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Biodegradation of two Azo dyes using *Dietzia* sp. PD1: process optimization using Response Surface Methodology and Artificial Neural Network

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ABSTRACT

A two level three factor (2^3) central composite design (CCD) was applied for optimization of the selected biodegradation process variables, i.e. initial solution pH (4–8), initial dye concentration (150–400 mg L^{-1}), and time consumed for degradation (14–48 h) for studying the biodegradation of Congo red (CR) and Indigo carmine (IC) using the isolated novel bacterial strain, *Dietzia* sp. PD1. This is the first report of dye degradation for the genus *Dietzia* obtained so far. The optimum dye degradation efficiency of *Dietzia* sp. PD1, obtained from RSM modeling for CR, was found to be 99.97% (at 177.63 mg L^{-1} of initial dye concentration at pH 5.56 for 42.64 h) and for IC it was found to be 99.95% (at pH 5.85, concentration of dye 135.62 mg L^{-1} and time 43.29 h) at temperature 303 K and stirring speed 150 rpm. A strong correlation existed between the input process variables and the output parameter for degradation of dye using Artificial Neural Network (ANN). The model developed using Response Surface Methodology (RSM) and ANN showed a close interaction (% deviation ~ 0.6%) with the experimental values for both the dye degradation. The ability of the isolated *Dietzia* sp. PD1 for degrading two dyes of different chemical structure and properties endorses the possibility of applying it for the treatment of real industrial effluent bearing a mixture of different dyes.

Keywords: Biodegradation; *Dietzia* sp. PD1; Congo red; Indigo carmine; Response Surface Methodology; Artificial Neural Network

1. Introduction

Rapid urbanization and industrialization have resulted in water pollution to a great extent through discharge of toxic dyes into surrounding water bodies. These dyes are considered as a major threat to neighboring water bodies, environment, and human health due to their mutagenic and carcinogenic properties. Azo dyes are one such group of dyes of immense concern. The presence of strong azo bonds in their chemical structures mostly renders these compounds as biorecalcitrant. The major sources of these dyes are industries that manufacture and process food stuff, textiles, pesticides, printing inks, rubber, plastic, etc. [1–5]. For example, Congo red, (CR; MF: $C_{32}H_{22}N_6Na_2O_6S_2$) an anionic benzidine based secondary diazo compound has been reported to act as skin

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and eye irritant, affect blood conditions, and cause respiratory problems. It also believed to possess mutagenic and carcinogenic properties [5]. Indigo carmine (IC) is another example of azo dye, (IC; MF: $C_{16}H_8N_2Na_2O_8S_2$) which shows similar toxic effects as CR. Besides its carcinogenic property, it can also give rise to hypertension, cardiovascular, and gastrointestinal disorders [6].

Due to the complex nature of these dyes, conventional methods like oxidation, coagulation, flocculation, hydrogen peroxide catalysis, membrane separation, and reverse osmosis are often found to be inefficient and insufficient for removal of these compounds from solution. This problem may only be effectively addressed by subjecting such compounds to biodegradation, as this process facilitates the conversion of innumerable toxic compounds to relatively harmless derivatives in both efficient and cost effective approach.

Existing studies show that various types of microorganisms (MOs) like actinomycetes, bacteria, fungi, etc. possess the potential of detoxification of azo dye compounds. Aerobic bacteria have also been reported to oxidize dyestuffs and thereby decolorize them. However, multifarious dye mixtures and other assisting agents often present in the color bearing industrial effluents often limit the possibility of utilizing biological treatment [7].

In most of the previous studies on dye removal using different MOs, the efficiency of different approaches have been investigated keeping individual and other factors constant [8–11]. To achieve maximum efficiency of dye removal, a number of experiments are required to be performed in order to determine the optimum experimental conditions. This will make the process tedious, time consuming, and complicated for determination of the combined effect of different vital parameters of the process in concern. To overcome these challenges and obtain appropriate optimization of the process being implemented, different statistical analogies are being developed. Response Surface Methodology (RSM) is one such useful experimental design which can be implemented to comprehend the combined effect of different parameters by a limited number of experiments only [4,12–19].

This technique can also aid in determining the combined effect of different parameters at optimized conditions inspite of complex interactions operating between them. RSM can be used for achieving a target of higher percentage (%) output by reducing the variability of the process. In addition to RSM, Artificial Neural Network (ANN) analysis has also been applied to this study, in order to predict the biodegradation of dye molecules using isolated MO. ANN is based on the structure of a biological neural system for solving problems. It is also utilizable for analysis of complex, noisy, and partial information systems [4,20,21]. In the present study, the process of biodegradation of both CR and IC by a bacterial strain isolated from real textile effluent has been optimized using RSM and modeled by ANN system and the results obtained in each case has been compared.

2. Material and methods

2.1. Dyes and chemicals

Congo red (CR; MF: $C_{32}H_{22}N_6Na_2O_6S_2$) and Indigo carmine (IC; MF: $C_{16}H_8N_2Na_2O_8S_2$) used in this study was of commercial grade and used as such without further purification. Other chemicals used were of analytical grade and obtained from Merck, India. Chemical structures of CR and IC have been discussed in a previous study [5,22,23]. The physico-chemical characteristics of both CR and IC are enlisted in Table 1.

2.2. Isolation and identification of bacterial strain

A bacterial strain capable of dye degradation was isolated from textile effluent procured from a textile industry near Kolkata, India. Serial dilution of the collected effluent was performed with 1% peptone water. Spread plates were prepared with the diluted sample and incubated overnight at 37 ± 2°C. Individual colonies obtained after overnight incubation were streaked onto basal salt agar (BSA) plates (separately containing CR and IC as carbon sources) and further incubated at 37 ± 1°C for three days. From the isolated bacterial strains, the one strain showing highest dye decolorization zone on the BSA plates was selected for further characterization, identification, and biodegradation studies. The chosen bacterial strain was designated as PD1 and maintained on nutrient agar slants. The sequence data recorded were aligned and analyzed for determining the closest homologous organism. Standard procedures [24] were used to record the morphological, physiological, and biochemical characteristics of the isolated strain.

2.3. Decolorization experiments

Cultures of the isolated strain incubated overnight were used for the dye degradation experiments [25]. 1 mL of culture was added to the basal salt medium broth containing the dye in different concentrations (mg L^{-1}) for various experiments. 10 mL aliquots of culture medium were harvested after specified time

Table 1
Physico-chemical properties of dyes selected for the study

Properties	Congo red	Indigo carmine
Formula	$C_{32}H_{22}N_6Na_2O_6S_2$	$C_{16}H_8N_2Na_2O_8S_2$
Molecular weight	696.66 g mol^{-1}	466.34 g mol^{-1}
Color	Red brownish-red (Dark)	Dark blue
Odor	Odorless	Odorless
Form	Solid	Solid
pH	6.7 at 10 g L^{-1} at 20°C (68°F)	5.6 at 10 g L^{-1} at 20°C (68°F)
Water solubility	25 g L^{-1}	1 g 100 ml^{-1}

intervals and centrifuged at 8,000 rpm for 15 min. The supernatant was collected in each case and its absorbance was recorded using a UV/VIS spectrophotometer (U-2800, Hitachi, Japan) at respective wavelengths (max λ) of the dyes used. The % decolorization of dye was calculated using the following Eq. (1):

$$\%\ \text{Decolorisation} = \frac{\text{Initial dye concentration} - \text{Final dye concentration}}{\text{Initial dye concentration}} \times 100 \quad (1)$$

2.4. Optimization of adsorption process by RSM

The optimization of CR and IC degradation was based upon three independent process variables including pH of the solution, initial dye concentration, and time of adsorption. The ranges and levels of different variables investigated in this study are given in Table 2 along with their units and notations. The % decolorization of CR and IC was considered as the output response of the system.

2.5. Experimental design

Biodegradation efficiency of a bacterial strain may be significantly affected by solution pH, initial concentration of dye, and duration of exposure. Standard RSM design central composite design (CCD) was applied for identification of the relationship between the response function (% decolorisation) and the process variables (pH, initial concentration, and contact time). A total of 20 experiments were performed in triplicate according to the CCD.

Matrix, Design Expert Version 7.1.6 (Stat-Ease, USA) and the mean of the results obtained experimentally were used for data analysis. The interaction among the different independent variables and their combined effect on the response was studied using the response surface contour plots.

2.6. ANN model

In recent years, ANN has received great importance for the purpose of process modeling. ANN is implemented to predict the behavior of a given process by analyzing the existing process. The architecture of an ANN model consists of innumerable hidden layers sandwiched between an input layer (independent variables) and an output layer (dependent variables). Interconnections of the layers are known as neurons which interact by transmitting signals to each other similar to biological systems.

In the present study, the inputs provided to the ANN model were identical to the factors considered in the RSM approach, i.e. pH of the solution, initial concentration of dye, and contact time. Similar to RSM modeling, the % decolorization of dye was considered as output (response) of the ANN modeling. The input–output data patterns required for modeling were obtained from batch experiments planned through CCD. A tan-sigmoid transfer function (tansig)

Table 2
Experimental range and levels of independent process variables for the dyes

			Range and levels (coded)				
Variables	Notation	Unit	$-\alpha$	−1	0	+1	$+\alpha$
Initial pH	A		2.63	4	6	8	9.36
Initial dye concentration	B	mg L^{-1}	64.77	150	40	400	485.22
Time	C	h	2.40	14	1,150	48	59.59

at hidden layer and a linear transfer function (purelin) at output layer were implemented. The Levenberg–Marquardt back-propagation algorithm was applied for network training. All the ANN calculations were executed using Neural Network Toolbox of MATLAB Version 7.9 (R2009b).

3. Results and discussion

3.1. Identification of the bacterial isolate

Phylogenetic analysis of the 16S rRNA gene sequence of strain PD1 suggested it to be an actinobacteria of genus *Dietzia*, which possessed 99% similarity with *Dietzia maris*. The isolated strain was therefore designated as *Dietzia* sp. PD1. The 16S rRNA gene sequence of *Dietzia* sp. PD1 isolate was deposited in the GenBank nucleotide sequence database under accession number JQ414030 [5]. The morphological, physiological, and biochemical characteristics of the isolate were reported in a previous study [5]. This is the first report where this micro-organism was used as a dye degrading agent.

3.2. Response surface optimization for maximum removal of CR & IC

3.2.1. RSM for CR

An empirical relationship between the response and the independent variables has been obtained from the following expressions:

$$\%\ \text{Removal} = -111.6 + 38.47A + 0.11B + 4.414C - 0.0118AB - 0.015AC - 0.0003BC - 3.21A^2 - 0.0001B^2 - 0.05C^2 \quad (2)$$

$$\frac{\partial R_1}{\partial A} = 38.47 - 0.0118B - 0.015C - 6.42A$$
$$\frac{\partial R_1}{\partial B} = 0.114 - 0.0118A - 0.0003C - 0.0002B \quad (3)$$
$$\frac{\partial R_1}{\partial C} = 4.414 - 0.015A - 0.0003B - 0.1C$$

$$\frac{\partial^2 R_1}{\partial A^2} = -6.42;\ \frac{\partial^2 R_1}{\partial B^2} = -0.0002;\ \frac{\partial^2 R_1}{\partial C^2} = -0.1 \quad (4)$$

where A represents the initial solution pH of the solution, B denotes the initial dye concentration (mg L^{-1}), C indicates the time taken for degradation (h), and R_1 is the % dye decolorization.

Eq. (3) was equated with zero for solving the partial differential equations. From the afore stated equations, A, B, and C were calculated to be 5.56, 177.63 mg L^{-1}, and 42.64 h, respectively, and % decolorization obtained under these conditions was 99.975%.

The statistical significance of the model implemented was evaluated using the F-test and p-values obtained from ANOVA analysis. It was noted that F value for this model was 412.14 and p-value was <0.0001. High F-value and low p-value obtained in this study demonstrated the significance of the regression model. Correlation coefficient value was determined to be 0.9973, which in turn signified that this regression was statistically significant and only 0.27% of the total variation remained unexplained using this model. The value of the adjusted multiple correlation was found to be 0.9949 and Predicted multiple correlation was estimated to be 0.9904. The non-significant value of lack of fit (more than 0.05) showed that the quadratic model remained valid for the study.

$$R_1 = -105.915 + 34.439A + 0.512B + 4.385C - 0.015AB - 0.0143AC - 0.0003AC - 2.715A^2 - 0.00018B^2 - 0.0492C^2 \quad (5)$$

$$\frac{\partial R_2}{\partial A} = 34.437 - 0.015B - 0.0143C - 5.428A$$
$$\frac{\partial R_2}{\partial B} = 0.151 - 0.015A - 0.0003C - 0.00037B \quad (6)$$
$$\frac{\partial R_2}{\partial C} = 4.385 - 0.0143A - 0.0003B - 0.0984C$$

$$\frac{\partial^2 R_1}{\partial A^2} = -5.428;\ \frac{\partial^2 R_1}{\partial B^2} = -0.00037;\ \frac{\partial^2 R_1}{\partial C^2} = -0.0984 \quad (7)$$

For this study, Eq. (6) was equated with zero for solving the partial differential equations. A, B, and C were evaluated to be 5.86, 135.6 mg L^{-1}, and 43.298 h, respectively, and % decolorization recorded under these conditions was 99.955%.

For IC, F value was evaluated to be 291.53 and p value was <0.0001. Correlation coefficient value was found to be 0.9962, which signified that this regression was statistically significant and only 0.38% of the total variation remained unexplained using this model. The value of adjusted multiple correlation in this case was 0.9928 and Predicted multiple correlation was 0.9702. The non-significant value of lack of fit (more than

0.05) showed that quadratic model remained valid for this study as well.

3.3. Effect of experimental parameters on dye degradation

The combined effect of initial dye concentration, initial solution pH, and time taken for dye removal of CR and IC was shown in the three-dimensional (3 D) surface plots of Figs. 1 and 2, respectively.

3.3.1. Effect of initial dye concentration with respect to degradation time

In both the cases [Figs. 1(a) and 2(a)], it was observed that an increase in the dye concentration resulted in a corresponding decrease in the rate of decolorization. This could possibly be due to the toxic effect of dyes on bacteria with or without insufficient biomass concentration (or inappropriate cell to dye ratio) in that dye solution [10].

3.3.2. Effect of initial solution pH with respect to degradation time

Figs. 1(b) and 2(b) represented the 3 D surface plot with respect to initial pH of the solution and the time of degradation by the bacterium. It was observed from the experimental data that pH of the medium had a greater influence on the removal of the dye with time. Solving the RSM model equation, it was observed that

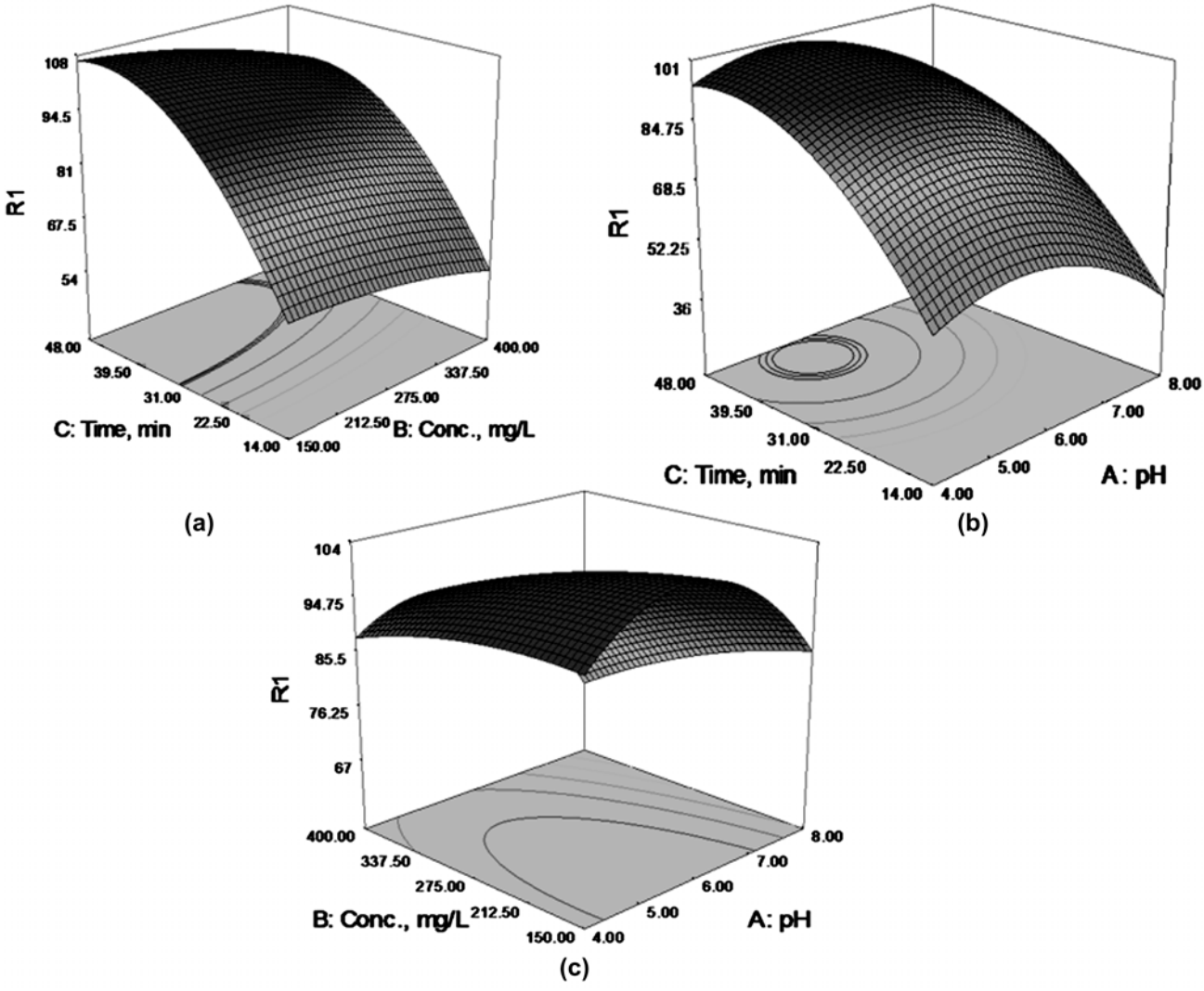

Fig. 1. 3 D surface plots showing interaction of different experimental parameters during removal of CR by *Dietzia* sp. PD1 [a: initial dye concentration vs. time; b: initial solution pH vs. time; and c: initial dye concentration vs. initial solution pH].

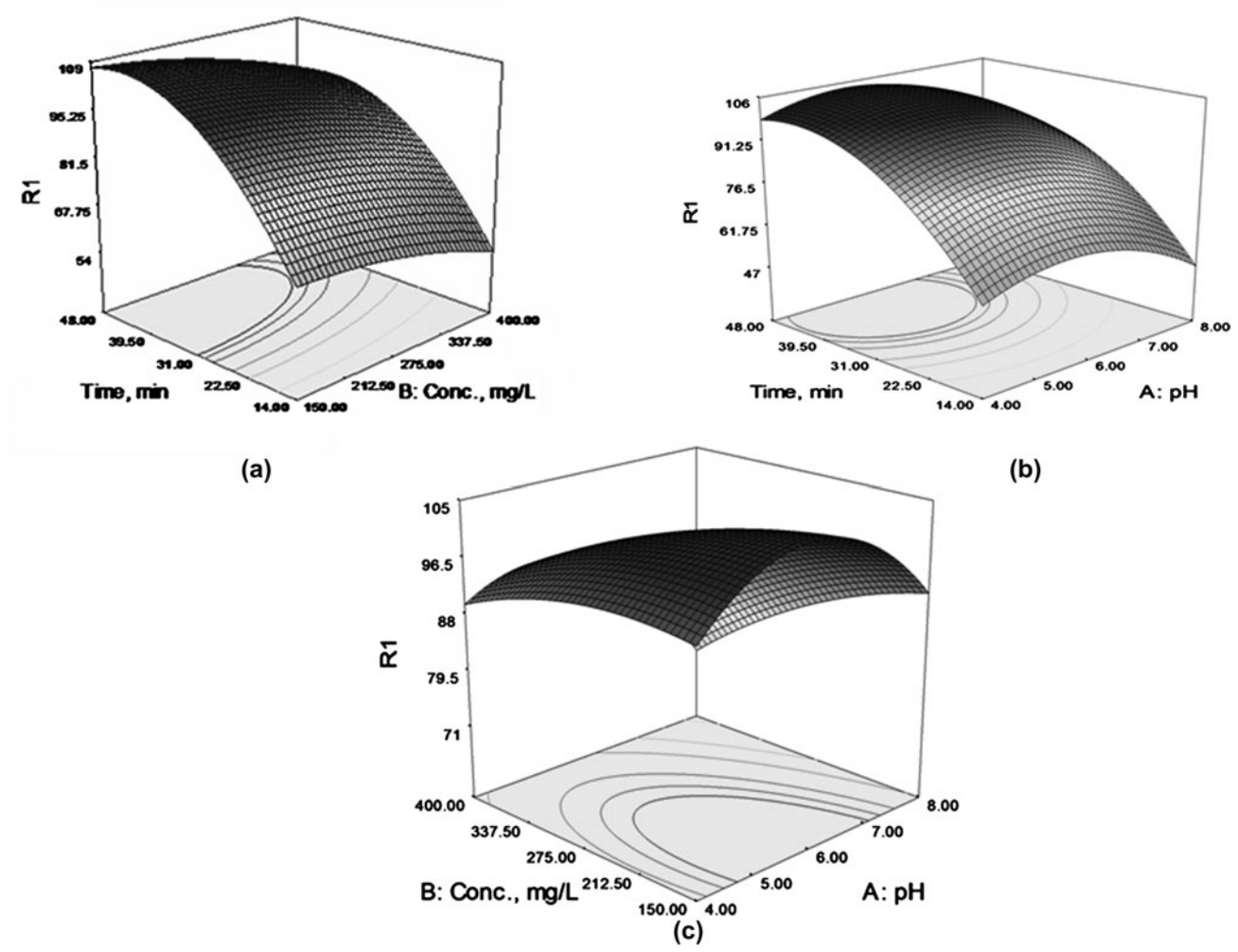

Fig. 2. 3 D surface plots showing interaction of different experimental parameters during removal of IC by *Dietzia* sp. PD1 [a: initial dye concentration vs. time; b: initial solution pH vs. time; and c: initial dye concentration vs. initial solution pH].

at pH 5.7, initial concentration of dye 177.63 mg L^{-1}, and degradation time of 42.64 h, the decolorization of CR dye was 99.97% and for IC, it was 99.95% at pH 5.86, in initial concentration of dye 135.6 mg L^{-1} and contact time of 43.298 h. These results indicated that pH of the medium was also an important factor regulating bacterial activity. The % dye decolorization was highest at the optimum pH, and was drastically reduced at strongly acidic or alkaline pH. pH of the surrounding medium influenced the transport of dye molecules across the cell membrane, thereby regulating the rate-limiting step for decolorization [7].

3.3.3. Effect of initial solution pH with respect to initial dye concentration

The effect of initial pH of solution and initial dye concentration of CR and IC using isolated microorganism was predicted from the 3 D surface plots [Figs. 1(c) and 2(c)]. It was observed that both the independent variables had a strong influence on the dye degradation study. A maximal removal efficiency of 99.975% was achieved at initial pH of 5.56 and initial dye concentration of 177.63 mg L^{-1} while the other variable was set at the middle value for CR and 99.95% for IC at pH 5.86. An increase in the Initial dye concentration decreased the % dye decolorization. This may be attributed to the fact that as initial concentration of dye increased, higher quantity of MO was required for dye degradation.

3.4. Modeling using ANN

In the present study, a three layer ANN-based model was developed to investigate the degradation of CR and CI using the isolated species. The RSM

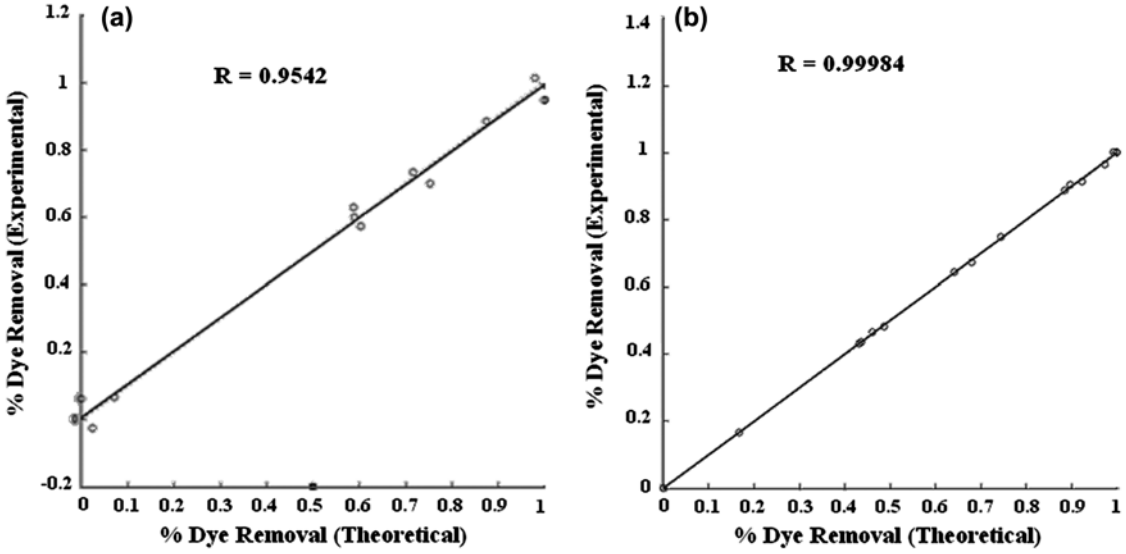

Fig. 3. Graph showing theoretical percent removal vs. experimental percent removal of dye from solution obtained using ANN [a: CR; b: IC].

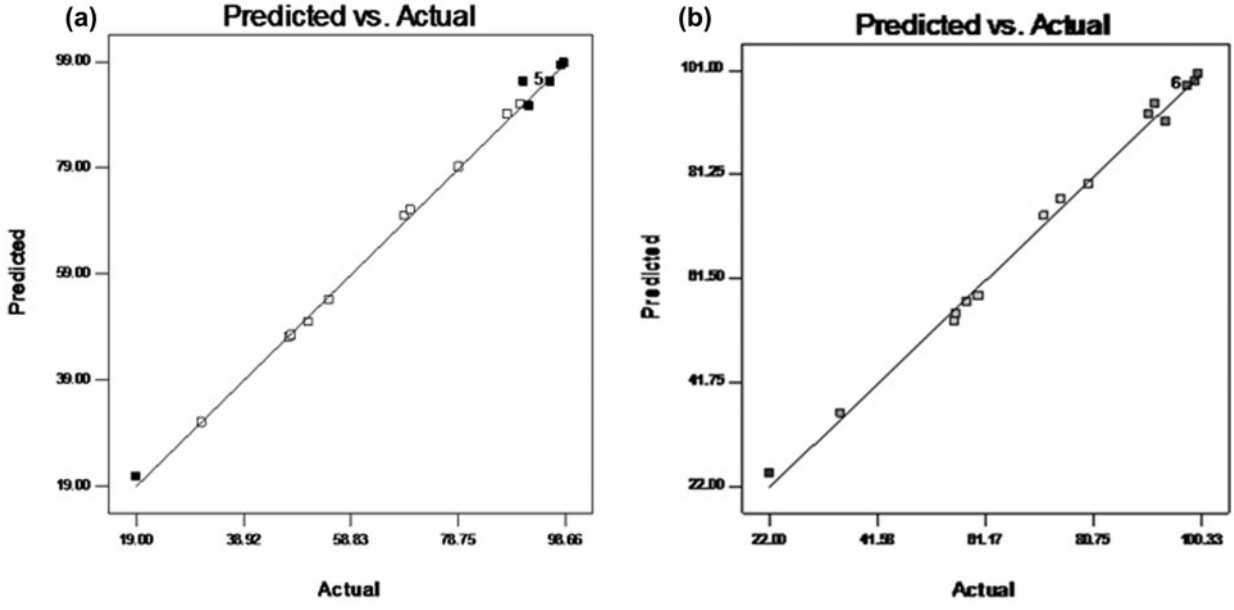

Fig. 4. Graph showing theoretical percent removal vs. experimental percent removal of dye from solution obtained using RSM [a: CR; b: IC].

design data was used for this purpose and an optimal architecture was designed. The original data was divided into three subsets including training (12 data points), validation (4 data points), and test sets (4 data points). The optimal architecture for the present study was trained using back propagation method based on Levenberg–Marquardt algorithm.

From the experimental and theoretical data ANN for CR and IC, it was observed that experimental and the predicted response given by the ANN model was significant and a correlation coefficient of 0.99 suggested the reliability of the developed ANN model for both the dye [Fig. 3].

3.5. Confirmation experiments of RSM and ANN

To validate the optimized data obtained by solving the numerical modeling under optimized conditions

for CR, confirmatory experiments were conducted with the parameters as suggested by the RSM model (pH 5.56, Concentration of dye 177.63 mg L^{-1}, time 42.64 h at temperature 303 K, and agitation speed 150 rpm) whereby the % decolorization was found to be 99.45% and the deviation from theoretical RSM analysis was 0.53%. From Fig. 4(a) also it was observed that predicted % decolorization using RSM was almost same as experimental % decolorization. Hence, the RSM model was considered significant for this study.

To validate the optimized data given by solving the numerical modeling under optimized conditions for IC, confirmatory experiments were also conducted with the parameters as suggested by the RSM model (pH 5.85, Concentration of dye 135.62 mg L^{-1}, and time 43.29 h at temperature 303 K, stirring speed 150 rpm) whence the percent removal was found to be 99.6% and the deviation from theoretical RSM analysis was 0.35%. From Fig. 4(b), it was observed that predicted % decolorization using RSM was similar to experimental % decolorization and so the RSM model was held significant for this study.

On the other hand, ANN analysis performed using the similar optimized conditions yielded % decolorization nearing 100% and so the deviation of experimental ANN from theoretical values was found to be 0.6% for both dyes studied. The fluctuation of the experimental data recorded with RSM model was smaller than that obtained using ANN model. As a reason, it may be suggested that RSM had better advantage of providing regression equation for prediction of experimental results than the ANN model, as it required lesser number of experiments in comparison to ANN analysis for building an efficient model. Our results were also supported by other similar findings reported previously [4,15].

4. Conclusion

To date, it is the first *Dietzia* strain reported so far for biodegradation of dyes from aqueous medium. The isolated specie *Dietzia* sp. PD1 is efficiently capable of degrading two dyes, namely CR and IC, possessing different chemical structure and properties and can thereby be utilized for treatment of real time effluents containing a mixture of different types of dyes. The present study was conducted to optimize CR and IC degradation using isolated MO, *Dietzia* sp. PD1. Combined effect of various process parameters on degradation of CR and IC was studied using RSM. It was observed that the initial solution pH, adsorbent concentration, and degradation time were vital parameters that significantly influenced the dye degradation efficiency of the MO. Optimization conditions for the maximum degradation efficiency of CR and IC were obtained by solving the RSM model expression. The level of the three variables, initial solution pH, 5.56; initial dye concentration, 177.63 mg L^{-1}; degradation time, 42.64 h were found optimum for maximum CR removal and the corresponding decolorization efficiency was found to be 99.975%. For IC, the optimized pH was 5.86, initial dye concentration of 135.6 mg L^{-1}, and 43.298 h and decolorization efficiency was found as 99.955%. Both RSM and ANN predicted degradation was in strong agreement with the experimental results, but the accuracy of prediction of RSM model was better than that of ANN model for both the dyes considered. Based on these findings, it may be concluded that there exists a non-linear quadratic relationship between the biodegradation efficiency of the isolated organism and the experimental factors selected for this study.

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