



Removal of methylene blue by *Saccharomyces cerevisiae*: process modelling and optimization

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ABSTRACT

Methylene blue (MB), as a thiazine cationic dye, has been commonly used in dye houses and textile industries. Long exposure to MB can cause health problems such as shocks, hypertension, anemia, nausea, serotonin syndrome, red blood cell breakdown, tissue necrosis and jaundice. The aim of this study was to optimize methylene blue (MB) removal from aqueous solutions by *Saccharomyces cerevisiae* using the response surface methodology. The influence of various factors including initial dye concentration (10–100 mg/L), solution pH (4–10), reaction time (10–60 min), and *S. cerevisiae* dosage (0.2–1.5 g/L), was investigated on the process. According to the findings, the obtained levels of MB removal were varied in the range of 36% to 96%. The maximum removal of MB (99.16%) occurred at pH 9.35, the reaction time of 50.81 min, dye concentration of 14.37 mg/L, and *S. cerevisiae* dose of 1.32 g/L. The results showed that the code of A with the coefficient of –11.41 was the most important factor in the removal process. The AD and D² with the coefficient of –11.30 and –7.76 had the maximum interaction impact, and the maximum square impacts on the process, respectively. The removal rate of MB was directly correlated with reaction time and *S. cerevisiae* dose. It can be concluded that *S. cerevisiae* can be considered as a low-cost adsorbent for the removal of MB in the aqueous media.

Keywords: Biodegradation; *Saccharomyces cerevisiae*; Methylene blue; Response surface methodology

1. Introduction

In recent years, the development of various industries including textiles, pharmaceuticals, cosmetics, plastics,

chemicals, etc. has substantial detrimental effects on the environment especially water resources [1]. The dyes used in the textile industry are harmful and biologically stable compounds due to their aromatic rings. Carcinogenic and

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non-biodegradable dyes such as sunset yellow and methyl orange released from the industrial effluent have caused a lot of concern [2]. Synthetic dyes are extensively applied in various industries and laboratories for dyeing products and for analytical purposes [3]. Among the various contaminants in the environment, synthetic dyes are released into the aquatic ecosystem via different industries such as paper, food, textiles, cosmetics, plastics, and rubber. These pollutants, even in low concentrations (<1 ppm), are visible and have detrimental impacts on various ecosystems and food networks [4]. Due to the prevention of sunlight penetration, the increment of dye pollution in aqueous environments such as lakes and rivers disturbs the ecological equilibrium, resulting in a decrease in photosynthetic activity of the autotrophic organisms [5]. Moreover, synthetic dyes can cause carcinogenic, mutagenic, and teratogenic impacts on humans [6].

Methylene blue (MB), as a thiazine cationic dye, is applied in dye houses and textile industries such as coloring paper, dyeing cotton, wools and paper coating [4]. This chemical compound is also used in medical practices including surgery, microbiology, and diagnostics [4]. The long exposure of humans to MB can result in various health problems such as shocks, hypertension, anemia, nausea, serotonin syndrome, red blood cell breakdown, tissue necrosis and jaundice [7]. Physical and chemical procedures like photocatalysis, ozonation, membrane filtration, adsorption, and electrocoagulation have been widely used to remove dye from wastewater. These processes can produce a lot of sludge and toxic by-products which need to be appropriately handled and treated [8,9].

Although physicochemical processes can typically reduce various azo dyes from wastewater, the biotechnology method using microbial species is an eco-friendly and effective process for the removal of these pollutants [10,11]. Compared to the physical and chemical methods, biotechnology procedures require less energy and produce less toxic sludge. *S. cerevisiae* (baker's yeast) is a type of fungi that can metabolize and biosorb dye contaminants from aqueous solutions [12]. *S. cerevisiae* can degrade synthetic dyes into the aromatic amines with side groups ($-\text{SO}_3$, $-\text{OH}$, $-\text{COOH}$, $-\text{Cl}$) [13].

The removal of azo dye by various microorganisms including bacteria and fungi was extensively reviewed by a study [14]. Aliasghar Navaei et al. [13] and Vatandoostarani et al. [15] successfully removed the azo dyes by *S. cerevisiae* at various times and they concluded that the decolorization process was dependent on the amount of the cell mass in the solutions. As mentioned earlier, MB is one of the major pollutants in industrial effluents. The removal of this pollutant by *S. cerevisiae* can significantly reduce the initial

investment and operation costs of the treatment. Farah and El-Gendy et al. [16] study reported that the expected costs for the removal of dye with dried biomass (baker's yeast) were about 18.79% of the commercial activated carbon. Therefore, due to advantages such as low cost, wide-spread distribution, non-pathogenic, simplicity, safety, cell rapid growth, and easy cultivation, *S. cerevisiae* was used for the removal of MB from aqueous solutions. The effect of various parameters (initial dye concentration, solution pH, reaction time, and *S. cerevisiae* dosage) was also optimized for the removal of this pollutant using the response surface methodology (RSM).

2. Materials and methods

2.1. Materials

The yeast *S. cerevisiae* (Persian Type Culture Collection, PTCC: 5052) was obtained from the Iranian Research Organization for Science and Technology (IROST), Iran. Methylene blue dye (MB), H_2SO_4 and NaOH were purchased from Merck Co., (Germany). Fig. 1 shows the chemical structure of the dye.

2.2. Preparing the reaction mixtures

To determine the removal rate of MB by *S. cerevisiae*, the effects of various factors including initial dye level (10–100 mg/L), solution pH (4–10), reaction time (10–60 min), and *S. cerevisiae* dosage (0.2–1.5 g/L) were investigated on the process (Table 1).

The experimental factors and their values were selected on the basis of the literature review and preliminary research [12]. The solutions were mixed into 250 mL Erlenmeyer flasks, containing 50 mL dye solution at 350 rpm. After completing the process, the samples were centrifuged (12,000 rpm for 12 min) to remove the cell mass in the mixture. Finally, MB concentration was determined using a UV-visible spectrophotometer (model T80+

Table 1
Range and levels of independent factors in the study

| Factor | Variable level | | | |
|-----------------------------------|----------------|-----|------|-----|
| | Code | -1 | 0 | +1 |
| MB (mg/L) | A | 10 | 55 | 100 |
| Reaction time (min) | B | 10 | 35 | 60 |
| <i>S. cerevisiae</i> dosage (g/L) | C | 0.2 | 0.85 | 1.5 |
| Solution pH | D | 4 | 7 | 10 |

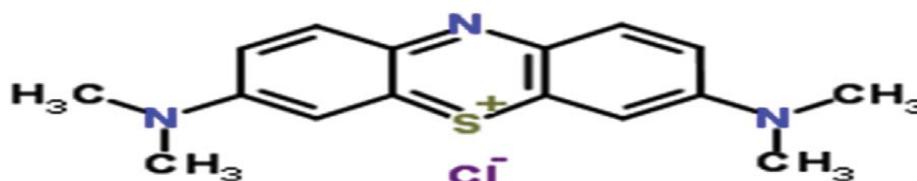


Fig. 1. Chemical structure of methylene blue.

(PG instrument Ltd., Leicester, UK)) at the wavelength of 660 nm. The removal rate (R , %) of the dye was obtained through the following equation:

$$R(\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (1)$$

where C_0 and C_e are the initial and final concentrations of the pollutant in the solutions (mg/L), respectively.

2.3. Experimental design and statistical analysis

The parameters in this study including initial MB concentration (mg/L), reaction time (h), dosage of *S. cerevisiae* (%) and solution pH was selected at high, medium and low concentrations with codes +1, 0 and -1, respectively. In this work, 29 runs including 16 real points, 8 pivot points, and 5 central focal points were considered by the design expert software. The influences of the factors in terms of linear, quadratic and cross products were calculated from the following equation [17]:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{1 \leq i < j} \beta_{ij} x_i x_j \quad (2)$$

where the predicted response, the constant coefficient, regression coefficients for linear impacts, quadratic coefficients, interaction coefficients, and the coded levels of the variables were illustrated by Y , β_0 , β_i , β_{ii} , β_{ij} and x_i or x_j , respectively. The fit of the models was assessed via calculating the coefficient of R^2 and the adjusted R^2 (R^2_{adj}).

3. Results and discussion

Fig. 2 shows the Fourier transform infrared spectroscopy analysis of *S. cerevisiae* before the dye removal. As seen in the figure, the peaks at 3,432.45; 3,322.45 and

334 cm^{-1} were attributed to the stretching vibrations of -OH, N-H, and C-H groups, respectively.

In this research, the effect of MB concentration, *S. cerevisiae* dosage, reaction time, and solution pH on the removal rate of the contaminant was also investigated. The findings are summarized in Table 2. The optimum condition in which the maximum MB occurred was determined to be 99.16% at pH 9.35, reaction time of 50.81 min, dye concentration of 14.37 mg/L, and *S. cerevisiae* dose of 1.32 g/L.

As seen, the lower and upper removal values of MB varied from 36.12% to 96.11%, respectively. The normality of the experimental data, which is essential for ANOVA, was confirmed by Design-Expert® Software.

A quadratic polynomial prediction formula was used to explain the relationship between the removal rate of MB and the independent parameters as follow:

$$\begin{aligned} \text{MB removal} = & 65.71 - 11.41A - 2.59B + 9.81C + 9.53D \\ & - 3.98AB - 7.59AC - 11.30AD + 1.81BC \\ & + 0.52BD + 2.72CD + 4.8A^2 - 7.14B^2 \\ & - 7.19C^2 - 7.74D^2 \end{aligned} \quad (3)$$

As can be seen in Eq. (3), each model has a fixed part and a variable part. Accordingly, the removal rate of MB (65.71%) was influenced by various parameters. In this study, the effects of A , B , C , and D parameters were obtained with the coefficients of -11.41, +2.59, +9.81, and +9.53, respectively. The code of A (MB concentration) with the coefficient of -11.41 was the most important factor that influenced the removal process. The AD and D^2 codes with the coefficient of -11.30, and -7.76 had the maximum interaction and square impacts, respectively in the process. Table 3 displays the statistical adequacy evaluation of the models. Table 4 indicates coefficients estimation for quadratic model of MB removal by *S. cerevisiae*.

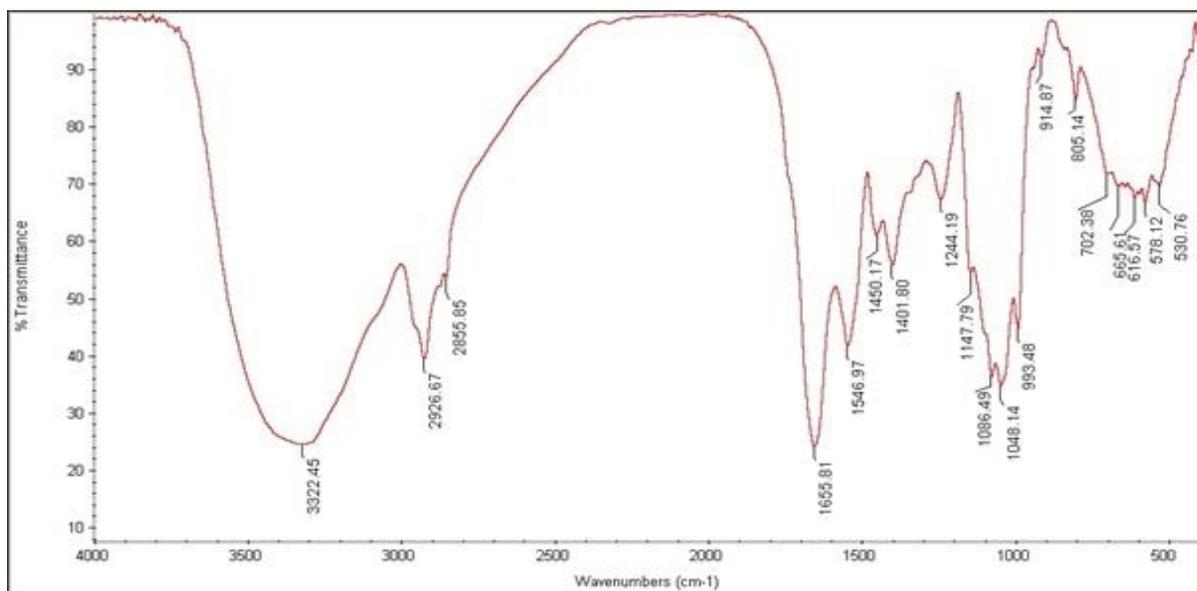


Fig. 2. Fourier transform infrared spectroscopy analysis for *S. cerevisiae* before the removal of MB.

Table 2
BBD matrix for MB removal by *S. cerevisiae*

| Run no | Coded variable | | | | Removal (%) | Run no | Coded variable | | | | Removal (%) |
|--------|----------------|----|----|----|-------------|--------|----------------|----|----|----|-------------|
| | A | B | C | D | | | A | B | C | D | |
| 1 | 1 | 1 | 0 | 0 | 47.12 | 16 | -1 | 0 | 0 | -1 | 52.03 |
| 2 | 0 | -1 | 0 | 1 | 62.13 | 17 | 0 | 1 | 0 | -1 | 38.03 |
| 3 | 0 | -1 | 1 | 0 | 63.15 | 18 | 0 | 0 | 0 | 0 | 63.24 |
| 4 | -1 | -1 | 0 | 0 | 72.01 | 19 | 0 | 0 | 0 | 0 | 65.01 |
| 5 | -1 | 1 | 0 | 0 | 77.03 | 20 | 0 | 1 | -1 | 0 | 36.15 |
| 6 | 0 | 1 | 1 | 0 | 61.23 | 21 | 1 | 0 | 0 | -1 | 52.16 |
| 7 | -1 | 0 | 1 | 0 | 93.14 | 22 | 0 | 0 | -1 | 1 | 49.36 |
| 8 | 0 | 0 | 0 | 0 | 66.05 | 23 | 0 | 1 | 0 | 1 | 56.12 |
| 9 | 0 | 0 | 1 | -1 | 47.12 | 24 | 1 | 0 | 0 | 1 | 51.02 |
| 10 | 1 | 0 | -1 | 0 | 48.12 | 25 | -1 | 0 | 0 | 1 | 96.11 |
| 11 | -1 | 0 | -1 | 0 | 57.09 | 26 | 0 | 0 | -1 | -1 | 36.12 |
| 12 | 0 | 0 | 0 | 0 | 67.19 | 27 | 0 | -1 | 0 | -1 | 46.13 |
| 13 | 0 | 0 | 1 | 1 | 71.23 | 28 | 1 | -1 | 0 | 0 | 58.03 |
| 14 | 1 | 0 | 1 | 0 | 54.02 | 29 | 0 | 0 | 0 | 0 | 67.08 |
| 15 | 0 | -1 | -1 | 0 | 45.29 | | | | | | |

Table 3
Statistical adequacy evaluation of models

| Source | Sequential <i>p</i> -value | Lack of fit <i>p</i> -value | Adjusted R^2 | Predicted R^2 |
|------------------|-------------------------------|--------------------------------|-------------------|--------------------|
| Linear | <0.0001 | 0.0014 | 0.5922 | 0.4743 |
| 2FI | 0.1129 | 0.0020 | 0.6763 | 0.4092 |
| Quadratic | <0.0001 | 0.4056 | 0.9840 | 0.9614 |
| Cubic | 0.1532 | 0.8814 | 0.9910 | 0.9803 |

Fig. 3 illustrates the distribution of the experimental vs. predicted biodegradation of MB by *S. cerevisiae*. There was a strong correlation between the removal values of MB with corresponding predicted values. However, the model efficacy was statistically investigated by ANOVA. The effect of the experimental factors (between -1 and +1 levels) on the removal rate of the pollutant is given in Fig 4.

The findings of ANOVA for 95% confidence interval are described in Table 5. As shown, the *F*-value for the model was 124.6 that confirmed the significance of the model.

The *p*-value for the lack of fit (LOF) test was 0.42, which revealed the fitness of the prediction model. Moreover, the regression R^2 was reasonable, because it was in the range of ± 0.2 to the adjusted correlation coefficient ($R^2_{adj}=0.98$).

The adequacy of the established model for MB removal prediction was verified by all the listed statistical parameters. As seen in Table 5, the *p*-value < 0.0001 implied that the effect of various parameters on the removal of MB was statistically significant for each model. As can be seen, all the independent factors and some first and second-order terms had substantial influences on the response. At the optimum condition, the results of similar experiments indicated high repeatability of the method to predict the actual removal rate of MB (relative deviation less than 2%).

3.1. Main and interaction effects

In this research, the quadratic model was found to be the best model to fit the experimental data with the independent parameters. ANOVA was applied to measure the significance of the model (*p*-values < 0.05). Overall, the findings indicated that this method was significant (*p*-values < 0.0001). Table 3 demonstrates that the values of R^2 , justified R^2 , and adequacy precision were 0.99, 0.98, and 45.81, respectively. The 3D plots clearly showed the possible interaction among the studied factors. Based on Fig 4, it should be noted that to assess the impact of one parameter on the main results, other parameters were fixed at the zero levels. For example, when the factor of pH was increased from level -1 (4) to +1 (10), the other three variables (MB concentrations (55 mg/L), *S. cerevisiae* dose (0.85 g/L), and reaction time (35 min)) were considered at the zero levels.

The findings of Fig. 4a showed that there was an indirect correlation between the removal rate of MB and its initial concentration. For example, with increasing MB value from -1 level to +1 level, its removal rate was decreased to about 19.44% while yeast dose, reaction time, and pH were at the zero level (*p*-value < 0.0001). As seen in Table 2, the maximum removal rate of the pollutant was observed at the initial MB concentration of 10 mg/L. According to the findings, by increasing MB level, the curve slope was decreased. This is due to the fact that the driving force for mass transfer was mostly enhanced with increasing dye levels. At low concentrations, the active sites onto the adsorbent surface are empty. But, the adsorption sites are occupied at higher concentrations of the pollutant and therefore, the removal efficiency was declined [18]. These findings are in agreement with the previous studies [19, 20]. Aksu reported that the maximum bioaccumulation capacity of *S. cerevisiae* was about 88.5, 84.6 and 48.8 mg/g for Remazol Black B, Remazol Blue and Remazol Red RB, respectively [21].

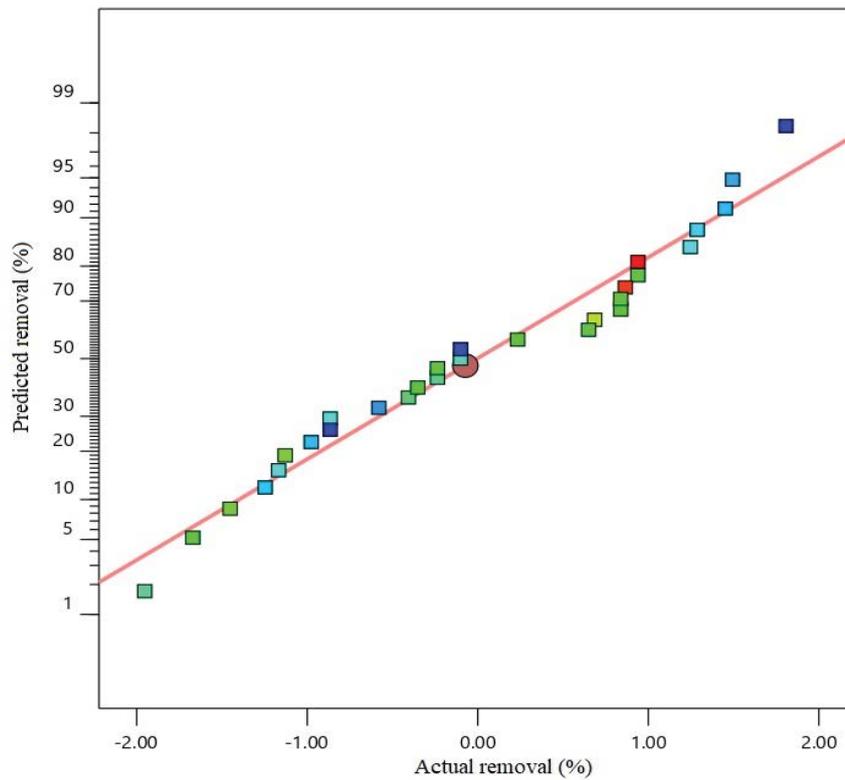


Fig. 3. Distribution of experimental vs. predicted removal for MB biodegradation onto *S. cerevisiae*.

Table 4
Coefficients estimation for quadratic model of MB removal by *S. cerevisiae*

| Factor | Coefficient estimate | df | Standard error | 95% CI low | 95% CI high | VIF |
|----------------|----------------------|----|----------------|------------|-------------|------|
| Intercept | 65.60 | 1 | 0.82 | 63.83 | 67.37 | |
| A-Conc. | -11.42 | 1 | 0.53 | -12.56 | -10.27 | 1 |
| B-Time | -2.58 | 1 | 0.53 | -3.73 | -1.44 | 1 |
| C-pH | 9.83 | 1 | 0.53 | 8.69 | 10.98 | 1 |
| D-Dose | 9.50 | 1 | 0.53 | 8.36 | 10.64 | 1 |
| AB | -4.00 | 1 | 0.92 | -5.98 | -2.02 | 1 |
| AC | -7.50 | 1 | 0.92 | -9.48 | -5.52 | 1 |
| AD | -11.25 | 1 | 0.9237 | -13.23 | -9.27 | 1 |
| BC | 1.75 | 1 | 0.9237 | -0.2312 | 3.73 | 1 |
| BD | 0.5000 | 1 | 0.9237 | -1.48 | 2.48 | 1 |
| CD | 2.75 | 1 | 0.9237 | 0.7688 | 4.73 | 1 |
| A ² | 4.87 | 1 | 0.7254 | 3.31 | 6.42 | 1.08 |
| B ² | -7.13 | 1 | 0.7254 | -8.69 | -5.58 | 1.08 |
| C ² | -7.26 | 1 | 0.7254 | -8.81 | -5.70 | 1.08 |
| D ² | -7.76 | 1 | 0.7254 | -9.31 | -6.20 | 1.08 |

Kumar et al.'s study successfully removed a reactive dye by *Pithophora* sp. [22].

According to Fig. 4a, there was an indirect correlation between the removal rate of MB and reaction time (p -value < 0.0001). The figure indicated that by enhancing the retention time from -1 level to +1 level, the removal rate was enhanced until 6.49%, while other factors were

at the zero level (p -value < 0.0001). This negative impact [Eq. (3)] was seen by the coefficient of -2.59 for the factor of A. Similar results have been reported for the removal of MB by Belala et al. [23] and Abdallah et al. [4].

The results of Fig 4b showed that with enhancing the yeast dose from -1 level to +1 level, the removal rate of the contaminant was increased (p -value < 0.0001). This can be

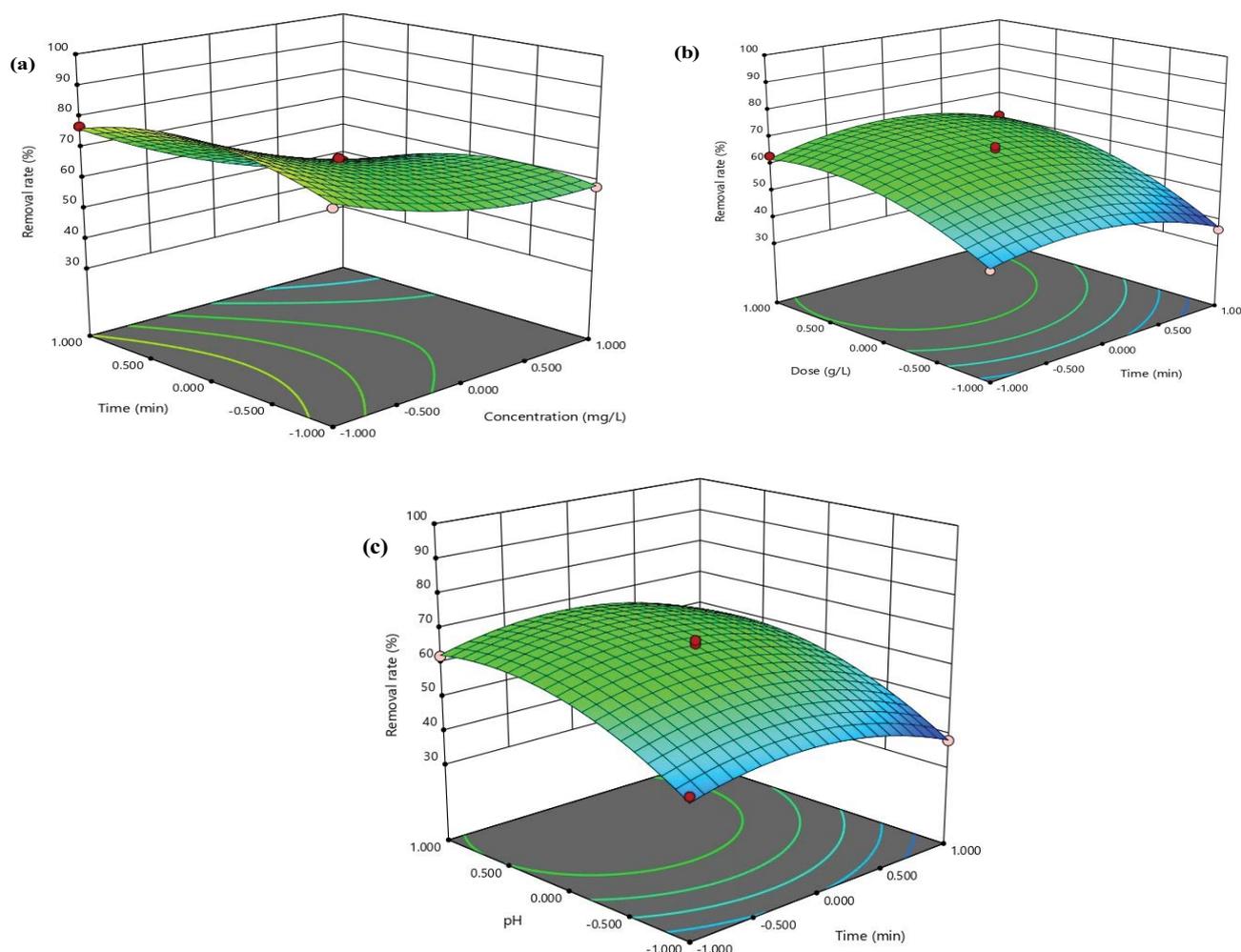


Fig. 4. Response surface plot about the effects of (a) pH vs. time, (b) *S. cerevisiae* vs. time and (c) *S. cerevisiae* vs. MB.

attributed to the increase in the availability of adsorbent sites for the sorption of the pollutant [24].

Solution pH can effect on the surface charge of the yeast and ionization state of MB molecules [25, 26]. The results of Fig. 4c demonstrated that there was a direct correlation between solution pH and MB removal rate. Therefore, the removal efficiency of MB was increased by increasing the solution pH from -1 level to $+1$ level (p -value < 0.0001). Carboxyl, phosphonate and amine groups play important roles in the biological activity of *S. cerevisiae*. In comparison with other groups, amines are more active in absorbing contaminants due to creating positive charges onto the yeast. Carboxyl and phosphonate groups and phosphonate, on the other hand, donate negative charges to the yeast [27].

MB dye gives positively charged ions when dissolved in water. In an acidic solution, the surface charge of the adsorbent is positive and thus, the adsorbent tends to repel the cationic dye. When the solution pH is enhanced, the yeast surface achieves a negative charge, thereby, due to an enhance in the electrostatic attraction between the negatively charged yeast and the positively charged MB, the sorption value is elevated [24,28]. In a study, the highest

removal rate of MB by *Lemna major* was observed at the solution pH of 5 [19]. Koyuncu and Kula [29] reported that the solution pH of 8 was favorable for MB removal using a novel activated carbon obtained from *Pseudevernia furfuracea*. Nayak et al. [30] study also expressed that activated charcoal from the fruit peel of the plant *Hydnocarpus pentandra* could successfully remove MB up to 95%. In a study, the photocatalytic activity of eggshell-based activated carbon for MB resulted in the maximum degradation efficiency of 83% [31].

4. Conclusion

In this paper, *Saccharomyces cerevisiae* was applied for the removal of methylene blue (MB) from the aqueous phase. The process of MB removal by the RSM is modeled and optimized. The findings demonstrated that the values of R^2 , justified R^2 , and adequacy precision were 0.99, 0.98, and 45.81, respectively. The optimum condition in which the maximum MB occurred was determined to be 99.16% at pH 9.35, reaction time of 50.81 min, dye concentration of 14.37 mg/L, and *S. cerevisiae* dose of 1.32 g/L. According to

Table 5
ANOVA for quadratic model of MB removal by *Spirulina platensis*

| Source | Sum of squares | df | Mean square | F-value | p-value |
|-------------------------|----------------|----|--------------------------|---------|---------|
| Model | 5,937.39 | 14 | 424.10 | 124.26 | <0.0001 |
| A-Conc. | 1,564.08 | 1 | 1,564.08 | 458.26 | <0.0001 |
| B-Time | 80.08 | 1 | 80.08 | 23.46 | 0.0003 |
| C-pH | 1,160.33 | 1 | 1,160.33 | 339.97 | <0.0001 |
| D-Dose | 1,083.00 | 1 | 1,083.00 | 317.31 | <0.0001 |
| AB | 64.00 | 1 | 64.00 | 18.75 | 0.0007 |
| AC | 225.00 | 1 | 225.00 | 65.92 | <0.0001 |
| AD | 506.25 | 1 | 506.25 | 148.33 | <0.0001 |
| BC | 12.25 | 1 | 12.25 | 3.59 | 0.0790 |
| BD | 1.0000 | 1 | 1.0000 | 0.2930 | 0.5968 |
| CD | 30.25 | 1 | 30.25 | 8.86 | 0.0100 |
| A ² | 153.63 | 1 | 153.63 | 45.01 | <0.0001 |
| B ² | 330.06 | 1 | 330.06 | 96.70 | <0.0001 |
| C ² | 341.73 | 1 | 341.73 | 100.12 | <0.0001 |
| D ² | 390.43 | 1 | 390.43 | 114.39 | <0.0001 |
| Residual | 47.78 | 14 | 3.41 | | |
| Lack of fit | 36.58 | 10 | 3.66 | 1.31 | 0.4287 |
| Pure error | 11.20 | 4 | 2.80 | | |
| Cor. total | 5,985.17 | 28 | | | |
| R ² | 0.9920 | | Predicted R ² | 0.9619 | |
| Adjusted R ² | 0.9840 | | Adeq. precision | 45.816 | |

the results, the code of A with the coefficient -11.41 was the most important factor that influenced the removal process. The parameter of AD with the coefficient of -11.30 had the maximum interaction impact, and D^2 with the coefficient of -7.76 had the maximum square impacts. The process of dye removal was desired under alkaline conditions. The removal rate of MB had a direct correlation with reaction time and *S. cerevisiae* dose, and an indirect correlation with initial MB concentration and solution pH. It can be stated that *S. cerevisiae*, as a low-cost microorganism, has a high ability for the removal of MB from aqueous solutions.

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