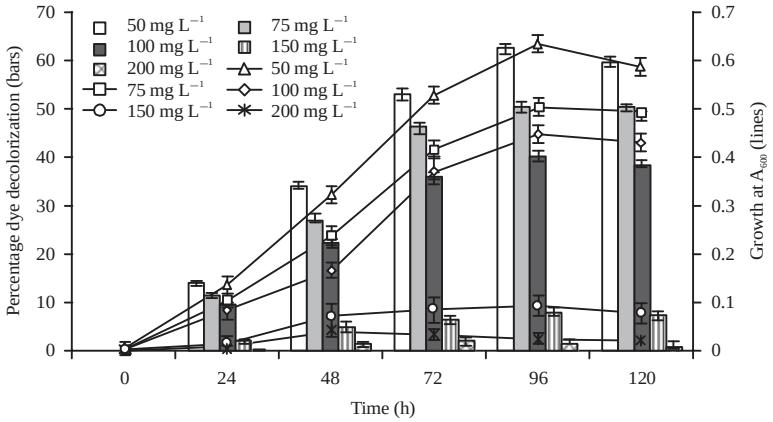

Fig. 1: Effect of reactive orange 4 dye concentration (50-200 mg L^{-1}) on decolorization and growth response of *B. cereus* at unoptimized pH 8.0, 33°C and 3.0% (v/v) inoculum under static culture conditions (Error bars depict standard deviation)

inhibitory effect could be attributed to toxicity of dye through blockage of enzyme active sites involved in dye decolorization (Tony *et al.,* 2009; Patel *et al.,* 2012). The dye decolorization and bacterial growth increased with time up to 96 h, followed by decrease up to 120 h at every dye concentration, except 200 mg dye L^{-1} MSM at which both the determinations increased only up to 72 h followed by decrease up to 120 h. The growth and dye decolorization were extremely poor at 200 mg dye L^{-1} MSM. The maximum growth (OD, 0.635) and dye decolorization (62.5%) were evident at 50 mg dye L^{-1} within 96 h (Fig. 1) and therefore, selected for further studies.

Similar to our findings, other researchers also reported inverse relationship between dye concentration and extent of decolorization in simulated MSM and the textile effluent (Khehra *et al*., 2006; Dayaram and Dasgupta, 2008; Saratale *et al*., 2013).

Effect of inoculum size: At every dose of inoculum (1.0-6.0%, v/v) under study, the growth response of *B. cereus* isolate and dye decolorization increased with time during 24-96 h incubation (Fig. 2). The bacterial growth and dye decolorization increased in synchrony at every incubation time up to 96 h, when inoculum dose was increased from 1.0-4.0%; thereafter, both the determinations decreased. The maximum bacterial growth (OD, 0.679) and dye decolorization (68.5%) were achieved at 4.0% inoculum within 96 h and was selected for further studies. Our results corroborate with Ponraj *et al*. (2011) who also reported 4.0% inoculum of *Bacillus* sp. optimum for maximum decolorization of orange 3R dye.

Effect of carbon and nitrogen sources: Figure 3a depicts the effect of easily metabolizable carbon (glucose, sucrose, starch) substrates on the growth of *B. cereus* and concomitant decolorization of reactive orange 4 at optimized 50 mg dye L^{-1} MSM and 4.0% (v/v) dose of *B. cereus* inoculum. All carbon sources under study effected dye decolorization at 1.0% (w/v) concentration (Fig. 3a); however, better growth response (OD, 0.618) and maximum dye decolorization (68%) were achieved at 1.0% (w/v) glucose concentration within 72 h incubation (Fig. 3b). The carbon sources may be arranged in the following decreasing efficiency of reactive orange dye decolorization: glucose (68%) >sucrose (39.6%) >starch (32.3%) at 72 h incubation. The bacterial growth and dye

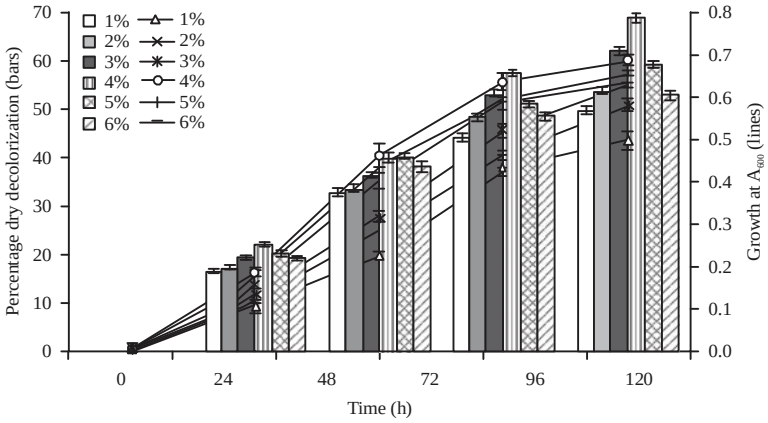

Fig. 2: Effect of inoculum size (1.0-6.0%, v/v) on dye decolorization and growth response of *B. cereus* at unoptimized pH 8.0, 33°C and optimized 50 mg reactive orange 4 dye L^{-1} concentration under static culture conditions (Error bars depict standard deviation)

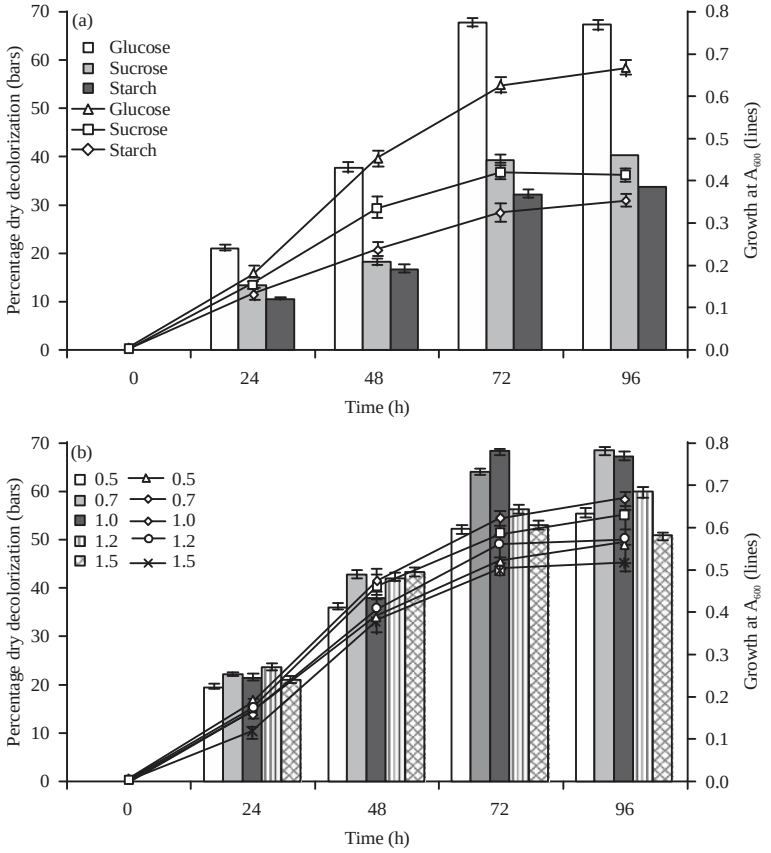

Fig. 3(a-b): (a) Effect of carbon sources [glucose (G), sucrose (S) and starch (ST) at 1.0%, w/v] on reactive orange 4 dye (at optimized initial 50 mg L^{-1}) decolorization and growth of *B. cereus* and (b) Effect of varied glucose concentrations (0.5-1.5%, w/v) on reactive orange 4 dye (at optimized initial 50 mg L^{-1}) decolorization and growth of RMLAU1 strain at unoptimized pH 8.0, 33°C and optimized 4.0% (v/v) inoculum under static culture conditions (Error bars depict standard deviation)

decolorization nearly halved at 1.0% level of starch as compared to glucose substrate (Fig. 3a). Any deviation in glucose concentration from optimum (1.0%, w/v) adversely affected both the determinations (Fig. 3b).

Other researchers also reported the requirement of glucose substrate for azo dye decolorization by different microorganisms (Meitiniarti *et al*., 2007; Wang *et al*., 2009; Liao *et al*., 2013). Contrary to our findings, sucrose has been reported as the best carbon/energy source for maximum decolorization of orange 3k by *Bacillus* sp. (Ponraj *et al*., 2011) and maroon V by consortium EDPA (Patel *et al*., 2012). On the other hand, Bayoumi *et al*. (2010) and Alalewi and Jiang (2012) reported starch as the best carbon substrate for decolorization of azo dyes by different bacterial strains. The dyes are deficient in readily metabolizable carbon/energy content and therefore biodegradation of dye without exogenous supplementation of carbon source is difficult (Padmavathy *et al*., 2003; Sarioglu and Bisgin, 2007). Ali *et al*. (2010) and Saratale *et al*. (2013) reported that sugars also serve as reducing agents (electron donors) for dye decolorization.

In general, inorganic nitrogen sources were better supplements for bacterial growth response and reactive orange dye decolorization by *B. cereus* isolate. The bacterial growth and extent of dye decolorization (within 96 h) were almost same when ammonium sulfate and ammonium nitrate were used (at 0.2%, w/v) as the nitrogen supplements. But since both the determinations (growth and color removal) with ammonium nitrate supplement were comparable at 72 and 96 h incubation, this nitrogen source was preferred over ammonium sulfate for further experimentation due to time wise economic consideration (Fig. 4a). When different concentrations (0.1-0.3%, w/v) of the best nitrogen source (ammonium nitrate) were employed, 0.2% (w/v) was found optimum for maximum growth (OD, 0.619) and dye decolorization (67%) within 72 h incubation (Fig. 4b) and was chosen for further research.

Among different organic and inorganic nitrogen sources employed, ammonium dihydrogen phosphate served as a better nitrogen source for decolorization of maroon V by consortium EDPA (Patel *et al*., 2012). Contrary to our results, Bayoumi *et al*. (2010) found peptone as the best nitrogen source for the purpose of dye decolorization under different culture conditions. Whereas, some recent studies reported yeast extract (at 0.15 g L^{-1}) as the best nitrogen supplement for decolorization of maroon V by *B. cereus* strain HJ-1.

Combined effect of pH and temperature: The RMLAU1 strain of *B. cereus* was able to grow and concomitantly decolorize the dye throughout the pH (7.0-9.0) and temperature (30-38°C) range under study. At every pH and temperature, both the determinations increased with time during 24-72 h incubation. Table 3 further reveals that increase in pH from 7.0-8.0 resulted in enhanced

Table 3: Combined effect of pH and temperature on reactive orange 4 dye decolorization and growth response of strain RMLAU1

	Incubation time (h)																			
	0					24					48					72				
	Initial pH																			
Temperature (°C)	7.0	7.5	8.0	8.5	9.0	7.0	7.5	8.0	8.5	9.0	7.0	7.5	8.0	8.5	9.0	7.0	7.5	8.0	8.5	9.0
Growth (A_{600})																				
30	0.002	0.003	0.002	0.003	0.002	0.187	0.245	0.272	0.229	0.172	0.405	0.431	0.450	0.415	0.382	0.495	0.560	0.582	0.543	0.470
33	0.003	0.002	0.003	0.003	0.003	0.225	0.263	0.290	0.257	0.188	0.422	0.460	0.498	0.459	0.452	0.530	0.598	0.621	0.582	0.577
35	0.002	0.004	0.002	0.004	0.002	0.239	0.281	0.305	0.272	0.205	0.564	0.583	0.537	0.563	0.540	0.529	0.630	0.643	0.635	0.609
38	0.004	0.003	0.002	0.002	0.003	0.176	0.227	0.259	0.218	0.163	0.480	0.476	0.502	0.480	0.469	0.488	0.558	0.605	0.570	0.483
Decolorization (%)																				
30	0	0	0	0	0	15.9	25.8	29.2	26.5	12.7	43.0	47.5	52.6	49.0	31.5	55.3	54.8	61.5	56.5	54.0
33	0	0	0	0	0	22.7	27.5	31.5	29.2	22.5	46.8	50.3	56.2	51.5	40.8	54.5	59.3	66.8	63.9	47.8
35	0	0	0	0	0	24.5	30.2	34.8	32.0	24.1	50.5	54.2	59.7	55.0	43.6	55.9	59.5	68.2	58.3	52.7
38	0	0	0	0	0	20.3	22.9	27.9	23.5	20.6	40.7	62.5	43.9	39.0	37.5	47.1	51.6	59.5	52.5	46.5

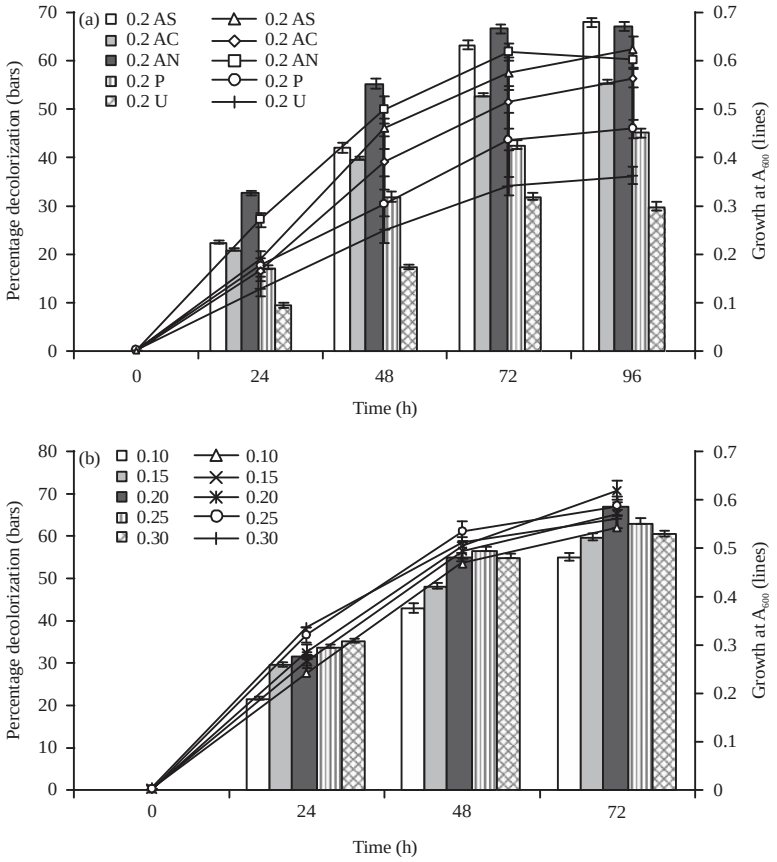

Fig. 4(a-b): (a) Effect of nitrogen sources [ammonium sulfate (AS), ammonium nitrate (AN), ammonium chloride (AC), peptone (P) and urea (U) at 0.2%, w/v] and (b) Effect of best nitrogen source (ammonium nitrate) concentration (0.1-0.3%, w/v) on reactive orange 4 decolorization and growth of *B. cereus* at optimized 50 mg dye L^{-1} concentration, 4.0% (v/v) inoculum, 1.0% (w/v) glucose, unoptimized pH 8.0, 33°C under static culture conditions (Error bars depict standard deviation)

bacterial growth and concomitant dye decolorization in harmony with time during 24-72 h, irrespective of incubation temperature employed. Further increase in pH from 8.0-9.0 was inhibitory for both the determinations. Maximum growth response (OD, 0.643) and dye decolorization (68.2%) achieved at optimum pH 8.0 and 35°C within 72 h incubation, was very close to dye decolorization (66.8%) at 33°C. With increase in temperature from 30-35°C, both the determinations increased with time, irrespective of initial pH of MSM. However, at 38°C the bacterial growth and color removal decreased and this slightly higher than optimum temperature was relatively more restrictive as compared to slightly lower (33°C) temperature. Thus, it may be inferred from the results that any slight deviation in pH and/or temperature from optimum decreased the extent of growth as well as dye decolorization (Table 3).

The pH and temperature are two important physical parameters which influence the efficiency of dye decolorization and the optimal conditions vary between pH 7.0-10.0 and 30-40°C, respectively (Asad *et al*., 2007; Chen *et al*., 1999; Ponraj *et al*., 2011). The *Lysinibacillus* sp. RGS exhibited 90% decolorization of remazol red in the broad pH (7.0-9.0) and temperature (30-35°C) range but

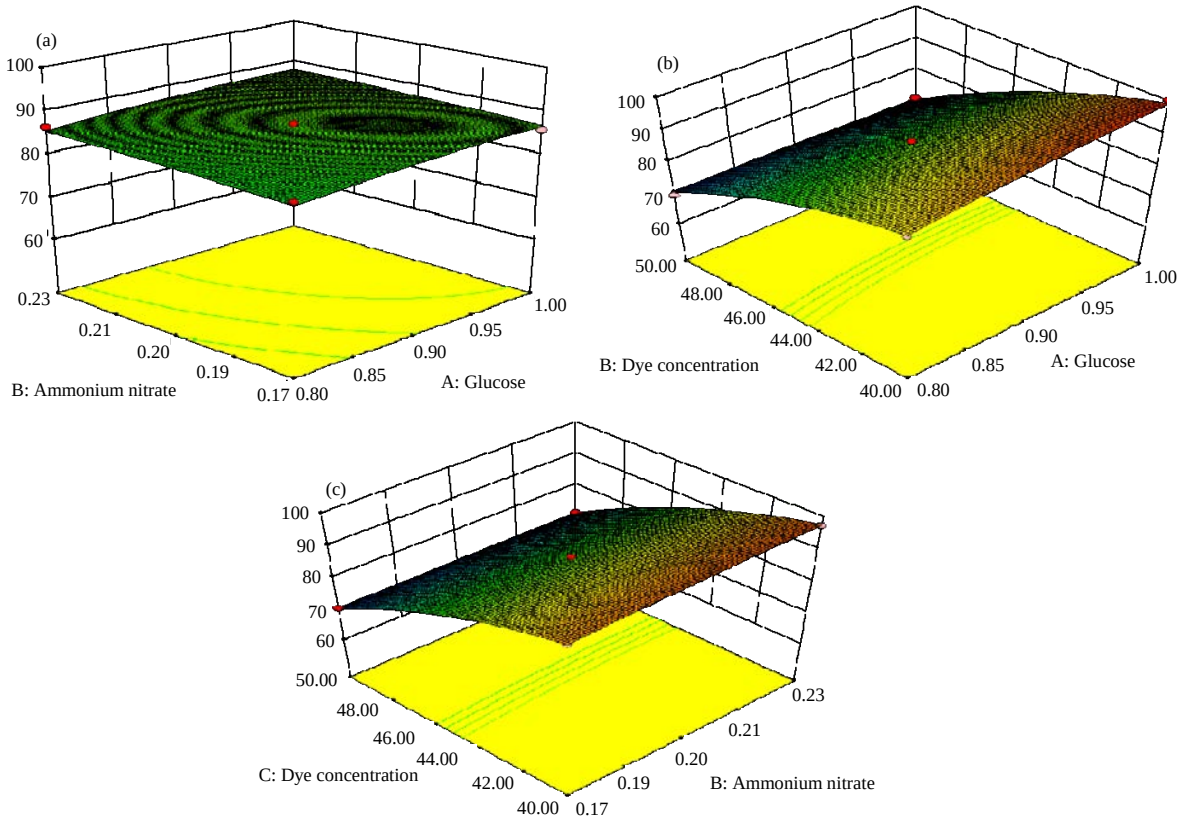

Fig. 5(a-c): Response surface curves showing interactive effects of (a) Ammonium nitrate and glucose (b) Dye concentration and glucose and (c) Dye concentration and ammonium nitrate, on reactive orange 4 decolorization by *B. cereus*

maximum decolorization was achieved at pH 7.0 and 30°C (Saratale *et al*., 2013). The optimal physical parameters required for reactive black B decolorization by *B. cereus* HJ-1 was pH 8.0 and temperature 25°C (Liao *et al*., 2013). Since the dye decolorization through azo bond reduction is an enzyme-mediated process, changes in pH will affect the degree of ionization of the enzyme (s) leading to alteration in protein conformation, thereby affecting the enzyme activity. Lourenco *et al*. (2000) reported that the major effect of pH may be attributed to the transport of dye molecule across the cell membrane, which may be considered as the rate limiting step for the decolorization process. Similar to pH, the temperature is also an important parameter for bacterial growth, thereby simultaneously affecting the enzymatic reactions necessary for dye decolorization.

Statistical optimization for dye decolorization: Interactive effects of relatively important conventionally optimized factors, viz., carbon (glucose), nitrogen (ammonium nitrate) sources and initial dye concentration were examined using Box-Behnken design. The results in Table 2 show the predicted responses of Box-Behnken design on the basis of polynomial equation.

Response surface curves constructed for the variation in dye decolorization are depicted in Fig. 5. Figure 5a shows the decolorization of reactive orange 4 with respect to ammonium nitrate versus glucose concentration. The flattened curve indicates no effect of varied levels of carbon (glucose) and nitrogen (ammonium nitrate) sources on dye decolorization at constant dye concentration. However, Fig. 5b illustrates that increased concentration of reactive orange 4 was

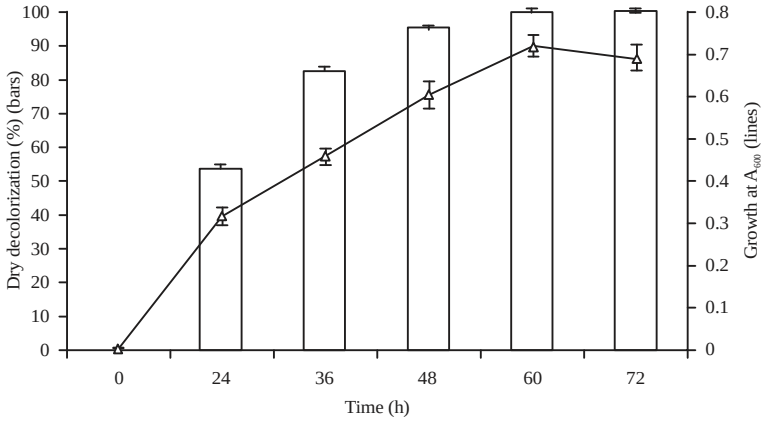

Fig. 6: Bioreactor trial for reactive orange 4 dye decolorization under optimized conditions of RSM using Box-Behnken Design (0.2% ammonium nitrate, 1.0% glucose and 40 mg reactive orange 4 dye L^{-1} MSM, pH 8.0, 35°C) during 72 h incubation

inhibitory for dye decolorization when ammonium nitrate was at constant level. Figure 5c shows the maximum dye decolorization (100%) with optimal 0.2% (w/v) ammonium nitrate, 1.0% glucose and 40 mg reactive orange L^{-1} MSM during 72 h incubation.

Dye discoloration at bench-scale bioreactor level: Figure 6 shows maximum bacterial growth and dye decolorization (100%) within 60 h under RSM optimized (pH 8.0, 35°C, 40 mg dye L^{-1} MSM, 1.0% glucose, 0.2% ammonium nitrate and 4.0% *B. cereus* inoculum) conditions. The results reveal that the complete dye decolorization was achieved in 12 h advance (within 60 h) compared to screw capped tubes level experiment using Box-Behnken design (100% within 72 h). Therefore, enhanced level of dye decolorization, in terms of time (saving of 12 h), was achieved by *B. cereus* RMLAU1 strain at bench-scale bioreactor level.

CONCLUSION

This study reveals that *B. cereus* isolate is capable of decolorizing reactive orange 4 dye effectively under wide range of cultural and nutritional conditions in a synthetic dye medium. Optimization of various process variables using conventional and response surface methodology suggests that dye concentration is the most important parameter for efficient dye decolorization. At bioreactor level, 100% dye decolorization was achieved within 60 h under optimum cultural (pH 8.0, 35°C, 40 mg dye L^{-1} MSM, 4.0% *B. cereus* inoculum) and nutritional (1.0% glucose and 0.2% ammonium nitrate) conditions with a time-wise economy of 12 h. Therefore, under specific range of cultural and nutritional conditions, the strain may be attempted for biodecolorization of reactive orange 4 containing textile dye effluent.

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