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Original Research

Response Surface Methodology and Artificial Neural Network for Modeling and Optimization of Distillery Spent Wash Treatment Using *Phormidium valderianum* BDU 140441

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Abstract

The aim of this work was to evaluate the capability of *Phormidium valderianum* BDU 140441 on biodegradation and decolorization of distillery spent wash. The effect of initial pH (6-10), temperature (24-32ºC), and light intensity (20-54 W/m^2) was studied using single factorial design and achieved a maximum decolorization of 74.5% with COD reduction of 83.48%. A 2^3 full factorial experimental central composite design (CCD) of response surface methodology (RSM) was used to investigate the interaction effect between these variables and the optimal values. The predicted results showed that a maximum decolorization of 85.5% and COD reduction of 87.29% was achieved under the optimum conditions of 8 pH, 30ºC, and light intensity of 36 W/m^2. It was observed that model predictions were in good agreement with experimental values (R^2 = 0.9830, Adj-R^2 = 0.9677), which indicated the suitability of the model and the success of the optimization tool. A non-linear artificial neural network (ANN) model was developed to predict the biological decolorization of the spent wash. The results indicated that ANN revealed reasonable performance (R^2=0.9999, y=0.9781x-0.5679).

Keywords: optimization, *Phormidium valderianum*, decolorization, response surface methodology, central composite design, artificial neural network

Introduction

During the last decade pollution control strategy implementation for industrial effluents has become one of the major concerns of society. The major problem prevailing in the ethanol production industry is the release of a large segregated quantity of dark brown colored distillery effluent with high pollutant characteristics remaining after the separation of the product from the fermentation broth known as spent wash. It was reported that spent wash discharge is 15 times of the total amount of ethanol produced. On average, 8-15 L of spent wash was generated for every liter of alcohol produced [1, 2]. As per the act of the Ministry for Environment and Forests, it was stated that distillery industries producing alcohol were ranked as the top in the "Red Category" [3]. The dark brown color of the spent wash was

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due to the presence of melanoidins, which were known to be a natural biopolymer produced through browning reactions. They are generated by the maillard reaction taking place between the amino and carbonyl groups in organic substances at high temperatures and low water activity. This reaction proceeds effectively at temperatures above 50ºC and pH 4-7 [4]. Several fungi such as *Phanerochaete chrysosporium* JAG-40, *Aspergillus* species, *A. gaisen*, *P. pinophilum*, and *Emericella nidulans* have been used for the treatment of distillery spent wash [5-9]. But these organisms were not able to maintain a consistency in the result and hence have not been implemented in the distillery industries. A report indicates that the use of hybrid methods involving a combination of electro coagulation and adsorption are more successful than the individual ones [10]. Recently microbial strains sequential treatment has gained importance in the treatment of distillery spent wash due to its high organic load. Fungi were used in the first two stages, followed by bacterial strain as the third stage [11]. Coupled biological treatment followed by photochemical processes was only implemented for treatment of bio-recalcitrant effluents from an agro-residue-based pulp and paper mill [12]. However, these sequential methods have their own technological advantages and yet are in their infancy, requiring economical considerations in order to apply it to large-scale distillery units It was reported that cyanobacteria has the ability to utilize melanoidine present in the spent wash as the sole carbon and nitrogen sources, and thereby decolorize the spent wash. Another advantage in using cyanobacteria is that apart from degradation of the melanoidine it also oxygenates water bodies, resulting in the reduction of biological oxygen demand (BOD) and chemical oxygen demand (COD) [13].

Government policies and norms on pollution control are becoming more stringent; distillery industries have been forced to look for an optimized effective technology for a treatment that should be cost effective and ecofriendly. The conventional single factorial method of optimization does not depict the combined interaction effect of all the factors involved. These limitations can be eliminated by optimizing all the affecting parameters collectively by statistical experimental design, such as response surface methodology (RSM) [14]. Hence this present investigation was focused toward the examination of an optimization tool for determining optimum process parameters for the treatment of distillery spent wash using cyanobacteria. To the best of our knowledge the application of RSM and ANN to predict the performance of biological degradation and decolorization of distillery spent wash by cyanobacteria has not been reported.

Materials and Methods

Anaerobically Treated Distillery Spent Wash (ADSW)

Molasses spent wash after biomethanation from an anaerobic digester was collected aseptically from the distillery division of Bannari Amman Sugars Limited, Periyapuliyur, Erode District, Tamil Nadu, India. The spent wash was centrifuged at 4200 × g for 15 min before removing the suspended solids and storage at 4ºC. The stored ADSW was filtered through Whatman No. 1 filter paper. Then the filtrate was diluted using distilled water for further studies.

Cyanobacteria

Three marine cyanobacterial species such as *Oscillatoria* sp (BDU 30602), *Phormidium valderianum* (BDU 140441), and *Synechocystis pevalekii* (BDU 21304) were acquired from germplasm of the National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The cultures were maintained in marine synthetic liquid ASN-III medium. Stock culture was maintained in marine synthetic liquid ASN-III medium with the composition as follows: NaCl (25 g/L), $MgSO_4 \cdot 7H_2O$ (3.5 g/L), $MnCl_2 \cdot 6H_2O$ (2 g/L), $NaNO_3$ (0.75 g/L), $K_2HPO_4 \cdot 3H_2O$ (0.02 g/L), $CaCl_2$ (0.5 g/L), KCl (0.5 g/L), Na_2CO_3 (0.02 g/L), Citric acid (0.003 g/L), EDTA (0.001 g/L), H_3BO_3 (2.860 g/L), $MnCl_2 \cdot 4H_2O$ (1.810 g/L), $ZnSO_4 \cdot 7H_2O$ (0.222 g/L), $Na_2MoO_4 \cdot 2H_2O$ (0.390 g/L), $CuSO_4 \cdot 5H_2O$ (0.079 g/L), and $Co(No_3)_2 \cdot 6H_2O$ (0.00494 g/L). The medium was prepared using tap water.

Screening of Cyanobacterial Culture for Treating ADSW

A batch experiment was performed with 250 mL Erlenmeyer flasks containing ASN-III medium with pretreated ADSW (ADSW collected from a bioreactor after treatment with *C. cladosporioides;* data not shown) and Cyanobacterial biomass in a photochemical orbitary incubator chamber (POIC). The initial pH of the medium was adjusted to 8 using 0.1 mol/dm^3 NaOH and 0.1 mol/dm^3 HCl. Mid log culture of the three strains from the MPR was transferred to the flask containing the above medium separately and incubated for 10 days under continuous illumination using fluorescent light at an intensity of 36 W/m^2, and the temperature was maintained at 28ºC. To each flask 4g of cyanobacteria was transferred from MPR. Uninoculated samples were served as controls. The measurements of light intensity incident on the flasks were carried out on the external surface of the flask using a digital luximeter (Minipa MLM 1010, country). At fixed time intervals equal volume of sample was collected and centrifuged. The supernatant was collected and the absorbance was measured at 475 nm. Then the % of decolorization was calculated as per Equation (1).

$$\% \text{ Decolorization} = \left[\frac{C_o - C_t}{C_o} \right] \times 100 \qquad (1)$$

Table 1. Levels of the variables of central composite design.

Factor	Variable	Low Level (-1)	High Level (+1)
A	pH	6.0	10.0
B	Light Intensity (W/m^2)	26.00	46.00
C	Temperature ºC	25.00	35.00

Optimization of Process Parameters Using Single Factorial Experimental Design

The effect of initial pH (6-10), temperature (24-32ºC) and light intensity (20-54 W/m^2) on % decolorization was studied using single factorial experimental design. In each experiment one factor was changed and other factors remained constant. Different numbers of lamps on each side of the POIC were combined to give the required light intensity. The experiment was conducted in a 250 mL Erlenmeyer flask containing 100 mL of ASN-III medium with pretreated ADSW. *P. valderianum* (4 g of 10^{th} day) was inoculated to the flask and incubated at 28ºC for 10 days in a POIC. Every 24 h a small amount the sample was withdrawn and centrifuged. The supernatant was collected and the absorbance was measured at 475 nm. The % of decolorization was calculated using Equation (1). During the optimization process, chlorophyll and protein estimation were carried out to determine the growth of Cyanobacteria. Chlorophyll and its quantitative estimation are important to study growth and photosynthetic rates. Chlorophyll was extracted completely using methanol as a solvent. The extraction process was carried out using a Soxchel apparatus. Protein concentration was estimated by Lowry's Method [15] using bovine serum albumin (BSA) as standard.

Optimization of Process Parameters Using Response Surface Methodology

Batch experiments were designed with three independent variables – pH (X_1), temperature (X_2) and light intensity (X_3) – at three coded levels (-1, 0, 1) as shown in Table 1. Assigning % of decolorization as dependent response variables, experiments were performed using the design obtained by the full factorial central composite design (CCD). The optimum parameter values obtained from the single factorial experiment were then converted to uncoded units using Equation 2 by assigning those values as center points. A 2^3 full factorial experimental design with 20 experiments were employed, which includes 8 trails for factorial design, 6 trails for axial points and 6 trails for replication of the central points based on the pattern generated through software. The experiment was conducted as per the design matrix with 100 ml of pretreated ADSW in 250 ml Erlenmeyer flask and incubated with 5 g of cyanobacteria for 10 days. During the process an equal volume of sample was collected at regular intervals and centrifuged. The supernatant was collected and the absorbance was measured at 475 nm. The analysis of variance (ANOVA; Table 4) and regression information were generated using Design Expert 8.0.4.1 software. Further based on the 'P' and 'T' values, the significant factors were determined. For statistical calculation independent variables were coded as:

$$x_i = \frac{X_i - X_0}{\delta X_i} \tag{2}$$

...where X_i is the experimental value of the variable, X_o is the midpoint of X_i, δX_i is the step change in X_i, and X_i is the coded value for X_i; i = 1-4. The experimental design with coded levels of variables using CCD is shown in Table 2. The behavior of the system was explained by the following second order polynomial equation:

$$y = \beta_o + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} x_i^2 + \sum\sum_{i<j} \beta_{ij} x_i x_j + \in \tag{3}$$

...where y is the percentage of color removal, β_o is offset term, β_i is the coefficient of individual effect, β_{ii} is the coefficient of squared effect, and β_{ij} is the coefficient of interaction effect [16]. Further, the optimal values were calculated using Equation (3). The goodness-of-fit of the regression model and the significance of parameters estimates were determined through the appropriate statistical method. Three dimensional response surfaces and contour graphs were plotted on the basis of the model equation to reveal the interaction effect among the variables and to determine the optimum value of each factor for the maximum % of decolorization. The experiment was repeated with the optimum process parameters to verify model validation.

Artificial Neural Network

Normally a neural network in its fundamentals is composed of several layers of neurons, there being one input layer, at least one hidden layer, and one output. The basic problem in constructing the neural network is to find the optimal number of hidden neurons. In the present work three neurons (n1, n2, n3) were used in the input layer, four in the hidden layer (w1, w2, w3, w4), and one in the output layer (o) as a transfer function to model the dependency of the decolorization process. The three input variables are (pH, temperature, and light intensity). Once the ANN was performed the experimental value and the output from ANN was fitted to explicate the result using regression value. The training parameter goal was set at 0.001, Epochs at 25,000, and a learning rate of 0.8 were used. The most common activation function in backward propagation neural network is the sigmoidal function.

$$y_i = \frac{2}{1 + e^{-Net_{iij}}} - 1 \tag{4}$$

$$Net_j = \sum W_i x + Bias \tag{5}$$

..where N_{et} is defined as the sum of the weighted input signal and the neuron is the weight connection to neurons of j, x denotes the input values, and bias is the bus of the neuron i.

Table 2. Experimental design and results of CCD.

Run	Factor 1: A – pH	Factor 2: B – light intensity (W/m^2)	Factor 3: C – Temperature °C	Response % decolourisation experimental	Response % decolorization predicted by RSM	Response % decolorization predicted by ANN
1	10.0	26.0	35.0	50.2	47.2	49.30
2	10.0	26.0	25.0	71.2	66.8	70.30
3	8.0	52.82	30.0	26.8	25.8	25.90
4	8.0	36.00	38.41	52.5	50.2	51.51
5	6.0	26.0	35.0	16.5	16.0	15.57
6	8.0	36.0	21.59	56.5	54.2	55.60
7	8.0	36.0	30	85.5	82.5	84.00
8	10.0	46.0	25.0	56.6	51.2	55.61
9	8.0	36.0	30.0	80.5	79.2	79.58
10	6.0	26.0	25.0	30.2	30.0	29.27
11	8.0	36.0	30.0	82.5	80.2	81.58
12	6.0	46.0	25.0	32.2	30.5	31.30
13	8.0	36.0	30.0	85.0	83.9	84.10
14	8.0	36.0	30.0	84.2	83.2	83.27
15	8.0	19.18	30.0	20.5	20.0	19.58
16	6.0	46.0	35.0	33.0	32.5	32.07
17	4.64	36.0	30.0	15.0	14.2	14.10
18	11.36	36.0	30.0	62.5	60.8	61.60
19	10.0	46.0	35.0	52.5	50.8	51.60
20	8.0	36.0	30.0	85.0	84.2	84.08

RSM and ANN Software

Experimental design was constructed by Design Expert Software (Version 8.0.1, State EaseInc., Minneapolis, USA) for RSM prediction. ANN calculations were determined using MATLAB 7 software with ANN toolbox after incorporating program coding for storing input data, training, response data, and giving an output layer. A three-layer network with transfer function using back propagation algorithm was used in this study.

Results and Discussion

Screening of Cyanobacterial Culture

From Fig. 1 it was observed that *Phormidium valderianum* showed the highest ability to decolorize spent wash compared to *Oscillatoria* sp., and *Synechocystis pevalekii*. A maximum decolorization of 48.5% was achieved at pH 9, 28ºC with light intensity of 40 W/m^2 for *Phormidium valderianum* on 7th day, and 42.6% and 29.25% for the other two species (Fig. 1). This effect was due to high photosynthetic reaction of this species toward the spent wash. Also due to enzymes segregated in the metabolic pathway with spent wash as substrate along with ASN-III medium. Hence investigation was focused with this species on treating distillery spent wash pretreated in a bioreactor with fungi, and also to determine the chlorophyll and protein content

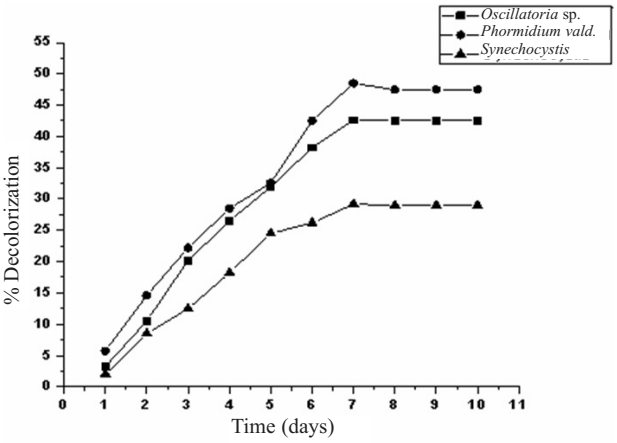

Fig. 1. Percentage decolorization of various cyanobacteria in the course of time.

released during photosynthesis. In order to reveal the efficiency of the strain in the decolorization process and also to optimize the process parameters, the experiment was continued with single and multi-factorial experimental design.

Single Factorial Experimental Design

Single factorial experiments have revealed the effects of pH, temperature, and light intensity on percentage decolorization of spent wash. Fig. 2a showed the effect of pH on percentage decolorization. It was observed that a maximum decolorization of 58.2% was obtained at pH 8 on day 8. However, the chlorophyll pigment was found to be 1.15 mg/mL and protein was estimated to be 0.862 mg/mL. Optimum growth was obtained at pH 8, which shows that cyanobacteria prefer to grow in alkaline pH rather than acidic pH. It was reported that decolorization and degradation of synthetic melanoidin was influenced by pH in the alkaline range due to hydroxyl ions [17]. In a report it was stated that *Phormidium valderianum* could remove the dye tested with more than 95% efficiency only at pH value above 11 [18]. But in this investigation it was observed that the organism could decolorize spent wash efficiently at pH 8. Fig. 2b shows the effect of temperature on percentage decolorization. It was observed that maximum decolorization of 61.8% was obtained at 28ºC. Chlorophyll pigment and protein was estimated to be 2.0 mg/mL and 0.58 mg/mL. This indicates that the organism could not withstand higher temperature. The color reduction was due to the active oxygen released by cyanobacteria during the photolysis of water. Fig. 2c shows the effect of light intensity on percentage decolorization. Optimum growth was obtained at light intensity of 36 W/m^2, indicating that *Phormidium valderianum* achieved a maximum decolorization of 74.5% with COD reduction of 83.48% (5,750 mg/L). However, the chlorophyll pigment obtained was found to be 3.2 mg/mL and protein was estimated and found to be 1.85 mg/mL. In a report it was stated that color reduction requires light for the consequent growth of the organism, but higher effluent concentration did not support the growth of the cyanobacteria *O. boryana* BDU 92181, which is due to the lack of light penetration because of the very dark color of the medium [19]. However, this problem was not faced in our study with *Phormidium valderianum,* since the spent wash is already been pretreated with fungi and hence it was observed that in all light intensities cyanobacteria were able to decolorize the spend wash efficiently.

Response Surface Methodology – Central Composite Design

A three level, CCD design was used to evaluate the relationship between the culture conditions (independent variables) and the percentage decolorization (dependent variable) of distillery spent wash. Three replicates at the central point were used to estimate the experimental error. CCD involves the following steps, such as performing the statistically designed experiments according to the design, factors, and levels selected. A similar study was reported by estimating the coefficients of the mathematical model to predict the response and check for its adequacy [20, 21]. Three factors such as pH, temperature, and light intensity were used to determine the key variables that significantly influence the percentage decolorization of distillery spent wash. Each factor was examined at two levels: -1 for low level and +1 for high level. Table 2 represents the CCD design matrix for 3 variables with coded values, along with the observed results. By applying multiple regression analysis on the experimental data, the following second order polynomial model was derived to explain the percentage decolorization.

$$\% \text{ Decolorization} = 83.57 + 14.53x_1 + 1.23x_2 - 3.28x_3 - 3.85x_1x_2 - 1.53x_1x_3 + 3.93x_2x_3 - 14.54x_1^2 \quad (6)$$

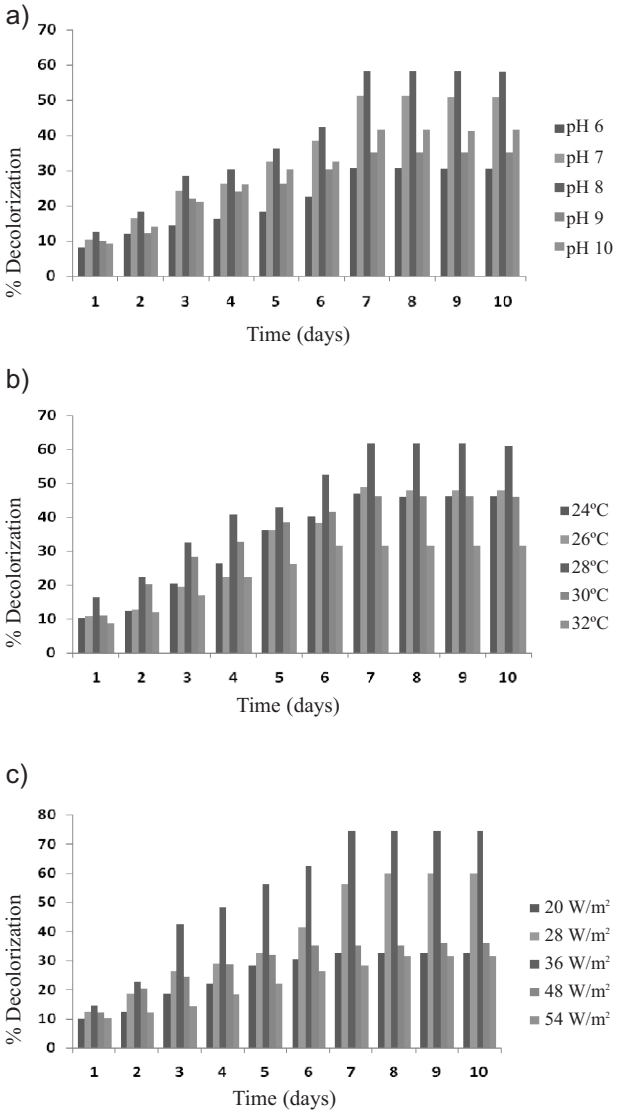

Fig. 2. Effect of (a) pH on percentage decolorization of ADSW at 26ºC with light intensity of 36 W/m^2, (b) temperature on percentage decolorization of ADSW at pH 8 with light intensity of 36 W/m^2, (c) and light intensity on percentage decolorization of ADSW at pH 8 at 28ºC.

Table 3. Estimated regression coefficient and corresponding *F* and *P* values.

Source	Sum of squares	Degrees of freedom	Mean square	*F*-value	*P*-value Prob>F
Model	11,808.81	9	1,312.09	64.33	<0.0001
A: pH	2,884.73	1	2,884.73	141.42	<0.0001
B: Light intensity	20.65	1	20.65	1.01	0.3380
C: Temperature	146.48	1	146.48	7.18	0.0231
AB	118.58	1	118.58	5.81	0.0366
AC	18.60	1	18.60	0.91	0.3621
BC	123.25	1	123.25	6.04	0.0338
A^2	3,046.33	1	3,046.33	149.35	<0.0001
B^2	5,694.25	1	5,694.25	279.16	<0.0001
C^2	1,159.70	1	1,159.70	56.85	<0.0001

Table 4. ANOVA second-order response surface model.

Source of variation	Sum of squares	Degrees of freedom	Mean squared	*F*-calculated
Residues	203.98	10	20.40	-
Lack of fit	185.47	5	37.09	10.02
Pure error	18.51	5	3.70	-
Total	12,012.79	19	-	-
Std. Dev	4.52	R^2	0.9830	
Mean	53.95	Adj R^2	0.9677	
C.V. %	8.37	Pred R^2	0.8810	
Press	1,429.87	Adequate Precision	21.505	

ANOVA and Regression Coefficient (R^2)

The analysis of variance of the quadratic regression model demonstrated that Equation 6 was a highly significant model, as was evident from Fisher's *F*-test with a very low probability value [(*P* model > *F*) =0.0001]. From Table 3 it was observed that the Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, AB, BC, A^2, B^2, and C^2 are significant model terms.Values greater than 0.1000 indicate that the model terms are not significant.The coefficient for the linear effect of pH (0.0001), and temperature (0.0231) was highly significant, and that of light intensity (0.3380) was least significant. It also was observed that the interaction effect AB (0.0366) and BC (0.0338) was highly significant compared to AC (0.3627) for the response. As per the reports, ANOVA separates the sum of variation of the result into two componenets: variation in connection with the model and variation associated with the experimental error. It is the tool required to test the significance and adequacy of the model derived for a particular process [22-24]. During the implementation of RSM for dye removal using macroalgae *Chara* sp. it was revealed that the regression coefficient (R^2) quantitatively evaluates the correlation between the experimental data and the predicted responses. Results of R^2 = 0.982 and Adj-R^2 = 0.966 obtained explicates that the predicted values were found to be in good agreement with experimental values. Since the R^2 value is closer to 1.0 it indicates that the regression line perfectly fits the data [25]. Similar to that in this investigation, R^2 obtained was 0.9830, which was close to 1. Results imply that the predicted values were found to be in good agreement with experimental values (R^2 = 0.9830 and Adj-R^2 = 0.9677), indicating the achievement of the RSM. The model's goodness of fit was checked by regression coefficient (R^2). In this case, the value of the coefficient (R^2=0.9830) from Table 4 indicated that only 1.77% of the total variance was not explained by the developed regression model. The obtained R^2 values suggest good adjustments to the experimental results since these indicate that 0.8810 of the variability in the response could be explained by the models.

Response Surface and Counter Plots

In order to analyze the regression equation of the model, three-dimensional surface and 2D contour plots were obtained by plotting the response (% decolorization) on the Z axis against any two variables while keeping the other variable at zero level. These plots are created to analyze the change in the response surface. The surface and contour plots of the quadratic model are shown in Fig. 3 (a-c). It was reported that Response surface plots provide a method to predict the decolorization efficiency for different parameter values of the tested variables and the contour plots help in identification of the type of interactions between these variables [26]. The axes of the contour plot are the experimental variables and the area within the axes is termed the response surface. Fig. 3(a) shows the response surface and contour plots developed as a function of pH and light intensity, while the temperature was kept constant at 30ºC.

It was observed that the percentage decolorization was found to be more sensitive to pH and it increased from 15 to 85.5% when the pH of the medium was changed from 6.0 to 8.0. When the pH value was higher than 8.0 the rate of decolorization decreased. The reason for this observation is due to the fact that the organic matter present in the spent wash gets precipitated and also due to inactivation of the enzyme. The decolorization rate increased faster with a small increase of pH at a constant temperature of 30ºC. Fig. 3(b) represents the response surface and contour plots developed as a function of light intensity and temperature, while pH was kept constant at 8.00 pH. It can be seen from the contour plot that the maximum decolorization of 85.5% occurs in the region of 30ºC temperature and 36 W/m^2 of light intensity. Biological decolorization increases with increases in light intensity and decreases in temperature. This was justified by the shape of the contour region. This fact is due to the growth of cyanobacteria with respect to chlorophyll and that the amount of protein released in the course of time decreases with increased temperature. Fig. 3(c) explicates the response surface and contour plots developed as a function of temperature and pH, while light intensity was kept at a constant value of 36 W/m^2. It can be seen from the figure that maximum decolorization occurs in the region of 29-31ºC and pH 8.

Overall, considering the main and interaction effects of the three factors, the optimum conditions were determined to be pH 8, light intensity of 36 W/m^2 and temperature 30ºC.

Experimental value and the predicted value from the model were found to be close, which indicates the second order polynomial equation was in good agreement (Fig. 4a). Hence it is proved that the interaction effect of pH and light intensity is higher than other combined effects. Results revealed that at the optimized process conditions of pH 8, 30ºC, and 36 W/m^2 a maximum of 85.5% and COD reduction of 87.29% (4,420 mg/L) were achieved.

Artifical Neural Network

ANN methodology was performed to provide a non linear mapping between input variables (pH, temperature, and light intensity) and the output variable (% decolorization)

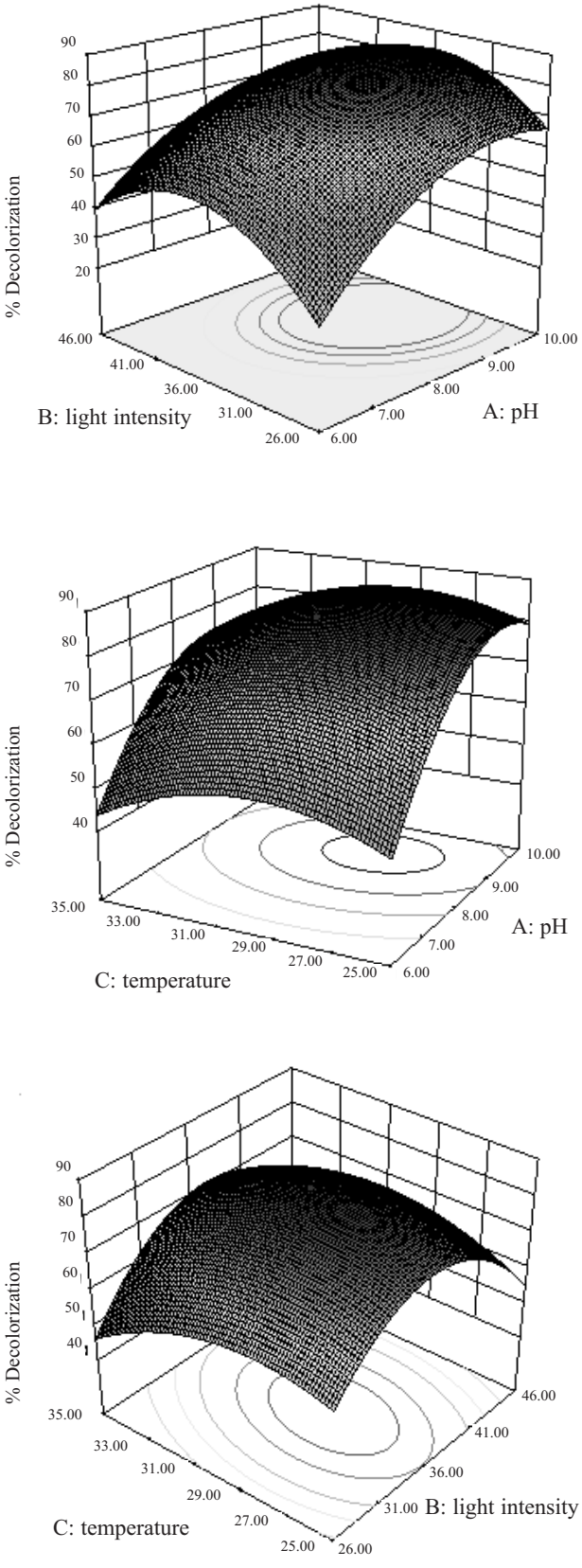

Fig. 3. Response surface and contour plots developed as a function of: (a) pH and light intensity while the temperature was kept at 30ºC, (b) pH and temperature while light intensity kept at 36 W/m^2, (c) light intensity and nutrient concentration while pH was 8.

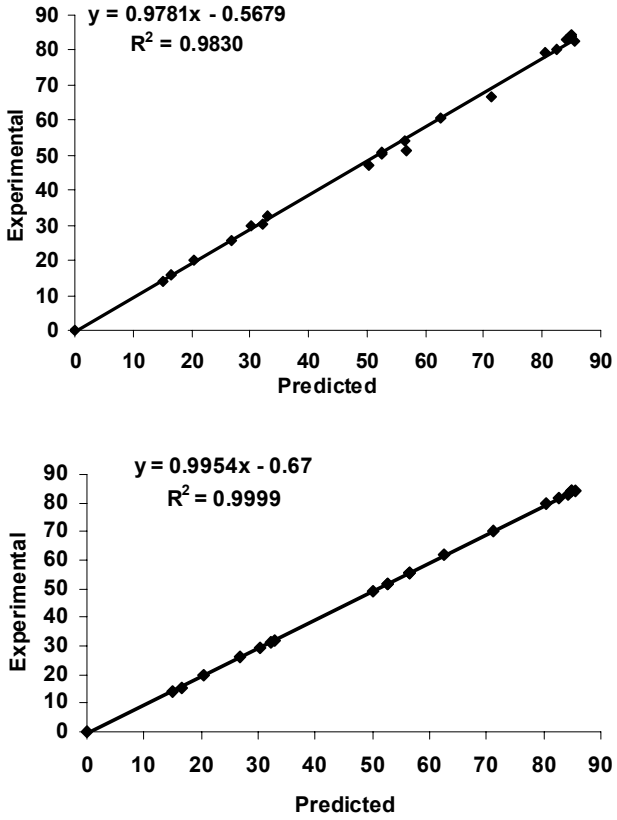

Fig. 4. Comparison of the experimental results with those predicted by (a) RSM and (b) ANN.

for the runs reported in Table 2. The configuration of the ANN network was developed with (3-4-1) structure, three input neurons, four hidden layers, and one output response by trial and error and topology of network. The training was done using the training scaled conjugate (trainscg). The number of epochs was set to 25,000 and the iterations involved for optimization were 88. The learning rate was fixed at 0.8 and the goal was 0.001. The following equation obtained is the response of the neural network training, relating the input variable (n1, n2, n2) to the output variable *y* in terms of weight and bases.

$$y = w_2 \cdot \left(\frac{2}{1.5 + e^{-3/(w_1 \cdot xv^1 \pm b_1)}} \right) + b_2 \quad (7)$$

...where w_1 and w_2 are the weights, b_1, b_2 are the bases, y is the predicted value from the neural network, and xv is the row vector of three independent variables. An expression was proposed based on the partitioning of the weights connected in the network path rsm [27, 28].

$$I_j = \frac{\sum_{m=1}^{m-Nn} ((|W_{jm}^{ih}| / \sum_{K=1}^{N1} |W_{km}^{ih}|) * W_{mo}^{ho})}{\sum_{K-1}^{k=N1} \{ \sum_{m-1}^{m=nk} (|W_{km}^{ih}| / \sum_{k-1}^{Ni} |W_{km}^{ih}|) * |W_{mn}^{ho}| \}} \quad (8)$$

...where I_j was the relative importance of the input variable on the output variable N_i and N_h. N_i is the number of input neurons, N_h is the number of hidden neurons, w is the connecting weight, and superscripts i, h, and o refer to the input hidden and output layers. Subscripts k, m, and n refer to the input, hidden, and output neurons. A regression analysis was carried out and the performance of ANN output was estimated through the regression coefficient, which is been calculated from:

$$R^2 = \frac{\sum_{m=1}^{N}(t_o - t_{mean})^2 - \sum_{m=1}^{N}(t_o - \lambda_p)^2}{\sum_{m=1}^{N}(t_p - \lambda_p)^2} \quad (9)$$

...where R^2 is the regression coefficient, N is the number of the patterns, m is the index number for pattern, t_p is the target value for the pattern, t_{mean} is the mean target value, and λ_p is the output of the pattern produced by the ANN model. Hence this model can be effectively utilized for the decolorization of distillery spent wash. Fig. 4a proposes that of the two optimization tools with model used, the ANN model (R^2=0.999) showed better results than RSM (R^2=0.983). A similar ANN network was performed for the biological decolorization of triphenylmethane dye by microalgae and obtained an R^2 of 0.982 [29]. ANN and RSM were used to build a model to describe the effect of process conditions for Cephalosporin C production [30]. Similarly, the sequential optimization strategy for design of an experimental and artificial neural network were applied to evaluate and optimize the media component for L-asparaginase production by *Aspergillus terreus* [31].

Model Validation

Based on the results of the single factorial and RSM, the optimum process conditions for spent wash degradation and decolorization were pH 8, 36 W/m^2 light intensity, and 30ºC temperature. In order to confirm the validity of the RSM model results a confirmation experiment with triplicate set was conducted at the above-specified optimum process conditions predicted by the model. Under these conditions percentage decolorization was found to be 85.5%, which were close to the RSM result of 85.2%. The experimental values were found to be close to the predicted values and hence the model was validated. The experiment was repeated in triplicate using the predicted optimal conditions determined by RSM and ANN models for validation. Thus the model was useful to predict the percentage decolorization and also to obtain optimum process parameters for decolorization of distillery spent wash.

Conclusion

The results of this present investigation have shown that *Phormidium valderianum* is the best type of cyanobacteria to treat distillery spent wash. The optimized process conditions for the treatment are pH 8, 30ºC, and light intensity of 36 W/m^2. Experimental value and the predicted value from the model were found to be close, which indicates the second order polynomial equation was in good agreement.

From RSM results it was proved that the interaction effect of pH and light intensity is higher than other combined effects. Apart from RSM, the ANN linked network was experimentally validated and determined to be effective for optimization of significant biological process parameters for decolorization of distillery spent wash.

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