Optimization of Methylene blue removal by mixed bacterial culture isolated from dye contaminated site

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The effluent discharge from industries such as textile, pharmaceutical, leather dyeing, and petroleum containing different dyes is one of the major sources of groundwater contamination. Dyes are toxic, mutagenic, and carcinogenic in nature, and adversely affect human beings and aquatic life. The remediation of dye is one of the major challenges to the researchers. In this work, an attempt has been made to solve the above concern. The potential bacterial species were isolated from dye contaminated site and used in the removal of Methylene blue (MB) dye. The most affecting process variable including process time, dye concentration, and pH were optimized using a central composite design (CCD) of response surface methodology (RSM) and obtained to be 4.0 days, 50 mg/L, and 7.0, respectively. The dye removal efficiency was found to be 78.2% at optimum conditions.

Keywords: Methylene blue, removal, optimization, isolation, RSM.

Introduction

The effluent discharge from industries such as textile, pharmaceutical, leather dyeing, and petroleum containing different dyes is one of the major sources of groundwater contamination, which leads to decreasing the quality of water[1,2]. In the last few decades, researchers and environmentalists have more attention on water quality and regulation to overcome groundwater contamination. However, due to the large application of dyes in all spheres of life, the concentration of dyes in the water bodies is increasing, which causes several adverse effect on the environment and human health. More than ten thousand types of dyes are applied in the textile industry, and approximately 0.8 million tons of dyes are produced annually worldwide[3]. Among different types of dye, azo dye shares approximately 70% of the total dyestuffs used in the textiles industry. These dyes are not only toxic, mutagenic, and carcinogenic in nature but also impede the rate of photosynthesis mechanisms of water bodies[4,5].

The physicochemical methods such as membrane separation, ion-exchange, coagulation/flocculation, adsorption, and advanced oxidation have been frequently applied for the treatment of dye from wastewater[6–8]. However, these methods are associated with operational difficulties, sludge disposal, and generation of secondary pollutants[6]. Therefore, it is essential to develop a cost-effective and environmentally benign technique for the treatment of wastewater containing dyes. In the last few decades, biological treatment has received omnipresent attention from the researchers. It is considered as a suitable option for the treatment of dye due to its cost-effective, environmentally-friendly, and ability to produce very less sludge[4,6,9]. The microorganisms such as *Bacillus* sp., *Pseudomonas entomophila* BS1, *Phormidium autumnale* UTEX1580, *Achaetomium strumarium*, *Oerskovia paurometabola*, *Rhodococcus* DSM 43066, *Brevibacillus parabrevis*, etc. have been successfully applied for the removal of dyes[1,2,7,10].

The efficacy of microorganisms towards removal of dyes mainly depends on the operating parameters such as substrate concentration, pH, temperature, process time, and types of bioreactor etc. For example, high substrate concentration impedes the rate of substrate removal due to substrate inhibition, whereas low concentration leads to starva-

tion[11,12]. Therefore, the determination of optimum condition is not only vital for the effective removal of the substrate but also helpful in the scale-up of the process. Previously, the one-factor-at-a-time (OFAT) technique has been used for the optimization of operating parameters. However, OFAT takes more time and unable to study the interactive effect of process variables[11,13,14]. To overcome the above concern, the response surface methodology (RSM) has been widely applied for the optimization of process variables[17–19]. Sutar *et al.*[20] have studied the removal of Malachite green by *Photobacterium leiognathi* and optimized the process variables such as pH, temperature, and NaCl (%) using Box-Behnken based RSM technique. In another study, RSM was applied to optimize the process variables such as pH and temperature for the biodegradation of Methyl orange dye using bacterial consortium[18].

In this work, Methylene blue (MB) dye was selected as a model pollutant. The soil sample collected from dye-contaminated site was enriched and acclimatized in the laboratory, and isolated bacterial culture was used for the removal of MB dye. The central CCD of RSM was used to optimize the process variables for the effective removal of MB dye.

Material and methods:

Chemicals and dyes:

Methylene blue dye was purchased from Sigma-Aldrich, India. The composition of mineral salt medium (MSM) reported by Alvarez *et al.*[15] was used in this work with some changes include (g/L): $Na_2HPO_4.2H_2O$ 2.5, KH_2PO_4 1.0, NaCl 0.5, NH_4Cl 0.5, $MgSO_4.7H_2O$ 0.3, $CaCl_2$ 0.02. MSM also contained trace elements as follows (mg/L): $CuSO_4$ 0.4, $MnSO_4.H_2O$ 4.0, $ZnSO_4.7H_2O$ 4.0, H_3BO_3 5.0, and $FeCl_3.6H_2O$ 2.0. Glucose (2 g/L) was also added to MSM as the carbon source. Prior to inoculation, the culture medium was autoclaved at 121°C for 15 min.

Microorganisms and culture condition:

The soil samples were collected from a dye-contaminated site located in Bhadohi, India, and used for the isolation of potential bacterial species. For this, initially, 5 g of soil sample was enriched in MSM (100 mL) containing glucose (0.5 g/L) and MB dye (25 mg/L) and kept in the incubator under 150 rpm at 30°C for 5 days. After 5 days, the 5 mL inoculum was transferred to fresh MSM contain glucose and dye with 50 mg/L and again kept under the same condition in the incubator. This process was repeated thrice with gradually increasing concentration of dye (100, 150, and 200 mg/L). The bacterial consortium was isolated by the serial dilution method and further used in the removal study.

Batch study:

Batch study was performed in the caped Erlenmeyer flasks (250 mL) contained 100 mL MSM with different concentration dye (10–50 mg/L). The enriched bacterial consortium was grown overnight and inoculated (2% v/v) into flasks. The flasks were kept in an incubator at 150 rpm and 30°C of temperature. The samples were taken at regular time intervals and centrifuged at 5000 rpm for 10 min to separate cell mass, and the clear supernatant was used to find out the residual dye. The absorbance of the dye was measured using a UV-Vis spectrophotometer at 665 nm, and the dye removal (%) was estimated by the following equation.

$$\text{Dye removal (\%)} = \frac{(C_i - C_o)}{C_i} \times 100 \qquad (1)$$

where C_i and C_o are the initial and final concentration of dye (mg/L), respectively.

Design of experiment (DOE) for optimization of MB:

The design of the batch experiments and their statistical study was carried out using CCD of RSM. For this, Design-Expert (Version 11) software was used[16]. The most affecting process variables namely; pH (5.0–9.0), dye concentration (10–50 mg/L), and process time (1–5 day) were selected (Table 1). On the basis of the factorial design, 20 runs were designed and summarized in Table 2. The coefficients were evaluated by a second-order polynomial equation (eq. (2)).

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ij} X_{ij} + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \cdots \qquad (2)$$

where, Y represents the response variable (% dye removal), β represents the correlation coefficient, and i and j represent the coefficients of linear multi-degree.

Table 1. Experimental ranges of independent variables used in optimization process

Factor	Name	Units	Minimum	Maximum	Mean
A	pH		5.0	9.0	7.0
B	Concentration	mg/L	10	50	30
C	Process time	day	1	5	3.0

Table 2. Experimental run for the removal of MB dye

Run	Time	Conc.	pH	Removal
1	3	30	7	82.4
2	1	50	5	11
3	5	10	5	32.4
4	3	50	7	71.5
5	5	50	5	25
6	3	30	7	66.6
7	1	50	9	7.8
8	5	30	7	91.1
9	3	30	5	32.6
10	1	10	5	12.9
11	3	30	9	24.2
12	3	30	7	83
13	3	10	7	79.5
14	1	10	9	15.9
15	3	30	7	82
16	5	10	9	26.5
17	3	30	7	82.3
18	5	50	9	14
19	1	30	7	43
20	3	30	7	83.5

Table 3. ANOVA analysis for quadratic model

Source	Sum of squares	Degree of freedom	Mean square	F-Value	p-Value
Model	17463.08	9	1940.34	25.87	< 0.0001
A-Time	968.26	1	968.26	12.91	0.0049
B-Conc.	143.64	1	143.64	1.91	0.1965
C-pH	65.02	1	65.02	0.8668	0.3738
AB	12.25	1	12.25	0.1633	0.6946
AC	34.86	1	34.86	0.4647	0.5109
BC	15.96	1	15.96	0.2128	0.6545
A²	292.26	1	292.26	3.90	0.0767
B²	9.50	1	9.50	0.1267	0.7293
C²	6591.73	1	6591.73	87.87	< 0.0001
Residual	750.17	10	75.02	–	–
Lack of fit	534.32	5	106.86	2.48	0.1712
Pure error	215.85	5	43.17	–	–

Dye removal = $78.9 + 9.84A - 3.97B - 2.55C - 1.24AB - 2.09AC - 1.41BC - 10.31A^2 - 1.86B^2 - 48.9C^2$ (3)

Effect of process variables as surface and contour plot:
Effect of MB concentration and process time:

The individual and interactive effects of initial MB concentration and process time on dye removal have been demonstrated with the surface and contour plot in Fig. 1(a, b). The surface plot (Fig. 1a) shows that the dye removal decreased with increasing MB concentration, while the dye removal increases with process time at pH 7.0. It was found that 83.5% of dye removal was obtained at 30 mg/L of dye, whereas 71.5% dye removal was observed at 50 mg/L under fixed time (3 days) and pH (7.0). The decrease in the removal efficiency beyond 30 mg/L may be due to substrate inhibition[11]. At fixed pH (7.0) and dye concentration (30 mg/L), the dye removal of 43% was obtained, which further increased with process time and reached to 91.1% in five days.

Effect of pH and process time:

The individual and interactive effects of pH and process time against dye removal have been demonstrated with the surface and contour plots in Fig. 1(c, d). At both acidic and alkaline condition, the dye removal efficiency was significantly decreased. The optimum dye removal efficiency was observed at pH 7.0. However, the process time shows the positive impact on dye removal efficiency. The removal of the substrate is a very slow process and generally requires some

Results and discussion

Analysis of variance (ANOVA) and experimental model:

The CCD of RSM examined the simultaneous effects of response (dye removal) against process variables (process time, initial dye concentration, and pH). The results obtained after running the experiments were fitted with a quadratic equation to explain the relation between response and process variables. ANOVA analysis for the removal of MB dye was accomplished to analyses the significance of each variable. The model F-value was obtained 25.87, and the corresponding p-value < 0.0001 revels that the model is well fitted with experimental data (Table 3). There is only 0.01% chance that a model F-value is large, which may be due to noise. The lack of fit value of 2.48 with p-value of 0.172 indicates that the lack of fit is not significant. The value adjusted R^2 (0.92) is in reasonable agreement with predicted R^2 (0.76). The value of R^2 (0.95) was larger than 0.8 indicate that the quadratic model is suitable for the optimization study. The results attained from the CCD were fitted with the quadratic model to explain the dependence of dye removal on the process variables, as shown in eq. (3).
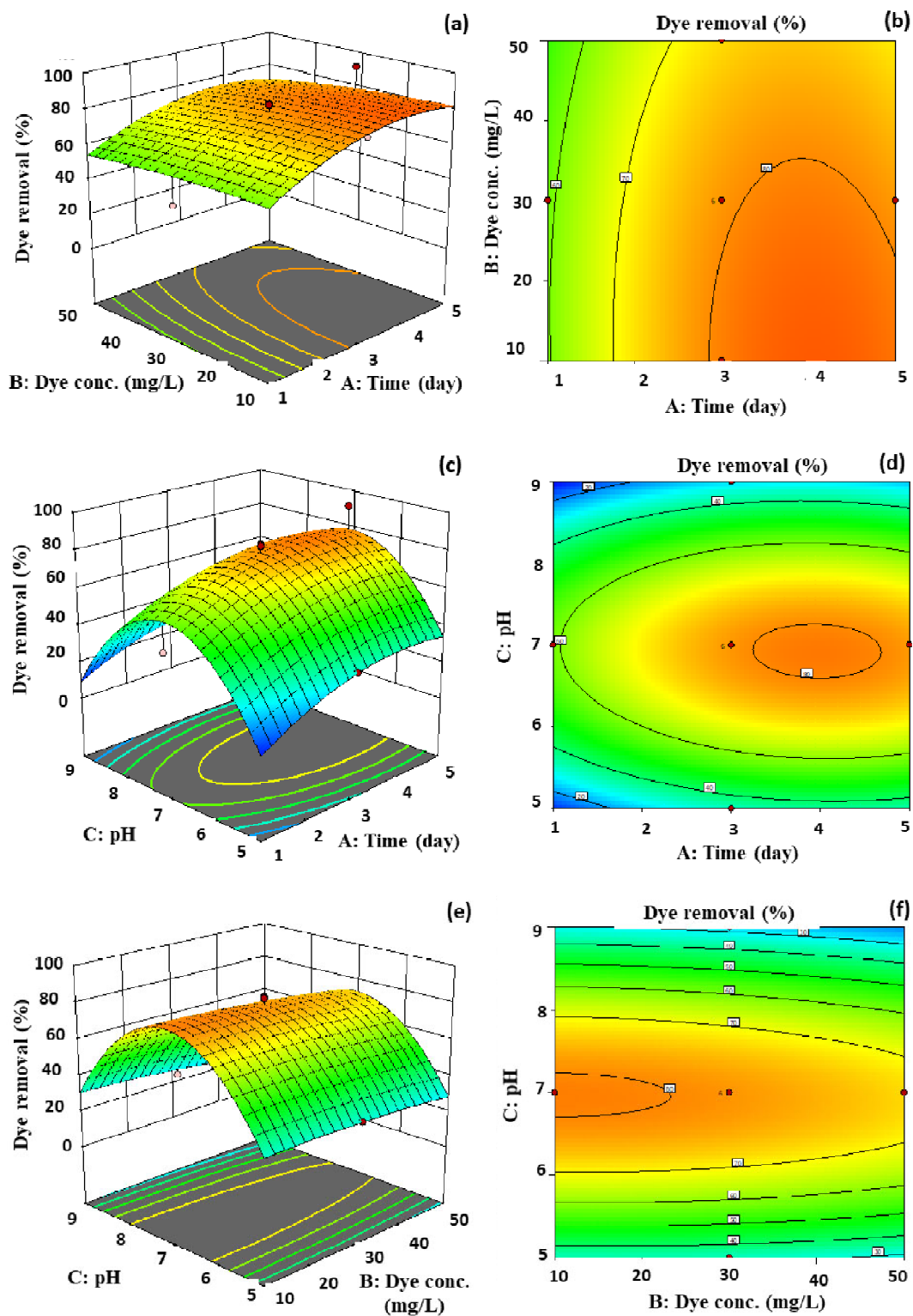
Fig. 1. Surface and contour of (a, b) effect of dye concentration and time; (c, d) effect of pH and time; (e, f) effect of pH and dye concentration against dye removal.

days for better growth and complete mineralization of substrate[2]. According to Padhi *et al.*[11], an elliptical contour indicates the significant interaction between the variables, while circular reveals insignificant interaction. In this work, the circular plot (Fig. 1d) shows less interaction between time and pH.

Effect of dye concentration and pH:

Surface and contour plots in Fig. 1(e, f) represents the simultaneous effect of dye concentration and pH with dye removal. The surface plot (Fig. 1e) shows that the acidic or alkaline condition shows the negative effect on dye removal, whereas optimum dye removal was obtained at neutral pH. The enzymes have ionic group on their active site, and these ionic group must be in suitable form (acid or base) to function. The variation of pH of the solution causes change in the activity ionic form of the active site, which leads to change the activity of enzyme and hence removal rate. The activity of the enzyme becomes lower at the acidic and alkaline conditions, which impede the removal efficiency of the substrate[16]. Similarly, the high concentration of dye adversely affects the dye removal efficiency. Generally, at the high concentration of pollutants, the removal efficiency decreased because of substrate inhibition and toxicity. The elliptical contour plot (Fig. 1f) shows the more significant interaction between pH process times.
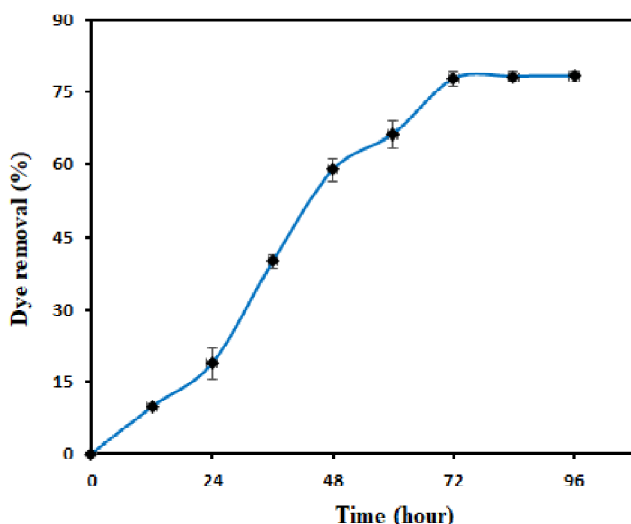
Model validation:

The process variables were optimized by targeting maximum removal of dye at the high concentration of MB with pH and process time in a moderate range. The optimum condition was predicted by CCD of RSM as; process time (3.87 days), dye concertation (50 mg/L), and pH (6.9) with 75.18% of dye removal. In model validation, process time, dye concertation, and pH were rounded off to 4.0 days, 50 mg/L, and 7.0 pH, respectively, and the experiment was performed in triplicate (Fig. 2). The attained result (78.2% of dye removal) was in agreement with predicted value and revealed only 1.5% of error.

Conclusion

Dyes are not only toxic, mutagenic, and carcinogenic in nature but also adversely affect the photosynthesis mechanisms of water bodies. The potential bacterial species were isolated from dye contaminated sites and successfully used in the removal of MB dye. The optimization study was performed by the CCD of RSM. The optimum conditions were found to be; process time (4.0 days), dye concentration (50 mg/L), and pH (7.0) for maximum removal of MB dye. This methodology will be helpful in the design and scale-up of the process with minimal efforts for the removal of dye in wastewater.

Acknowledgement

Fig. 2. Removal of Methylene blue dye at optimum condition (pH 7.0; Conc. 50 mg/L).

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