Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2018.22842

130 (2018) 226–231
October

New approach for the biodecolorization of Remazol Black-B (RB-B) by *Streptomyces hygroscopicus* strain PTCC1132

Tayyebeh Tahamtan[a], Vida Alvani[b], Amit Bhatnagar[c], Mostafa Leili[d], Ehsan Gharehchahi[e], Mohammad Ansarizadeh[f,*]

[a]*Department of Environmental Health Engineering, Mamassani Higher Education Complex for Health, Shiraz University of Medical Sciences, Shiraz, Iran, email: tahamtan_tayebeh@yahoo.com*
[b]*School of Engineering and Information Technology, Murdoch University, Murdoch 6150, Western Australia, Australia, email: vida.alvani@gmail.com*
[c]*Department of Environmental and Biological Sciences, University of Eastern, Finland, P.O. Box 1627, FI-70211, Kuopio, Finland, emails: amit.bhatnagar@uef.fi, dr.amit10@gmail.com*
[d]*Department of Environmental Health Engineering, Research Center for Health Sciences, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran, email: mostafa.leili@gmail.com*
[e]*Kavar Scientific Center of Applied Medical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran, email: e.gharehchahi@yahoo.com*
[f]*Department of Environmental Health Engineering, Sepidan Higher Education Complex for Health, Shiraz University of Medical Sciences, Shiraz, Iran, Tel. +987142541385; Fax: +987142541387; email: mansarizadeh@yahoo.com*

Received 2 February 2018; Accepted 22 July 2018

ABSTRACT

The textile industry is a rapidly developing industrial sector that produces – wastewater laden with various pollutants including synthetic dyes and heavy metals. Remazol Black-B (RB-B) is a group of azo dyes that are extensively used in textile industries. The objective of this study was to evaluate the potential of the *Streptomyces hygroscopicus* PTCC1132 to remove RB-B and to determine optimal conditions for decolorizing. The Taguchi optimization approach was used to reduce the number of experiments and the time needed to find optimum conditions. The effect of operating parameters such as temperature, pH, initial RB-B concentrations, and salt dosage were evaluated by defined factor 4 on four-level Taguchi L16 orthogonal array. Qualitek-4 software was used for data analysis. Maximum efficiency for RB-B decolorization was obtained at 33°C; wherein the solution pH was set to 9.0; and RB-B concentration and salt concentration were 5,000 mg/L and 1%, respectively. The corresponding decolorization efficiency was obtained as 95.27% at under optimized conditions confirming that the bacteria exhibits resistance against the toxic effects of RB-B, especially at high dye concentrations. Our findings indicate of the effectiveness of the evaluated microorganisms for the removal of RB-B from dye-containing effluents.

Keywords: *Streptomyces hygroscopicus* PTCC1132; Remazol Black-B; Azo dye; Biodecolorization; Taguchi method

1. Introduction

Declining water quality has become a global challenge due to population growth, urbanization, industrialization, agricultural activities, and climate change [1,2].

Industrial wastewater accounts for enormous burden on natural water resources due to the effects of direct discharges into water pathways without proper wastewater treatment. Textile industry is a highly polluting industry that generates large quantities of dye-containing effluents (DCEFs) and the former is considered as one of the main source of water pollution, having many adverse effects on the aquatic ecosystems [3]. Some of these adverse effects included

* Corresponding author.

diminishing photosynthetic activity, depletion of dissolved oxygen, and decline in water quality due to reduction in sunlight penetration upsetting the balance of aquatic flora and fauna [1,4]. Moreover, residues of reactive dyes increase total organic carbon, biological oxygen demand, and chemical oxygen demand of receptive waters [5].

Synthetic dyes are often highly toxic, and have carcinogenic and mutagenic effects on mammals [2,5,6]. Textile dye effluents generally contain high levels of various organic and inorganic compounds [5,7]. One of the main class of organic colorants is the azo dyes that contain one or more nitrogen–nitrogen double bonds linked to aromatic rings. These chemicals have been widely applied as colorants for a variety of consumer goods including food, leather, cosmetics, paper, rubber, plastic, and especially textiles [4,8,9]. Owing to their harmful health effects, it is crucial to treat dye-containing wastewaters through an appropriate method, before its discharge into the water resources. Various physicochemical methods have been evaluated to treat DCEFs including filtration, sedimentation, membrane separation, ion exchange, oxidation, adsorption, photocatalysis, coagulation-flocculation, electrocoagulation, electrofloatation, precipitation-flocculation, electrochemical treatment [10–12]. However, these methods have some limitations such as energy consumption, high operational costs, and excessive sludge formation. Even though, adsorption processes are commonly used to treat the polluted water and even air, the main challenges using these processes are as follows: they are merely a phase-transfer technique, which does not cause any change in the structure of the contaminant, and they produce high volume of concentrated contaminants, which requires specific consideration to be managed. Meanwhile, in some cases, the management of sludge is also a critical problem [13,14].

Commonly, azo dyes contain one or more sulfonic acid groups attached to the aromatic rings. Due to their structural properties, dye can act as detergents and thereby inhibit the growth of the microorganisms [15,16]. Nevertheless, a pure strain from a single-species or mixed-population of microorganisms, including bacteria, fungi, algae, actinomycetes, and plants have exhibited great potential to decolorize and degrade the azo dyes [1,3,5,7,16].

During the past two decades, several studies have been conducted to evaluate the ability of different species of bacteria to decolorize the synthetic organic dyes [1,5,12,17–20]. Biological treatment for decolorization of DCEFs is cost-effective, eco-friendly, and accompanied by lesser sludge production in comparison with the physicochemical treatment techniques [1,5]. Therefore, biological process can be considered as a feasible treatment alternative.

The Taguchi model can predict influence and optimum levels of operating parameters via specific number of the experiments. The Taguchi's orthogonal array (OA) method has been employed to investigate the optimal design of experiments (DoE) and the effects of multiple variables as well as interactions among them. With this model, it is possible to minimize the whole testing time, thereby significantly reducing experimental costs to find optimum conditions. Therefore, it was used as an acceptable way to optimize design variables [21]. In this regard, the effects of temperature, pH, initial RB-B concentration, and salt dosage were investigated. An analysis of variance (ANOVA) was performed for the raw and signal-to-noise (S/N) ratio data in order to indicate the significant parameters affecting the process, and their effects on the response characteristics were quantified.

Thus, the main focus of this work was to develop new method for biodecolorization of Remazol Black-B (RB-B) by *Streptomyces hygroscopicus* strain PTCC1132 (Persian Type Culture Collection) from aqueous medium using the Taguchi optimization approach to reduce the number of experiments and time required to find the optimum conditions.

2. Material and method

2.1. Bacterial strain and cultivation conditions

S. hygroscopicus PTCC1132 bacteria was prepared from Persian Type Culture Collection (PTCC). FZmsn medium was used to cultivate *S. hygroscopicus* PTCC1132 bacteria. Table 1 shows the main ingredients of culture medium.

2.2. Dye and chemicals

The chemical structure of nonhydrolyzed RB-B with molecular weight of 991.82 g/mol and absorbance wavelength (λ_{max}) of 597 nm is shown in Fig. 1 [22–24]. The RB-B (Color Index (CI): 20407) used in this study was obtained from Ciba Geigy GmbH representative in Iran. The aqueous solution of RB-B dye with desired concentrations for the experiments was prepared by serial dilution of the stock solution. Doubly deionized water (18 MΩ/cm) was used to prepare the solutions throughout all experiments.

2.3. Experimental design and statistical analysis

In this investigation, Taguchi method (TM) design was applied to predict the optimal combination of design factors affecting RB-B decolorization by *S. hygroscopicus* PTCC1132. The DoE methodology has generally been adopted to optimize a predetermined response by controlling design variables. TM is a statistical approach to improve response rate and the quality of products or to determine the optimum conditions in the field of engineering and biotechnology. Therefore, Taguchi experimental design is a useful method for determining the effects of factors or variables or the combination of them to achieve the best result [25–28]. Thus, an analysis of the S/N ratio is required to compute the experimental results. The purpose of this study was to maximize the removal efficiency, where the S/N ratio with higher the

Table 1
Culture medium ingredients

Ingredients	Concentration (g/L)	Purity (%)
NaCl	5	98
Soy flour	20	95
$CaCO_3$	2	98.5
Agar	16	85
Mannitol	20	95
Nystatin	1	95

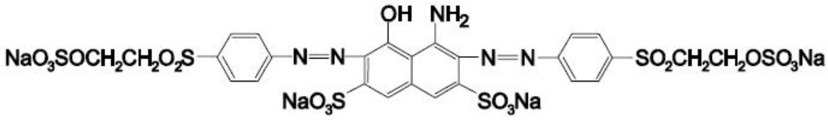

Fig. 1. Chemical structure of Remazol Black B [22].

better (HB) characteristics [29] is required, which is given by Eq. (1) [30] as follows:

$$\text{SNL} = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \quad (1)$$

where in SNL is the signal to noise ratio or the performance characteristics as "the larger the better," y_i is the comparative variable in experiment *i* for a certain combination of control factor levels (response), and *n* is the number of replicate under the same experimental conditions.

The DoE is based on the layout of an L_{16} OA in four levels, that is, L_{16} (4^4), and was designed as presented in Table 2, and the experimental conditions were obtained. The mean from duplicate measurements of the efficiency and *S/N* ratio for each test condition are given in Table 3. Data was analyzed using Qualitek-4 software (version 17.1.0, Nutek Inc., USA).

2.4. Decolorization assay

For each experiment 20 mL of the FZmsn medium was added to 100 mL Erlenmeyer flask. Then, pH, RB-B, and salt concentrations were adjusted according to different levels shown in Table 1. The entire content was sterilized by autoclaving for 15 min at 15-PSI pressure and 121°C. Then, 5% of contamination was added to each Erlenmeyer flask. The flasks were then incubated for 5 d at four different temperatures, listed in Table 1.

To estimate the ability of bacteria to remove RB-B dye, the supernatant was separated by centrifugation at 13,000 rpm for 4 min. The supernatant was measured at 595 nm by the UV-visible spectrophotometer. The efficiency of RB-B removal (*R*) was calculated using Eq. (2) [31] as follows:

$$R(\%) = \frac{(C_0 - C_t)}{C_0} \times 100 \quad (2)$$

where in C_0 and C_t are the RB-B concentrations at the feed solution and at reaction time *t*, respectively.

Table 2
Controllable factors and their levels used for design of experiments

Factors	Level			
	1	2	3	4
Temperature (°C)	28	30	33	35
pH	6	7	8	9
RB-B (mg/L)	500	1,000	5,000	10,000
Salt (%)	0.5	1	1.5	3

3. Results and discussion

In this study, the effect of temperature, pH, dye, and salt concentration on bioremediation of RB-B by *S. hygroscopicus* PTCC1132 was investigated under an aerobic condition. The L_{16} (4^4) OA was designed, and the experimental conditions were obtained by combining Table 2 and the L_{16} (4^4) OA. The OA of the Taguchi experimental design including the values obtained for different parameters are presented in Table 3. Various levels of the parameters had different effects on the efficiency and % average decolorization was found to be 72.24(±11.39)%. The OA results (Table 3) indicated that *S. hygroscopicus* PTCC1132 exhibits the highest decolorization (91.56%) in experiment No. 12 wherein the experimental conditions were: temperature: 33°C, pH: 9.0, RB-B and salt concentration: 5,000 mg/L and 1%, respectively. Minimum decolorization (50.28%) was obtained for experiment 15, wherein the experimental conditions were set as: temperature: 35°C, pH: 8.0, RB-B and salt concentration: 10,000 mg/L and 3%, respectively.

In Table 4, the effect of factors and interaction at different levels on RB-B bioremoval are presented. The magnitude of the difference between the average effects of different factors shows the relative influence of the factor or interaction to the variability of results. It is clear from Table 4 that, the

Table 3
The orthogonal array of Taguchi experimental design and corresponding RB-B decolorization

Experiment number	Temperature	pH	Salt	RB-B	Decolorization (%)
1	1	1	1	1	61.62
2	1	2	2	2	70.40
3	1	3	3	3	82.40
4	1	4	4	4	69.38
5	2	1	3	2	66.26
6	2	2	4	1	54.63
7	2	3	1	4	78.20
8	2	4	2	3	74.46
9	3	1	4	3	79.03
10	3	2	3	4	76.03
11	3	3	2	1	83.63
12	**3**	**4**	**1**	**2**	**91.56**
13	4	1	2	4	81.74
14	4	2	1	3	77.05
15	4	3	4	2	50.28
16	4	4	3	1	59.12
Average					72.24
Standard deviation					11.39

The bolded run exhibits the highest decolorization efficiency.

Table 4
Main effects (average effect of factors and interaction) of the factors on RB-B decolorization

Factor	Level 1	Level 2	Level 3	Level 4	L2-L1	L3-L1	L4-L1
Temperature	70.95	68.89	82.57	67.04	–2.56	11.61	–3.90
pH	72.17	69.53	72.63	74.13	–2.63	1.46	1.46
RB-B (mg/L)	64.75	69.63	78.74	76.34	4.87	13.48	11.58
Salt (%)	77.11	78.06	70.96	63.33	0.45	–6.15	–13.77

RB-B concentration in level 3 has the highest effect on decolorization, where, the maximum removal efficiency (78.74%) was obtained at initial RB-B concentration of 5,000 mg/L. Furthermore, the obtained data confirms that increasing the RB-B concentration (from level 1 and 2 to level 3 and 4) leads to an increase in dye removal.

Many studies [16,32–34] in which the ability of bacteria to decolorize DCEFs in anaerobic conditions has been investigated to infer that most of the bacterial strains have the ability to decolorize at dye concentrations below 100 mg/L which are significantly lower than the concentrations applied in this study. To further evaluate the microbial survival ability, the bacteria were exposed to high dye concentrations (5,000 and 10,000 mg/L) and it was observed that even at such high concentrations, these microbial cells acclimate themselves to the toxic effects of dye which could be of immense value.

The increase of temperature from 28°C up to 33°C leads to an increase in decolorization from 70.95% to 82.57% (Table 3). However, further increases in temperature have negative effects on decolorization and led to a decrease of decolorization efficiency. Thus, these organisms have a dominant effect on the temperature of 33°C.

The solution pH also affects microbial growth and decolorization of DCEFs. Prior studies have shown that microorganisms, including *Micrococcus* sp., *Micrococcus luteus* and *Paenibacillus polymyxa* have the ability to decolorize RB-B over a wide pH range: 6.5–8.0 [32,35,36]. The results from this study indicate that decolorization of RB-B occurs in the pH range of 6.0–9.0 with the maximum remediation capacity occurring at pH 9.0 (Table 3).

In Table 5, all possible interactions between two factors are calculated. As evident, the severity index (SI) for RB-B concentration and salt concentration is equal to 52.28, which demonstrate the highest interaction effects on dye remediation, while the temperature and RB-B concentration have the lowest interaction effects.

ANOVA for all factors is presented in Table 6. As shown, temperature, salt, and dye concentration have the highest contribution on RB-B decolorization by *S. hygroscopicus* PTCC1132, whereas pH has minimum influence on the decolorization efficiency.

The results of the *S*/*N* ratio for various designed experiments after ANOVA calculations are shown in Table 7. The third level of temperature (33°C), the fourth level of pH (9.0), the third level of RB-B concentration (5,000 mg/L), and the second level of salt concentration (1%) were considered as optimum conditions for RB-B deodorization by *S. hygroscopicus* PTCC1132, in which the expected removal efficiency was 95.27%.

3.1. Application of the optimized procedure

As suggested by Taguchi [25,26], a crucial step is the confirmation test which is conducted to verify the experimental results. As can be verified from Table 7, the optimum combinations of factor levels were not previously tested, thus, a series of checking experiments were carried out using the initial solution and the different noise levels, as well as solutions from student laboratories in order to check their reliability. The decolorization efficiency, in terms of the observed efficiency, was found to be 92.15%, as an average value, which validates the proposed conditions.

3.1.1. Analysis of variance

The contribution of individual factors is the deciding control key to be enforced to attain high wastewater treatment efficiency. In Taguchi approach, the main purpose of the ANOVA was to assess the effect of each parameter on

Table 5
Interaction between two factors for RB-B bioremoval

Column	Interaction factor pairs	Columns	SI%[a]	Col[b]	Opt.
1	RB-B conc. × salt conc.	3 × 4	52.28	7	[2,1]
2	pH × salt conc.	2 × 4	33.42	6	[4,1]
3	Temperature × pH	1 × 2	24.72	3	[3,4]
4	Temperature × salt conc.	1 × 4	15.16	5	[3,1]
5	pH × RB-B conc.	2 × 3	13.48	1	[4,2]
6	Temperature × RB-B conc.	1 × 3	3.45	2	[3,2]

[a]SI-Interaction severity index (100% for 90° angle between the lines, 0% for parallel lines).
[b]Show columns that should be reserved if this interaction effect were studied.

Table 6
Analysis of variance (ANOVA) for all factors

Col.	Factor	DOF (*f*)	Sum of squares (*S*)	Variance (*V*)	*F*-Ratio (*F*)	Pure sum (*Ś*)	Percent (*P*)
1	Temperature	3	600.09	200.03	1.964	294.66	15.15
2	pH	3	44.88	14.96	0.146	0	0
3	RB-B	3	462.66	154.22	1.514	157.24	8.03
4	Salt	3	532.02	177.35	1.714	226.62	11.65
Other/error		3	305.43	101.81			65.12
Total:		15	1,945.12				100.0

Table 7
The optimum condition for RB-B decolorization

Parameter	Level description	Level	Contribution
Temperature (°C)	33	3	10.33
pH	9	4	1.39
RB-B (mg/L)	5,000	3	5.99
Salt (%)	1	2	5.32
Total contribution from all factors		23.07	
Current grand average of performance		72.24	
Expected result for optimum conditions		95.27	
Observed result for optimum conditions		92.15	

the variance of the OA experiments results to determine the variation in the contribution of each factor with respect to total variance of all the parameters. From the calculated ratios (*F*) (Table 6), it can be concluded that all factors and interactions considered in the experimental design are statistically significant at a 95% CI, indicating that the variability of the experimental data can be explained in terms of significant effects. The general trend of influencing factors can be characterized by studying the main effects of each factor. Table 6 depicts the results of the ANOVA analysis, with the percentage contribution of each factor, which was calculated by the ratio of the sum of squares of that factor to the total sum of squares, and their interactions.

4. Conclusions

Due to the presence of the aromatic ring in the chemical composition of many dyestuffs such as RB-B, these compounds are resistant to biological degradation or uptake, hence, the purpose of this study was to examine the capability of *S. hygroscopicus* PTCC1132 to remove RB-B from the industrial effluent. An L_{16} OA was applied to investigate the effect of main operational parameters including initial RB-B concentration, pH, and salt concentration on decolorization efficiency. According to the Taguchi model, through few numbers of well-defined experimental sets, as well as the results obtained from the evaluation of each parameter effect using ANOVA, temperature and solution pH were shown to have, respectively, the most and least contribution on RB-B removal efficiency. The removal efficiency of about 92.15% was observed under the optimal conditions which reveal that *S. hygroscopicus* PTCC1132 can tolerate the harsh environmental conditions such as toxicity, salinity, relatively high azo dye concentration, and high solution pH. In conclusion, our findings show that *S. hygroscopicus* PTCC1132 has the ability to remove RB-B from the DCEFs. However, the mechanism of dye removal through such bacteria could be evaluated in future research works.

Acknowledgment

The authors are grateful for the support given by the Vice-Chancellor for Research and Technology of Shiraz University of Medical Sciences and Razi University.

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