Statistical modeling and optimization of Toluidine Red biodegradation in a synthetic wastewater using *Halomonas* strain Gb

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Abstract

Background Synthetic dye wastewater is a group of environmental pollutants that are widely used in some industries like textile, printing, dyeing and etc. Traditional treatment methods for wastewaters containing synthetic dyes are considered as expensive and time consuming approaches due to the chemical stability of these pollutants. Therefore, in recent years, biodegradation by means of capable microorganisms has been considered as an effective way to remove these pollutants. Hence, the present study has aimed at examining the decolorization of Toluidine Red (C.I. no.12120), which is an oil soluble azo dye, as the sole sources of carbon and energy from a synthetic dye wastewater by the halophilic *Halomonas* strain Gb bacterium. In order to model, optimize, and investigate the individual factors affecting the biodegradation capacity of this dye by *Halomonas* strain Gb, for the first time response surface methodology (RSM) and central composite design (CCD) were applied.

Methods In this research, statistical modeling and optimization were performed by Design Expert software version 10 and the degradation capacity was considered by carrying out 30 tests using RSM method. For this purpose, the effect of 4 variables included dye concentration (10–30 ppm), salt concentration (2–10%), pH (5.5–9.5), and temperature (20–40) at different times of 2nd, 4th, and 10th days have been studied. Then, a second-order function was presented for the amount of dye removal in terms of the four selected variables, based on statistical modeling.

Results According to the obtained results and analysis of variance, all main variables were found to be significantly effective on the biodegradation capacity. With regard to the results, the highest amount of biodegradation between different days was 81% and observed at the 4th day, while the optimum conditions for the maximum biodegradation of this time has been determined at pH of 6.5, temperature of 35 °C, and salt and dye concentrations were equivalent to 4% and 25 ppm, respectively. There is 11% relative error between the experimental and predicted results in the selected experiments, which confirms the reliability of the obtained correlation for calculating the decolorization capacity.

Conclusion In accordance with the results, the proposed model can provide a good prediction of the effect of different conditions on the biodegradation of Toluidine Red, and the optimization results in this study have been consistent with the previous studies conducted with the IP8 and D₂ strains by the OFAT method. Moreover, the proposed model may help in better understanding the impact of main effects and interaction between variables on the dye removal. Overall, the results indicated that the halophilic bacterium used in dye removal can be more effective in high-salinity environments.

Keywords Halophilic bacteria · Dye wastewater · Biodegradation · Toluidine red · Statistical modeling and optimization

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Introduction

Environmental contamination due to detrimental hazardous dyes from dye manufacturing and dyeing industries remains important environmental issue. It is estimated that approximately 15% of total dyes production are lost and discharged in the effluent during production and dyeing process which causes serious risks and detrimental effects on human and various ecosystems [1]. Synthetic dyes are widely used in advanced technologies in

Fig. 1 Chemical structure of Toluidine Red [45]

various fields such as textile, paper, leather tanning, food processing, plastics, cosmetics, rubber, printing and dye manufacturing industries. Also, artificial dyes are also used to track groundwater flow [2–8]. It is worth mentioning that there are about 9000 different types of commercial dyes and pigments and with worldwide production capacity estimated to be more than 7×10^5 tons annually. Synthetic dyes are chemically diverse, and with regard to their industrial application divided into azo, triphenylmethane, and heterocyclic/polymeric structures [9–14]. Azo dyestuffs are the largest class of industrial dyes that are not only used for textile, plastic, surface coating paints, and ink, but also in new technologies and most of these dyes have a complex chemical structure and are stable in the environment [15–18]. In this regard, different physical, chemical, and biological methods for dye wastewater treatment have been identified [19, 20]. The common methods such as adsorption [21–23], coagulation and flocculation [24–26], membrane separation [26–28] and photocatalytic and electro-Fenton process [29, 30] have been applied. These physicochemical methods due to their high operational cost, low efficiency for a wide range of dyes and formation of poisonous byproducts are not sufficient to remove the azo dyes from the polluted effluent [31, 32].

Bioremediation is one of the most effective techniques because of its adaptation to the nature without need to additive chemicals, easy handling and low costs and capability to simultaneously use with physicochemical methods, as well as the possibility of complete degradation of pollutants [33]. Until now, many researches have been carried out on biodegradation of water soluble azo dyes with biological methods [34, 35], but only a few numbers of them have been performed on oil soluble azo dyes [36, 37]. Previous researches have indicated that halotolerant bacteria, which are able to live in hypersaline environments may have a great potential in pollutants degradation. Halophilic microorganisms have the

Table 2 Selected variables and their levels in Toluidine Red biodegradation process

Factor	Name	Unit	Type	−2	−1	0	+1	+2
A	Color Conc.	ppm	Numeric	10	15	20	25	30
B	NaCl Conc.	%	Numeric	2	4	6	8	10
C	pH	—	Numeric	5.5	6.5	7.5	8.5	9.5
D	Temperature	°C	Numeric	20	25	30	35	40

ability to grow and perform their metabolic pathway in a medium with up to 30% salinity [38–42].

Removal of Toluidine Red, an oil soluble azo dye [43], from a synthetic dye wastewater by IP8 and D₂ strains has been investigated in our previous researches [44, 45]. One-factor-at-a-time (OFAT) experiments have been used to determine color removal efficiency, which could not necessarily lead to finding optimal conditions. On the other hand, it is not possible to investigate the interaction effects of factors using OFAT, so statistical methods such as the response surface can be utilized for solving these problems [46].

To our knowledge, no report exists in applying RSM technique for biodegradation of Toluidine Red as well as correlation between dye removal and effective variables. Therefore, the aim of present research is to systematically study of Toluidine Red removal using a new *Halomonas* strain Gb with RSM at three time intervals (2nd, 4th and 10th days) for the first time. In this survey, modeling, optimization, and evaluation of factors affecting the biodegradation capacity of this oil soluble dye have been studied.

Materials and methods

Dye and chemicals

The dye used in this study was Toluidine Red with a Color Index of 12,120. Toluidine Red is a water-insoluble azo pigment with the chemical formula of C₁₇H₁₃N₃O₃ and molecular weight of 307 g/mol. The chemical structure of the Toluidine Red is shown in Fig. 1. Dimethylformamide (DMF) was used as a solvent for preparation of dye stock solution. Tween 80 was also added to the mineral salt medium (MSM) because of the insolubility of the dye. NaOH and HCl was used to adjust pH of culture media. Chemical compounds were obtained from Merck Company.

Table 1 The composition of mineral salts in the culture

Formula	KNO ₃	(NH ₄) ₂ SO ₄	K ₂ HPO ₄ ·3H ₂ O	KH ₂ PO ₄	MgSO ₄ ·7H ₂ O	CaCl ₂ ·2H ₂ O	FeSO ₄ ·7H ₂ O	MnSO ₄ ·H ₂ O
g/lit	5	1	0.87	0.54	0.2	0.02	0.01	0.005

Microorganism and culture medium

The microorganism used in this survey was *Halomonas* strain Gb as a halophilic bacterium which previously isolated from saline regions of Iran by a research group of the Department of Microbiology in Tehran University [32]. Nutrient agar containing 7.5% (w/v) sodium chloride salt was used for preservation of bacterial strain. Mineral salt medium for bacterial growth contains the following compounds and quantities as shown in Table 1.

The pH of the medium was adjusted to desired amount by addition of 1 N NaOH and 1 N HCl after autoclaving (121 °C, 15 min). The amount of 2–10% (w/v) sodium chloride was added to the culture medium due to the halophilic behavior of

the bacterium. The dye solution was sterilized separately and working concentrations of 10–30 ppm of dye have been used.

Toluidine red biodegradation experiments

The mineral culture medium for decolourization was prepared according to the method described in section 2.1. The amount of 15 ml of decolourization medium was poured into the test tubes with 35 ml volumes and after adjusting the pH and adding the dye to the desired amount (10 to 30 ppm), the inoculation of the *Halmonas* strain Gb was carried out within the test tubes. For inoculation, 1% of 1.5×10^8 CFU/ml of the bacterial suspension was added to the nutrients medium and the tubes were incubated in 35 °C for 10 days under static

Table 3 Results of the colour biodegradation by *Halmonase* strain Gb bacterium in 2, 4 and 10 days intervals

Run	Factor 1 A:Color Conc.	Factor 2 B:NaCl Conc.	Factor 3 C:pH	Factor 4 D: Temp.	Response 1 De-colorization %	Response 2	Response 3
					(Day 2)	(Day 4)	(Day 10)
1	15	4	8.5	25	14.2	13.4	20.6
2	20	6	7.5	30	20.2	21.6	32.7
3	15	8	6.5	35	24.6	39.34	38.4
4	20	6	7.5	30	19.8	27.5	36.1
5	15	4	8.5	35	49	49.97	44
6	25	4	8.5	25	16.5	15.9	28.9
7	25	4	8.5	35	69.7	79.8	65.9
8	20	6	7.5	30	20.7	22.1	35.4
9	25	4	6.5	35	65.7	84.3	66.5
10	30	6	7.5	30	42.6	51.4	61.8
11	10	6	7.5	30	13.8	19.5	30.7
12	20	10	7.5	30	17.8	15.4	12.1
13	20	6	7.5	20	19.9	28.4	31.3
14	25	8	6.5	35	44.6	70.2	58.6
15	15	4	6.5	35	30.3	32.05	31.6
16	15	8	8.5	35	30.7	37.3	35.7
17	25	4	6.5	25	18.8	24.4	32.6
18	20	6	7.5	40	65.4	87.13	65.7
19	20	2	7.5	30	28.9	25.4	32.5
20	20	6	9.5	30	16.3	18.9	35.8
21	25	8	6.5	25	27.6	33.5	39.7
22	15	4	6.5	25	14.8	19.2	15.9
23	20	6	5.5	30	25.7	29.4	36.2
24	15	8	6.5	25	21.1	26.7	32.4
25	25	8	8.5	25	12.9	10.8	19.2
26	20	6	7.5	30	22.3	31.2	35.4
27	25	8	8.5	35	38.9	37.2	37.3
28	15	8	8.5	25	18.1	20.9	28.7
29	20	6	7.5	30	21.3	23.7	40.1
30	20	6	7.5	30	26.4	24.9	38.4

Table 4 ANOVA results for second-order models and effectiveness status of variables on decolourization

Source	Sum of squares	df	Mean square	F value	p value 10th day	Influence status †	p value 2th day	p value 4th day ‡	Influence status °
Model	5453.04	14	389.50	29.22	< 0.0001	HS ^a	< 0.0001	< 0.0001	HS
A-Color Conc.	1115.21	1	1115.21	83.65	< 0.0001	HS	< 0.0001	< 0.0001	HS
B-NaCl Conc.	134.43	1	134.43	10.08	0.0063	HS	0.0002	0.0278	S ^b
C-pH	54.60	1	54.60	4.10	0.0612	PS ^c	0.3572	0.0049	HS
D-Temp.	2181.23	1	2181.23	163.61	< 0.0001	HS	< 0.0001	< 0.0001	HS
AB	241.80	1	241.80	18.14	0.0007	HS	0.0330	0.0100	HS
AC	201.64	1	201.64	15.12	0.0015	HS	0.0122	0.0036	HS
AD	194.60	1	194.60	14.60	0.0017	HS	< 0.0001	0.0001	HS
BC	232.56	1	232.56	17.44	0.0008	HS	0.0183	0.0097	HS
BD	225.00	1	225.00	16.88	0.0009	HS	< 0.0001	0.0016	HS
CD	7.56	1	7.56	0.57	0.4630	NS ^d	0.0070	0.3324	NS
A ²	145.89	1	145.89	10.94	0.0048	HS	0.0095	0.0090	HS
B ²	371.70	1	371.70	27.88	< 0.0001	HS	0.2653	0.4781	NS
C ²	1.80	1	1.80	0.14	0.7183	NS	0.7842	0.8432	NS
D ²	225.73	1	225.73	16.93	0.0009	HS	< 0.0001	< 0.0001	HS
Residual	199.98	15	13.33						
Lack of Fit	166.52	10	16.65	2.49	0.1630	NS	0.1490	0.1453	NS
Pure Error	33.45	5	6.69						
Cor Total	5653.01	29							

† The above ANOVA results (sum of squares, mean square, F value, influence status) are related to 10th day.

‡ p values are only shown for 2th and 4th days.

^a Highly significant at level of 1%

^b Significant at level of 5%

^c Possibly significant at level of 5–10%

^d Not significant

° Influence status for 4th day

Fig. 2 The 3D graph for the optimum conditions of biodegradation on the fourth day after incubation

conditions to allow decolourization, and removal was examined at different time intervals (the 2nd, 4th and 10th days).

Experimental design approach

In order to study the process variables influence on Toluidine Red biodegradation as the selected response, RSM with CCD was applied. RSM is a statistical design approach as a practical method to investigate the relationship between response function and a group of independent variables. A second-order model can be built by this method based on a polynomial function with quadratic terms as given in Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^K \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{K-1} \sum_{j=i+1}^K \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

Where Y represents the response, X_i and X_j are the independent variables, β_0 , β_i , β_{ii} , β_{ij} and ε are constant term, linear coefficient, squared effects and interaction effects, and statistical error, respectively. For estimating the β 's parameters in this model, least squares method can be used [46, 47]. Experimental variables of this study and their levels are given in Table 2.

For modelling and optimization studies, Design Expert software (Version 10) was applied. The total number of trials in RSM with CCD calculated with $2^k + 2k + n_0$, where k and n_0 are the number of the independent variables and replicate number of the central point, respectively. In this survey, k and n_0 are 4 and 6 respectively which resulted in 30 treatments as shown in Table 3. Therefore, based on the proposed experimental design by the RSM, the number of 30 experiments were performed and in each treatment, the objective function of biodegradation by *Halmonas* strain Gb (Y) was measured. All of the experiments were performed with 3 repetitions. Control experiment was also performed without microbial inoculation in center point of the independent variables and according to the results, an insignificant dye removal of 4% was observed due to physico-chemical effects.

Measuring the concentration of colour in the wastewater

In this study, the spectrophotometric method was used to measure the residual colour concentration in the aqueous phase, according to our previous research [44]. For this purpose, after mixing the tubes and blending sufficiently, 3 ml of culture medium was sampled and centrifuged using microcentrifuge (Hettich, Micro 200R, Germany) at 7500 rpm for 5 min and the bacterial cells was separated, light absorption of the supernatant was read at the wavelength of 490 nm by the spectrophotometer (Varian Cary 100, USA) and the

colour removal capacity has been calculated using the following equation (Eq. 2):

$$\text{Dye removal}(\%) = (A_0 - A_t) / A_0 \times 100 \quad (2)$$

Where A_0 and A_t represent the absorption of the sample at the time 0 and the time t .

Results and discussion

Biodegradation of toluidine red by *Halomonas* strain Gb

In this survey, the colour biodegradation efficiency by halophilic *Halomonas* strain Gb bacterium using RSM with 30 experiments was evaluated. The percentages of de-

Table 5 Results of Toluidine Red biodegradation which calculated with both experimental method and proposed computational model

Standard	Actual	Predicted
Order	Value	Value
1	19.2	9.63
2	24.4	28.07
3	26.7	30.13
4	33.5	32.99
5	13.4	16.81
6	15.9	17.01
7	20.9	21.65
8	10.8	6.27
9	32.05	35.46
10	84.3	81.01
11	39.34	35.69
12	70.2	65.66
13	49.97	47.94
14	79.8	75.25
15	37.3	32.51
16	37.2	44.24
17	19.5	22.19
18	51.4	52.37
19	25.4	27.49
20	15.4	16.98
21	29.4	33.10
22	18.9	18.86
23	28.4	27.69
24	87.13	91.50
25	23.7	25.17
26	31.2	25.17
27	21.6	25.17
28	24.9	25.17
29	22.1	25.17
30	27.5	25.17

colorization at three time intervals of 2, 4, and 10-day are shown in Table 3.

Analysis of variance

Analysis of variance (ANOVA) results for examination of variables effect on decolourization efficiency was studied. The results of ANOVA and therefore status of the effectiveness of each main and binary variables on the biodegradation capacity are shown in Table 4. In this survey, accuracy of the predicted models and variables effectiveness were examined by Fisher's F-test. F-test results in a probability value (p value) less than 0.05 and 0.01 indicating significance and highly significance, respectively. The ANOVA results of this survey reveal that the quadratic model is the best-fit equation for prediction of the selected response in the 2nd, 4th and 10th days. As it can be seen in Table 4, for 10th day, F-test ($F_{\text{model}} = 29.22 > F_{14,15,\alpha=0.01} = 3.6$) results in p value less than 0.01 which indicates that the model is highly significant. According to the ANOVA results and p value, it can be concluded that all of the selected variables have affected the

decolorization capacity after 4th day and all variables are also highly significant after 10th day except pH with possibly significant effect. Moreover, the most effective variables were temperature, dye and salt concentrations respectively. The effect of pH was lower than other variables, even at the beginning of the biodegradation process (the second day), pH changes were not effective in dye removal. Due to production of intermediate metabolites, it was observed that pH has also been effective over time from 2th to 10th day. Accordingly, the effectiveness status of variables at 4th and 10th day is shown in Table 4.

Optimization and statistical modeling of toluidine red biodegradation

The optimum conditions which result in the maximum colour biodegradation were found based on the obtained quadratic models using Design Expert software. Based on the RSM results, the highest amount of biodegradation in the 2nd, 4th and 10th days was 63.2%, 81%, and 68% respectively. According to the results as shown in Fig. 2, the highest

Fig. 3 Effective status of main variables on the Toluidin Red biodegradation on the fourth day after incubation

removal of 81% on the 4th day after incubation was observed at color concentration of 25 ppm, NaCl concentration of 4%, pH of 6.5, and temperature of 35 °C. Therefore, the effectiveness statuses of the variables and the corresponding graphs have been investigated for the fourth day in the following.

In a similar study carried out by Mohareri et al. (2012) for the biodegradation of Toluidine Red by *Halomonas* strain D₂ bacterium, the OFAT method was used to reach the optimum point. Similarly, their results showed that the highest biodegradation capacity of 80% at temperature of 35 °C, pH of 6.5, 25 ppm dye concentration and an optimum salt concentration of 5% [45]. Similar results are also obtained for the removal of Cibacron Black w-55 by *Halomonas* sp. IP8 strain conducted by Pourbabaie et al. (2011) [48]. Selvakumar et al. (2013) have used *Ganoderma lucidum*, a white rot fungi, for the textile wastewater treatment in a batch reactor and optimiza-

tion of pH, temperature, agitation speed and color concentration was examined by RSM. In their research, a maximum of 81.4% color removal has been observed after 6 days. Their results indicated that enzyme activity increased till the 4th day and reached a maximum thereafter, the enzyme activity decreases [49]. It is worth mentioning that however different researches have been carried out on modelling and optimization of colour biodegradation from dye wastewater by RSM approaches [50–52], but to the best of our knowledge, none of these studies has been so far applied to biodegradation of oil-soluble azo dyes by the RSM statistical method.

According to the results obtained by the RSM, the amount of biodegradation after 4 days of incubation, which has the highest level of biodegradation, is presented with the second-order polynomial function below:

$$\begin{aligned} \text{De-colorization (4}^{\text{th}} \text{ day)} = & +156.30 - 2.29 \text{ Color Conc.} + 38.57 \text{ NaCl Conc.} \\ & + 15.45 \text{ pH} - 21.835 \text{ Temp.} - 0.39(\text{Color Conc.} \times \text{NaCl Conc.}) - 0.91(\text{Color Conc.} \times \text{pH}) \\ & + 0.27(\text{Color Conc.} \times \text{Temp.}) - 1.96(\text{NaCl Conc.} \times \text{pH}) - 0.51(\text{NaCl Conc.} \times \text{Temp.}) \\ & + 0.26(\text{pH} \times \text{Temp.}) + 0.12 \text{ Color Conc.}^2 - 0.18 \text{ NaCl Conc.}^2 + 0.20 \text{ pH}^2 + 0.34 \text{ Temp.}^2 \end{aligned}$$

The accuracy of the model and the polynomial function in fitting the data is expressed by R^2 . The coefficient of determination between measured data and simulated results (R^2) and adjusted R^2 were 0.9668, 0.9359, respectively. The high R^2 -value suggested the reliability of the proposed quadratic model. Results of Toluidine Red biodegradation, which calculated with both experimental method and proposed model are presented in Table 5.

According to Table 5, the average error in the study of Toluidine Red biodegradation has been 11% based on the proposed model and experimental data, which indicates that the results of the obtained model and the experimental data are in good agreement with each other and the model can be appropriately used with proper certainty.

Effect of main factors on biodegradation capacity

Temperature

As shown in Fig. 3a, on the fourth day, with a rise in temperature from 20 to 40 °C, the colour removal has significantly increased. The color removal is significantly higher in high temperature (91.5%) than in low temperature (17.8%) with p value of <0.0001 as shown in Table 4. Mohareri et al. (2012) have studied Toluidine Red biodegradation by *Halomonas* strain D₂ and observed that the increment of temperature in

the range of 25–35 °C led to increasing bacterial activity and maximum color removal was 80% at the temperature of 35 °C [45]. Similarly, Asad et al. (2007) showed that Remazol Black B biodegradation by *Halomonas* sp. D₂ bacteria has raised by the increase of temperature from 25 to 35 °C, with a maximum removal of 45% at 35 °C. However, according to their results, the increment of temperature to 40 °C has decreased the amount of biodegradation [32]. Similar results were also obtained by Kolekar et al. (2008) for biodegradation of Disperse Blue 79 and Acid Orange 10 by *Bacillus fusiformis* KMK5 [53].

Dye concentration

By increasing the dye concentration from 10 to 30 ppm as shown in Fig. 3b, the biodegradation has been enhanced and the highest activity of the bacterium was observed at a concentration of 30 ppm (p value = 0.0278). Similarly, the results of biodegradation of Toluidine Red by the *Halomonas* strain D₂, showed that the highest amount of biodegradation (75%) was obtained at a concentration of 25 ppm after 144 h [45]. The results of similar studies showed that by increasing the initial concentration of dye, the amount of biodegradation also increases [54, 55], which can be directly attributed to the bacterial consumption of dye as the sole sources of carbon and energy for its growth and activity [45].

Fig. 4 The 3-D graphs and interaction for the mutual effects on the biodegradation capacity (a) dye and salt concentration (AB); (b) dye concentration and temperature (AD); (c) color concentration and pH (AC), (d) NaCl concentration and temperature (BD)

pH

With an increase in pH from 5.5 to 9.5 (Fig. 3c), slightly change in the bacterial activity and biodegradation capacity was observed, the maximum colour removal was found in acidic condition. Mohareri et al. (2012) showed that *Halomonas* strain D₂ have a good performance in biodegradation of Toluidine Red in a wide range of pH from 4.5 to 10.5 and it was found that this bacterium was able to biodegrade this dye in the mentioned range of pH and the highest bacterial activity was observed at pH 6.5 after 96 h [45]. Similarly, Hassan-Kiadehi et al. (2017) had studied the effect of pH on biodegradation of azo dyes for both halophilic *Halogeometricum borinquense* and *Haloferax mediterranei* bacteria in the range of 5–9 and concluded that an optimal pH was 7 [56]. Jain et al. (2012) from a study on the biodegradation of Reagent Violet 5R using a bacterial consortium (SB4) isolated from contaminated soil, have concluded that

the highest removal was observed at a pH of 7, and the biodegradation would decrease by an increase in the amount of basicity [31].

Salt concentration

Although the bacterium used in this study have the ability to biodegrade the colour in high salt concentrations (2–10%), with an increase in salt concentration from 4 to 10%, the colour biodegradation has been decreased (Fig. 3d). This biodegradation decreasing trend by increasing the amount of salt concentration can be due to the lack of cellular activity or its plasmolysis [45]. Mohareri et al. (2012) have shown that by increasing the salt concentration from 2.5% to 5%, the biodegradation of the Toluidine Red has been increased by *Halomonas* strain D₂ bacterium and then declined [45]. Asad et al. (2007) expressed that with an increment of salt concentration from 2% to about 8%, the biodegradation of

Fig. 4 (continued)

Halomonas sp. Gb was increased and then declined to a concentration of 20% [32]. Taran and Foreidin (2013) have focused on the biodegradation of Remazol Black-B in a wide range of salt concentrations up to 20% by *Halomonas* sp. PTCC 1714, which had been isolated from Lake Urmia in Iran, so that the highest dye biodegradation nearly 54% occurred at 10% concentration of salt [57].

The effect of mutual factors on biodegradation capacity

In order to investigate the interaction effects of dye concentration, salt concentration, pH and temperature (AB, AC, AD, BC, and CD), three-dimensional graphs have been plotted to reveal the effects of significant variables and their interaction on the biodegradation capacity as the response variable in this research (Fig. 4). Evaluation of these figures clearly demonstrated the interaction of variables and their role in the removal of colour by the bacterium qualitatively.

Figure 4 shows the interaction effect of dye concentration and salt concentration (AB), dye concentration and temperature (AD), and NaCl concentration and temperature (BD). Also, there was a similar effect for the interaction effect of AC and BC variables. As it is shown on the Figs. 4a-d, these binary variables influence each other, so effect study of the main variables is not sufficient for in-depth conclusions about the biodegradation capacity of this bacterium.

As indicated in Fig. 4a, it is concluded that the biodegradation would increase with increasing the dye concentration from 10 to 30 ppm in most salt concentrations, while in lower concentrations of dye, the biodegradation was higher with increasing salt concentration. The results showed that with a simultaneous increase in the colour and salt concentration, the color removal percent has been decreased, which was probably due to the inhibitory effect. Figure 4b showed the interaction effect of the two variables of dye concentration and temperature (AD). According to the Fig. 4b, these two variables

affected each other, and the increment of temperature had a significant role on bacterial activity and biodegradation, and the highest amount of biodegradation has occurred in higher dye concentration and temperature.

Regarding the interaction effect of other variables, it can be concluded that the biodegradation percent has been increased by increasing the dye concentration (A) from 10 to 30 ppm at pH (C) 5.5, but by increasing the pH value above 7, the pH has not been effective in biodegradation (Fig. 4c). Concerning the interaction effect of two variables of salt concentration (B) and temperature (D) (Fig. 4d), it can be mentioned that the biodegradation has been increased with increasing temperature in all salt concentrations which is more sensible in lower concentrations of salt. The reason for this can be attributed to the activity of bacteria, as the temperature increases the activity of the bacteria has been increased, thereby increasing the biodegradation.

Also, according to the results obtained from the analysis of variance on the fourth day with the software, the interaction effects of pH (C) and temperature (D) can be ignored in the removal process (Figure not shown).

Conclusion

In this research, the statistical modeling and optimization of the biodegradation of Toluidine Red, which is an oil-soluble dye, by *Halomonas* strain Gb using a RSM was carried out for the first time. Biodegradation capacity was tested by carrying out 30 tests using statistical analysis. For this purpose, the effect of 4 variables included dye concentration (10–30 ppm), salt concentration (2–10%), pH (5.5–9.5), and temperature (20–40) at different times of 2nd, 4th, and 10th days have been studied. Then a mathematical function for the biodegradation containing the four selected variables, based on statistical modeling was proposed. According to the obtained results and analysis of variance, all main variables and most of their interaction have been influenced by bacteria on biodegradation. Given to the results, the highest amount of biodegradation from different days at the 4th day calculated as 81%, and the optimum conditions for the highest biodegradation of this day has been determined at pH of 6.5, temperature of 35 °C, and salt and dye concentrations of 4% and 25 ppm, respectively. So, the proposed model can provide a good prediction of the effects of different conditions on the biodegradation of Toluidine Red, and the optimization results in this study have been well. Therefore, the proposed model can be of fairly good integrity. Also, the proposed model for calculating the biodegradation capacity was very good matched with experimental results with an average error of 11%. Overall, the results from the optimization of color

biodegradation revealed potential of *Halomonas* strain Gb in high saline environments.

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Compliance with ethical standards

Declaration Not applicable.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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