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Application of Face-Centered Central Composite Design (FCCCD) in Optimization of Enzymatic Decolorization of Two Azo Dyes: A Modeling vs. Empirical Comparison

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ABSTRACT

Biological treatment, especially enzymatic methods, can be employed for effective and environmental- friendly treatment of dye effluents. Laccase, belonging to the blue multi-copper oxidases category, can oxidize a wide variety of substrates, especially synthetic dyes. In this study, laccase was used to biodegrade two azo dyes, i.e., Direct Red 23 and Acid Blue 92. Before conducting the experiments, the laccase used in this study was enzymatically characterized. Face-centered central composite design (FCCCD) was used to optimize the main parameters of the decolorization process. The optimum conditions to maximize the bio-decolorization process of AB92 were X_1=11.85 mg L^{-1}, X_2=5.10, and X_3=98.37 mg L^{-1}, with a decolorization extent of 92.30%. These conditions for DR23 were X_1=17.68 mg L^{-1}, X_2=3.70, and X_3=97.89 mg L^{-1}, with a decolorization extent of 95.60%. The optimum conditions for both dyes showed that the enzymatic decolorization favored lower dye concentration. Considering the ANOVA analysis data, the synergetic interactions orders were as follow: AB92: dye concentration-enzyme dosage> dye concentration-pH> enzyme dosage-pH; DR23: dye concentration-pH> dye concentration-enzyme dosage> enzyme dosage-pH. Prog. Color Colorants Coat. 12 (2019), 179-190© Institute for Color Science and Technology.

1. Introduction

The presence of synthetic dyes in aquatic environments, even at minimal quantities, is extremely evident and undesired. Colored wastewaters from textile, paper, etc. industries including synthetic dyes have attracted great consideration due to their toxic effects on living organisms, especially human health, and compatibility with living in aquatic conditions. Moreover, reports have revealed the carcinogenicity and mutagenicity properties of this kind of pollutants [1-5]. The contribution of synthetic dye-containing effluents on global water pollution is up to 20%; hence, their uncontrolled release is one of the greatest threats to the living environments and human health [6]. Consequently, colored wastewaters treatment before its discharge into the recipient waters is inevitable. Currently, the application of physicochemical techniques such as adsorption, advanced oxidation processes (AOPs), coagulation, etc. for treatment of colored wastewaters has not been satisfactory because of the resistant nature of synthetic dyes, the obligation of concentrated formed residue disposal, and the high

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capital cost [3, 7]. Adsorption is one of the most interesting, effective, and practical techniques for dye removal [8]. For instance, reusability, treatment of massive amounts of colored wastewaters, low cost, and avoiding from producing hazardous materials while generating pure effluents, are considered as the main advantages of adsorption processes [8-11]. However, some drawbacks, such as demanding longterm contact times and low yields, has restricted its application. Therefore, the development of effective and eco-friendly treatment methods is inevitable to meet the strict discharging constraints [3].

Impressive biodegradation and discoloration of dye-containing effluents could be achieved using biological treatment, especially enzymatic methods [12]. Biological treatment processes are less expensive, selective, easy to control, and environmentally friendly [6]. Enzymatic bioremediation of colored wastewaters is accompanied with high performance and reaction rate [13]. Some of the other beneficial characteristics of enzymatic treatment are remarkable yield and selectivity, naturally acquired, and catalyzing complex reactions in moderate circumstances [3]. Oxidation, desulfonation, and also the aromatic formations breaking are the possible procedures for enzymatic biodegradation of resistant dyes [14]. Toxic dyes are converted or degraded into non-toxic or harmless outputs using enzymatic decolorization procedures; hence, avoids the production of subsequent pollution [6]. Various enzymes including manganese peroxidase, horseradish peroxidase, and laccase have employed for this purpose [15].

Laccase (E.C.1.10.3.2., oxygen oxidoreductase), belonging to the multi-copper blue oxidases category, can catalyze the one-electron oxidation of various polyphenolic and nonphenolic compounds, including synthetic dyes, and commonly can be found in plants, insects, and bacteria [15, 16]. However, the usage of laccase for direct oxidation of several substrates with high redox potential is not feasible [16]. Because of great non-specific catalytic capacity, laccase has a prominent potential for industrial and environmental purposes [7]. Bioremediation, detoxification of different xenobiotics, and textile and paper pulp bio-bleaching are some of the possible applications of this enzyme [17, 18]. Within the oxidation reaction, laccase reduces molecular oxygen, as the terminal electron receiver, which represents a green and environmental- friendly technique for colored wastewater treatment [19].

The efficient enzymatic decolorization process is usually affected with various parameters. One of the fundamental optimization techniques is the one factor at a time, which takes too much time and is effortful and imperfect [20]. The application of Response Surface Methodology (RSM) can overcome these shortcomings [21]. RSM is an efficient tool for investigating the impacts of various independent factors on the dependent variable(s) using a collection of mathematical and statistical methods [22]. Furthermore, the adoption of suitable methodology in the RSM has a controlling impact on response and the accuracy of the empirical prediction [6]. The application of face-centered central composite design (FCCCD), as an ideal, reliable, and standard design, on the biodegradation of colored wastewaters is not mature enough [23].

In this study, laccase from genetically modified *Aspergillus* was employed for biodegradation of two potentially toxic dyes, i.e., Direct Red 23 (DR23) and Acid Blue 92 (AB92). Reports have recorded the carcinogenicity, toxicity, slowly biodegradation, and broad application of DR23 and AB92 in the textile industries [24, 25]. The decolorization experiments were designed using face-centered central composite design (FCCCD) based on response surface methodology (RSM). Central Composite Design (CCD), also called Box-Wilson design, promotes the highest data achievement concerning a process by conducting a minimum quantity of trials [26]. To the best our knowledge, this is the first report on making a comparison between enzymatic biodegradation of two azo dyes using FCCCD.

2. Materials and Methods

2.1. Materials

Laccase (14.2 U/mg) from genetically modified Aspergillus and 2,2′-Azino-bis (3- ethylbenzthiazoline-6-sulfonic acid) (ABTS) were obtained from Novo Nordisk and Sigma-Aldrich (St. Louis, MO, USA), respectively. Azo dyes, i.e., DR23 and AB92, used in this research and were purchased from Alvan Paint & Resin production. All other chemicals were of analytical grade and used without any purification.

2.2. Methods

2.2.1. Standard Enzymatic Evaluations

The protein concentrations were specified using Bradford's method [27]. The activity of the free laccase

was spectrophotometrically estimated by the assessment of absorbance increase at 418 nm for 90 seconds chasing the generation of $ABTS^+$ at room temperature (ε=36000 $M^{-1}cm^{-1}$). A 5 mM solution of ABTS, in 100 mM sodium acetate buffer at pH 5 formed the reaction mixture. Then, 200 µL of the enzyme solution (0.1 g L^{-1}) was added to the 1.8 mL of ABTS to start the enzymatic reaction. One unit of laccase activity (U) is the quantity of the laccase which can oxidize 1 µmol ABTS per minute.

The Michaelis-Menten kinetic parameters values of ABTS were assessed through the evaluation of laccase activity using different ABTS concentrations ranging from 0.1- 6.0 mM in sodium acetate buffer (100 mM, pH 5) at room temperature. Moreover, the kinetic parameters (K_m and V_{max}) were calculated employing the non-linear regression (enzymatic reaction rate versus substrate concentration) analysis, the Lineweaver–Burk plot [28].

2.2.2. Face-centered Central Composite Design (FCCCD)

Using CCD, also named Box-Wilson design, makes possible the maximum data attainment regarding a process by managing a minimum number of experiments [26]. In this study, FCCCD was used to determine the optimal circumstances of the main variables affecting the extent of enzymatic decolorization (the response parameter), including dye concentration (X_1: 10-50 mg L^{-1}), pH (X_2: 3.6±0.1-4.6±0.1), and enzyme dosage (X_3: 30-100 mg L^{-1}). After conducting initial examinations for determination of the range of the variables, the experiments were established using FCCCD with three elements at three levels. Table 1 shows the designated (X_1, X_2, and X_3) and coded values (-1, 0, and +1) of the independent parameters at three levels, which is the same for both of dyes. Parameters were coded using the following relation [29]:

$$\varphi_k = \frac{X_k - X_0}{\Delta X_k} \qquad k = 1,2,3,\ldots,n \tag{1}$$

in which the independent factor actual value, the central point of the independent factor actual value, the corresponding step variation of the independent factor actual value, and the dimensionless value of the independent factor are represented by X_k, X_0, ΔX_k, and φ_k, respectively [30]. In this survey, ±α=±1, which means the location of the star points at the center of each factorial space face.

By stating of n and n_c as the number of independent factors and the number of center point tests, the whole number of the needed experiments, N, was calculated using the following relation [31]:

$$N = 2^n + 2n + n_c \tag{2}$$

Accordingly, the total number of the decolorization experiments for each dye was equal to 20; 8 factorial points, 6 star points, and 6 center point tests for the absolute error calculation. The random sequencing of the experiments was employed to prevent the influence of the unexpected variations of the decolorization extent (response factor). Implementing a general model to the data achieved from FCCCD (three factors at three level) resulted in obtaining the optimal conditions of the decolorization process.

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^{\,2} + \sum_i \sum_{<j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \tag{3}$$

Table 1: FCCCD Design Matrix.

Factors	Levels				
	Low (-1)	Central (0)	High (+1)	-α	+ α
X_1: Dye Concentration (mg L^{-1})	10	30	50	10	50
X_2: pH	3.6	4.6	5.6	3.6	5.6
X_3: Enzyme Dosage (mg L^{-1})	30	65	100	30	100

In the above-mentioned equation, the response; the independent parameters; the intercept, linear, quadratic, and interaction terms (the regression coefficients of the model); the number of the independent parameters, here k=3; and the residual, are designated by Y; X_i, X_j; β_0,β_j, β_{jj}, β_{ij}; k; and ε, respectively [29].

Design-Expert Statistical Software package 7.0.0 trial version (Stat Ease Inc., Minneapolis, USA) was employed to process the FCCCD-achieved data. The experimental data were investigated by the least squares method. The ordinal total summation of the squares and a review of the model statistics were applied to obtain the adequacy of different models. Various proposed models, such as linear, quadratic, and interaction were examined using the analysis of variance (ANOVA) by analyzing the corresponding acquired regression coefficients. The coefficient of determination (R^2), adjusted coefficient of determination ($R^2_{adj.}$), and the model lack of fit examination were employed to find the sufficiency of the achived models. Moreover, F-test at probability levels ($p \leq 0.05$) was exploited to determine the statistically valid terms of the model.

2.2.3. Enzymatic Decolorization Experiments

Enzymatic decolorization processes were conducted in a 250 mL vessel (a reaction capacity of 150 mL) containing 100 mM sodium acetate buffer, employing a magnetic heater stirrer at 45 ºC. The addition of the laccase initiated the decolorization reaction (t=0), and samples were collected every 10 minutes from zero to 60 minutes. A double-beam spectrophotometer was utilized to analyze the dye concentration in each sample, and hence, the overall decolorization yield (Y):

$$Y(\%) = \frac{A_i - A_f}{A_i} \times 100 \quad (4)$$

where, A_i and A_f are initial (t=0) and final (t=60) absorbances of each experiment.

3. Results and discussion

3.1. Enzyme Characterization

3.1.1. The Influence of pH on Laccase Activity

Before conducting any enzymatic decolorization processes, it is crucial to find the optimal range of pH, in which laccase has the highest activity. Figure 1 represents the relative activity of the laccase over a range of pH of 3.0 to 8.0, measured at room temperature. Over this operational pH range, relative activity increases by increasing the pH value and then decreases. Therefore, the optimal pH value is pH 5.0 and laccase have higher activity at acidic to neutral pH values. Moreover, the reduction of relative activity is more drastic in more acidic medium. In general, fungal-based laccases express superior stability in acidic medium [32].

3.1.2. Enzymatic Kinetic Studies

Michaelis-Menten model kinetic parameters, K_m and V_{max}, were specified at optimum pH value (room temperature). Table 2 presents these parameters. The calculated K_m and V_{max} values of the enzyme are of 0.71 mM and 62.11 µM min^{-1}. K_m expresses the association of the enzyme to its substrate; the higher value of K_m is corresponding to the lower affinity [33, 34]. Turnover number, k_{cat}, remarks the efficient first-order breakdown reaction of the enzyme-substrate compound [33]. Besides, the catalytic yield of the enzyme is indicated by k_{cat}/K_m factor.

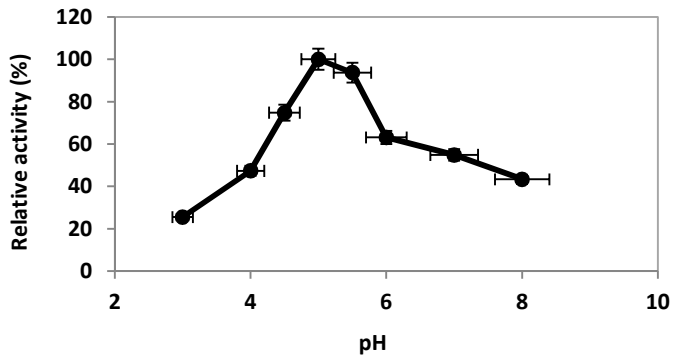

Figure 1: Relative activity of free laccase at different pH values (at 25 ºC).

3.2. Enzymatic Decolorization of AB92 and DR23

3.2.1. The Utilization of FCCCD for Statistical analysis and Process Optimization

The laccase potential in bio-decolorization of synthetic dyes has been approved [14]. In this survey, free laccase was applied for biodegradation of two azo dyes (goal contaminants: AB92 and DR23). The effect of the main independent factors (dye concentration, pH, and enzyme dosage) affecting the response parameter (decolorization yield) was explored by employing FCCCD; resulted in the specification of the optimum process operating conditions and their interactions. By adopting FCCCD and considering 3 parameters at 3 levels, a total number of 20 decolorization tests were established for each dye. Table 3 outlines the empirical, predicted, and the residual values of each enzymatic decolorization experiments for both of dyes.

Table 2: Michaelis-Menten model kinetic parameters.

Enzyme	K_m (mM)	V_{max} (μM min^{-1})	k_{cat} (μmol s^{-1} g^{-1})	k_{cat}/K_m (L s^{-1} g^{-1})
Free laccase	0.71±0.06	62.11±3.8	103.52±4.4	0.14±0.01

Table 3: FCCCD experimental design versus empirical results.

Run	Dye Conc. (mg/L) (X_1)	pH (X_2)	Enzyme Dosage (mg/L) (X_3)	Decolorization (%)					
				Actual (Y)		Predicted (Y')		Residual (Y-Y')	
				AB92	DR23	AB92	DR23	AB92	DR23
1	50	4.6	65	58.52	42.96	63.43	41.59	-4.91	1.37
2	50	5.6	100	68.67	77.37	66.28	75.31	2.39	2.06
3	10	4.6	65	80.52	46.23	76.08	45.77	4.44	0.46
4	30	4.6	65	71.9	66.28	69.75	65.57	2.15	0.71
5	30	4.6	65	68.77	65.8	69.75	65.57	-0.98	0.23
6	10	3.6	30	45.35	50.69	48.77	53.21	-3.42	-2.52
7	50	3.6	30	46.9	40.12	43.99	39.24	2.91	0.88
8	30	3.6	65	62.73	77.04	61.20	75.28	1.53	1.76
9	50	5.6	30	52.03	60.52	50.09	62.85	1.94	-2.33
10	10	5.6	100	82.87	68.73	86.81	69.71	-3.94	-0.98
11	10	5.6	30	47.31	43.5	43.72	41.97	3.59	1.53
12	30	4.6	65	65.34	56.98	69.75	65.57	-4.41	-8.59
13	30	4.6	65	66.56	67.87	69.75	65.57	-3.19	2.30
14	30	4.6	65	74.37	67.04	69.75	65.57	4.62	1.47
15	30	4.6	30	51.65	63.7	54.93	61.26	-3.28	2.44
16	10	3.6	100	92.18	94.25	91.86	92.38	0.32	1.87
17	50	3.6	100	58.85	61.14	60.18	63.13	-1.33	-1.99
18	30	4.6	100	88.86	86.46	84.57	87.07	4.29	-0.61
19	30	4.6	65	71.04	65.77	69.75	65.57	1.29	0.20
20	30	5.6	65	57.74	75.82	61.72	75.75	-3.98	0.07

FCCCD acquired the decolorization predicted values through a second-order correlation (quadratic polynomial) between independent and the response variables. Equations (5 and 6) expresses these relations.

$$Y\,(AB92) = -124.40 - 0.33X_1 + 72.35\,X_2 + 0.71X_3 + 0.14\,X_1X_2 - 0.01X_1X_2 - 8.30X_2^2 \quad (5)$$

$$Y\,(DR23) = +259.61 + 1.53X_1 - 99.04X_2 - 0.004X_3 + 0.44X_1X_2 - 0.005X_1X_3 - 0.08X_2X_3 - 0.05X_1^2 + 9.95X_2^2 + 0.007X_3^2 \quad (6)$$

The coefficients of the proposed regression models supply the estimation of the relative contribution of every parameter on the response extent. Each coefficient of the linear term with a positive sign designates its relative positive impact on the efficient enzymatic biodegradation of the azo dyes.

Table 4 and 5 indicate the ANOVA data for both of the second-order regression models, considering the biodegradation of AB92 and DR23. F-value and p-value affirm the compatibility of the model [35].

The model F-values for AB92 and DR23 were 34.83 and 35.02, respectively, which implies the significance of both models. There is only a 0.01% chance that a “Model F-value” this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The significant model terms for AB92 were X_1, X_3, X_1X_3, and X_2^2. While, X_3, X_1X_2, X_1X_3, X_2X_3, X_1^2, X_2^2, and X_3^2 were the significant model terms for DR23. Values greater than 0.1000 indicate the model terms are not significant. It is worth mentioning that the reason to maintain the not significant terms of the models is to track the straight impact of single parameters or to visualize the effect of interactive parameters, even though they have not remarkable impact on the predicted values of the models. Also, the "Lack of Fit F-value" were 1.62 and 0.53 for AB92 and DR23, respectively, which implied the Lack of Fit is not significant relative to the pure error. There is a 30.78% and 74.87% chance, for AB92 and DR23 in order, that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good; we want the model to fit. Moreover, the "Predicted R^2" of 0.8032 and 0.7945 were in reasonable agreement with the "Adjusted R^2" of 0.9144 and 0.9416 for biodegradation of AB92 and DR23, respectively. A suitable value of R^2 shows the high potential of the established regression model in the defined experimental domain [14]. Based on the obtained correlation coefficient values (0.9414 for AB92and 0.9692 for DR23), the established regression models are not able to describe only 5.86% and 3.08% of the total variation [1].

Table 4: The ANOVA Data of AB92 Biodegradation.

Source	Sum of Squares	Degree of Freedom	Mean Square	F-Value	p-value Prob > F	F-Value Assessment
Model	3364.48	6	560.75	34.83	< 0.0001	significant
X_1	400.18	1	400.18	24.86	0.0002	-
X_2	0.68	1	0.68	0.04	0.8402	-
X_3	2196.03	1	2196.03	136.40	< 0.0001	-
X_1X_2	62.16	1	62.16	3.86	0.0712	-
X_1X_3	361.81	1	361.81	22.47	0.0004	-
X_2^2	343.62	1	343.62	21.34	0.0005	-
Residual	209.29	13	16.10	-	-	-
Lack of Fit	151.12	8	18.89	1.62	0.3078	not significant
Pure Error	58.17	5	11.63	-	-	-
Cor Total	3573.77	19	-	-	-	-
Std. Dev.	4.01	-	R^2	0.9414	-	-
Mean	65.61	-	*Adjusted* R^2	0.9144	-	-
C.V. %	6.12	-	*Predicted* R^2	0.8032	-	-
PRESS	703.27	-	*Adequate Precision*	20.280	-	-

Table 5: The ANOVA Data of DR23 Biodegradation.

Source	Sum of Squares	Degree of freedom	Mean Square	F-Value	p- value Prob > F	F-Value Assessment
Model	3836.72	9	426.30	35.02	< 0.0001	significant
X_1	43.81	1	43.81	3.60	0.0871	-
X_2	0.55	1	0.55	0.04	0.8363	-
X_3	1665.65	1	1665.65	136.81	< 0.0001	-
X_1X_2	607.26	1	607.26	49.88	< 0.0001	-
X_1X_3	116.74	1	116.74	9.59	0.0113	-
X_2X_3	65.32	1	65.32	5.37	0.0430	-
X_1^2	1317.45	1	1317.45	108.21	< 0.0001	-
X_2^2	272.11	1	272.11	22.35	0.0008	-
X_3^2	203.26	1	203.26	16.70	0.0022	-
Residual	121.74	10	12.17	-	-	-
Lack of Fit	42.17	5	8.43	0.53	0.7487	not significant
Pure Error	79.58	5	15.92	-	-	-
Cor Total	3958.47	19	-	-	-	-
Std. Dev.	3.49	-	R^2	0.9692	-	-
Mean	63.90	-	*Adjusted* R^2	0.9416	-	-
C.V. %	5.46	-	*Predicted* R^2	0.7945	-	-
PRESS	813.63	-	*Adequate Precision*	21.537	-	-

"Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratios of 20.280 and 21.537 indicated an adequate signal. Therefore, this model can be used to navigate the design space [36]. The coefficients of variation (*C. V.*) of the enzymatic decolorization experiments were 6.12% and 5.46% for AB92 and DR23, respectively. These relatively low quantities represent the delicacy and reliability of the conducted empirical values [37]. Moreover, the depiction of the predicted versus actual decolorization values for each dye confirms the conformity of the regression model with empirical results (Figure 2).

Desirability function value equal to 1.0 results in optimal conditions as well as the maximum decolorization yield [1]. The optimum conditions to maximize the bio-decolorization process of AB92 were X_1=11.85 mg L^{-1}, X_2=5.10, and X_3=98.37 mg L^{-1}, with a decolorization extent of 92.30% (Figure 3 (a)). These conditions for DR23 were X_1=17.68 mg L^{-1}, X_2=3.70, and X_3=97.89 mg L^{-1}, with a decolorization extent of 95.60% (Figure 3 (b)). It can be seen that laccase shows a higher affinity towards DR23, which can be assigned to the lower redox potential of DR23 and the limited accessibility of the enzyme the hydroxyl groups in AB92 [38, 39].

The optimum conditions for both dyes showed that the enzymatic decolorization favored lower dye concentration. Reports claimed that laccase poisoning or passing the enzyme-dye saturation point could be considered as the possible reasons [40, 41]. Furthermore, decolorization yield is higher in more acidic medium. It proves the strong effect of pH on enzymatic decolorization process, which is related to the attachment of hydroxly anions to the copper ions located at the active site of the enzyme and hindering the internal electron transfer of laccase [42]. As mentioned before, laccase shows higher affinity to DR23, as the substrate, than AB92. Using the regression models and setting the decolorization yield to 75% and dye concentration to 30 mg L^{-1} (at their corresponding optimum pH value) can verify this claim. Figure 4 indicates that for purpose AB92 requires more enzyme dosage.

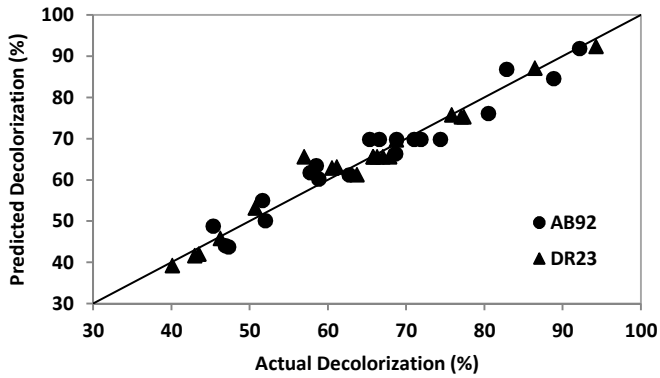

Figure 2: The Predicted to Actual decolorization extents (%) for AB92 and DR23.

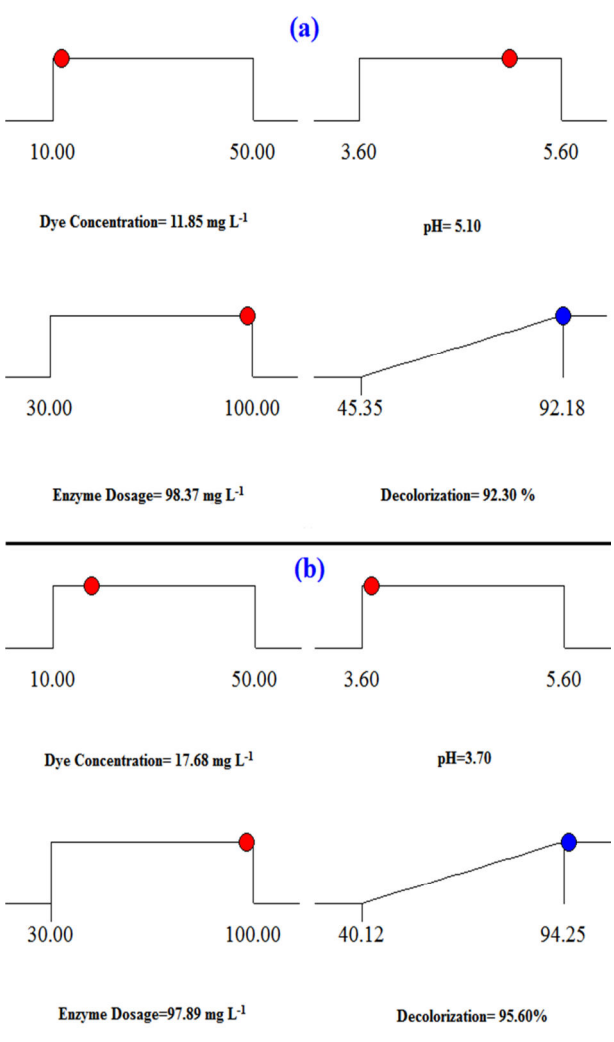

Figure 3: The optimum conditions for biodegradation of: (a) AB92, (b) DR23 at their related optimum conditions.

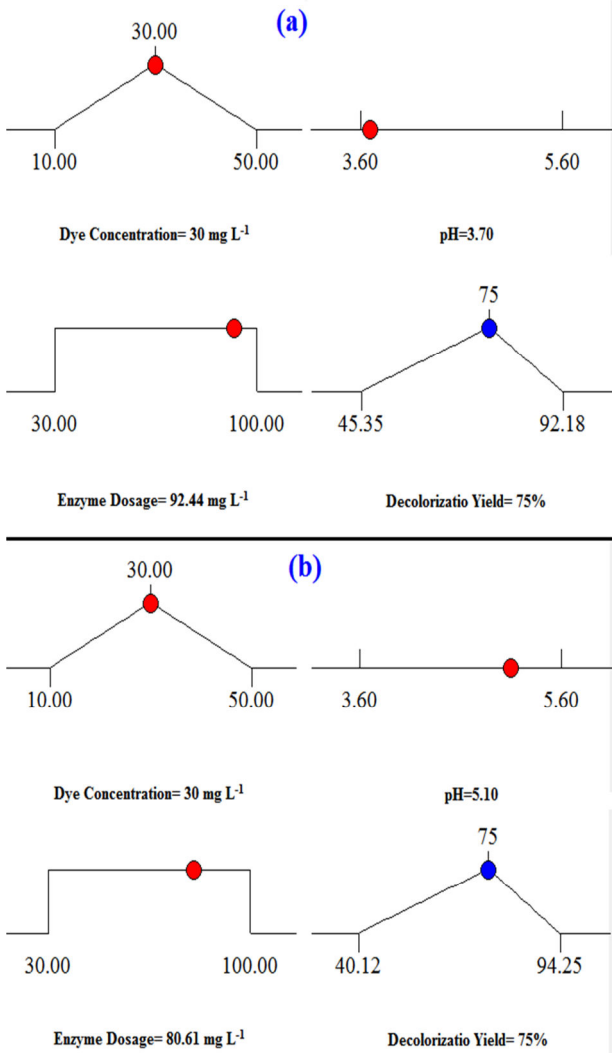

Figure 4: The identification of enzyme affinity towards different substrates: (a) AB92, (b) DR23.

3.2.2. The Synergetic Effects of the Parameters on Enzymatic Decolorization Yield

Figure 5 and 6 illustrate 3D response surface plots (3D-RSPs) along with counter schemes (CS) of the synergetic impact of independents factors for enzymatic decolorization of AB92 and DR23, which provides a predictor technique for the effect of the individual factors and, more importantly, the type of their synergetic interactions on the response parameter [6]. Each couple of the illustrations (3D-RSPs and CSs) was generated by changing two parameters, while the third one is preserved constantly at the central value. Considering the ANOVA analysis data, the synergetic interactions orders are as follow:

AB92: dye concentration – enzyme dosage> dye concentration – pH> enzyme dosage – pH

DR23: dye concentration – pH > dye concentration – enzyme dosage > enzyme dosage – pH

In the case of DR23, dye concentration-pH synergetic effect is of great importance. By increasing the substrate concentration (dye), higher decolorization yields are achievable in lower pH values and vice versa. In both situations (AB92 and DR23), the synergetic interaction effect of enzyme dosage and pH has the lowest significance level, which indicates that enzyme dosage is less affected by pH. Furthermore, for both of dyes, enzyme dosage plays a fundamental role in high decolorization yields, by providing more enzyme active sites [6, 40, 41]. Also, more quantities of the enzyme certify the enzyme-substrate equilibrium [43].

According to Figure 5 and 6, the highest decolorization yield, in both cases, occurs in a moderately acidic environment, as mentioned in the section of finding the optimum decolorization yield conditions. One of the possibilities is that in more acidic mediums the hydrogen ions (H^+) are abstained from the enzyme, due to the ionic attraction of H^+ to anionic dyes. Moreover, a more appropriate open configuration of the enzyme could take place in an acidic environment [32].

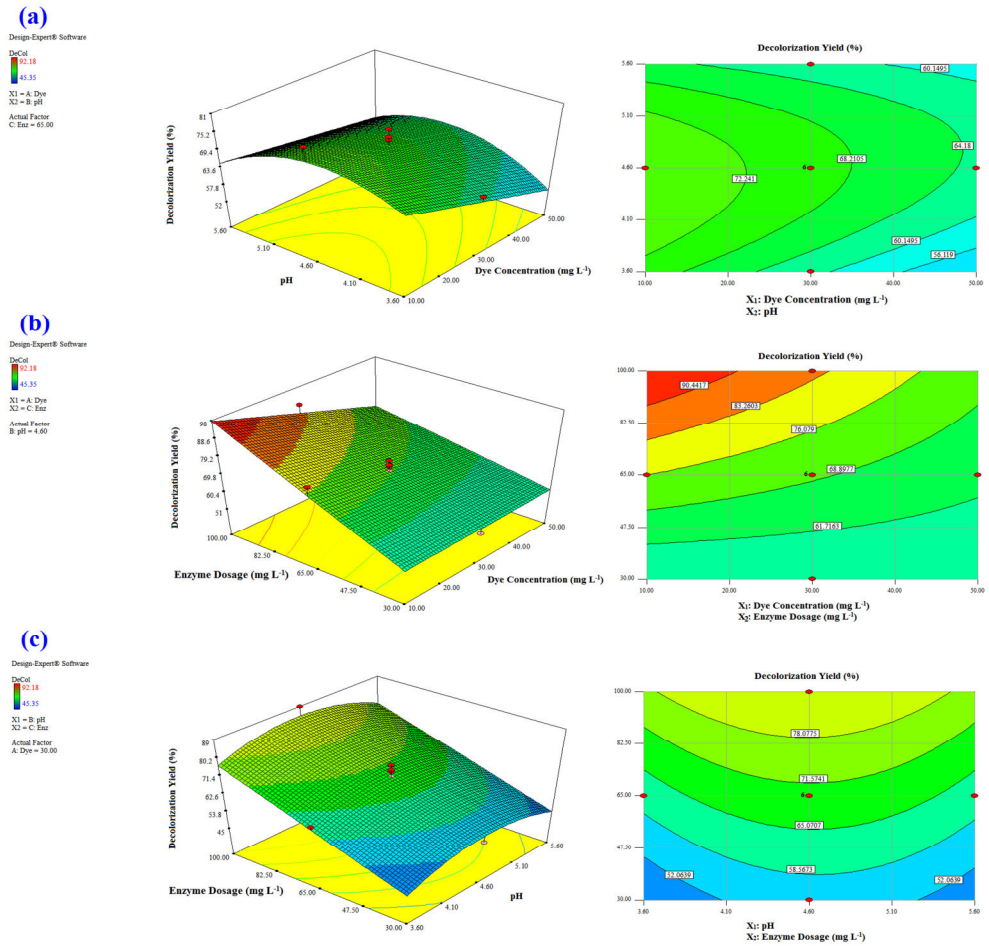

Figure 5: 3D response surface plots along with counter schemes of the synergetic factors for enzymatic decolorization of AB92: (a) dye concentration- pH, (b) dye concentration- enzyme dosage, and (c) pH- enzyme dosage.

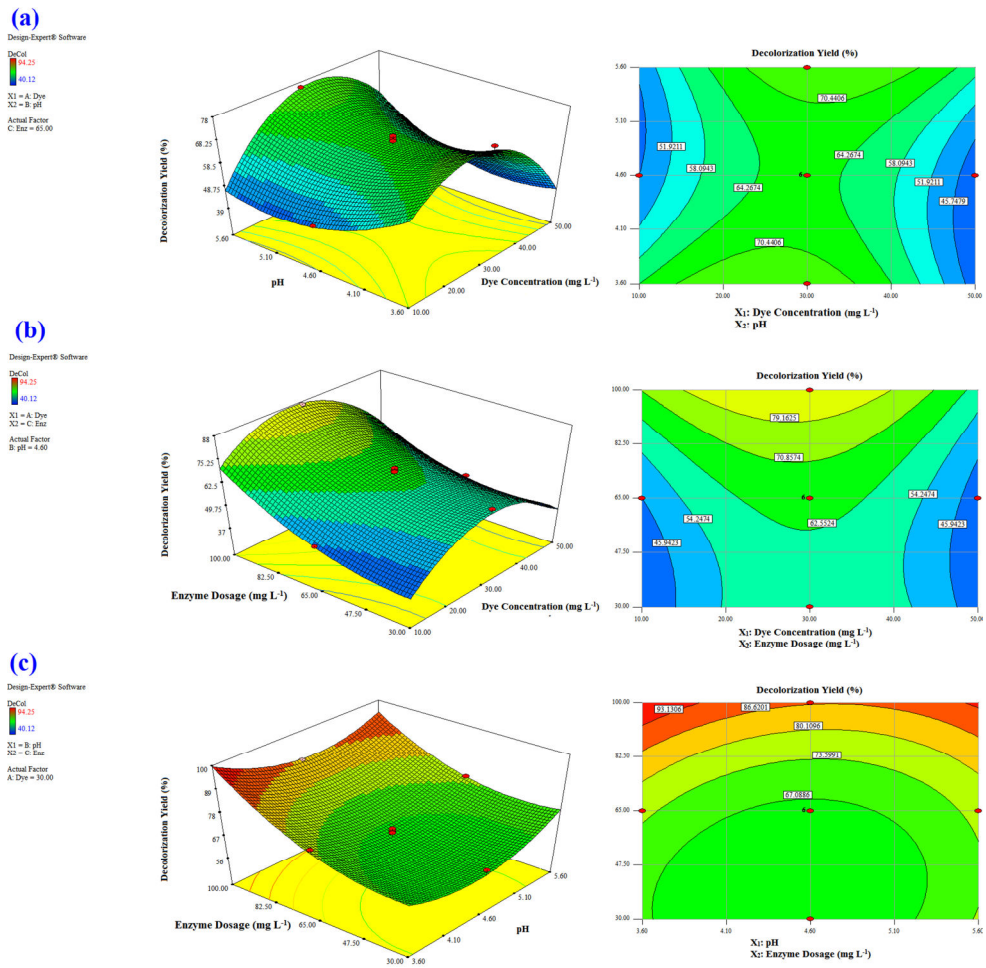

Figure 6: 3D response surface plots along with counter schemes of the synergetic factors for enzymatic decolorization of DR23: (a) dye concentration- pH, (b) dye concentration- enzyme dosage, and (c) pH- enzyme dosage.

Briefly, ANOVA analysis and 3D-RSPs are helpful tools in the extraction of the maximum data from a set of empirical experiments. In this survey, the importance order of the independent parameters, in their related experimental range, is as follows for enzymatic biodegradation of both of dyes: Enzyme dosage > dye concentration > pH. It means that high decolorization yield could be achieved in high enzyme dosage, low dye concentration, and acidic pH value.

4. Conclusions

Enzymatic decolorization of two recalcitrant azo dyes was optimized using FCCCD. The obtained models showed excellent agreement with empirical results for both of dyes. ANOVA analysis and 3D-RSPs are helpful tools in the extraction of the maximum data from a set of empirical experiments. The optimum conditions to maximize the bio-decolorization process of AB92 were X_1=11.85, X_2=5.10, and X_3=98.37 mg L^{-1}, with a decolorization extent of 92.30%. These conditions for DR23 were X_1=17.68, X_2=3.70, and X_3=97.89 mg L^{-1}, with a decolorization extent of 95.60%. The optimum conditions for both dyes showed that the enzymatic decolorization favored lower dye concentration. Laccase shows higher affinity to DR23, as the substrate, than AB92. The importance order of the independent parameters, in their related experimental range, is as follows for enzymatic biodegradation of both of dyes: Enzyme dosage > dye concentration > pH. Based on the optimum operational conditions obtained for both of dyes, high decolorization yield could be achieved in high enzyme dosage, low dye concentration, and acidic pH value.

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