Optimization of Hydrolysis Degradation of Neurotoxic Pesticide Methylparathion Using a Response Surface Methodology (RSM)

Doumandji Lotfi [2], Moussiden Anissa [1], Benmabrouka Hafsa [1],
Boufades Djamila [1], Zaher Ihdene [1], Pemanos yelda Bakos [3], Hamada Boudjema [1].

[1] *Laboratory of Petrochemical synthesis, Faculty of Hydrocarbons and Chemistry, University M'hamed Bouguara-Boumerdes, Algeria.*
[2] *Laboratory of Chromatography analysis, National Institute of Criminalistics and Criminology (INCC), Bouchaoui,Algiers Algeria.*
[3] *Department of Chemistry,College of Science, University of ZaKho, Kurdistan, Iraq*

Abstract : *The use of chemometric methods such as response surface methodology (RSM) based on statistical design of experiments (DOEs) is becoming increasingly widespread in several sciences such as analytical chemistry, engineering and environmental chemistry. The optimization of the hydrolysis degradation of the neurotoxic organophosphorus pesticide methyl parathion (MP) was carried in presence of aqueous solution decontaminant containing Monoethanolamine (MEA).For the optimization,an experimental design was used based on the surface response methodology; it was applied to assess the individual and interaction effects of several operating parameters (Temperature, pH and ratio R [MEA]/[MP]) on the yield of hydrolysis degradation of methyl parathion. A composite face-centered (CCF) experimental design was employed.Based on the experimental design data, a semi-empirical expression was obtained, allowing to predict and to optimize the yield of hydrolysis degradation of methyl parathion. This model was very consistent with experimental results (correlation factor: 99.70%). Optimal experimental conditions found for hydrolysis of methyl parathion (30 mg.l^{-1}) were temperature (≈95°C), pH (9.20) and ratio R([MEA]/[MP]:5.4/1). Graphical response surface and contour plots were used to locate the optimum point.*

Keywords*: Experimental design, Response surface methodology (RSM),neurotoxic; methyl parathion, hydrolysis, degradation.*

I. Introduction

Organophosphorous compounds are widely used as pesticides,insecticides and chemical warfare agents [1–3]. Protection against organophosphorus nerve agents is amatter of international concern. 'Nerve agent', or 'nervegas', represents a generic name for a class of highly toxiccompounds with marked inhibitory activity on theenzyme acetylcholinesterase.

Despite several decades of research, the need for new and improved methods for the detoxification of highly neurotoxic organophosphorous compounds is still there. In this preliminarycommunication, we have interested on hydrolysis of a model of neurotoxic pesticide organophosphorus such as methyl parathion (Fig. 1).

Methyl parathion is a neurotoxic insecticide, which is registered to control insect pests on food,feed and fiber crops. Its toxicity is largely due to the inactivation of the enzymeacetylcholinesterase (AChE) in insects and mammals [2]. Methyl parathion requires metabolicactivation to its oxon,methyl paraoxon, to yield an anticholinesterase activity. AChE isresponsible for the hydrolysis of acetylcholine (ACh) at cholinergic synapses, which is necessaryfor the control of the neurotransmission. The majordegradation product of methyl parathion is para-nitrophenol. The principle routes of dissipationare microbial degradation, aqueous photolysis, hydrolysis, and incorporation into soil organicmatter.
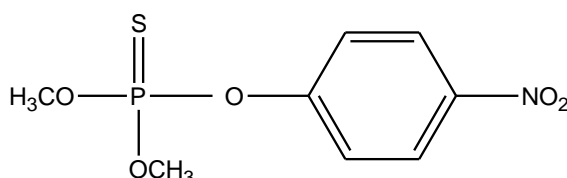

Fig.1. Chemical structure of Methyl parathion.

In this paper, we have used the response surface methodologyto study the influence of experimental parameters (temperature, pH and ratio R [MEA]/[MP]) on the yield of hydrolysis degradation of methyl parathion by aqueous solution containing monoethanolamine and to determine the optimalconditions of hydrolysis of methyl parathion.

The yield of hydrolysis degradation (%) was selected as the response for optimization and the functional relationship between the response and the most significantindependent variables (factors) was established by means of experimentaldesign. Optimization of experimental parameters is usuallyassessed by systematic variation of one parameter while the othersare maintained constant. However, this approach is unable topredict the best conditions of the process. In this respect, experimentaldesigns are appropriate tools for this purpose. In fact, theexperimental design allows considerable reduction of experimentsnumber and a fast interpretation. In the experimental design, it ispossible to study a large number of factors and to detect the possibleinteractions between them. All the parameters are simultaneouslyapplied in order to calculate their relative effect [4]. Response surface methodology has been applied to model and optimize several treatment processes including adsorption Fenton'soxidation, photocatalytic processes and hydrolysis degradation.

II.   Materials And Methods

2.1. Materials

Methyl parathion, (MP)O,O-dimethyl-O-(4-nitrophenyl)-phosphorothioate(98% purity) was obtained as a solid powderfrom the Algerian company of products phytosanitary (ALPHYT of BENI-MARED, BlidaAlgeria) andused as received. Pesticide solutions were preparedby dissolving the appropriate amount of methyl parathion in doubledistilledwater. Dilute H_3PO_4 or NaOH were used to obtain the desired pH values. Monoethanolamine (99.9 % purity)was purchasedfromPanreac. Working MEA solutions wereprepared by diluting the stock solution with double-distilled water. Solvent such as dichloromethane (Quality HPLC grade) were purchased from Prolab.Distilled water was used for preparation of solutions, which was prepared in the laboratory obtained from *NüveND12* water purification system, is characterized by a pH value between 6.2 and 6.8.All chemicals wereused as obtained.

2.2. Hydrolysis experiments

For a typical experiment of hydrolysis degradation, 200 ml aqueous solution ofdesired methyl parathion concentration was placed in cylindrical Pyrex-glass reactor and it was adjusted to the desirable pH (8, 9 and 10) values by addition buffer solution.Then a solution of monoethanolamine (MEA) at concentration 200 mg/l was introduced in the reactor. The temperature of the whole reactor was kept at desired temperature (75°C, 85°C and 95°C) with a water-cooling jacket around its outside. An electric stirrerwas used toinducesatisfactory mixing of the solution in the reactor (500 rmp).Experiments were performed up to 90 min of timereaction and samples for analysis were removed at regular time intervals.Each experiment was duplicated at least under identical conditions.The samples were extracted with dichloromethane and quantified by spectrophotometric analysis UV-visible (UV-1800 SHIMADZU piloted by a micro-computer HP brand) at λ max =275.4 nm.
The equation used to calculate the yield of hydrolysis degradation (Y) experiments was:

$$Y(\%) = 100x\frac{C_0 - C_t}{C_0} \qquad (1)$$

Where De is the rate of hydrolysis degradation of methyl parathion after t min of reaction, C_0 and C_t are the solute concentrations in water at time t=0 and t=t, respectively.
Where Y is the yield of hydrolysis degradation of methyl parathion after t min of reaction, C_0 and C_t are the solute concentrations in water at time t=0 and t=t, respectively.

2.3 Experimental design

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. It is commonly used for the modeling and analysis of problems in which a response of interest is influenced by several operating variables [5]. This technique has 4 major steps, which are experimental design, model fitting, model validation and condition optimization. In this study, Face Centred Composite Designs (CCF) were generated by MODDE 6.0 (Umetrics AB, Umea, Sweden)for the investigation of hydrolysis degradation of methyl parathion was employed in order to optimize the yield of hydrolysis degradation (%). Three factors were considered: temperature (T), pH and ratio R [MEA]/[MP] . Table 1summarizes the levels for each factor involved in the design strategy. The levels for the three main variables X_1 (Temperature), X_2 (pH), X_3 (ratio R [MEA]/[MP]), were chosen according to previous experiments carried out at our lab, the experimental setup capacity and also from data in the literature for similar laboratory experiments [6,7]. The variables $X_I$were coded as $x_i$according to the following relationship:

$$x_i = \frac{X_I - X_0}{\Delta X} \qquad (2)$$

Where X_0 is value of the $X_i$at the centre point and ΔX presents the step change.

Table 1: Experimental range and levels of independent process variables.

Independent variable	Factor x_i	Range and level		
		Low (-1)	Center (0)	High (+1)
Temperature (°C)	X_1	75	85	95
pH	X_2	8	9	10
ratio R ([MEA]/[MP])	X_3	2	5	8

The total number of experiments of the designs employed in this study consisted of a total of 17 ($2^k + 2\,k + n_c$)experiments divided into three blocks:

(i) a two level full factorial design 8 (all possible combinations of codified values +1 and -1); (ii) three central (n_c), replicates of the central point (0); (iii) six axial or star points (α= +1) located at the center and both extreme levels of the experimental models. Experiments were undertaken in random order to provide protection against the effects of lurking variables [8].

From experimental observations, it was assumed that the higher order interactions were small relative to the low order interactions, because a system with several process variables is conducted primarily by some of the main effects and low order interactions [9]. Therefore, the present work considers only the two-way interactions.

III.   Results And Discussion

The most important parameters, which affect the yield of hydrolysis degradation are temperature, pH and ratio R [MEA]/[MP]. The main effects ofeach parameter on degradation yields (%) are given in Figs. 2-4 for hydrolysis of methyl parathion.From the figures, it was observed that the optimum temperature,pH and ratio R [MEA]/[MP] were near 95°C, 9 and 5, respectively.

Based on Face Centred Composite Designs (CCF), optimization design process involves mainly four major steps:
i.   Perform statistically designed experiments according to the experimental plan.
ii.   Estimating ofcoefficients in proposed the mathematical model based on the experimental results and elaborate the result of analysis of variance (ANOVA). (iii) Check the adequacy of the model through diagnostic plots;
iii. Predict the response and confirm the model. The following response equation was used to correlate the dependent and independent variables.

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3$$
$$+ b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + b_{12} x_1 x_2$$
$$+ b_{13} x_1 x_3 + b_{23} x_2 x_3. \quad (3)$$

Where Y is the predicted response (yield of hydrolysis degradation), b_0 is a constant coefficient, b_1, b_2, and b_3 are the coefficients for the linear effects, b_{11}, b_{22} and b_{33} are the quadratic coefficientsand b_{12},b_{13} and b_{23} are the interaction coefficients. The model was fitted by Partial Least Squares (PLS) included in MODDE 6.0 software was used for statistically fitting the second order model in equation (3) to experimental data. The three factors and three levels CCF experimental results and predicted values for yield of hydrolysis degradation are presentedin Table 2.
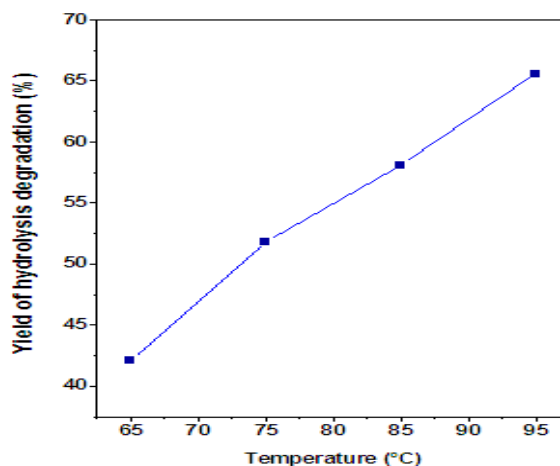

Fig. 2: Effect of temperature on the yield of hydrolysis degradation of a solution with concentration of methyl parathion= 30mg. l^{-1}, volume of the solution = 250 ml, pH=8, reaction time = 90 min.
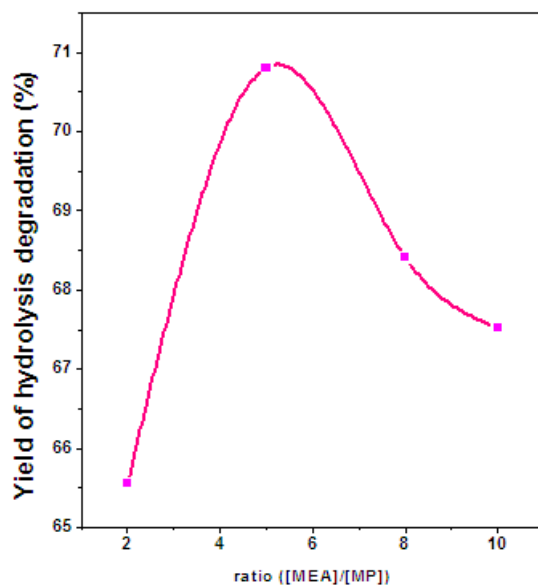
Fig. 3: Effect ratio R on the yield of hydrolysis degradation of a solution with concentration of MP = 30mg. l^{-1}, volume of the solution = 250 ml, pH=8, reaction time = 90min.
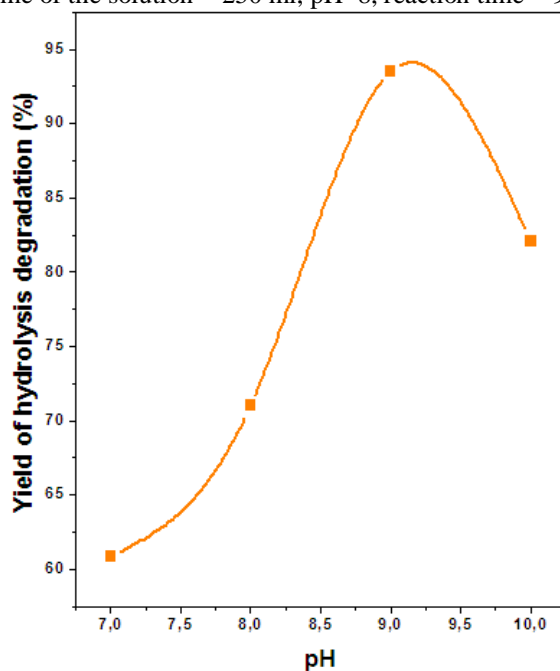

Fig. 4. Effect of pH on the yield of hydrolysis degradation of a solution with concentration of the methyl parathion= 30mg. l^{-1}, volume of the solution = 250 ml, R ration (5:1), reaction time = 90 min.

3.1 Evaluation and validation of model

The regression coefficients values, confidence intervaland p-value are given in Table 3. The p-value was used as a tool to check the significance of each of the regression coefficients, which in turn indicated the pattern of the interactions between the variables. b_0, b_1, b_2 and b_3 are significant at (p value<0.05) and the interaction coefficient $b_{12}$is alsosignificant at (pvalue<0.05).Also, the coefficients of the quadratic effects (b_{22}, b_{33}), of the variables are significant at (pvalue<0.05).

With the MODDE software significance of the coefficients can be plotted as a stick diagram (Fig.5), a coefficient is considered influential when its confidence interval does not cross the x-axis.

Table.2. A 2^3 full factorial CCF design with three replicates of the centre point

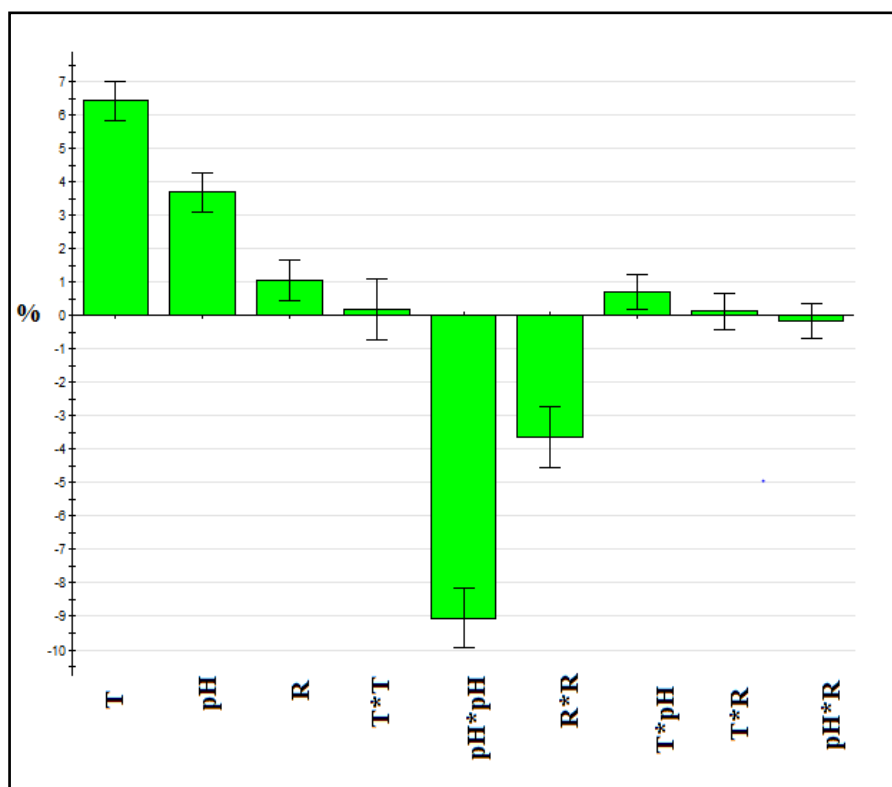| Run	Temperature (°C)	pH	Ratio R [MEA]/[MP]	Y Experimental (%)	Y Predicted (%)
1	-1	-1	-1	51.78	51.70
2	1	-1	-1	65.55	65.296
3	-1	1	-1	60.04	59.291
4	1	1	-1	77.14	77.457
5	-1	-1	1	55.12	54.519
6	1	-1	1	68.42	68.885
7	-1	1	1	61.04	61.01
8	1	1	1	80.15	79.946
9	-1	0	0	75.45	76.909
10	1	0	0	93.5	9.175
11	0	-1	0	65.12	65.589
12	0	1	0	74.25	74.915
13	0	0	-1	76.84	77.605
14	0	0	1	79.89	80.259
15	0	0	0	85.82	84.732
16	0	0	0	85.6	84.732
17	0	0	0	85.02	84.732

Fig 5. Significance of the regression coefficients of model.

A positive sign designated a synergetic factor effect and indicated that the response was improved when the factor increased, whereas a negative sign designated an antagonistic factor effect and indicated that the response was not enhanced with the factor level. It could be concluded from the model applied for the hydrolysis degradation of methyl parathion in the present study that:

(i)Temperature, pH and ratio R [MEA]/[MP] (individual factor) and interaction between temperature and pH represented positive effects,

(ii) pH and ratio R [MEA]/[MP] (quadratic factors effects) represented negative effects.

In fact, pH, temperature, ratio R [MEA]/[MP] and the interactions betweentemperature and pH were noted to exert relatively more effects on hydrolysis degradation of methyl parathion.

Table.3 Estimated regression coefficients of the central composite design (CCF) of hydrolysis of methyl parathion.

Regression coefficients	value	p-value	Confidence interval(±)
b_0	84.732	2.22e-014[a]	1.012
b_1	6.429	3.45e-008[a]	0.591
b_2	3.686	1.58e-006[a]	0.591
b_3	1.049	0.004a	0.591
b_{11}	0.193	0.62	0.903
b_{22}	-9.049	6.08e-008[a]	0.903
b_{33}	-3.624	3.02e-005[a]	0.93
b_{12}	0.714	0.014a	0.522
b_{13}	0.12	0.60	0.522
b_{23}	-0.171	0.46	0.522

[a]Significant at 5% level (p<0.05).

Application of RSM offers, on the basis of parameter estimation (Table 4), the following empirical relationship between the yield of hydrolysis degradation (*Y*) and independent studied variables,

$$Y\,(\%) = 84.732 + 6.429\,X_1 + 3.686\,X_2 + 1.049\,X_3 - 9.049\,X_2^2 - 3.624\,X_3^2 + 0.714\,X_1X_2 \quad (4)$$

The yield of hydrolysis degradation Y (*%*) have been predicted by Eq. (4)and presented in Table 3. These results indicated good agreements between the experimental and predicted values of yield of hydrolysis degradation of methyl parathion.

Using the experimental results, analysis of variance andfitting quadratic response model are determined by ANOVA(Table 4). This statistical tool is required to test the significanceand adequacy of the model. The mean squares (MS) areobtained as follows:

MS=SS/DF where:
SS=sum of squares (SS) of each variation sources
DF=the respective degrees of freedom (DF).

The Fischer variation ratio (F-value) is a statistically validmeasure of how well the factors describe the variation in the dataabout its mean.It can be calculated from ANOVA as follows:

F-value=MS (due to the model variation) /MS (due toerror variance).

The greater F-value from unity explains adequately thevariation of the data around its mean, in addition the estimatedfactor effects are real. In general, the calculated F-value should be several timesgreater than the tabulated value for the model to be consideredgood.In fact, when evaluating the validity of the fitted model with ANOVA (Table 4), results show that p < 0.05. Therefore, the regression model is statistically significant with the 95% confidence level in the range studied. In addition, the lack of fit is not significant in

Table. 4: Analysis of variance (ANOVA) for the selected quadratic model.

Source of variation	DF	SS	MS	F value	Probability (p)	SD
Total corrected	16	2246.16	140.385			11.8484
Regression	9	2239.15	248.794	248.411	0.000	15.7732
Residual	7	7.01079	1.00154			1.00077
Lack of fit	5	6.69619	1.33924	8.514	0.108	1.15725
Pure error	2	0.314597	0.157298			0.396609
N=17	**Q2=**	0.915	Cond.no=	4.141		
DF=7	**R2=**	0.997	Y-miss =	0		
Comp.=3	**R2adj=**	0.993	RSD=	1.0008		

[a]SD: standard deviation.
Neither of the developed models with the 95% confidence level (p > 0.05).

On the other hand,the quality of the obtained mathematical model can be evaluated by two statisticalcriteria which are given directly by software MODDE 6.0 to check experimental (*R*2criterion) and predictive (*Q*2 criterion) quality of the mathematical model (Fig.6). When values of *R*2and *Q*2 are close to the unit, the model is considered as good and can be used for optimizationand prediction. As values of these two criteria, according to the model given by equation(4), are respectively $R2 = 0.997$ and $Q2 = 0.915$ the model can thus be used to predict andoptimize the process.
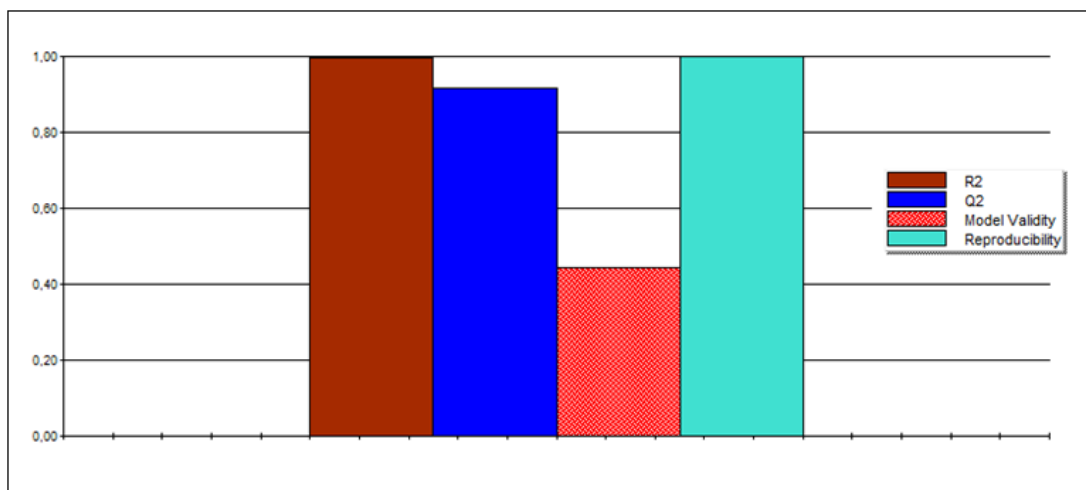
Fig.6: Representation of adjustment coefficients of model.

Thus, the third column in the Fig. 6 represents the validity of the model, and is a measure of the validity of the model. When the model validity column is greater than 0.25, there is no lack of fit model. This means that the error model is in the same range as pure error. When the validity model is less than 0.25 you have a significant lack of fit and error model is significantly larger than the pure error (reproducibility).an value valid model equal to the unit, the model was considered perfect. The validity of this model is greater than 0.40 (>0.40), is meaning that this model is valid. Then the reproducibility: is the variation of the response in the same conditions (pure error), often at center points, compared to all the variation of the response.The perfect reproducibility was obtained where a value of reproducibility close to the unit. In this case the reproducibility of model is 0.999.

3.2 Adequacy check of the model

Adequacy check of the proposed model is an important part of the analysis procedure. Good adequacy can ensure that the approximating model provides an adequate approximation to the real system,otherwise it may give poor or misleading results [26].

Fig. 7a exemplifies the plot of the residual vs. predicted values of the yield of hydrolysis degradation (%), showing that residuals are randomly distributed around the mean due to the good agreement of the model and discarding the existence of systematic errors.

Fig. 7b depicts the good linear correlation found between the predicted and experimental values of the yields of hydrolysis degradation (%). This plot has correlation coefficient of 0.996. Results confirm that the experimental values are in good agreement with the predicted values.
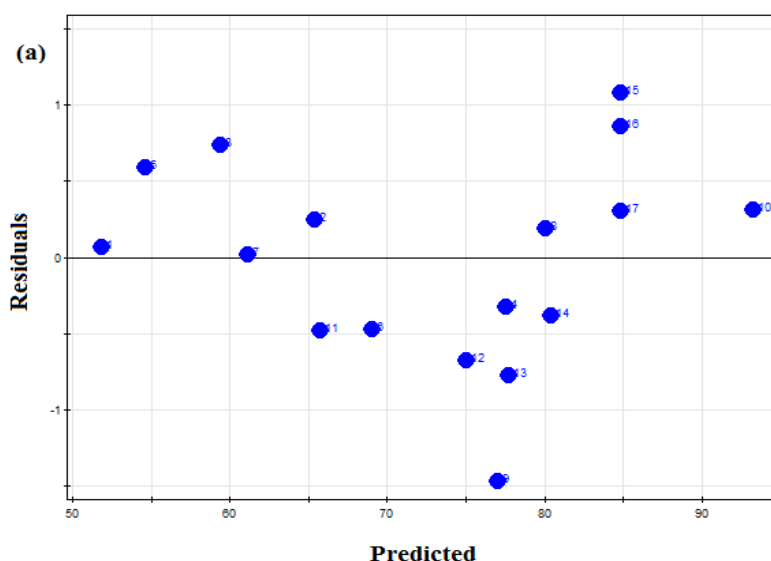

Fig.7 (a) Residual-predicted for the yield of hydrolysis degradation (%) by response surface methodology (RSM). Predicted values were calculated from Eq. (4) and residual values as the difference between the observed and predicted ones.

3.3 Response surface and contour plotting

The response surface and contour plots of the model predicted responses, while two variables kept at constant and the others varying within the experimental ranges, were obtained by the MODDE software and utilized to assess the interactive relationships between the process variables and rate of hydrolysis degradation outputs for the process of hydrolysis of methyl parathion.

Response surface plots provide a method to predict the yield degradation for different values of the tested variables and the contours of the plots help in identification of the type of interactions between these variables [11]. Each contour curve represents an infinite number of combinations of two tested variables with the other two maintained at their respective zero level.
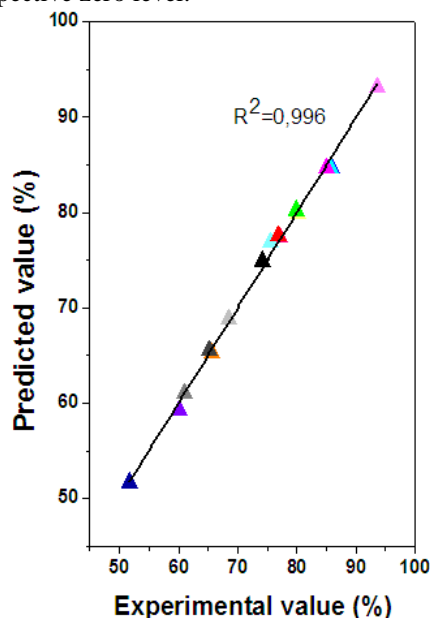

Fig. 7b: Comparison of the experimental results of the rate of hydrolysis degradation with those predicted via response surface methodology (RSM) resulted equation (4).

A circular contour of response surfaces indicates that the interaction between the corresponding variables is negligible. In contrast, an elliptical or saddle nature of the contour plots indicates that the interaction between the corresponding variables is significant [10].

3.3.1 Effect of pH and molar ratio on rate of hydrolysis degradation

Fig. 8 shows the response surface and contour plots for yield of hydrolysis degradation (%) as a function of pH and ratio R ([MEA]/[MP]) for temperature 85°C. As can be seen from Fig. 8, that degradation rates increased with increasing molar ratio R from 2 to 5,and then decreased slightly. This is caused by the solvation phenomenon that can be generated by the monoethanolamine (MEA) on the methyl parathion. However, when ratio R increases over optimum value (a large excess), it can become a limiting factor.Similar trend was also observed in the case of the effect of pH value on yield degradation of methyl parathion. The figure shows that the hydrolysis yield increases with increase in the pH. When the pH is higher than pH 9, this compound was resistant to hydrolysis.

3.3.2 Effect of temperature and molar ratio on rate of hydrolysis degradation

Fig. 9 illustrates the response surface and contour plots for yield degradation (%) as a function of ratio (R) and temperature (T) under pH 9. As can be seen from Fig.5, the highest yield of degradation (93%) occurred when the ratio R maintained at optimum value about 5 and under the maximum value of the temperature.
When increasing the temperature of reaction, the particles of reagents acquire a greater kinetic energy. The increase in displacement leads to more effective collisions and, consequently, a faster reaction rate involving the highest yield of degradation (the probability between Pollutant and aqueous solution decontaminant increases).
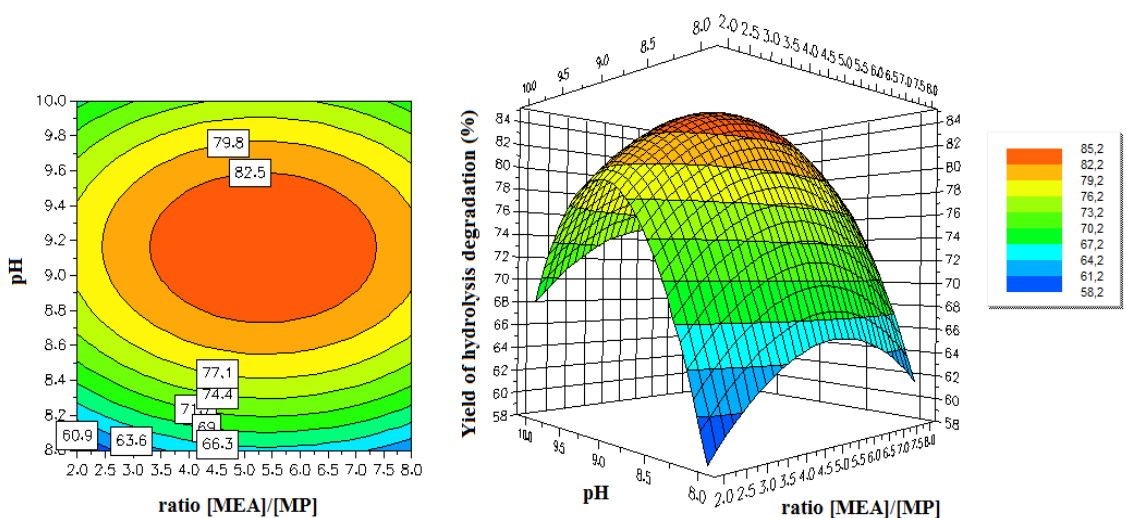
Fig. 8 The response surface plot and contour plot of the yield of hydrolysis degradation (%) as the function of ratio R ([MEA]/[MP]) and temperature (°C).
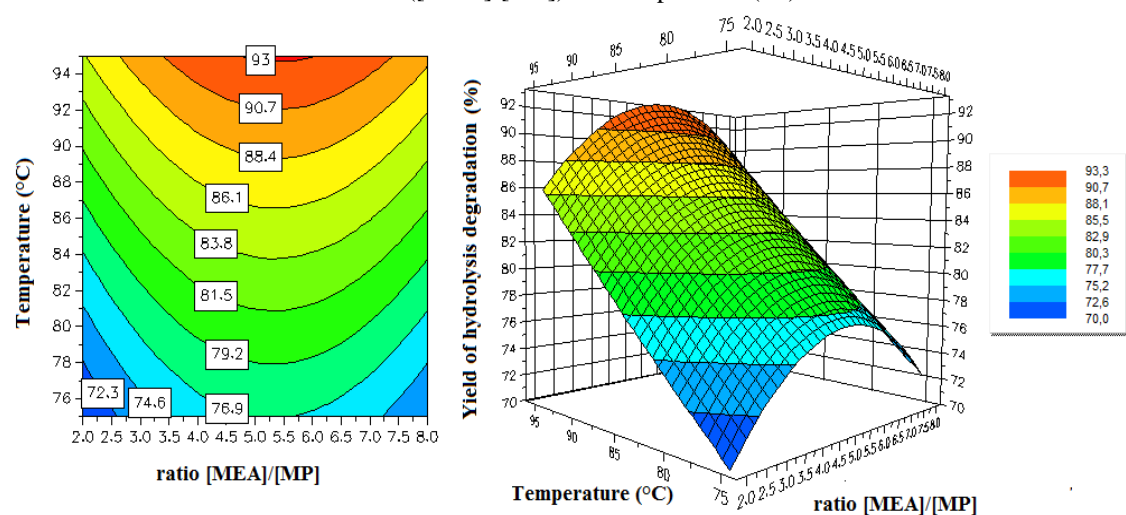

Fig. 9 The response surface plot and contour plot of the yield of hydrolysis degradation (%) as the function of ratio R ([MEA]/[MP]) and temperature (°C).
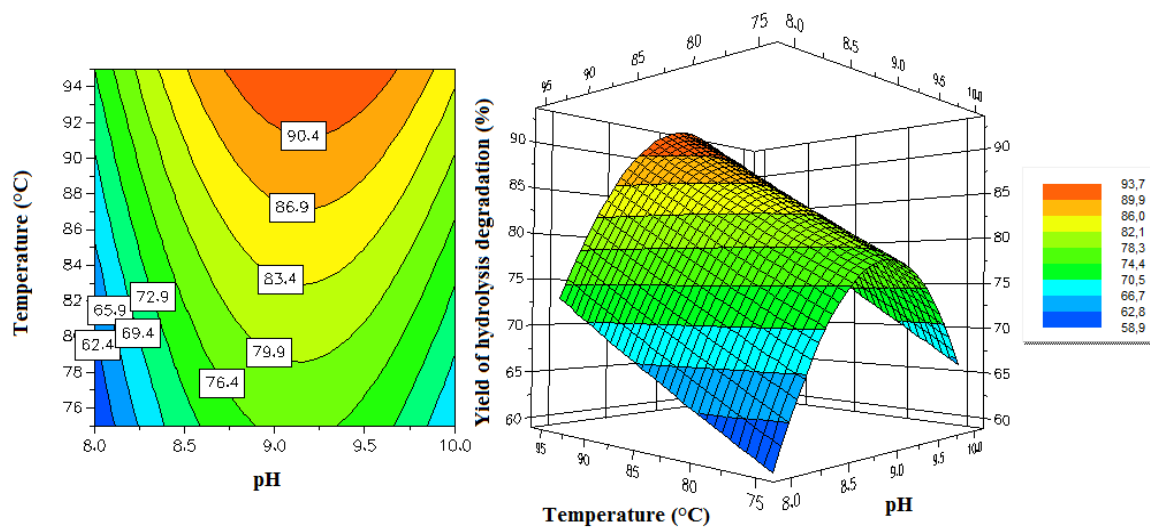

Fig. 10 The response surface plot and contour plot of the yield of hydrolysis degradation (%) as the function of ratio R ([MEA]/[MP]) and temperature (°C).

Table 5.Optimum values of the process parameters for maximum the yield of hydrolysis degradation (%).

Temperature (°C)	pH	Ratio R ([MEA]/[MP])	Predicted value (Y) (%)	Experimental value (Y) (%)
95	9.20	5.40	93.85	92.82

3.3.3 Effect of temperature and pH on degradation yield

Fig. 10 displays the 2D and 3D plots for yield degradation (%)as a function temperature (T) and pH (at a fixed ration R of 5). As it is clear fromthe response surface and contour plots, rates degradationreached to high values (>90%) when pH of solution was kept about 9 at high value of temperature.

3.4 Determination of optimal conditions for the hydrolysis of methyl parathion

Optimal conditions according to the models were determined with the help of the MODDE 6.0 software, running the optimizer for the system. The optimizer uses a Nelder Mead simplex method with the fitted response functions to optimize an overall desirability function combining the individual desirability of each response.

Optimal conditions were characterized by maximizing the yield of hydrolysis degradation of methyl parathion. The optimum values of the process variables for the maximum of rate of hydrolysis degradation are shown in Table 5. After verifying by a further experimental test with the predicted values, the result indicated that the maximal of rates of degradation was obtained when the values of each parameter were set as the optimum values, which was in good agreement with the value predicted from the model. It implies that the strategy to optimize the hydrolysis conditions of methyl parathion and to obtain the maximal rates of degradation by RSM for the hydrolysis of methyl parathion with the use decontaminating aqueous solution

IV.   Conclusion

The degradation of hydrolysis of methyl parathion using an experimental design methodology was studied.An empirical relationship between the response and independent variables was attained and expressed by the second-order polynomial equation based on results.Under optimal value of reaction parameters, high yield of degradation (>93%) was obtained for hydrolysis of methyl parathion. A CCF design was successfully employed for experimental designand analysis of results. This study clearly showed that responsesurface methodology was one of the suitable methods to optimizethe operating conditions and maximize the yield of degradation (%).Analysis of variance showed a high coefficient of determinationvalue ($R2 = 0.997$), thus ensuring a satisfactory adjustment ofthe second-order regression model with the experimental data..Graphical response surface and contour plots were used to locatethe optimum point. Satisfactory prediction equation was derivedfor hydrolysis of methyl parathion using RSM to optimize the parameters.

Acknowledgements

References

[1].   M. M. HURLEY, Chemico -Biological Interactions, CBI-5195, 1-5 (2005).
[2].   A. BARET, V. DANEL, Chemical warfare agents, EMC-Toxicology Pathology 1 (2004) 117-123.
[3].   R. Grover, A.J. Cessna (Eds.), Environmental Chemistry of Herbicides, CRC Press, Boca Raton, FL, 1991.
[4].   Myers RH, Montgomery DC. Response surface methodology: process and product optimization using designed experiments. 2nd ed. New York: John Wiley and Sons;2002.
[5].   ZaroualZ, ChaairH,EssadkiAH,ElAssK, AzziM. Optimizing the removal of trivalent chromiumby electrocoagulation using experimental design. Chem Eng J 2009;148:488–95.
[6].   Convention International for the prohibition of chemical weapons: (www.opcw.org).
[7].   W.T. Ford, Catalysis in polymer latexes, React. Funct. Polym. 33 (1997) 147–158.
[8].   D.C. Montgomery, Design andAnalysis of Experiments, third ed., Wiley,New York, 1991.
[9].   A. Gurses, M.Yalcin, C. Dogar, Electrocoagulation of some reactive dyes: astatistical investigation of some electrochemical variables, Waste Manage.
[10]. H.L. Liu, Y.R. Chiou, Optimal decolorization efficiency of Reactive Red 239 byUV/TiO2 photocatalytic process coupled with response surface methodology,Chem. Eng. J. 112 (2005) 173–179.
[11]. D.C. Montgomery, Design and Analysis of Experiments, fifth ed., John Wiley andSons, New York, 2001.