*Algerian Journal of Environmental Science and Technology*
*September edition. Vol.7. N°3. (2021)*
*ISSN : 2437-1114*
*www.aljest.org*

# The use of alternative sustainable biomaterials for dye removal and chemical oxygen demand reduction by a bio coagulation/ bio flocculation process Optimization through response surface methodology

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## ARTICLE INFO

***Article History:***

*Received* ***: 10/03/2020***
*Accepted* ***: 11/10/2020***

***Key Words*:**

*Bio coagulation;*
*Bio flocculation;*
*Dye;*
*COD;*
*Performance.*

## ABSTRACT/RESUME

***Abstract:*** *In this study, the performance of a new bio coagulant (the carob) and a bio flocculent (cactus) was investigated for methylene blue dye and chemical oxygen demand removal from aqueous solution. For this purpose, response surface methodology (RSM) was applied to optimize four operating variables of coagulation/flocculation process which are: coagulant dosage, flocculent dose, initial pH and initial dye concentration. The results showed that the decrease of coagulant dose was always beneficial for enhancing dye removal. The optimum carob dosage, cactus dosage, initial pH and initial dye concentration were found to be 830mg/L, 64mg/L, 2 and 40mg/L, respectively. Dye removal of 94.63% and COD reduction of 74.12% were observed and confirm close to RSM results. It can be concluded that using the carob as a low-cost material in the coagulation/flocculation process for dye and COD elimination can offer some advantages such as high treatment efficiency and economic savings on overall treatment plant operation costs.*

## I. Introduction

Water is the principal constituent of all living organisms, however this precious resource is being menaced by climate change and human actions [1], [2]; especially the rapid growth in industrialization and urbanization. Industrial activity is accountable for generating a large volume of hazardous effluents. Dye is one of the most important hazards in industrial sector [3]. It is widely used in the production processes of the paint, textiles, rubber, leather, paper, plastics, printing, cosmetics, mineral processing, pulp mill and pharmaceutical industries that require large quantities of water and, in turn, generate a high volume of wastewater with varying physicochemical characteristics [4]. In most developing countries of the world, industrial effluents are generally discharged significant quantities in the adjacent river or land. These effluents contain a number of substances that are harmful to the environment. As result, it can create heavy pollution from effluents containing high levels of salinity, organic loading, dissolved matter and suspended solids and inorganic matter. Such effluents are also reported as potential sources of mutagenic and carcinogenic substances [5, 6].

Methylene blue (MB), chemically known as 3.7 bis (dimethylamino) phenothiazine-5-ium chloride, is a cationic dye and one of the most dyes found in the waters discharged by industrials activities and histologic microbiologic. Although MB is seen in some medical uses in high quantities, it can also be usually used in coloring paper, dyeing, coating for paper stocks, rubber, plastics, leather, cosmetics … etc. Severe toxicity is imposed by this pollutant: premature Neonate toxicity and central nervous system toxicity causing gastritis, vomiting, diarrhea, headache, etc., either by direct contact or ingestion [7-9]. For that, Legal restrictions in organized industrial zone and environment conservation make it obligatory that the effluent is treated before its discharge into the environment because of their biodegradation difficulty when they are directly released [10]. Most countries have determined their requirements in terms of pH value, sulfide, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total chromium, oil, etc. Furthermore, few countries have also stated the limits for nitrogen produced from ammonia, an important toxic product of wastewater [11].

Numerous methods have been proposed to remove color from dye wastewater such as physical–chemical treatment, bio-oxidation, biological treatment, activated sludge treatment, microfiltration, coagulation–flocculation, Fenton oxidation, adsorption, electrochemical oxidation and electro-coagulation. Among these, coagulation/flocculation has historically attracted a considerable attention for its high removal efficiency [12].

Coagulation is a widely practiced process due to its relatively simple operation and low cost [13]. In coagulation process; stable colloidal particles are destabilized, such that they can agglomerate into settleable flocs. It can be accomplished by inorganic coagulant or organic polymers, and both have been succeeded in dye removal. Conventional coagulants in waste water treatment are alum, Ferric chloride, sodium aluminate, aluminum chloride and ferric [14], they showed high performance in the process of destabilizing the suspended matter and colloids but they have some inconvenient; such as the production of large amounts of non-biodegradable sludge and high level of toxicity [15-17]. Mishra and Bajpai also have pointed out that excessive polymer used might be toxic to bioassay test organisms [18]. Synthetic coagulants are basically salts of a strong acid (e.g., HCl or $H_2SO_4$) and a low base $Al_2(OH)_3$ or $Fe(OH)_3$), they are a mixture of cation (from base) and anion (from acid). However, recent research has reported several serious drawbacks of employing aluminum salts, such Alzheimer's disease. There is also the problem of alum reaction with natural alkalinity present in the water leading to a reduction of pH [19- 21].

Recently, Industrialization and sustainable development are the essential needs of developing as well as developed countries , for that reason, substitution of conventional coagulants by bio materials is a main strategy of sustainable development and a potential alternative to overcome the principal disadvantage of coagulation process using synthetic coagulants[22]. These bio materials (bio coagulant/bio flocculent) are biodegradable, non-toxic, produce very little residual sludge and respect the roles of green chemistry [23, 24]. Various research has showed the turbidity removal efficiency in different types of water, using Moringa oleifera, gaur, mesquite seed gum okra, bacteria isolates and Plantago psyllium [25- 28].

Cactus (Opuntia spp) has proved its competence in the coagulation process of drinking water treatment and wastewater treatment including heavy metals, dyes and organic materials elimination from aqueous environment. It has the ability to remove bacteria [29-34].

The optimization of the significant parameters in the coagulation/flocculation process through the classical method is based on varying a single factor while keeping all others constant at a specific set of conditions. This is extremely time-consuming (it requires many experiments runs), expensive, unlikely to reach the true optimum due to the neglected interactions between variables [35- 37]. These limitations of the conventional method can be overcome by applying statistical experimental design techniques using the response surface methodology (RSM). RSM uses an experimental design such as the central composite design (CCD) to fit a model by least squares technique. Adequacy of the proposed model is then revealed using the diagnostic checking tests provided by analysis of variance (ANOVA) [38, 40].

The focus of the present work was to assess the potentiality of the dried carob as a bio coagulant and the dried prickly pear cactus cladodes as a bio flocculent for the treatment of synthetic dye solution (methylene blue) using coagulation/flocculation process, which was optimized applying RSM/CCD approach. The influence of factors such as carob dosage, cactus dosage, initial pH and initial dye concentration was investigated in terms of dye and chemical oxygen demand (COD) removal efficiencies.

## II. Materials and methods

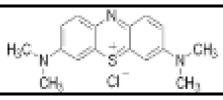
### II.1. Material

Methylene Blue (Analytical Reagents) was taken as a pollutant model. Its molecular structure is given in table 1. Stock solution was prepared by dissolving requisite quantity of dye without further purification in distilled water, and the concentrations used were obtained by dilution of the stock solution. The residual concentration was measured by UV–Visible spectrophotometry for dye concentration at the wavelength of 660 nm (UV–VIS spectrophotometer Shimadzu UV-160 A.

Biomaterials cactus and carob were collected from Constantine, a region in the northeast of Algeria. They were repeatedly washed with distilled water to remove dirt particles. Carob powder was prepared by drying carob at 48°C for 24 h then removing seeds manually. After that, the wings were ground in a coffee grinder and sieved to get particles of a size of $63.10^{-6}$m. Cactus powder was prepared by cutting fresh cactus *species (pads) into* strips of $10^{-2}$ m width followed by drying at 48°C for 24 h, then ground and sieved.

***Algerian Journal of Environmental Science and Technology***
***September edition. Vol.7. N°3. (2021)***
***ISSN : 2437-1114***
***www.aljest.org***

***Table 1.*** *Physicochemical characteristics of methylene blue.*

| Name | Molecular structure | $M_w$ (g/mol) | $\lambda_{max}$ (nm) |
|---|---|---|---|
| Methylene Blue |  | 319.85 | 660 |

### II.2. Jar Test

Solution pH was adjusted according to pH levels in table 2 before dosing the coagulant. The jar test was used to carry out the coagulation/flocculation process using 1 L beaker jars, with six paddle stirrers (Phipps and Bird model). The time and the speed for rapid and slow mixing were set with an automatic controller. Samples were mixed at 250 rpm for 5 min after coagulant addition to provide coagulation. Rapid mixing was reduced to 30 rpm after flocculent addition for 25 min to provide flocculation. After 30 min of settling, the supernatant from each beaker was withdrawn from the sampling port and analyzed for the residual concentration of methylene blue, and the COD determination. The removal efficiencies were measured in terms of methylene blue concentration or chemical oxygen demand (COD).

The COD removal efficiency ($Y_{COD}$ %) and methylene blue removal efficiency ($Y_{dye}$ %) were calculated using equation (1) and equation (2), respectively.

$$Y_{COD}(\%) = \frac{COD_0 - \mathrm{COD}}{\mathrm{COD}_0} \times 100 \qquad \textbf{(1)}$$

Where, $COD_0$ and COD represent the initial and final COD (mg $O_2$/l) of wastewater, respectively.

COD was determined in laboratory according to the methods given in the series of standard methods for the examination of water and wastewater

$$Y_{dye}(\%) = \frac{[\mathrm{BM}]_0 - [\mathrm{BM}]}{[\mathrm{BM}]_0} \times 100 \qquad \textbf{(2)}$$

Where $[BM]_0$ and [BM] represent the initial and final dye concentration, respectively.

The pHs of the samples were adjusted to pH ranging from 2 to 12 using 0.1 M HCl and 0.1 M NaOH solutions and measured with the pH meter apparatus "JENWAY 3505".

### II.3. Response Surface Methodology

In order to determine the most critical factors and their region of interest, a preliminary study on the effect of bio coagulant dosage effect, bio flocculent dose, pH, mixing speed and retention time on the coagulation-flocculation process was carried out.

It was studied the influence of the four selected factors: the bio-coagulant dose of carob powder (***$X_1$***), the bio flocculent dosage of cactus powder (***$X_2$***), the initial solution pH (***$X_3$***) and the initial dye concentration (***$X_4$***) on the yield of two important responses: the elimination of color $Y_{dye}$ (%) and the reduction of the chemical oxygen demand $Y_{COD}$ (%). All the experiments are conducted in triplicate for each run.

In order to describe and determine the relationship between factors affecting output responses of coagulation/flocculation process, a central composite design (CCD) which is the standard response surface methodology was employed, it is a very efficient design tool for fitting the second-order models also to search an optimum combination of four independent factors.

The variables ranges and levels investigated in this study are presented in table 2 in coded and actual values.

Each independent factor had five significant levels; factor levels were selected such that the upper level corresponds to +1, the lower level to −1 and the center level to zero.

A CCD containing 31 experiments with 16 cube points, 8 axial points, and 7 replicates at the center point. The response can be expressed as second-order polynomial equation showing the effect of the factors in terms of linear, quadratic and cross product terms according to equation (3).

$$Y_m = b_0 + \sum_{i=1}^{k} b_i X_i \sum_{i=1}^{k} \sum_{j=i+1}^{k} b_{ij} X_i X_j + \sum_{i=1}^{k} b_{ii} X_i^2 + \varepsilon \qquad \textbf{(3)}$$

Where $Y_m$ is the predicted response to be modeled,

$X_i$ and $X_j$ the independent coded variables which influence $Y_m$,

$b_0$, $b_i$, $b_{ii}$ and $b_{ij}$ are the offset terms (regression coefficients),

the *i*th is linear coefficient and the quadratic coefficient, the *ij*th is interaction coefficient,

ε is the statistical error.

In this case (*k*= 4 input variables), the empirical model in term of coded factors can be written as:

$$Y_{m=} b_{0+} b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 \qquad \textbf{(4)}$$

In order to determine if a relationship existed between factors and responses, the collected data was analyzed statistically using statistical software, Minitab 17. Statistical testing and the proposed models adequacy were performed using the diagnostic checking tests provided by the fisher's statistical test for analysis of variance (ANOVA). The coefficient of determination $R^2$ was used to express the fitted polynomial model quality. Model terms were rejected or selected based on the *p-value* (probability) with *95%* confidence level. Finally, the optimal values of the critical parameters were given by analyzing the surface and counter plots and the Optimizer response of Minitab.

***Table 2.*** *Experimental range and level of the independent variables in coded and actual values.*

| Factors | Range and levels | | | | |
|---|---|---|---|---|---|
| | -1 | -0.5 | 0 | 0.5 | 1 |
| **$X_1$, coagulant dose[a]** | 0.1 | 0.325 | 0.55 | 0.775 | 1 |
| **$X_2$, flocculent dose[a]** | 0.05 | 0.0625 | 0.075 | 0.0875 | 0.1 |
| **$X_3$, pH** | 2 | 4.5 | 7 | 9.5 | 12 |
| **$X_4$,initial concentration[a]** | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 |

[a] Unit of dose: $gL^{-1}$

The MATLAB 2009b was used also to demonstrate the 3D surface and 2D contour plots of the response model.

For statistical calculations, the variables $X_i$ were coded as $x_i$ according to the following equation:

$$X_i = \frac{X_i - X_0}{\delta X} \quad \textbf{(5)}$$

Where $X_i$ : is the actual value of the independent variable,

$x_0$ : is the value at the center point of the investigated area

$\delta$x: is the step change.

## III. Results and discussion

### III.1. Model Regression Development and Validation

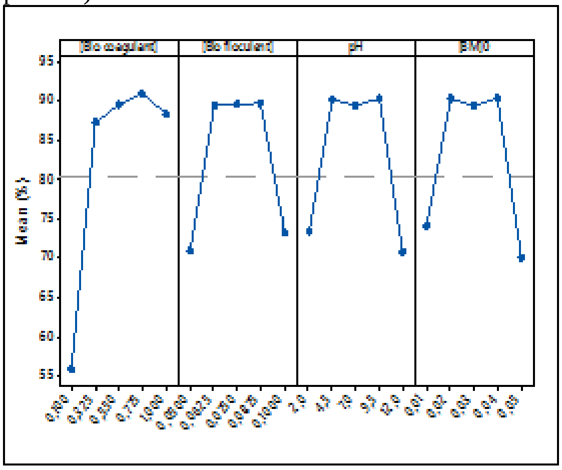
The CCD shown in table 3 allowed the mathematical equations development where each response *Y= f(X1,X2,X3,X4)* was assessed as a function of coagulant dose, flocculent dose, pH and the initial dye concentration, and calculated as the sum of a constant, four linear effects, four quadratic effects, and six interaction effects according to equation (4). The results of the fitted models for dye removal efficiency and COD removal efficiency are given in equations (6) and (7) in terms of coded factors.

$$Ydye\ (\%) = 90.04 + 15.90X_1 + 1.08X_2 - 1.21X_3 - 2.02X_4 - 7.28X_1^2 - 5.76X_2^2 - 2.64X_3^2 - 2.68X_4^2 - 1.06X_1\,X_2 - 0.02X_1\,X_3 + 1.61X_1\,X_4 - 0.008\,X_2\,X_3 - 0.22X_2\,X_4 + 0.15\,X_3\,X_4 \quad \textbf{(6)}$$

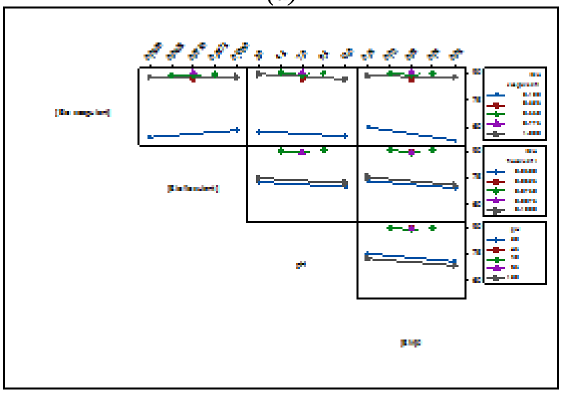
#### III.1.1. Dye removal efficiency analysis $Y_{dye}(\%)$

Main and interaction effects diagrams of bio coagulant dosage, bio flocculent dosage, pH and initial dye concentration on dye reduction efficiency are shown in figure 1 (a, b), respectively.

A main effect occurs when the mean response changes through the level of a factor. It is used to compare the relative strength of the effect across factors. By analyzing the graph of figure 1(a), it seems that the behavior of these factors varies from one's response to another; it is clearly shown that all factors were important in the coagulation /flocculation treatment performance. It is seen higher dye removal was obtained at low initial pH values and the maximum removal was obtained when the initial pH was between 4.5 and 9.5. Interaction effect plots are represented in figure 1(b). The interaction between factors occurs when the change in response from the low level to the high level of one factor is different from the change in response at the same two levels of a second factor. From the graphs it can be shown that there was no significant interaction between all factors (the interaction curves are almost parallel).




*(a)*



(b)

***Figure 1.*** *Main effect (a) and interaction effect (b) plots for dye removal efficiency.*

To determine the regression coefficient significance of factors, the Student's t-test was engaged. An experimental significance level was calculated from the values known as the Student's coefficient *t* [42]. The regression coefficients values, t-statistics, significance level p-values and standard errors are shown in table 4. To verify and confirm the significance of each coefficient, it is recommended to use *P-value* or Student's *t- test* as a tool in order to understand the mutual interaction motif among the factors. The larger the Student's t-test and the smaller P-value are the more significant is the corresponding coefficient [42, 43]. If the coefficient probability *p-value* was greater than 0.05, it can be concluded that the term did not have a significant effect on the predicted response [44].

*Algerian Journal of Environmental Science and Technology*
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*ISSN : 2437-1114*
*www.aljest.org*

***Table 3.*** CCD experimental design in coded values.

| Run | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $Y_{dye}$(%) | $Y_{dye}$(%) predicted | $Y_{COD}$(%) | $Y_{COD}$(%) predicted |
|---|---|---|---|---|---|---|---|---|
| 1 | -1 | -1 | -1 | -1 | 59.03 | 58.78 | 38.20 | 43.53 |
| 2 | 1 | -1 | -1 | -1 | 87.42 | 89.51 | 73.10 | 62.42 |
| 3 | -1 | 1 | -1 | -1 | 62.53 | 63.51 | 41.50 | 40.80 |
| 4 | 1 | 1 | -1 | -1 | 90.89 | 90.01 | 43.50 | 48.91 |
| 5 | -1 | -1 | 1 | -1 | 52.14 | 56.10 | 43.20 | 40.22 |
| 6 | 1 | -1 | 1 | -1 | 90.35 | 86.76 | 30.00 | 37.83 |
| 7 | -1 | 1 | 1 | -1 | 61.87 | 60.81 | 42.50 | 39.57 |
| 8 | 1 | 1 | 1 | -1 | 86.53 | 87.23 | 28.00 | 26.40 |
| 9 | -1 | -1 | -1 | 1 | 49.98 | 51.66 | 35.50 | 32.21 |
| 10 | 1 | -1 | -1 | 1 | 90.05 | 88.83 | 65.00 | 72.82 |
| 11 | -1 | 1 | -1 | 1 | 54.23 | 55.53 | 43.60 | 40.66 |
| 12 | 1 | 1 | -1 | 1 | 90.05 | 88.46 | 72.40 | 70.49 |
| 13 | -1 | -1 | 1 | 1 | 50.98 | 49.58 | 25.50 | 24.98 |
| 14 | 1 | -1 | 1 | 1 | 85.29 | 86.68 | 48.50 | 44.31 |
| 15 | -1 | 1 | 1 | 1 | 53.14 | 53.43 | 29.70 | 35.50 |
| 16 | 1 | 1 | 1 | 1 | 86.05 | 86.29 | 44.50 | 44.06 |
| 17 | -0.5 | 0 | 0 | 0 | 87.23 | 80.26 | 49.60 | 53.88 |
| 18 | 0.5 | 0 | 0 | 0 | 90.97 | 96.16 | 65.44 | 60.74 |
| 19 | 0 | -0.5 | 0 | 0 | 89.35 | 88.05 | 59.52 | 60.67 |
| 20 | 0 | 0.5 | 0 | 0 | 89.61 | 89.14 | 61.50 | 59.93 |
| 21 | 0 | 0 | -0.5 | 0 | 90.17 | 89.98 | 63.22 | 64.94 |
| 22 | 0 | 0 | 0.5 | 0 | 90.35 | 88.77 | 59.64 | 57.50 |
| 23 | 0 | 0 | 0 | -0.5 | 90.35 | 90.47 | 58.44 | 58.87 |
| 24 | 0 | 0 | 0 | 0.5 | 90.35 | 88.46 | 61.30 | 60.45 |
| 25 | 0 | 0 | 0 | 0 | 89.25 | 90.03 | 60.24 | 60.66 |
| 26 | 0 | 0 | 0 | 0 | 89.00 | 90.03 | 59.86 | 60.66 |
| 27 | 0 | 0 | 0 | 0 | 89.41 | 90.03 | 61.00 | 60.66 |
| 28 | 0 | 0 | 0 | 0 | 89.00 | 90.03 | 60.33 | 60.66 |
| 29 | 0 | 0 | 0 | 0 | 89.00 | 90.03 | 60.33 | 60.66 |
| 30 | 0 | 0 | 0 | 0 | 89.00 | 90.03 | 61.40 | 60.66 |
| 31 | 0 | 0 | 0 | 0 | 89.00 | 90.03 | 59.97 | 60.66 |

From the results in Table 4, it can be deduced that bio coagulant dosage ($X_1$) (g $L^{-1}$) and initial dye concentration ($X_4$) ($gL^{-1}$) have *p-values* smaller than 0.05 which indicates that the terms in the model are significant with 95% confidence level. Furthermore, concerning quadratic terms effect, it can be observed that all of them are insignificant (*P-value* > 0.05). While, bio coagulant dosage ($X_1$) with initial dye concentration ($X_4$) had a small effect on the coagulation flocculation process since its *p-values* is equal to 0.048.

In order to evaluate the adjustment quality and to examine the efficiency and the statistically significance of the model, statistical testing of the model was performed with Fisher's statistical test for analysis of variance (ANOVA); Fisher variation ratio (*F-value*) is the ratio between the mean square of the model and of residual error, which is a statistical measure utilized to know how well the factors represent the variation in the data with respect to its mean [45].

***Table 4.*** *Estimated regression coefficients with corresponding t values for dye removal efficiency*.

| Term | Coefficients | t-value | P-value | Standard error |
|---|---|---|---|---|
| Constant | 90.04 | 111.95 | 0.000 | 0.804 |
| $X_1$ | 15.09 | 21.45 | 0.000 | 0.741 |
| $X_2$ | 1.08 | 1.46 | 0.163 | 0.741 |
| $X_3$ | -1.21 | -1.64 | 0.121 | 0.741 |
| $X_4$ | -2.02 | -2.72 | 0.015 | 0.741 |
| $X_1^2$ | -7.28 | -0.99 | 0.339 | 7.38 |
| $X_2^2$ | -5.76 | -0.78 | 0.447 | 7.38 |
| $X_3^2$ | -2.64 | -0.36 | 0.725 | 7.38 |
| $X_4^2$ | -2.68 | -0.31 | 0.761 | 7.38 |
| $X_1$*$X_2$ | -1.06 | -1.41 | 0.178 | 0.753 |
| $X_1$*$X_3$ | -0.02 | -0.02 | 0.982 | 0.753 |
| $X_1$*$X_4$ | 1.61 | 2.14 | 0.048 | 0.753 |
| $X_2$*$X_3$ | -0.008 | -0.01 | 0.992 | 0.753 |
| $X_2$*$X_4$ | -0.22 | -0.29 | 0.779 | 0.753 |
| $X_3$*$X_4$ | 0.15 | 0.20 | 0.845 | 0.753 |

*Table 5. Analysis of variance (ANOVA) for dye removal efficiency (%).*

| Source | DF | SS | MS | F-value | F-tab |
|---|---|---|---|---|---|
| Model | 14 | 6682,79 | 477,34 | 52,64 | 2.4446 |
| Residual | 16 | 145,09 | 9,07 | | |
| Total | 30 | 6827,88 | | | |

***$R^2$=97.88%, $R^2_{adj}$=96.02%.***

The model is suitable and good predictor of the experimental results, when the *F-value* is greater than the tabulated value of *F-distribution* for a certain freedom degrees number in the model at a level of significance α [46].

Table 5 groups the results provided by ANOVA for the dye removal efficiency.

The model is found to be significant at 95% confidence level by the *F-test* as shown in table 5 (*F-value* is higher than $F_{(14,\ 16)\ 0.05}$ (*F-tab* equal to 2.4446). It is validated from a statistical standpoint and it gives a good prediction of the experimental data. Further, the $R^2$-value is **0.978**, which is desirable and implies that just 2.1 % of data deviation can't be explained by the developed polynomial model.

The diagnostic plot of predicted values versus actual values of dye removal efficiency has been illustrated in figure 2. Data points are closely spread around the first bisector indicating a very good fit between the simulated and the experimental values.

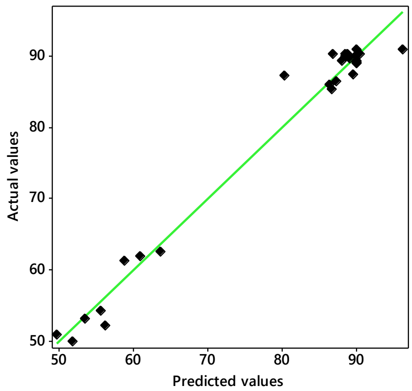


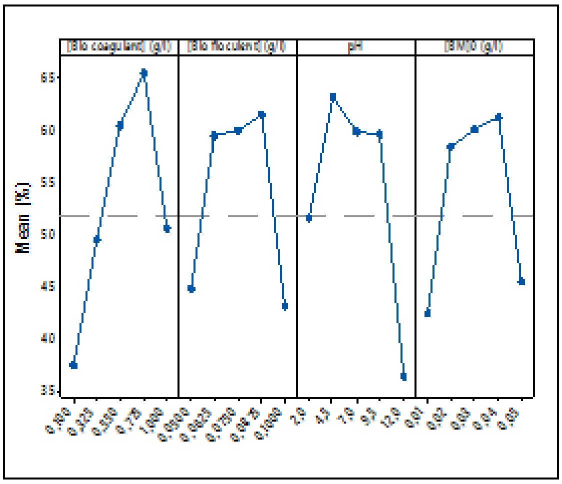

***Figure 2.** Predicted values vs. experimental values for dye elimination efficiency.*

### III.1.2. COD Removal Efficiency Analysis $Y_{COD}$ (%)

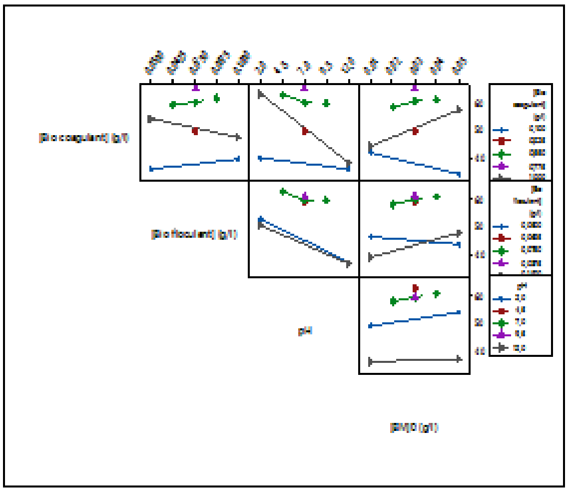
The COD removal of the coagulation flocculation experiments are shown in table 3 and the empirical model in coded terms of factors is written as:

$$Y_{COD}\ (\%) = 60.67 + 6.86X_1 - 0.75\ X_2 - 7.44\ X_3 + 1.58\ X_4 - 13.4\ X_1^2 - 1.4\ X_2^2 + 2.2\ X_3^2 - 4\ X_4^2 - 2.70\ X_1X_2 - 5.32\ X_1X_3 + 5.43\ X_1\ X_4 + 0.52\ X_2\ X_3 + 2.80\ X_2\ X_4 - 0.98\ X_3\ X_4 \quad (7)$$

As shown in diagrams represented in figure 3(a, b) the effectiveness of the bio coagulation/bio flocculation process in removing COD is highly dependent on bio coagulant dosage and initial pH of solution and reached to 60% at bio coagulant dosage of 0.775g/L and at pH of 4.5. From figure 4(b) it is clearly shown that factors interaction has a weak effect.




*(a)*



*(b)*

***Figure 3.**Main effect (a) and interaction effect (b) plots for COD reduction efficiency.*

According to Student's t- test given in table 6, the coefficient's terms: $b_0$, $X_1$and $X_3$ have values of *p-value* equal to 0.000 which shows the meaning of these terms. It can be observed that the interaction effect of bio coagulant dose with bio flocculent dose ($X_1$*X3) and bio coagulant dose with initial dye concentration ($X_1$*$X_4$) was very significant on

*Algerian Journal of Environmental Science and Technology*
*September edition. Vol.7. N°3. (2021)*
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***Table 6.*** *Estimated regression coefficients and corresponding t values for COD removal efficiency.*

| Term | Coefficients | t-value | P-value | Standard error |
|---|---|---|---|---|
| Constant | 60.67 | 43.10 | 0.000 | 1.41 |
| $X_1$ | 6.86 | 5.29 | 0.000 | 1.30 |
| $X_2$ | -0.74 | -0.58 | 0.573 | 1.30 |
| $X_3$ | -7.43 | -5.73 | 0.000 | 1.30 |
| $X_4$ | 1.58 | 1.22 | 0.240 | 1.30 |
| $X_1^2$ | -13.40 | -1.04 | 0.315 | 12.9 |
| $X_2^2$ | -1.44 | -0.11 | 0.912 | 12.9 |
| $X_3^2$ | 2.23 | 0.17 | 0.865 | 12.9 |
| $X_4^2$ | -4.00 | -0.31 | 0.761 | 12.9 |
| $X_1*X_2$ | -2.69 | -2.04 | 0.058 | 1.32 |
| $X_1*X_3$ | -5.31 | -4.04 | 0.001 | 1.32 |
| $X_1*X_4$ | 5.43 | 4.12 | 0.001 | 1.32 |
| $X_2*X_3$ | 0.51 | 0.39 | 0.699 | 1.32 |
| $X_2*X_4$ | 2.79 | 2.12 | 0.05 | 1.32 |
| $X_3*X_4$ | -0.98 | -0.74 | 0.467 | 1.32 |

COD removal efficiency.
ANOVA of COD reduction efficiency is shown in table 7.

The quadratic regression indicates that the model was significant because $F_{statistic}$ (12.71) is greater than tabulated $F_{(14, 16), 0.05}$ (2.444).

The value of the correlation coefficient ($R^2 = 0.917$) is desirable, this indicates that more than 91.75% of data deviation can be explain by the developed empirical model. Moreover, the predicted $R^2$ value is in agreement with adjusted statistics ($R_{adj}^2 = 0.845$), this implies that significant term have been inclusive in the regression model.

The predicted values versus actual plots for COD removal efficiency are shown in figure 4. The observed points of this plot reveal that the actual values are distributed relatively near to the straight line and the model adequacy can be judged.

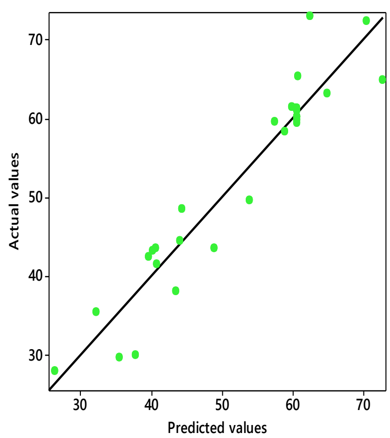



***Figure 4.*** *Predicted values vs. experimental values for COD reduction efficiency.*

**III.2. Optimization Process**

Contour plots of the RMS (3D) plots are drawn as a function of two variables at a time, holding up all other variables at fixed levels (at the zero level). Contour plots are useful for better explanation of the two independent variables and their interactive effects on dye elimination and COD reduction efficiencies.

3D plots and there contours for dye removal efficiency and COD reduction performance are presented in figures 5 and 6, respectively.

Figures 5(c, d) and 6(a, b) show symmetrical mound shape contours with the maximum response occurring within the center of contour. The relative circular curve reveals that the bio coagulant dosage has a little interaction with bio flocculent dosage.

The saddle contour in the 3D plots in figures 6(c, d) indicates that the dye removal and COD reduction efficiencies decrease at the center of the region, also it implicates the interaction between pH with coagulant dose. Since the elaborated factors did not have the same effect on the considered responses, an optimization study was necessary to determinate the optimum operating conditions for any fixed objective. The advantages of using RMS were to be able to set several objectives and also to carry out various numerical experiments, changing the objectives to be achieved each time.

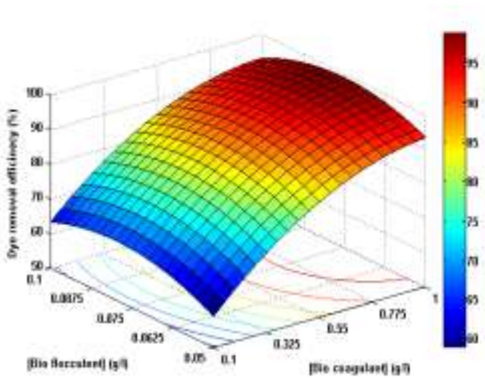
Maximum elimination of dye, maximum COD reduction were targeted and Minitab Optimizer of the considered RMS design gave the results of mono-objective optimization presented in figure 7(a, b). Optimum values of operating conditions for maximum responses are shown in table 8 in actual values, carob powder dose was 0.83g.L$^{-1}$, cactus

***Table 7.*** *Analysis of variance (ANOVA) for COD removal efficiency (%).*

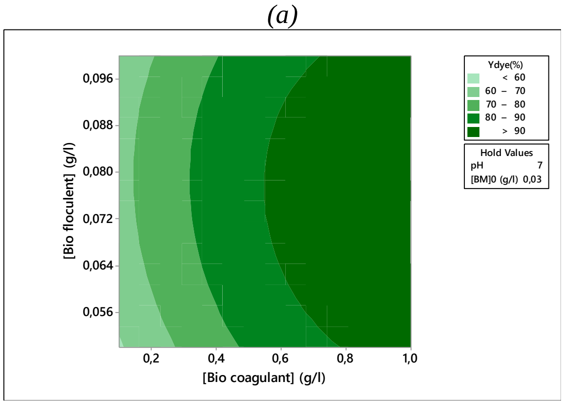
| | DF[a] | SS[b] | MS[c] | F-value | F-tab |
|---|---|---|---|---|---|
| Model | 14 | 4943,21 | 353,086 | 12,71 | 2.4446 |
| Residual | 16 | 444,41 | 44,256 | | |
| Total | 30 | 5387,62 | | | |

***$R^2$=97.88%, $R^2_{adj}$=96.02%.***

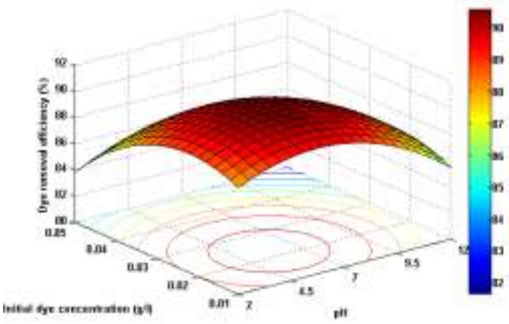
powder dose was 0.06 $g.L^{-1}$, pH equal to 2 at initial dye concentration of 0.04$g.L^{-1}$. While predicted responses were 92.93% and 75.13% of dye removal and COD reduction efficiencies, respectively.



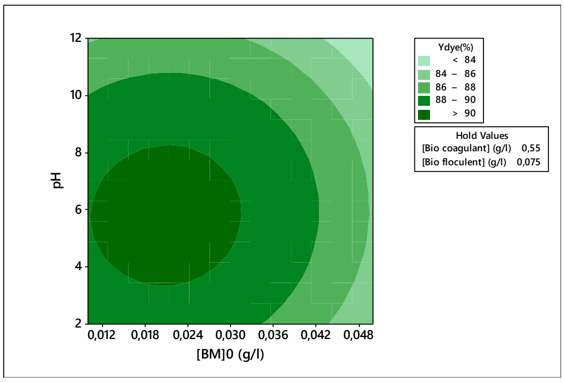

*(a)*




*(b)*

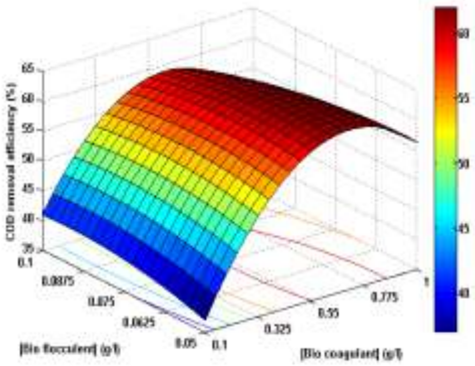



*(c)*

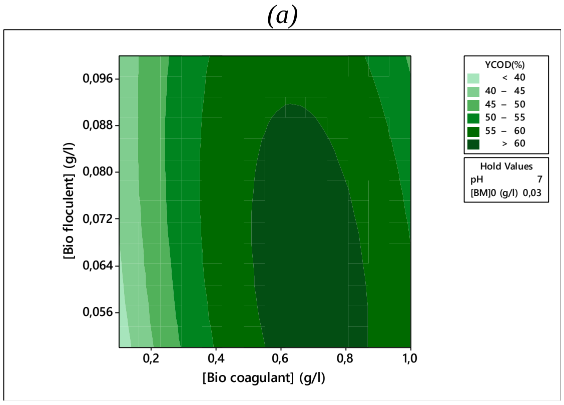



*(d)*

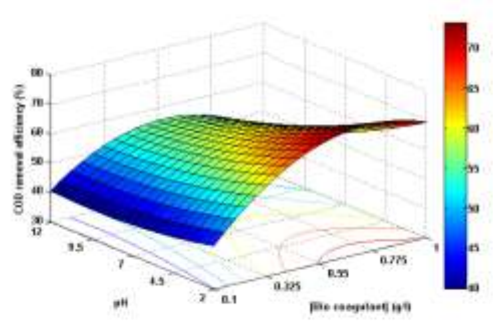
***Figure 5.*** *3D plots(a, d) and Contour plots (b,d) for dye removal efficiency.*
*In 5 (a, b) (initial pH=7, $[BM]_0$=30mg$L^{-1}$)*



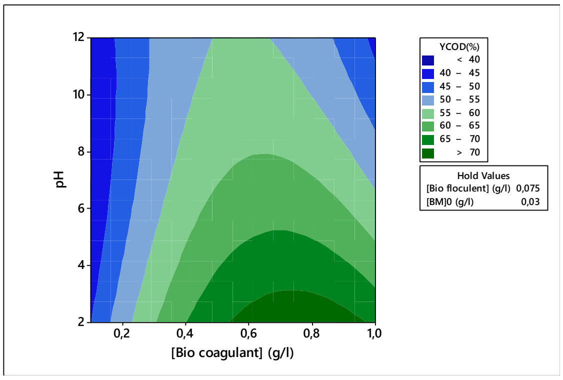

*(a)*




*(b)*



*(c)*




*(d)*

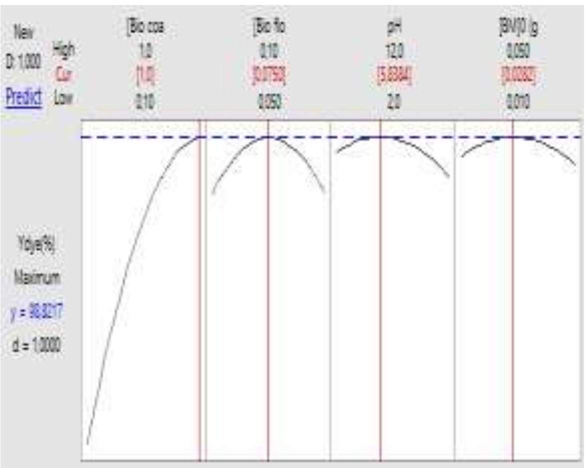
***Figure 6.*** *3D plots (a, d) and Contour plots (b,d) for COD reduction.*
*In (a, b) (initial pH=7, $[BM]_0$=30mgL$^{-1}$)*
*In( c, d)(cactus dose =75mgL$^{-1}$, $[BM]_0$=30mgL$^{-1}$).*

To verify the accuracy of the regression models obtained, a validation experiments were performed and tests are carried out at the optimum conditions from Minitab response optimizer.

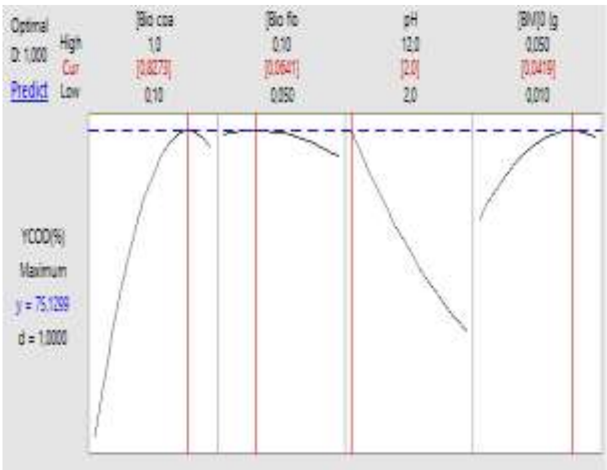
***Table 8**. Confirmation experiments for optimum region.*

| | bio coagulant dose | bio flocculent dose | pH | $[BM]_0$ | predicted values | Experimental values | Error |
|---|---|---|---|---|---|---|---|
| $Y_{dye}$(%) | | | | | 92.93 | 93.63 | 0.7 |
| | 830 (mg/L) | 64 (mg/L) | 2 | 0.04 (mg/L) | | | |
| $Y_{COD}$(%) | | | | | 75.13 | 74.12 | 1.01 |

The experiments were conducted in triplicates and the mean value was calculated. The mean of three tests of dye removal efficiency was 93.63% and the mean of COD reduction was 74.12%. By the comparison, the mean values and predicted values represented in table 8, it is evident that the model is validated.



*(a)*



*(b)*

***Figure 7.*** *Minitab results from the mono-objective optimizer for dye removal efficiency (a) and COD removal efficiency (b).*

**IV. Conclusion**

In this research, a bio coagulation/flocculation process was studied to remove methylene blue dye and COD from solutions by using ecofriendly materials the carob as a coagulant and the cactus as a flocculent. Statistical optimization method (a central composite (CCD) designed with response surface methodology (RSM)) overcomes the limitations of classical methods and was successfully employed to obtain the optimum process conditions while the interactions between process variables were demonstrated. From the optimization multi objective, the maximum dye removal efficiency was obtained at initial pH of dye solution of 2, carob dosage of 830mg$L^{-1}$, flocculent dosage of 64mg$L^{-1}$ and initial dye concentration of 40mg$L^{-1}$. ANOVA showed a high $R^2$ value of regressions model equation ($R^2$ = 0.978 for dye removal and $R^2$=0.917 for COD removal efficiencies), thus ensuring a satisfactory adjustment of the second-order regression models with the experimental data. Therefore, RSM has been proved to be a powerful tool for optimizing the coagulation/flocculation process for the methylene blue dye removal and COD reduction from synthetic wastewater. Carob and cactus can be considered as an appropriate alternative for conventional costly biomaterials.

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***Algerian Journal of Environmental Science and Technology***
***September edition. Vol.7. N°3. (2021)***
***ISSN : 2437-1114***
***www.aljest.org***

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**Please cite this Article as:**
Ayat A., Arris S., Abbaz A., Bencheikh-Lehocine M., Meniai A.H., The use of alternative sustainable biomaterials for dye removal and chemical oxygen demand reduction by a bio coagulation/ bio flocculation process Optimization through response surface methodology, ***Algerian J. Env. Sc. Technology, 7*:3 (2021) 1985-1995**