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Optimization of medium for decolorization of Congo red by *Enterobacter* sp. SXCR using response surface methodology

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ABSTRACT

Response surface methodology based central composite design (CCD) was employed to interpret the decolorization of sulfonated azo dye Congo red by newly isolated bacteria *Enterobacter cloacae* SXCR. The aim of this work was to evaluate the effect of various components of medium such as KH_2PO_4, Na_2HPO_4, NaCl, $MgSO_4$, and trace elements on the dye decolorization. The effects of different sources of carbon and nitrogen sources were also considered for the better performance on the decolorization of Congo red. The influence of these factors on decolorization experiments were performed by newly isolated bacterial strain which was isolated from petroleum contaminates soil samples. However, the effects of salts, carbon, and nitrogen sources were investigated using classical methods while influence of interacting parameters like glucose, peptone, and beef extract on dye removal were examined using CCD. A second-order polynomial model successfully described the effect of independent variables on the dye removal. The value of the adjusted determinant coefficient ($R^2 = 0.998$) indicates a reasonably good model for practical implementation. Our results show the optimal concentration of KH_2PO_4 is 2.2 g/l, Na_2HPO_4 is 3.5 g/l, NaCl is 2 g/l, $MgSO_4$ is 0.5 g/l, and trace elements is 0.01 g/l; glucose is 2 g/l, peptone is 3 g/l, and beef extract is 3 g/l were required for maximum decolorization (~97%).

Keywords: Congo red; CCD; Decolorization; *Enterobacter* sp. SXCR; Optimization

1. Introduction

It has been estimated over 7×10^5 metric tons of synthetic dyes are produced worldwide every year for different uses in textile, paper, leather, cosmetics, food, and pharmaceutical industries [1]. Among all the known dyes, more than 2000 different types of azo dyes are currently being used in dying and textile industries worldwide [2]. Azo dyes, which are characterized by one or more R_1-N=N-R_2 bonds, are the largest and most diverse groups of synthetic dyes. These dyes are widely used in number of industries [3]. Textile processing consumes large amount of water during dyeing and finishing operation. During these processes about 5–10% of dyes are discharged with wastewater without any further treatment. Thus, the industrial effluent often contains residual dye, which appears to be xenobiotic and often render them recalcitrant [4]. The reactive azo dyes-containing effluents from these industries have caused serious water pollution affecting aquatic life [5]. Therefore,

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industrial effluent containing dyes must be treated before their discharge into the environment. An efficient treatment would make possible the water to be reused for other industrial process, resulting in substantial economy. These dyes can be hardly degraded due to stable aromatic ring structure and resistance to oxidizing agents. Various physical and chemical techniques, such as chemical precipitation, ion exchange, reverse osmosis, ozonation [6], and solvent extraction have been extensively used to treat the dye containing wastewater. Moreover, these methods are effective but their operational costs are relatively high [7] and lead to other disadvantage like huge quantity of sludge depositions resulting in secondary pollution [8]. Biological methods are generally considered economical and eco-friendly. As a biological method, the microbial decolorization and degradation of dyes has been of considerable interest due to inexpensive, eco-friendly, and less disposal of sludge. Furthermore, the decolorizations of azo dyes by micro-organism are attributed by reductive cleavage of azo bond under anaerobic condition. The breakage of azo bond is associated with enzymatic steps which ultimately lead to formation of aromatic amines. These intermediate products are usually carcinogenic and mutagenic [9]. Presence of these dyes in aqueous environment affects the photosynthetic activity because reduced light penetration impedes oxygen solubility and may also be toxic to aquatic organisms due to presence of aromatic, metal ions, chloride, etc. It is quite undesirable to discharge these azo dyes containing wastewater into the environment directly due to their high pollutant in nature and toxic intermediate product [10]. A large number of microbial strains [11–13]), fungi [10], [14], and algae have been reported for decolorization of dyes. Although bacterial decolorization is usually faster compared to fungus it may require a mixed community to mineralize azo dyes [15]. Bacterial degradation of azo dye is usually catalyzed by azoreductase under anaerobic condition. The resulting products such as aromatic amines are further degraded aerobically or anaerobically by multistep process.

Usually, the influence of environmental factors such as temperature, pH, initial dye concentration, oxygen requirements, and basic medium composition on microbial decolorization of azo dyes is tested with single-factor optimization using classical methods which are often cost effective, time consuming, and does not enable an effective optimization process. To overcome the limitation of classical methods, statistical optimization methods including response surface methodology (RSM) are being applied to explore the relationship between a response and a set of design variable [16]. RSM is a powerful tool that represents a set of experimental design and multiple regression methods to study the interaction between the experimental variables through different combination of factors and selecting the optimal conditions of variables or desirable responses [17]. The main advantage of RSM is to reduced number of experimental trial needed to evaluate multiple parameters and their interaction [18].

The main objective of the present study was to investigate the optimum composition of medium ingredients required to decolorized azo dye Congo red by newly isolated bacterial strain *Enterobacter cloacae* SXCR in liquid system under anaerobic condition. The three-factorial central composite design (CCD) was applied to evaluate the different operational medium components and optimized using different combinations of selected parameters for enhanced removal of sulfonated azo dye Congo red by bacterial strain *Enterobacter cloacae* SXCR. Although the different compositions of medium ingredients affect the growth and decolorization activity of isolated micro-organism in batch culture such as KH_2PO_4, Na_2HPO_4, NaCl, $MgSO_4{\cdot}7H_2O$, trace elements, beef extract, peptone, and glucose, beef extract and peptone are costly and effective items and mainly contribute to growth and activity of enzyme azoreductase in culture condition (34˚C, pH 7), therefore emphasis was given to optimize the effect of these two components alone with glucose on decolorization and degradation efficiency by using RSM. The effects of other ingredients such as KH_2PO_4, Na_2HPO_4, NaCl, metals such as $MgSO_4{\cdot}7H_2O$, trace elements, and different carbon and nitrogen sources on decolorization were optimization using classical methods. The CCD clearly indicated that there are interactions between glucose, peptone, and beef extract on decolorization of Congo red. The effect of salts, carbon, and nitrogen sources were also noticed; however, the effect of $MgSO_4{\cdot}7H_2O$ was not very significance.

2. Materials and methods

2.1. Dye and chemicals

The dye Congo red used in this study was supplied from LOBA (Chennai). All other chemicals were of analytical grade, available commercially, used without further purification and were purchased from HiMedia, Mumbai, India. Congo red, sulfonated azo dye, was used as model azo dye for characterizing dye decolorization activity of *Enterobacter cloacae* (Fig. 1).

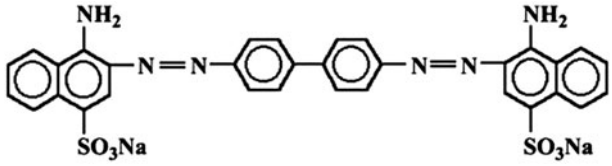

Fig. 1. Structure of Congo red.

2.2. Isolation and identification of decolorizing bacterial strains

Micro-organism used in the present study was *Enterobacter cloacae*, isolated from polluted petroleum soil sample. About 5 gm of petroleum contaminated soil sample was mixed with 100 ml of sterilized nutrient broth medium (pH 7.2) of following composition (g/l): peptone-5, yeast extract-1.5, Beef extract-1.5, NaCl-5 in 250 ml Erlenmeyer flask, and incubated at 37˚C in orbital shaker at 150 rpm for 2 h. The supernatant was collected after allowing the soil to settle down for one hour. The micro-organism having the ability to decolorized dye was screened by inoculating 1 ml of supernatant on nutrient agar plate containing 0.1 g/l of Congo red and incubated at 37˚C for five days. The colony showing maximum decolorization zone was transferred to the 100 ml broth containing minimal salt medium of the following composition (g/l): KH_2PO_4–2.2, Na_2HPO_4–3.5, NaCl-2, $MgSO_4$–0.5, and beef extract-3.0, peptone-3.0, glucose-2 supplemented with 100 mg/l of the dye Congo red. The pure culture was maintained on nutrient agar and stored at 4˚C. Identification of the isolated strain was performed by 16S rDNA sequence analysis (Samved Biotech. Pvt., India).

2.3. Micro-organism and culture media

The micro-organism used in this study was *Enterobacter cloacae* SXCR isolated from petroleum polluted soil with the ability of degrading azo dye (Congo red) and triarylmethane dyes (Methyl violet). The minimal salt medium (MS) used in the experiment has following composition (g/l)—KH_2PO_4–2.2, Na_2HPO_4–3.5, NaCl-2.0, $MgSO_4{\cdot}7H_2O$-0.5, and beef extract-3.0, peptone-3.0, glucose-2.0 supplemented with 0.5 g/l of the dye Congo Red .The temperature was maintained at 34˚C and pH 7 using 0.1 N HCl or 0.1 N NaOH. The desired concentration of dye was made by dilution method from stock solution (1.0 g/l) which was prepared by dissolving dye in deionized water. The stock solution of trace elements contains (g/l): $ZnSO_4{\cdot}7H_2O$-0.1, $MnSO_4{\cdot}2H_2O$-1.0, $CuSO_4{\cdot}5H_2O$-4.0, and $CoCl_2{\cdot}6H_2O$-2.5. The pure culture was maintained on nutrient agar and stored at 4˚C.

2.4. Batch decolorization experiments

The decolorization experiments were optimized by studying the effects of different factors on percentage decolorization of dye Congo red by bacterial isolate *Enterobacter* sp. SXCR. The method used for optimization of different components of the media as followed, varying one component at a time and maintaining the other at constant level. Batch experiments were carried out to evaluate the decolorization efficiency of isolated strain in 250 ml of Erlenmeyer flask containing 100 ml of minimal salt medium (pH 7) supplemented with 0.5 g/l of dye Congo red at 34 C in static condition. The influence of temperature and pH were studied previously by author. The effects of salts (KH_2PO_4, Na_2HPO_4, and NaCl) metals such as $MgSO_4$ and trace elements ($ZnSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$, and $CoCl_2{\cdot}6H_2O$) were studied by adding varying concentration of one component keeping the other constant in minimal salt medium supplemented with 0.5 g/l of Congo red dye at 34 C, pH 7 in static condition for 96 h. The concentration of KH_2PO_4 varied in range of 2–9 g/l while the value of Na_2HPO_4 was selected in range of 1–5 g/l. The varying amount of salts concentration was also considered in these experiments such as NaCl in range of 1–9 g/l, MgSO4 in range of 0.1–0.9 g/l, and concentration of trace elements varied from 0.2 to 3 g/l. Decolorization efficiency was also obtained on different carbon source (2% of glucose, lactose, maltose, sodium acetate, and starch) and different nitrogen sources (2% of urea, potassium nitrate, ammonium nitrate, peptone, and yeast extract). The aliquot (2 ml) of culture media was withdrawn at different time interval, centrifuged at 8,000 rpm for 10 min. Decolorization was monitored by measuring the absorbance of culture supernatant at 497 nm by using UV–visible spectrophotometer and the percentage decolorization was calculated as follows:

$$\text{Percent decolorization} = A_o - A/A_o \times 100 \quad (1)$$

where A_0 is the initial absorbance and A is the absorbance at incubation time t. Percentage decolorization was calculated from the absorbance values obtained against the uninoculated (without micro-organism) controls. Abiotic controls (without micro-organism) were always included.

2.5. Optimization using RSM

RSM is a combination of mathematical and statistical techniques used to evaluate the relationship between a set of controllable experimental factors and

observed results [19]. Design-expert 8.1 software was used to analyze the obtained results. In this study, three independent variables or factors were chosen to maximize decolorization, including glucose (factor A), peptone (factor B), and beef extract (factor C). The CCD was used to evaluate the relative importance of process parameters for Congo red decolorization by strain *Enterobacter* sp. SXCR. All three experimental parameters and CCD levels were used to evaluate dye decolorization study. The specific range for each parameter is as follows (g/l): glucose (0.4–1.8), peptone (0.4–1.8), and beef extract (1.0–4.0). To formulate experimental results for mathematical models, least square methodology was used for regression analysis. The significance of the model equation and model terms was evaluated by *F*-test. A regression method was used to fit the second-order polynomial equation and analyze the contour response surface plot. Analysis of variance (ANOVA) was used to evaluate the statistical significance of the model.

3. Results and discussion

3.1. Optimization of decolorization by RSM

The following independent process parameters are represented in quantitative form while using CCD of RSM [20].

$$Y = f(X_1, X_2, X_3, X_4 \ldots X_n) \pm \varepsilon. \quad (2)$$

where Y is the response, f is the response function, ε is the experimental error, and X_1, X_2, X_3, X_4 … X_n are independent selected parameters. The following equation are used where a second-order polynomial model are fitted in experimental results while using RSM in CCD model:

$$Y = C_0 + \sum_{i=0}^{n} C_i X_n + \sum_{i=0}^{n} di{X_i}^2 \pm \varepsilon \quad (3)$$

where Y is the estimated target function, C_0 is constant, C_i is a linear coefficient, and ε is an error.

The optimization using the CCD method showed that three important costly components of minimal salt medium affecting the decolorization of Congo red dye were glucose, peptone, and beef extract. The range and levels of these three independent variables are presented in Table 1. A total of 20 experiments with different combination of these variables were carried out to evaluate maximum decolorization (Table 2). By applying multiple regression analysis, the observed decolorization response was fitted with a second-order polynomial equation as:

Table 1
Independent factors and their range used for optimization

Factors	Lower value	Upper value
Glucose (g/100 ml)	0.04	0.18
Peptone (g/100 ml)	0.04	0.18
Beef extract (g/100 ml)	0.10	0.40

$$Y = +82.68 + 9.33A + 10.67B + 24.63C + 2.81AB - 6.85AC - 10.94BC - 1.43A^2 - 1.36B^2 - 9.54C^2 \quad (4)$$

In this equation, Y was the predicted response and A, B, and C were the coded values of the test factors.

The statistical significance of the model equation was evaluated by *F*-test and ANOVA as shown in Table 3. The regression used for optimization of media constituents showed that quadratic model was found to be the most significant model among four studied model. The significance of coefficient was analyzed by the p value. Probability > F (0.001) indicates that the model considered statistical significance. The fit of model was tested by the estimated higher determination coefficient ($R^2 = 0.998$), which indicates good relationship between the experimental and predicted values of response.

3.2. Combination effect of variable

The decolorization efficiency of dye Congo red was investigated using different combination and interaction of essential and costly medium constituents (glucose, peptone, and beef extract) by RSM which are difficult to be observed in conventional methods. The dye decolorization efficiency was evaluated by using RSM and contour plots. A linear contour plot showed the interaction is negligible between the corresponding variable while an elliptical or saddle contour plot showed the interaction between the corresponding variable [21].The influence of three factors simultaneously on the dye decolorization is graphically represented as three-dimensional (3D) response surface plots as shown in Fig. 2(a–c). The interactions of glucose, peptone, and beef extract were clearly identified through CCD method. The optimal concentration of glucose was 2 g/l, while for beef extract and peptone were 3 g/l, respectively.

3.2.1. Combined effect of glucose and peptone

The RSM was used to investigate the interaction of different concentration of glucose and peptone on dye

Table 2
The actual design of experiments and response for Congo red decolorization

Run number	Glucose (g/100 ml)	Peptone (g/100 ml)	Beef (g/100 ml)	Decolorization (%) (expeimental)
1	0.18	0.04	0.40	94.78
2	0.11	−0.01	0.25	63.45
3	0.11	0.11	0.25	82.62
4	0.04	0.04	0.10	12.57
5	0.04	0.04	0.40	93.22
6	0.04	0.18	0.40	92.93
7	0.18	0.18	0.10	88.2
8	−0.01	0.11	0.25	61.15
9	0.11	0.11	0.25	82.6
10	0.11	0.11	0.25	81.97
11	0.18	0.18	0.40	97.69
12	0.11	0.11	0.25	78.53
13	0.11	0.11	0.25	87.5
14	0.18	0.04	0.10	33.5
15	0.04	0.18	0.10	48.02
16	0.23	0.11	0.25	96.85
17	0.11	0.11	0.50	97.69
18	0.11	0.23	0.25	94.95
19	0.11	0.11	0.25	82.76
20	0.11	0.11	−0.00	14.4

decolorization by *Enterobacter* sp. and obtained results are represented in form of contours and 3D plot (Fig. 2(a)). The decolorization efficiency was increased with an increase in the concentrations of glucose and peptoneto the optimal level. Further increase in concentration of peptone has not much effect on dye decolorization at that extent. This result shows the best condition for decolorization of Congo red by *Enterobacter* sp. at peptone 3 g/l and glucose 2 g/l, respectively.

3.2.2. Combined effect of glucose and beef extract

The interaction of glucose and beef extract is shown in Fig. 2(b). This result indicates that the interaction of glucose and beef extract on decolorization activity by *Enterobacter* sp. was positive and statistically significant, as conformed by the contour lines and residual plots.

3.2.3. Combined effect of beef extract and peptone

Fig. 2(c) has shown the interaction of beef extract and peptone on dye decolorization. The optimum value of these two variables can be investigated by saddle point or by testing the maxima formed by the X and Y coordinates. This condition was obtained at the saddle point for best response. The optimum value of beef extract and peptone are 3 and 3 g/l, respectively.

It is evident from the observed 3D contour plots that the optimum concentration for decolorization of glucose is 2 g/l, peptone is 3 g/l, and beef extract is 3 g/l. At this concentration about 96% decolorization activity of Congo red was shown by isolated bacterial strain.

The analysis of model adequacy is an important procedure of statistical optimization method. If the model is not satisfactorily fitted, it would give poor and misleading results. This is verified by looking at the residual plots. The fitness of model is usually analyzed using coefficient of multiple regressions (R^2). In this model, R^2 was 99.2% which shows the fitness of model. The studentized residual and normal% probability plot are shown in Fig. 3. To best of our knowledge, this is the first report which shows the interaction of glucose, peptone, and beef extract on decolorization of Congo red by *Enterobacter* sp.

3.3. Effect of carbon sources

The growth of micro-organism and decolorization of dyes without any carbon sources is very difficult [22]. Hence, to determine the effect of carbon sources on the decolorization of dye by isolated bacterial strain, various carbon sources such as glucose,

Table 3
ANOVA for RSM parameter fitted to quadratic model for decolorization of Congo red using *Enterobacter* sp.

Source	Sum of squares	df	Mean square	*F* value	Prob > *F*
Model	13745.07	9	1527.23	142.90	<0.0001 significant
A	1189.78	1	1189.78	111.32	<0.0001
B	1555.41	1	1555.41	145.53	<0.0001
C	8286.63	1	8286.63	775.35	<0.0001
AB	63.00	1	63.00, 5.89	0.0356	
AC	375.24	1	375.24, 35.11		
BC	957.69	1	957.69, 89.61	<0.0001	
A^2	29.30	1	29.30, 2.74	0.1288	
B^2	26.46	1	26.46, 2.48	0.1467	
C^2	1312.04	1	1312.04	122.76	<0.0001
Residual	106.88	10	10.69		
Lack of fit	65.90	5	13.18	1.61	0.3074 not significant
Pure error	40.97	5	8.19		
Cor total	13851.95	19			

Note: $R^2 = 0.9923$; Predicted $R^2 = 0.9559$; Adjusted $R^2 = 0.9853$; CV = 4.40, and PRESS = 610.19.

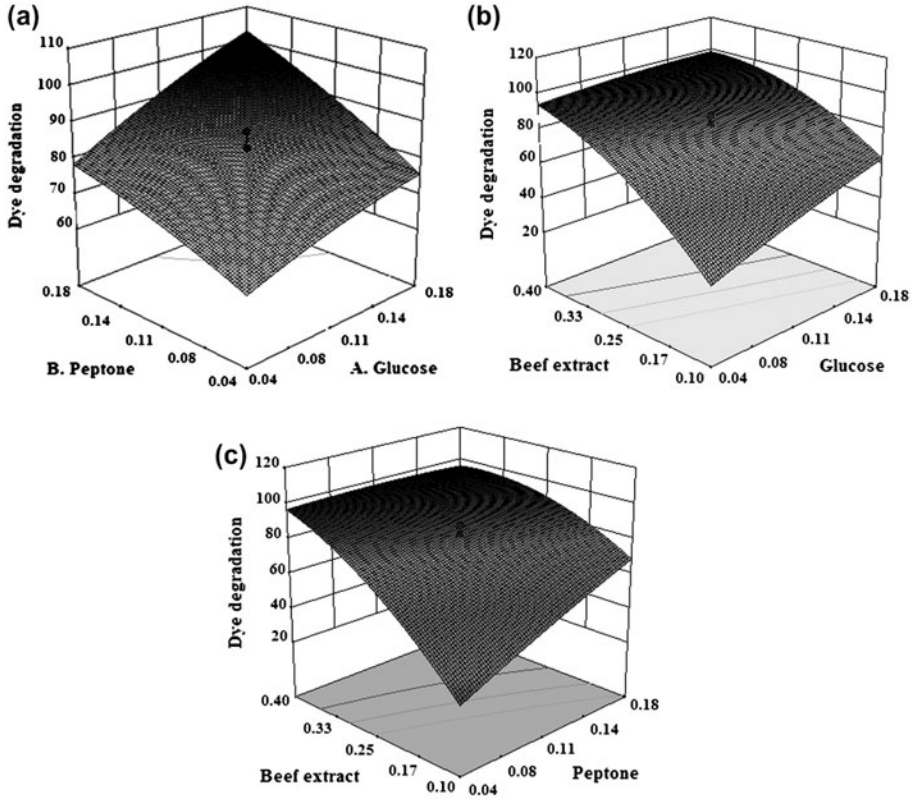

Fig. 2. Three-dimensional response surface plot for the effect of (a) peptone and glucose (b) beef extract and glucose, and (c) beef extract and peptone on decolorization of Congo red by *Enterobacter cloacae* SXCR.

maltose, lactose, starch, and sodium acetate were added separately at a concentration of 2 g/l in minimal salt medium containing 0.5 g/l of dye Congo red. As shown in Fig. 4, the decolorization potential of glucose was the best among all the selected carbon sources. Bacterial strain shows dye decolorization (91%) at the end of 118 h. The results in this report are in agreement with those of Chen et al. (2011) [11].

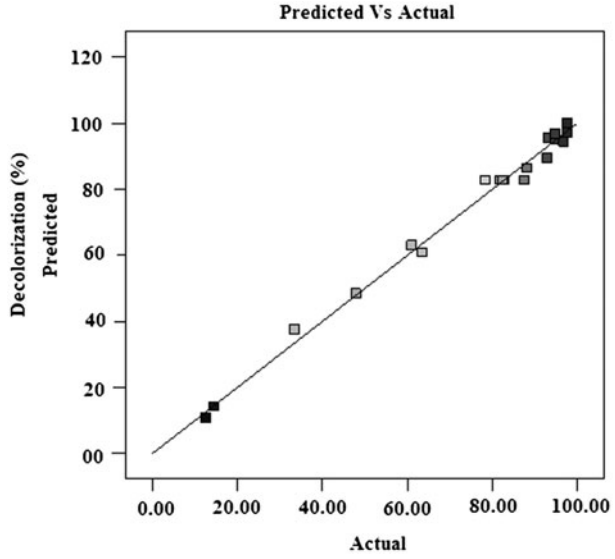

Fig. 3. A plot of actual and predicted value for% decolorization. Solid line represents predicted value while unfilled symbol represent actual value.

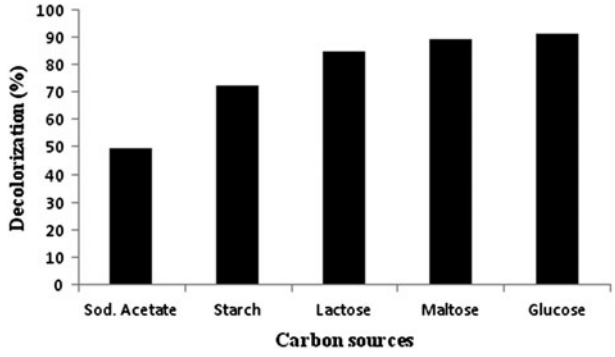

Fig. 4. Effect of different carbon sources on Congo red decolorization by *Enterobacter cloacae* SXCR.

They showed the decolorization of Reactive black 5 by *Enterobacter* sp. GY-1 in different medium. When these medium were supplemented with 1% glucose as carbon sources, decolorization activity was more obvious. Moosvi et al. (2007) [23] observed that when the concentration of glucose increased from 0.5 to 1 g/l, the decolorization percentage increased from 25 to 93%; and with further increase in concentration of glucose from 1 to 2.5 g/l, decolorization was decreased and the observed value was about 90%. Hence, 1 g/l of glucose can consider optimum for decolorization of RV5R. A complete decolorization of dye Solar golden yellow R was also noted with 1% of glucose in medium after 48 h by Asgher et al. (2008) [24].

3.4. Effect of nitrogen sources

The effects of different nitrogen sources on dye decolorization by *Enterobacter* sp. in minimal salt medium were evaluated. The effect of various nitrogen sources: peptone, yeast extract, urea, potassium nitrate, and ammonium nitrate were analyzed by adding each nitrogen sources separately at concentration of 2 g/l in minimal salt medium supplemented with 0.5 g/l of dye Congo red at 34˚C, pH 7 in batch culture. Results are illustrated in Fig. 5 which indicated that yeast extract was the best nitrogen sources among the other selected nitrogen sources. Similar results were reported by Xu et al. (2006) [25]; however, 0.5 g/l exhibited maximum 94% decolorization of Reactive Violet 5R [22]. The decolorization percentage was observed to be 97% with yeast in minimal salt medium at 118 h. However, in our studies, both the yeast extract and the peptone showed equal effects on decolorization. Hence either peptone or yeast extract may be selected as nitrogen source.

3.5. Effect of salts

In order to develop an efficient economic method for dye decolorization, medium optimization was carried out by conventional procedure for one factor at a time which involves changing an independent variable while the other parameters are fixed at certain range. The effects of salts such as KH_2PO_4, Na_2HPO_4, and $MgSO_4$ on dye decolorization were investigated and results are shown in Fig. 6. Strain SXCR could sustain the toxic effect of dye Congo red at concentration of 0.5 g/l and decolorized 97% in 120 h. The optimum concentration of KH_2PO_4 and Na_2HPO_4 was observed 2.2 g/l (Fig. 6(a)) and 3.5 g/l (Fig. 6(b)), respectively. Lin-Na Du et al. (2010) [26] observed high decolorization efficiency with increased concentration of Na_2HPO_4 in slightly acidic condition.

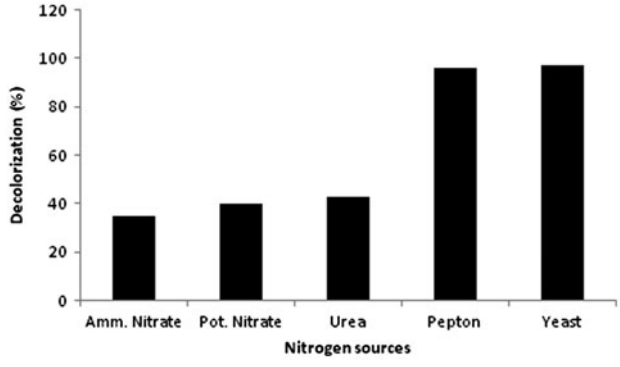

Fig. 5. Effect of different nitrogen sources on Congo red decolorization by *Enterobacter cloacae* SXCR.

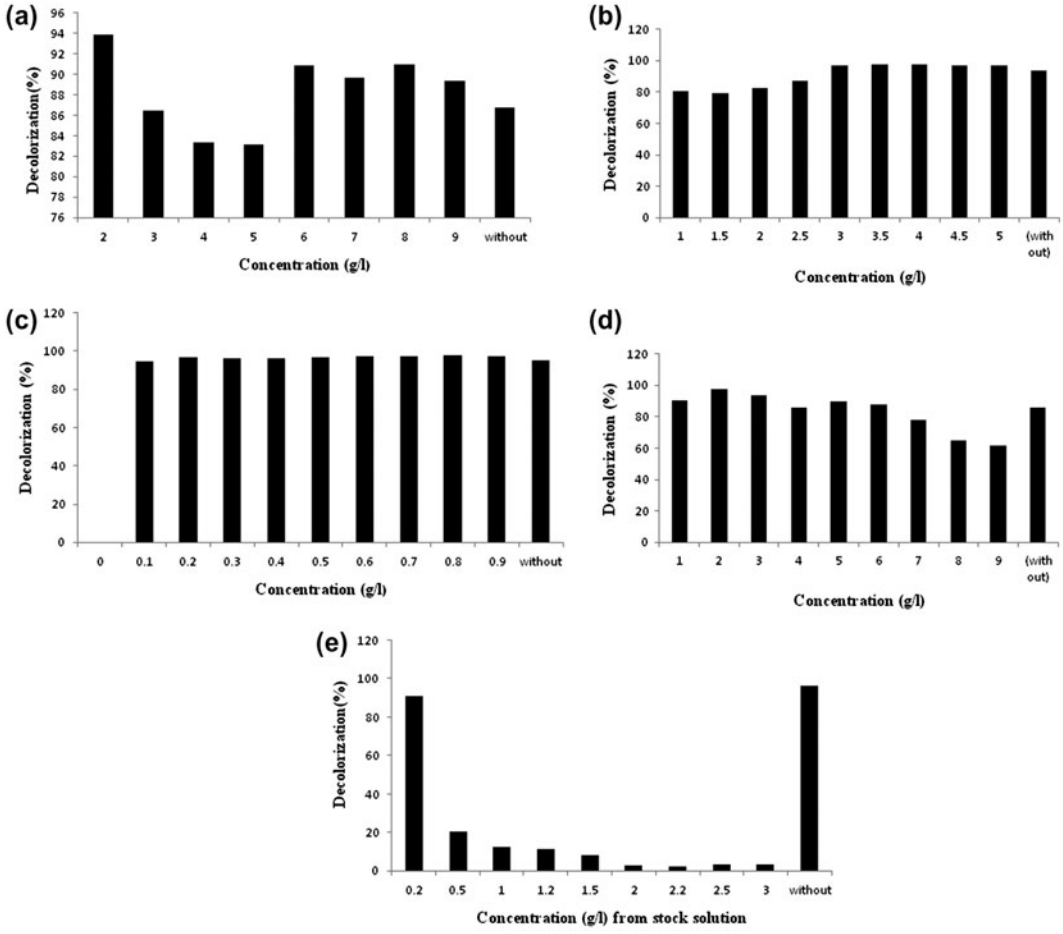

Fig. 6. (a) Effect of KH_2PO_4 on Congo red decolorization by *Enterobacter cloacae* SXCR (b) Effect of Na_2HPO_4 on Congo red decolorization by *Enterobacter cloacae* SXCR (c) Effect of $MgSO_4$ on Congo red decolorization by *Enterobacter cloacae* SXCR (d) Effect of NaCl on Congo red decolorization by *Enterobacter cloacae* SXCR, and (e) Effect of Trace element on Congo red decolorization by *Enterobacter cloacae* SXCR.

The effect of $MgSO_4$ is almost negligible for dye decolorization by bacterial strain as shown in Fig. 6(c). The results obtained for the addition of NaCl at various concentrations are shown in Fig. 6(d). A similar result was observed by Chen et al. (2011) [11] while Zhao et al. used 3% of NaCl for optimum decolorization of dye Acid Red GR by strain LA-4 [27]. The results showed that optimal concentration of NaCl is 2 g/l, the decolorization rate decreased when the concentration of NaCl was more than this value. Generally, sodium concentration above 3 g/l can cause moderate inhibition of most bacterial activities [28].

The addition of trace elements was found to inhibit the dye decolorization (Fig. 6(e)). The rate of decolorization decreases with an increase in the metal ions concentration. Zhao et al. (2010)[27] shown that metal ions such as Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, and Ni^{2+} had no effects on the dye decolorization, while Zn^{2+} and Cu^{2+} could inhibit the decolorization of dye. Similar inhibitory effects of Zn^{2+} and Cu^{2+} were noted by Gopinath et al. (2009)[29] at the concentration 0.1 g/l and 0.2 g/l, respectively, by *Bacillus* sp. on Congo red decolorization, but presence of Ca^{2+}, Mg^{2+}, and Na^{+} up to a concentration of 4 g/l enhanced the decolorization efficiency of dye. The inhibitory effects of metal ions are significantly affected on the growth and degradation potential of micro-organism in industrial wastewater [30]. Here, we observed that 1 g/l concentration of Zn^{2+}, Cu^{2+}, and Co^{2+} are significantly inhibit the decolorization of Congo red by *Enterobacter* sp.

4. Conclusions

Decolorization of sulfonated azo dye Congo red was investigated by using newly isolated bacterial strain *Enterobacter cloacae* SXCR. The RSM-based CCD was applied for optimizing the operational parameter

of decolorization. The optimal decolorization of Congo red was found to be at concentration of KH_2PO_4 is 2.2 g/l, Na_2HPO_4 is 3.5 g/l, NaCl is 2 g/l, $MgSO_4{\cdot}7H_2O$ is 0.5 g/l, trace elements have inhibitory effects, glucose is 2 g/l, peptone is 3 g/l, and beef extract is 3 g/l. A quadratic model was obtained by using Design-expert 8.1 software. The value of the adjusted determinant coefficient ($R^2 = 0.998$) was close to 1, indicating a high significance of the model. The model employed provides good quality of prediction for the above variable in terms of effective dye decolorization. The results obtained in the present study indicate RSM based on CCD can save the time and effort by the estimation of the optimum condition of the maximum removal of dye, and was a suitable approach to optimize the selected parameter for achieving maximal decolorization of the dye. Hence, these results show the potential of *Enterobacter* sp. for further application towards treatment of textile-dyeing effluents.

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