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Advanced oxidation of commercial herbicides mixture: experimental design and phytotoxicity evaluation

Alejandro López[1] · **Andrea Coll**[1] · **Maia Lescano**[1,2] · **Cristina Zalazar**[1,3]

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Abstract In this work, the suitability of the UV/H_2O_2 process for commercial herbicides mixture degradation was studied. Glyphosate, the herbicide most widely used in the world, was mixed with other herbicides that have residual activity as 2,4-D and atrazine. Modeling of the process response related to specific operating conditions like initial pH and initial H_2O_2 to total organic carbon molar ratio was assessed by the response surface methodology (RSM). Results have shown that second-order polynomial regression model could well describe and predict the system behavior within the tested experimental region. It also correctly explained the variability in the experimental data. Experimental values were in good agreement with the modeled ones confirming the significance of the model and highlighting the success of RSM for UV/H_2O_2 process modeling. Phytotoxicity evolution throughout the photolytic degradation process was checked through germination tests indicating that the phytotoxicity of the herbicides mixture was significantly reduced after the treatment. The end point for the treatment at the operating conditions for maximum TOC conversion was also identified.

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Keywords Herbicides mixture · UV/H_2O_2 process · Total organic carbon · Response surface methodology · Phytotoxicity test

Introduction

Glyphosate (N-phosphonomethyl glycine) is the herbicide most widely used in the world. In Argentina, glyphosate use increased from 1 million to more than 200 million liters (Binimelis et al. 2009; Lupi et al. 2015). The widespread use of this herbicide causes two important problems: water pollution, due to its high solubility, and the emergence of resistant weeds. In order to prevent the growth of glyphosate resistant weeds, a typical recommended management strategy is to use mixtures (Diggle et al. 2003; Bonny 2015). Application of aqueous commercial herbicides mixture is nowadays a common practice in agriculture-intensive South American countries (Simoniello et al. 2008; Soloneski et al. 2016). Glyphosate is combined with other herbicides that have different modes of action and soil residual activity, for example, 2,4-D (2,4-dichlorophenoxyacetic acid) and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) (Baldé et al. 2011; Viglizzo et al. 2011). The herbicides widely differ in their aqueous solubility. Two of them present similar hydrophobicity (2,4-D and atrazine). On the other hand, glyphosate is a hydrophilic herbicide (Mackay et al. 2006). The disposal of aqueous herbicides wastewater is still an unresolved environmental issue in many countries. Wastewaters are frequently produced through rinsing operations of empty herbicide containers or spray equipment. The resulting complex mixture may represent a potential long-term impact in human health (Groten et al. 2001; Hernández et al. 2013). As Ikehata and Gamal El-Din (2006) explained, commercial herbicides formulation contains additives apart from active ingredients such as solvents, surfactants, carriers and intensifiers. Even so, degradation studies are generally performed employing pure compounds at higher concentrations than the values that can be found in the environment (Sarmento and Miranda 2014; Murcia et al. 2015). Few researches report mixtures degradation (Huston and Pignatello 1999; Jiménez et al. 2011).

Advanced oxidation processes (AOPs) are defined as processes based on the in situ generation of powerful oxidizing agents, such as the hydroxyl radical (HO•), to effectively decontaminate waters (Gogate and Pandit 2004). Indeed, they constitute promising, efficient and cost-effective methods for recalcitrant pollutants degradation, like herbicides (Wang and Xu 2012). An advanced oxidation technology like the UV/H_2O_2 process is a potentially effective and efficient alternative to treat herbicide wastewaters (Nienow et al. 2008; Gao et al. 2009). Even when it could be an extensive electricity consumer, the process can be carried out under natural conditions and has certain advantages in comparison with the most renowned AOPs: relatively low capital and operating costs and simplicity in its operation (Aleboyeh et al. 2008). It can reach appreciable rates of contaminants oxidation and offers a wide range of applications (Stefan et al. 1996; Sindelar et al. 2014). It also appears to be one of the most feasible AOP for full-scale applications (Juretic et al. 2015).

Murcia et al. (2015) proposed a possible reaction pathway focusing on the formation of chlorophenols for 2,4-D oxidation by the UV/H_2O_2 process. The authors then derived a model based on pseudo-first-order kinetics. In the same direction, the work by Sarmento and Miranda (2014) dealt with the formulation of a mechanistic kinetic model for atrazine degradation employing the UV/H_2O_2 process. In addition, Nienow et al. (2008) and Gao et al. (2009) explored the UV/H_2O_2 process optimization. These works are focused on pure contaminant degradation.

Response surface methodology (RSM) is a set of mathematical and statistical methods to design experiments, build models and evaluate the effects of independent variables (factors) on a dependent variable (response) (Rauf et al. 2008). RSM is widely used for experimental design and is widely applied to model several water treatments (Rosales et al. 2012). In this sense, availability of computer RSM software has turned RSM applications to an issue rather simpler (Nair et al. 2014). Estimation of linear, interaction and quadratic effects of input factors and the establishment of a mathematical model for prediction of the response are also accounted by this technique (Kasiri and Khataee 2011). Response surface models require little mechanistic knowledge to be developed and they can be derived relatively quickly. However, despite their potentialities, these models can be used to predict the system behavior only within the studied experimental region (Troup and Georgakis 2013).

Response surface models have been derived to model and predict the behavior of different kinds of AOPs. Examples can be found ranging from the oxidation of synthetic organic dyes solutions by UV/H_2O_2 process (Körbahti and Rauf 2008; Zuorro et al. 2013) to complex industrial wastewaters degradation by Fenton-like processes (Bianco et al. 2011; Sekaran et al. 2014). Nevertheless, the UV/H_2O_2 process modeling by RSM for commercial herbicides mixture degradation has not been reported yet.

In most cases, AOPs do not achieve contaminant total degradation (i.e., mineralization) and the evaluation of the toxicity due to remnant oxidation intermediates should be an essential task (Karci et al. 2012). For instance, to evaluate treatment quality, biological toxicity assays should complement the chemical ones (Pérez-Moya et al. 2007). This is certainly a relevant issue in the treatment of herbicide wastewaters since it has been demonstrated that some partial degradation byproducts can be more toxic than the parent compounds (Mariani et al. 2015; Vidal et al. 2015). Bioassays rely on measuring the effect on living organisms (i.e., microorganisms, plants and algae, invertebrates and fishes) exposed to contaminants (Rizzo 2011). They are reliable, cost-effective, fast and reproducible methods (Valerio et al. 2007). Toxicity evaluation based on bioassays can be a defining resource in order to ensure safe discharge into receiving water bodies or promote a subsequent biological treatment stage (Karci et al. 2012). Bioassays represent a very useful tool, also, to determine the end point for water treatment and reducing the operating costs of the involved AOP (Junges et al. 2013).

In this work, the suitability of the UV/H_2O_2 process for commercial herbicides mixture degradation (glyphosate, 2,4-D, and atrazine) was studied. Modeling of the process response related to specific operating conditions like initial pH and initial H_2O_2 to total organic carbon molar ratio was assessed by the RSM technique. Also, germination tests employing seeds of *Eruca sativa* Mill were employed in order to evaluate the phytotoxicity of the herbicides mixture during an extended run at the operating conditions for maximum total organic carbon (TOC) conversion.

Materials and methods

Reagents

The following reagents were used: (a) commercial glyphosate formulation, 67.9% ww^{-1} as acid (Round Up Ultra Max, N-phosphonomethyl glycine salt), (b) commercial 2,4-D formulation, 50 g/100 mL as acid (Chemotécnica, dimethylamine salt), and (c) commercial atrazine formulation, 50 g/100 mL. Glyphosate, 2,4-D and atrazine typical physical-chemical properties can be appreciated in Table 1.

Table 1 Herbicides physical-chemical properties (Mackay et al. 2006)

	Glyphosate	2,4-D	Atrazine
Solubility (25 °C)	~12,000 mg L^{-1}	~750 mg L^{-1}	~30 mg L^{-1}
Log K_{OW}	(−) 2.75	(+) 2.25	(+) 2.5

Hydrogen peroxide solution (Ciccarelli, 30% ww^{-1}) was used as the source of H_2O_2. Catalase enzyme from bovine liver (Fluka, >2000 units mg^{-1}) was employed for H_2O_2 decomposition (1 unit decomposes 1 μmol H_2O_2 per minute at pH 7.00 and 25 °C). Ultrapure water (0.055 μS cm^{-1}) was used in all experimental runs.

Equipment, operating conditions, and procedure

The reaction was carried out in a 110 cm^3 batch cylindrical photoreactor made of Teflon® closed with two flat, circular quartz windows. Each window permitted the interposing of one shutter to block the passage of light when necessary. Radiation was supplied by two low-pressure mercury vapor lamps with one emission wavelength at $\lambda = 253.7$ nm. The reactor was part of a closed recycling system including: a 2000 cm^3 glass storage tank with mechanical stirring and provisions for sampling, pH and temperature measurements and a high flow rate recirculation pump (2 L s^{-1}). The entire system was operated at a high recirculation flow rate thus good mixing in the reactor was achieved. A heat exchange system is also included to keep a constant reaction temperature (25 °C). A simplified scheme of the experimental setup is shown in Fig. 1.

Experimental runs were performed by varying two of the most significant factors related to the efficiency of the UV/H_2O_2 process: initial pH (between 3 and 10) and initial H_2O_2 concentration (between 120 and 1500 mg L^{-1}) at constant TOC initial concentration (30 mg L^{-1}) and spectral fluence rate (22.4×10^{-9} Einstein cm^{-2} s^{-1}). The spectral fluence rate at the reactor windows (i.e., the incident photon flux) was experimentally measured by potassium ferrioxalate actinometry based on the work by Murov et al. (1993) and calculated according to Zalazar et al. (2005). Initial pH and H_2O_2 concentration ranges were selected according to previous works where the UV/H_2O_2 process was applied for commercial herbicide degradation under similar experimental conditions (Mariani et al. 2015; Vidal et al. 2015). It has also been found, in previous UV/H_2O_2 and UV/O_3 process experiences that: direct photolysis of glyphosate, as acid, is negligible (Manassero et al. 2010); direct photolysis of 2,4-D is not of great importance (Gilliard et al. 2013); atrazine is decomposed mainly by HO• radical attack (Sarmento and Miranda 2014).

The total reaction time was 8 h. It must be noted that due to the type of equipment used in this work (a recycle with a tank), this time does not represent the one effectively corresponding to the irradiation time of the total system volume. Thus, the actual exposure to radiation must take into account the ratio given by the photoreactor volume over the total volume ($V_R/V_T = 0.11$).

For each run, the following procedure was followed: with shutters on, the lamps were turned on and they were allowed for at least 30 min to reach electrical stability. A working mixture (1000 cm^3) of glyphosate, 2,4-D and atrazine was prepared employing ultrapure water. Initial concentration of each herbicide was 30 mg L^{-1}. These are concentrations obtained after rinsing operations of empty herbicide containers (Femia et al. 2013). pH was adjusted with H_2SO_4 (1 N) or NaOH (1 N). The mixture was added to the reactor and the recirculation was established. Once reaction temperature was constant, the shutters were removed indicating the time $t = 0$ of the reaction. Samples (35 mL) were taken each 1 h. After a typical run, the equipment was carefully washed.

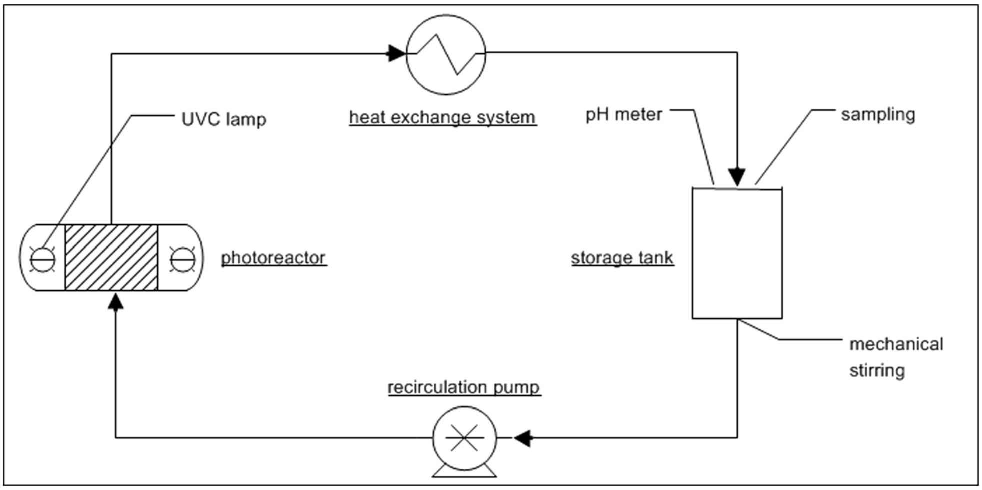

Fig. 1 Experimental setup scheme

Analyses

The following analyses were performed: H_2O_2 concentration was analyzed by a spectrophotometric method at 350 nm (Allen et al. 1952) employing a Perkin Elmer® spectrophotometer. Immediately after sampling and prior to the analysis, a catalase enzyme solution was added to each sample in order to decompose remnant H_2O_2 and to avoid further (direct) oxidation. The pH was measured with a HQ 40 d Hach® pH meter (accuracy: ±0.1) and TOC was analyzed with a Total Elementar® organic carbon analyzer. Atrazine and 2,4-D were measured by HPLC-UV Waters® (λ = 221 nm and λ = 236 nm, respectively) and glyphosate was analyzed employing HPLC (Waters®) equipped with a conductivity detector.

Experimental design

Modeling of the process response related to specific operating conditions as initial pH and initial H_2O_2 to TOC molar ratio (R) was assessed by RSM. A minimum set of assays adequately distributed in the experimental region was tested ($3 \leq \text{pH} \leq 10$, $1.4 \leq R \leq 17.6$). Design-Expert Software® (V10) was used for regression analysis and coefficients estimation. TOC conversion (%) at 8 h was defined as the response.

A three-level full factorial design with two factors (pH, R) was selected. Being k the number of factors, it is common to specify the design as a 3^k design and the level of each factor as low, center, and high (coded as −1, 0 y + 1, respectively). For two factors and three levels, 12 experimental runs were performed.

Second-order equations are often used in RSM problems (Montgomery 2001). Therefore, a second-order polynomial model was derived for the correlation of the response:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 + e \qquad (1)$$

Y states for the response, X_1 and X_2 are the factors in its coded form (initial pH and R ratio, respectively), b_i are the regression coefficients for linear (main) effects, b_{ii} reflect the quadratic (curvature) effects, and the b_{ij} account for the interaction (cross-factor) effects. The term "e" denotes the random error component that represents different sources of variability, including (Vera Candioti et al. 2014): effects such as measurement error on the response, non-studied factors, and other sources of variability of the system itself.

In order to evaluate the statistical significance, adequacy and quality of fit of the second-order regression model, analysis of variance (ANOVA) statistics (Fisher F-test, adequate precision ratio, determination coefficient) and diagnostic plots (residuals) were examined (Montgomery 2001). Actual (i.e., experimental) and predicted responses were then compared to validate the model.

Phytotoxicity evolution

The phytotoxic effects of commercial herbicides mixture on seeds of *Eruca sativa* Mill were studied during germination stage of seeds through the germination index (%) and the root elongation (mm).

Bioassays were carried out in plastic dishes (8 cm diameter, 3 cm height) with filter paper on the bottom. Each dish contained a defined volume (2 mL) of the working mixture or control (i.e., distilled water). Samples were taken within 2, 6, 8, 10, 12, and 16 h during an extended run at the operating conditions for maximum TOC conversion (see *Full factorial design model* subsection). Remnant H_2O_2 present in the samples was removed using catalase solution prior to bioassays. Dishes were wrapped with Parafilm® and placed in a growth chamber at 25 ± 1 °C in the dark. All assays were carried out in triplicate (i.e., three replicates per sample of 20 seeds each). After 48 h, the number of germinated seeds and the root elongation were measured. Germination index was calculated according to Eq. 2 (Komilis et al. 2005).

$$\text{GI}(\%) = (N/N_C) \times (L/L_C) \times 100 \qquad (2)$$

Where: N = number of seeds germinated in a sample, N_C = number of seeds germinated in the control, L = average root elongation in a sample, and L_C = average root elongation in the control.

Seeds were considered germinated when a root length greater than 2 mm was observed. Dead or rotten seeds were not considered germinated. Percentages were relative to the controls (i.e., controls always had a GI equal to 100%). A germination index lower than 60% was indicative of phytotoxicity.

Single-factor ANOVA was applied for the whole of the root elongation data (i.e., all the replicates). Obtained summary data was then employed for Dunnett's statistical test with a 95% of confidence level (Montgomery 2001).

Results and discussion

Full factorial design model

The experimental grid is presented in Table 2. Surprisingly, TOC conversion variation seemed to be minor (less than 10%) with the switching in R ratio between center and high level, for a fixed-low pH level (i.e., run 5). On the other hand, one of the observed responses for center level of both factors (i.e., run 8) was abnormally high. Accordingly, the grid was built

Table 2 Full factorial design grid. Actual and predicted responses

Run	pH	R	Exp.[a]	Pred.
1	3 (−1)	1.5 (−1)	10.5	11.01
2	10 (+1)	1.6 (−1)	26.9	26.7
3	5 (0)	17.1 (+1)	41.4	40.47
4	10 (+1)	14.65 (+1)	41.45	42.93
5	3 (−1)	17.6 (+1)	36.5	–
6	10 (+1)	6.9 (0)	47.3	46.02
7	5 (0)	1.4 (−1)	42.4	–
8	5 (0)	8.3 (0)	61.2	–
9	5 (0)	7.85 (0)	53.6	53.91
10	5 (0)	7.9 (0)	56.5	53.98
11	5 (0)	7.8 (0)	50.7	53.84
12	3 (−1)	8.6 (0)	33.9	33.39

[a] Discarded: runs 5, 7, 8

excluding, at first, two runs: 5 and 8. The regression analysis was then applied. According to the externally studentized residuals plot (Vera Candioti et al. 2014), run 7 could be a potential outlier (i.e., an abnormal data point) which negatively influence the quality of fit of the first derived model. The run 7 was hence discarded.

The regression analysis was again applied. This time, according to the externally studentized residuals plot, no potential outliers were identified (see *Model adequacy check* subsection). A new model was derived and the significance of each term was evaluated by ANOVA. Not significant terms were removed through backward elimination as the chosen strategy, with a 95% confidence level. The fitted second-order model (without interaction) to describe and predict the system behavior is in its non-coded form:

$$X_{\mathrm{TOC}}(\%) = 24.37329\ \mathrm{pH} + 6.28237\ R - 1.70840\ \mathrm{pH}^2 - 0.31005\ R^2 - 55.45583 \quad (3)$$

As it can be appreciated, factors are not involved in any significant interaction (i.e., the initial pH effect is not dependent on the R level).

An R level for maximum TOC conversion is predicted whatever the level of the pH factor. In AOPs, it has been extensively demonstrated that organic matter degradation usually increases at higher H_2O_2 concentrations (Gogate and Pandit 2004). However, degradation reactions progress up to a certain limit in which H_2O_2 starts to inhibit the photolytic degradation process (Wang and Xu 2012). Once this limit is exceeded, H_2O_2 begins to act as a HO• scavenger. The scavenging effect and the existence of an R ratio for maximum TOC conversion are shown through the corresponding quadratic term of the model, preceded by a minus sign.

The existence of an initial operating pH for maximum TOC conversion can also be predicted, for each initial H_2O_2 concentration (i.e., for each R level). In this sense, for a given initial H_2O_2 concentration TOC conversion increases for higher pH-levels up to a certain value where it begins to decrease. The occurrence of an initial H_2O_2 concentration for maximum TOC conversion was already observed by Mariani et al. (2015) for the mixture of glyphosate/2,4-D. In the same work, it was observed that X_{TOC} (pH 10) > X_{TOC} (pH 3) for a fixed R ratio. Arántegui et al. (1995) noted, also, a positive effect in atrazine conversion for a given initial H_2O_2 concentration with a switching in pH between 4.8 and 9. An analogous effect was observed by Khan et al. (2014), when the pH was changed from 3 to 5.7. These tendencies are clearly captured by the regression model: for low, center, and high level of the R factor it follows that X_{TOC} (pH 10) > X_{TOC} (pH 3). Also, for the whole R span the model predicts declining TOC conversions for an initial pH >7. This is an issue which could demand further investigation. For illustrative purposes, TOC and H_2O_2 conversions for center level of both factors are shown in Fig. 2.

Operating conditions to maximize the TOC conversion were evaluated employing a numerical technique based on the fitted model and the factors in their critical range as the constraints. For 63.3% TOC conversion, conditions found were: pH =7.13 and R = 10.13.

In order to show the effect of each factor in TOC conversion, a 3-D plot based on the fitted model is presented in Fig. 3.

Model evaluation: variance analysis

The statistical significance of the regression model to describe and predict the system behavior was evaluated by typical ANOVA statistics (Fisher F-test, adequate precision ratio). Table 3 shows ANOVA results of the fitted model for TOC conversion.

Fisher F-test relies in the calculation of the F-value which is computed through the ratio between the mean square of the model (df = 4) and the mean square of the residual error

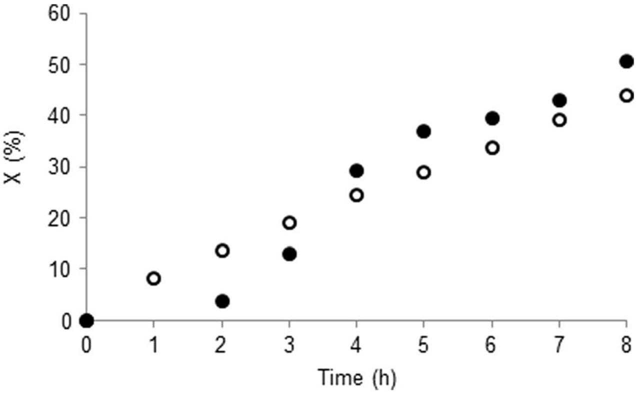

Fig. 2 TOC (●) and H_2O_2 (○) conversions (%) vs. time for pH =5 and R = 7.8

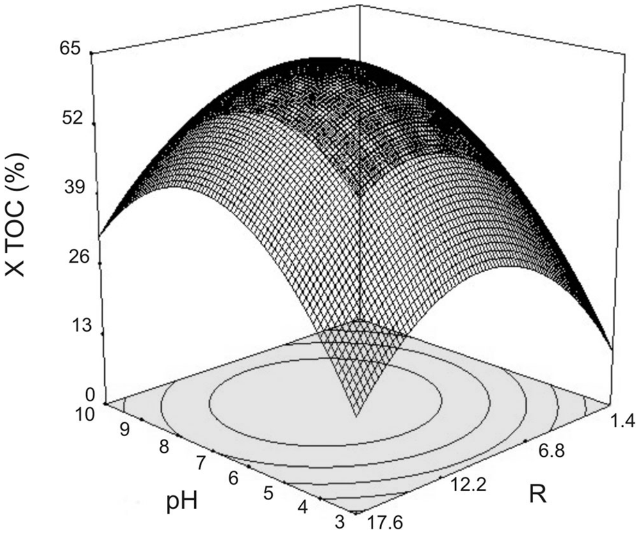

Fig. 3 Response surface plot for TOC conversion (%) as a function of initial pH and *R* ratio

(d*f* = 4). The value (78.2) is greater than the tabulated critical one which is $F_{4,4;0.05}$ = 6.39 for a 95% of confidence level (Montgomery 2001). Therefore, according to the computed *F* value and the low probability value for calculated Fisher F-test ($p < 0.05$) it can be observed that the model is statistically significant. The model terms can be considered statistically significant because their respective *p* values are not greater than 0.1. The standardized effect of each factor on the process response (i.e., the factor contribution) was checked on the basis of a Pareto chart (Yetilmezsoy et al. 2009). As it can be seen in Fig. 4, the interaction effects are not potentially important because the length of the corresponding bar remained behind the reference line (i.e., the line for $p = 0.05$). For the same reason, pH and *R* effects seem to be potentially important because the length of their bars extend beyond the same line.

Taking into account these two factors, initial pH is particularly relevant (see Table 3). It is well known that pH have a marked effect in the speciation and hence in the reactivity of an organic compound (Schwarzenbach et al. 2003). Thus, for 2,4-D and atrazine with pK_a values of 2.64 and 1.6, respectively (Yao and Haag 1991), ionization percentages higher than 99% can be obtained in both cases for pH >5. At the same time, according to pK_a values of glyphosate (Yao and Haag 1991), it can be observed that between 5.9 < pH < 10.4 the phosphonate group is totally ionized. Therefore, the breakdown of the C-P bond could be more favorable in this ionic form. In fact, at the conditions for maximum TOC conversion, 2,4-D and atrazine are nearly 100% ionized and the totally ionized glyphosate form is present in a percentage higher than 90%.

According to ANOVA results, it is expected that this model would produce a signal comparatively larger than the total of the variability. The adequate precision ratio (i.e., a signal-to-variability ratio) reinforces these findings. In this sense, it could be said that the model is an appropriate tool to describe the system behavior when an operator navigates within the completely experimental region (adequate precision >4).

Model adequacy check: diagnostic analysis

Even though it was demonstrated that the model is statistically significant, it becomes essential to check diagnostic plots and determination coefficient value (R^2) in order to determine if the model correctly explains the variability through the experimental data. The adequacy of the regression model was first assessed by checking residuals diagnostic plots. As it was mentioned, residuals (i.e., the random error component) represent other sources of variability not accounted for the fitted model and it is expected that they follow a normal distribution. The normal probability plot is the appropriate graphical proof to decide whether the residuals occur according to a normal distribution, which is indeed one of the basic assumptions for ANOVA validation (Vera Candioti et al. 2014). The normal probability plot is shown in Fig. 5. It can be seen that internally studentized residuals are distributed around a straight line; at the same time, residuals (vs. ascending predicted responses) do not describe a defined shaped pattern (see Fig. 6). Therefore, the existence of a homogeneous variance (other basic assumption for ANOVA validation) is positively evaluated (Vera Candioti et al. 2014).

Table 3 ANOVA results for predicted TOC conversion (%)

Source	Sum of squares	Degrees of freedom	Mean square	*F*	*p*
Model	1686.00	4	421.50	78.20	0.0005
pH (X_1)	262.62	1	262.62	48.73	0.0022
R (X_2)	107.33	1	107.33	19.91	0.0111
pH^2	431.25	1	431.25	80.01	0.0009
R^2	562.17	1	562.17	104.30	0.0005
Residual error	21.56	4	5.39	–	–

R^2 = 0.9874, Adeq. precision = 24.831, RMSE = 0.5159 mg L^{-1}

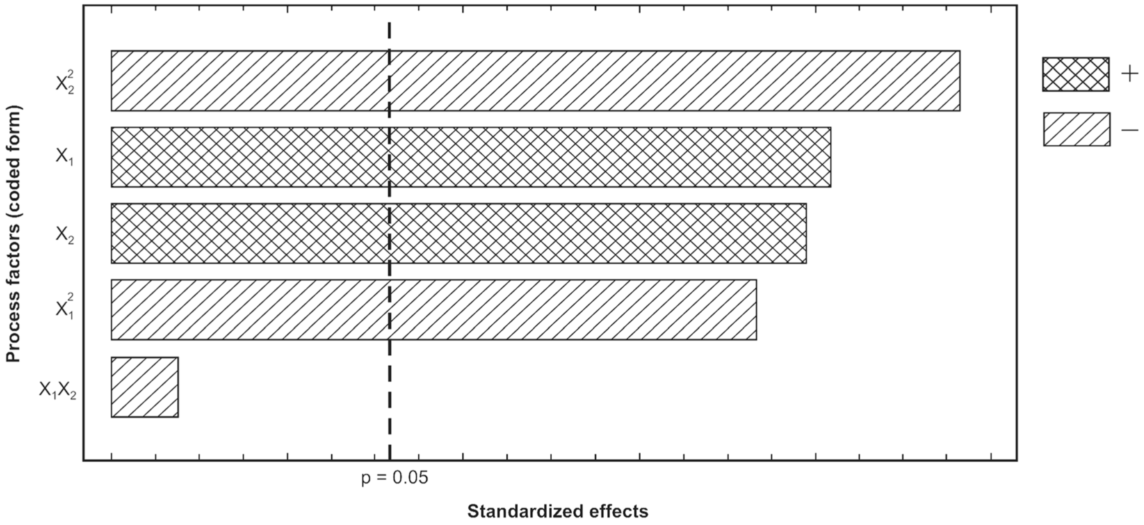

Fig. 4 Standardized effects on TOC conversion (%). pH (X_1), R (X_2), pH^2 (X_1^2), R^2 (X_2^2), pH R (X_1X_2)

Qualitatively, according to the externally studentized residuals plot (figure not shown) no apparent deviations of data points from the straight line are observed; therefore, there are not potential outliers with a negative influence on the quality of fit of the regression model.

The model quality of fit was evaluated, quantitatively, by computing R^2 statistic (see Table 3). Good correlation between the experimental data and the predicted responses was obtained indicating that the fitted model explains 98.74% of the total variability that affects the response. Another supplementary statistic, the root mean square error (RMSE), reinforces this result (see Table 3). The RMSE (i.e., the positive square root of the sum of squares of the residual error divided by the effective total number of experimental runs) is a measure of the total variability that is not explained by the model (Mendes et al. 2015).

TOC conversion was finally validated by comparing experimental and predicted responses. As it is shown in Fig. 7, the majority of the experimental data were in good agreement with the predicted responses under the operating conditions studied, with a correlation coefficient $r = 0.99$.

Phytotoxicity evaluation

Changes in the phytotoxicity of treated herbicides mixture during an extended run, at the operating conditions for maximum TOC conversion, are presented below (see Table 4).

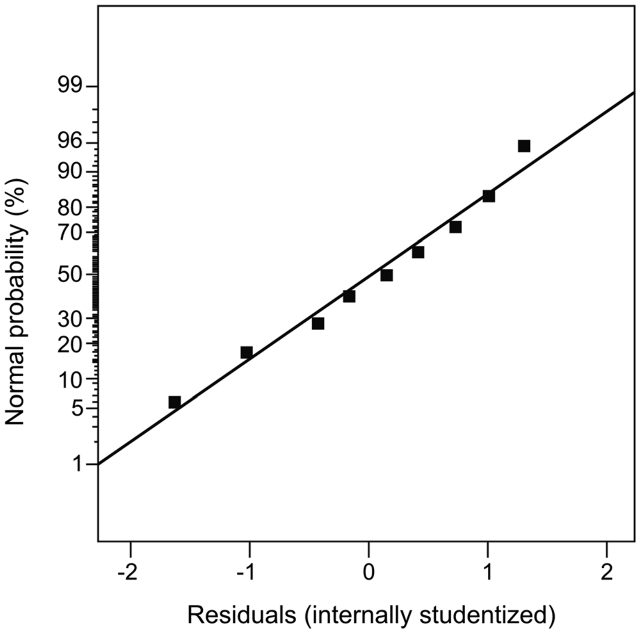

Fig. 5 Normal probability plot vs. residuals

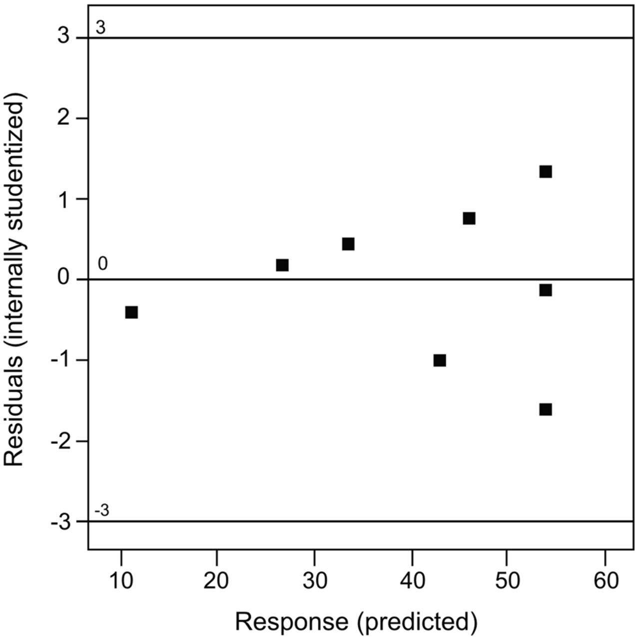

Fig. 6 Residuals vs. predicted responses

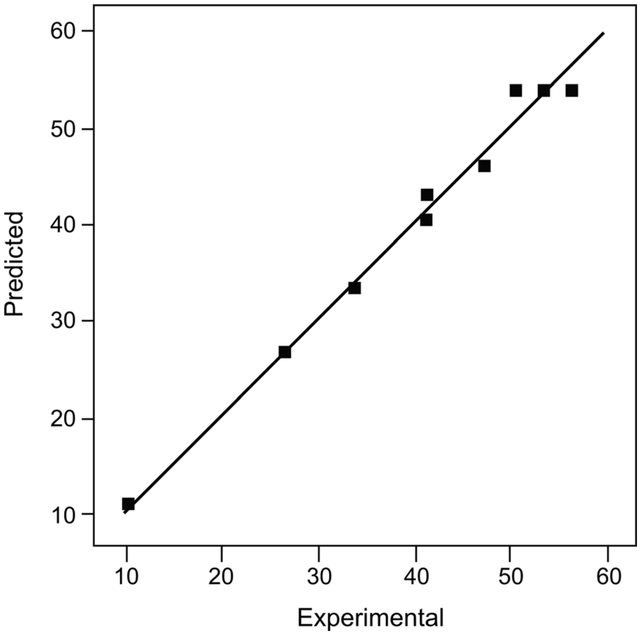

Fig. 7 Predicted TOC conversion (%) vs. experimental

As it can be seen, root elongation appears to be practically constant along the first 6 h of treatment; towards 8 h, germination index reduces markedly (~60%) indicating toxicity existence (note that root elongation follows the same tendency). As the reaction progresses (10 h) root elongation increases while germination index level suggests the lack of toxicity. Finally, towards 16 h, both indicators reach considerably higher levels than the control.

It should be mentioned that, at the beginning (0 h), the phytotoxicity was at its highest value (germination index = 0%, root elongation = 0 mm). This was clearly due to the presence of the parent compounds (i.e., glyphosate, 2,4-D and atrazine) in the non-treated herbicides mixture. After 2 h of treatment, 2,4-D and atrazine were not detected while glyphosate was present in decreasing concentrations until the end of the reaction (data not shown). Hence, at 8 h of reaction time, the observed toxicological response (i.e., the raise of toxicity) could be attributed to the production of oxidation intermediates, which can be even more toxic (Oller et al. 2011; Rizzo 2011). The reaction intermediates would probably include oxalic acid, a typical final degradation by product of more complex molecules (Garcia-Segura and Brillas 2011) and cyanuric acid, which is the ultimate oxidation by product of atrazine (Balci et al. 2009). For longer reaction times (16 h), mineralization progress and inorganic anions production (i.e., nitrates, phosphates) would allow understanding the remarkable phytotoxicity reduction as well as the establishment of a positive environment for growing and development of the assayed seeds.

Table 4 Phytotoxicity evolution during an extended run (16 h)

Reaction time (h)	Germination index (%)[a]	Root elongation (mm)[b]
0	0	0
2	66.2	10.2 ± 3.41
6	77.1	10.1 ± 2.76
8	60.6	8.8 ± 3.10
10	82.3	10.5 ± 3.78
12	88.5	10.8 ± 4.36
16	138.9	15.6 ± 5.24

[a] 100% for control

[b] 11.6 mm for control

Dunnett's statistical test, based on single-factor ANOVA, supports previous findings. The test revealed that the difference in terms of the average root elongation between the control and sample 4 (8 h) is statistically significant, with a 95% confidence level (see Table 5). At the same time, the difference in terms of the average root elongation between the control and sample 8 (16 h) is statistically significant for the same confidence level.

From the toxicity study, it can be concluded that, at the operating conditions for maximum TOC conversion it is necessary to extend the treatment at least until 10 h (i.e., end point). With nearly 71% of TOC conversion, relatively small remnant toxicity confirms the capability of the UV/H_2O_2 process to detoxify the herbicides mixture.

Conclusions

In this work, the suitability of the UV/H_2O_2 process for commercial herbicides mixture degradation was studied. Modeling of the process response related to specific operating conditions was assessed by RSM. A second-order polynomial model was derived for the correlation of the response. This model could well describe the effects of the selected factors (initial pH, *R* ratio) on TOC conversion. Its structure has shown that the factors were not involved in any significant interaction under the operating conditions studied. TOC conversion was highly influenced by the initial pH while the influence of the *R* ratio was smaller.

Table 5 Dunnett's statistical test (95% confidence level)

Sample-control	msd (mm)	adba (mm)	adba > msd
S1-C	0.2039	0.1380	False
S3-C	0.1992	0.1460	False
S4-C	0.2027	0.2807	True
S5-C	0.2015	0.1128	False
S6-C	0.1992	0.0752	False
S8-C	0.1952	0.4030	True

msd minimum significant difference, *adba* absolute difference between averages

The statistical significance, adequacy and quality of fit of the model were evaluated. The model could well describe and predict the system behavior within the tested experimental region. It also correctly explained the variability in the experimental data. TOC conversion was validated by comparing experimental and predicted responses. Experimental values were in good agreement with the modeled ones confirming the significance of the model and highlighting the success of RSM for UV/H_2O_2 process modeling.

Phytotoxicity evolution along the photolytic degradation process was checked through germination tests employing seeds of *Eruca sativa* Mill. The result indicated that the phytotoxicity of the mixture of glyphosate, 2,4-D and atrazine was significantly reduced after the treatment. The end point of the treatment at the operating conditions for maximum TOC conversion was also identified.

The UV/H_2O_2 process could be suitable for aqueous commercial herbicides mixture degradation. Operating conditions found for maximum TOC conversion could be useful for real-field applications.

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