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# Fenton and photo-Fenton degradation of 2-chlorophenol: Multivariate analysis and toxicity monitoring

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**Abstract**

The simultaneous occurrence of Fenton and photo-Fenton reactions is an attractive process for contamination remediation involving high toxicity and low biodegradability species. In this work, a multivariate experimental design was applied to the treatment of 2-chlorophenol, as representative of chlorinated aromatic compounds, in order to evaluate the use of the Fenton reagent under light irradiation (wavelength close to 400 nm for photo-Fenton reactions and up to 550 nm for Fenton-like reactions). Hence, the aim of this work is to study the evolution of toxicity through all the degradation process by characterizing the toxicity level of 2-chlorophenol and its reaction intermediates as a function of temperature and the Fenton reagent loads. Factorial experimental design was used to assign each variable's weight in TOC removal after 30 min of reaction. Hydrogen peroxide concentration appears as a main direct effect for a favorable TOC reduction. Temperature has also an important effect in the 2-chlorophenol degradation, especially when the ratio of Fenton reagents is not correctly chosen. More than 90% TOC reduction could be achieved in only 30 min of treatment. In addition, the HPLC analysis showed the elimination of the studied compound and its byproducts under specific working conditions. Finally, results indicated the importance of taking into account toxicity evolution.


*Keywords:* 2-Chlorophenol; Byproducts; Multivariate analysis; Photo-Fenton; Toxicity

## 1. Introduction

High electrical energy demand and excessive consumption of chemical reagents are common problems among all Advanced Oxidation Process (AOP) [1]. However, combining Fenton reagent with a light source results in a low-cost process for wastewater treatment [2]. Since a photo-Fenton reaction can use photons with a wavelength close to 400 nm, and given that mixtures of Fe(III) and $H_2O_2$ (known as Fenton-like reactions [3]) have shown photon absorption up to 550 nm [4,5], the photo-Fenton process can also be run under solar irradiation [6,7].

The generally accepted Fenton reaction mechanism is where hydroxyl radicals $OH^{\bullet}$ are produced by interaction of $H_2O_2$ with ferrous salts (Eq. (1)). Additionally, Fe(III) can react with $H_2O_2$ in the so-called Fenton-like reaction (Eqs. (2) and (3)), regenerating Fe(II) and thus supporting the Fenton process [3]:

$$\mathrm{Fe(II)} + \mathrm{H_2O_2} \rightarrow \mathrm{Fe(III)} + \mathrm{OH^{\bullet}} + \mathrm{OH^-}, \quad k = 53\text{–}76\ \mathrm{L\,mol^{-1}\,s^{-1}} \tag{1}$$

$$\mathrm{Fe^{3+}} + \mathrm{H_2O_2} \rightarrow \mathrm{Fe^{2+}} + \mathrm{HO_2^{\bullet}} + \mathrm{H^+}, \quad k = (1\text{–}2) \times 10^{-2}\ \mathrm{L\,mol^{-1}\,s^{-1}} \tag{2}$$

$$\mathrm{Fe^{3+}} + \mathrm{HO_2^{\bullet}} \rightarrow \mathrm{Fe^{2+}} + \mathrm{O_2} + \mathrm{H^+}, \quad k = (0.33\text{–}2.1) \times 10^{6}\ \mathrm{L\,mol^{-1}\,s^{-1}} \tag{3}$$

The degradation rate of the organic pollutants by Fenton reaction could increase when an irradiation source is present. The positive effect of irradiation on the degradation rate is due to the photoreduction of Fe(III) to Fe(II) ions, a step that produces new $OH^{\bullet}$ radicals and regenerates Fe(II) ions that can further react with more $H_2O_2$ molecules. The photoreduction of

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Fe(III) follows equation:

$$FeOH^{2+} + h\nu \rightarrow Fe^{2+} + OH^{\bullet} \quad (4)$$

with $Fe(OH)^{2+}$ being the dominant Fe(III) species in solution at pH 2–3. Recently, it has been proven that the irradiation of Fe(III) + $H_2O_2$, also called Fenton-like reaction, enhances the reaction rate of oxidant production through the involvement of high valence Fe intermediates responsible for the direct attack to organic matter [5,8]. Absorption of visible light by the complex formed by Fe(III) and $H_2O_2$ seems to be the cause of formation of such high valence Fe-based oxidants.

Chlorophenols are important intermediates in the chemical industry. They are widely used as fungicides as well as in the synthesis and in the transformation of herbicides and insecticides. Due to their toxicity and high solubility in water, chlorophenols are ranked as severe environmental pollutants [9]. It is important to study both their transformation rate in the aqueous phase as well as identify the byproducts produced by partial degradation, which may result in increasing toxicity levels, because they may pose an important trade-off.

We have focused on 2-chlorophenol ($pK_a = 8.11$) due to its high toxicity as well as the need to remove 2-chlorophenol from industrial effluents. Previous works already proved the efficiency of Fenton reactions in the reduction of the most important parameters (such as AOX, COD, and TOC) that account for the environmental impact of chlorophenols [10]. The purpose of this study is to study and characterize the Fenton, Fenton-like and Photo-Fenton reactions applied to reduce 2-chlorophenol concentration in aqueous solution.

HPLC analyses of the samples were performed throughout the reaction to determine the presence and relative concentrations of 2-chlorophenol and related degradation intermediates. In order to ensure the quality of the treated solution, the toxicity was studied in those cases where the HPLC analyses clearly showed the presence of intermediate compounds.

The growth of *Escherichia coli* is inhibited by the presence of 2-chlorophenol in the culture medium [11]. Thus, to monitor the toxicity decay during the degradation process, we have studied the growth of *E. coli* bacteria in presence of Fenton and Photo-Fenton reaction products.

Experimental $2^3$ design with the adequate central points will result in a polynomial equation and a three-dimensional representation of the phenomena where the percentage TOC removal is the response factor [12]. The study is addressed to determine the influence of Fenton reagent loads (Fe(II), $H_2O_2$) and the influence of the temperature. The study was performed considering two levels (low and high) and the interaction between the variables was assessed. Using this technique, it is possible to find the most adequate conditions for the reaction system. Applications of this statistical methodology have been reported for pulp mill effluent treatment with heterogeneous photocatalytic oxidation [13], ozone [14] and in the Fenton reaction of industrial wastewaters containing aromatic amines [15] among others.

## 2. Experimental

Analytical grade 2-chlorophenol was purchased from Sigma–Aldrich and was diluted to $10^{-3}$ M. Analytical grade hydrogen peroxide and heptahydrated ferrous sulfate were purchased from Panreac and Sigma–Aldrich, respectively, and were used as received. The rest of the chemicals used were, at least, of reagent grade. Solutions were prepared with deionized water obtained from a Millipore Mili-Q system.

Experiments were conducted in a thermostatic cylindrical 130 mL Pyrex cell. The reaction mixture inside the cell, consisting of 100 mL of 2-chlorophenol solution and the precise amount of Fenton reagent, was continuously stirred with a magnetic bar. Temperature was maintained at $T \pm 0.1$ °C. 2-Chlorophenol solution pH is 5.5, after adding heptahydrated ferrous sulfate pH decreased down to 4.5 and the addition of hydrogen peroxide reduce pH down to $2.5 \pm 0.2$. Temperature and pH were on-line measured to ensure they kept constant along all the reaction time.

The experiments were carried out with a sunlight lamp (Ultra-Vitalux®, Osram, 300 W). The Ultra-Vitalux® sunlamp consists of a quartz burner and a tungsten filament which are blended in such a way that the radiation emitted is practically the same as natural sun radiation. The spectral radiation, supplied by Osram, presents wavelengths closes to 400 nm (appropriate for photo-Fenton reaction) and up to 550 nm (useful for the Fenton-like reaction). The distance between the lamp and the reactor's center was 15 cm.

Total organic carbon (TOC) of samples was determined with a Shimadzu 5000 TOC analyzer. The residual hydrogen peroxide concentration was determined spectrophotometrically after reaction with ammonium metavanadate [16].

For the analysis of low molecular weight compounds dissolved in the solution after the reactions, an HPLC Mettrohm 690 equipped with a UV–Vis detector (model 795A) from Applied Biosystems and a Hewlett-Packard electronic integrator (model 3394A) was used. A Shimadzu LC-10AT high pressure pump with the capability to provide four different solvents by means of a proportional system valve was used. The liquid chromatography analysis was carried out using methanol (Riedel-de Häen HPLC gradient grade)/water (bi-distilled) 0/100 volume percentage at initial time, and 100/0 at 15 min. A Hypersil ODS column of 200 mm long, 4.6 mm diameter and 5 μm particle diameter was used. The flow rate was 1 mL $min^{-1}$ and the recorded value were fitted at 254 nm.

The toxic effect of 2-chlorophenol and their degradation products were evaluated by the inhibition growth of selected bacteria. *E. coli* bacteria was grown in a Luria-Bertani (LB) batch culture medium containing (per liter) 3 g of $K_2HPO_4$, 1 g of $KH_2PO_4$, 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl. The assays were carried out in 96 well microtitre plates, which contained $10^4$ bacteria in suspension in LB per well. Tests were carried out by adding 40 μL of the reaction sample and 160 μL of LB broth (final volume of 200 μL). Controls consisted of LB broth alone. After incubation at 37 °C for 10 min, the bacterial growth was determined by reading the absorption at 600 nm in an Anthos Labtec microtitre plate

reader at 0, 24, 48 and 72 h incubation. The growth inhibition was assessed by comparing the differences between the absorption (optical density) values of the treatment and the respective control at 0, 24, 48 and 72 h.

## 3. Results and discussion

The Fenton, Fenton-like and photo-Fenton reactions were performed on $10^{-3}$ M 2-chlorophenol aqueous solutions (TOC 74 ± 2 ppm, pH 5.5 ± 0.08, $LD_{50} = 10^{-3}$ M ($10^4$ bacteria of *E. coli* DH5α)).

As expected from Eqs. (1)–(4), the complex reactive systems are pH dependent processes. However, each reaction has its optimum performance at different pH values. For example, data concerning TOC degradation of paper mill effluents indicate that the fastest TOC removal takes place at pH 2.8 [7]. On the other hand, several authors reported optimal pH for monochlorophenol degradations to be between 2 and 4 [10,17]. Thus, the pH used in all the experiments presented in this study was around 2.5 which is natural 2-chlorophenol (pH 5.5) $10^{-3}$ M solution pH after adding the Fenton reactants.

The concentration of Fenton reagent has an important influence in chlorophenol degradation. The stoichiometric coefficient for Fenton reaction has been found to be approximately 0.5 mol organic compound per 1 mol hydrogen peroxide (corresponding to 36 ppm TOC for 2-chlorophenol/36 ppm hydrogen peroxide [10]). A typical range for Fenton reactants is about 1 part of iron per 5–25 parts of hydrogen peroxide (w/w) [18]. However, the dose of hydrogen peroxide normally reported for Fenton and photo-Fenton systems applied to the degradation of pure compounds or complex effluents is increased about 2–10 parts for 1 part of contaminant [1,10,19,20].

On the other hand, it should be noted that an excess of $H_2O_2$ or $Fe^{2+}$ might be detrimental since these species can react with some of the intermediates, such as the OH radical, responsible for the direct oxidation of the organic load (Eqs. (5) and (6)).

$$Fe(II) + OH^{\bullet} \rightarrow Fe(III) + OH^{-}, \quad k = (2.6\text{–}5) \times 10^8\ L\,mol^{-1}\,s^{-1} \tag{5}$$

$$H_2O_2 + OH^{\bullet} \rightarrow HO_2^{\bullet} + H_2O, \quad k = 2.7 \times 10^7\ L\,mol^{-1}\,s^{-1} \tag{6}$$

With the aim to evaluate the effect of the Fenton reagent concentrations in the 2-chlorophenol degradation, a factorial experimental design ($2^3$) was applied. Variables studied were the iron and hydrogen peroxide concentrations and temperature, varying in the ranges 20–70 mg $L^{-1}$, 200–700 mg $L^{-1}$ and 25–65 °C, respectively. The low and high levels were coded as (−1) and (+1). Fifteen experiments were performed including the central points to assure adequate statistical consistency. Temperature was also tested because previous studies of our group clearly indicated that temperature has a great influence on the reaction system [20]. The matrix shown in Table 1 was resolved using the software FATORIAL [12].

The TOC removal percentage after 15 and 30 min of reaction was used as the response factor ($Y$ in the polynomial of Eqs. (7) and (8)). The coefficients of the quadratic model in the polynomial expression were calculated by multiple regression analysis. In Eqs. (7) and (8), $X_1$, $X_2$ and $X_3$ represent the variables [Fe(II)], [$H_2O_2$] and temperature, respectively. Values in parenthesis describe the relative error associated to each coefficient.

In the polynomial representing 15 min of reaction the explained variance for a 95% confidence level obtained by the $F$-test is 99.97%.

$$\begin{aligned} Y(\text{TOC removal after 15 min}, \%) &= 63.9(\pm5.1) + 4.0(\pm2.4)X_1 + 43.5(\pm3.1)X_2 \\ &\quad + 2.4(\pm2.4)X_3 + 1.6(\pm2.8)X_1^2 - 4.1(\pm4.6)X_2^2 \\ &\quad - 9.9(\pm2.8)X_3^2 \end{aligned} \tag{7}$$

Table 1
Factorial experimental design of the 2-chlorophenol treated by Fenton and Photo-Fenton reactions

| Experiment number | Codified values | | | Variable levels | | | TOC | |
|---|---|---|---|---|---|---|---|---|
| | [Fe(II)] (ppm) | [$H_2O_2$] (ppm) | Temperature (°C) | [Fe(II)] (ppm) | [$H_2O_2$] (ppm) | Temperature (°C) | Reduction after 15 min (%) | Reduction after 30 min (%) |
| 1 | −1 | −1 | −1 | 20 | 200 | 25 | 0.2 | 0.8 |
| 2 | +1 | −1 | −1 | 70 | 200 | 25 | 8.00 | 11.00 |
| 3 | −1 | +1 | −1 | 20 | 700 | 25 | 94.90 | 96.87 |
| 4 | +1 | +1 | −1 | 70 | 700 | 25 | 93.00 | 94.50 |
| 5 | −1 | −1 | +1 | 20 | 200 | 65 | 16.07 | 17.30 |
| 6 | +1 | −1 | +1 | 70 | 200 | 65 | 8.00 | 8.50 |
| 7 | −1 | +1 | +1 | 20 | 700 | 65 | 95.80 | 95.98 |
| 8 | +1 | +1 | +1 | 70 | 700 | 65 | 96.40 | 96.40 |
| 9 | 0 | 0 | 0 | 45 | 450 | 45 | 64.50 | 73.30 |
| 10 | 0 | 0 | 0 | 45 | 450 | 45 | 65.00 | 78.00 |
| 11 | 0 | 0 | 0 | 45 | 450 | 45 | 62.30 | 72.00 |
| 12 | −1.68 | 0 | 0 | 3 | 450 | 45 | 51.80 | 73.50 |
| 13 | 1.68 | 0 | 0 | 87 | 450 | 45 | 85.20 | 88.40 |
| 14 | 0 | 0 | −1.68 | 45 | 450 | 11 | 32.30 | 34.70 |
| 15 | 0 | 0 | 1.68 | 45 | 450 | 79 | 39.70 | 40.20 |

In the polynomial representing 30 min of reaction the explained variance for a 95% confidence level obtained by the $F$-test is 99.89%.

$$Y(\text{TOC removal after 30 min}, \%) = 74.4(\pm 3.1) + 1.8(\pm 1.5)X_1 + 43.3(\pm 1.9)X_2 + 1.8(\pm 1.5)X_3 + 2.3(\pm 1.7)X_1^2 - 11.0(\pm 2.8)X_2^2 - 13.1(\pm 1.7)X_3^2 \quad (8)$$

Fig. 1(A and B) shows a comparison between the experimental values and the predicted values of the TOC removal percentage obtained from the polynomial empirical models. The experimental assay are related to the matrix design shown in Table 1.

For times longer than 30 min the second order polynomial equations were unsuccessful. As recent studies notice, the main reason for the failure of this methodological approach is probably the wide range of results that the model must cover [21]. Especially at longer times, two different tendencies are clearly appearing: from one side, a group of experiments for which the TOC degradation is complete, and another set of experiments for which the system conditions do not allow farther degradation. The very recent work by Gernjak et al. [21] proposes other empirical models for describing the photo-Fenton system. Due to the complexity of the system and depending of the studied contaminant the correlations are likely to be somewhat different for every particular case.

From the polynomial equation (8) it can be concluded that TOC removal is quite high during the 30 min reaction time reaching values close to 100% under the appropriate conditions. Again, the polynomial fitting is revealed inaccurate for those regions close to complete TOC removal. The model is not applicable in these cases since predicted values over 100% could be obtained. Such a model mismatch is given by the inability of a polynomial model to describe the saturation behavior of the real system under some conditions, as well as by

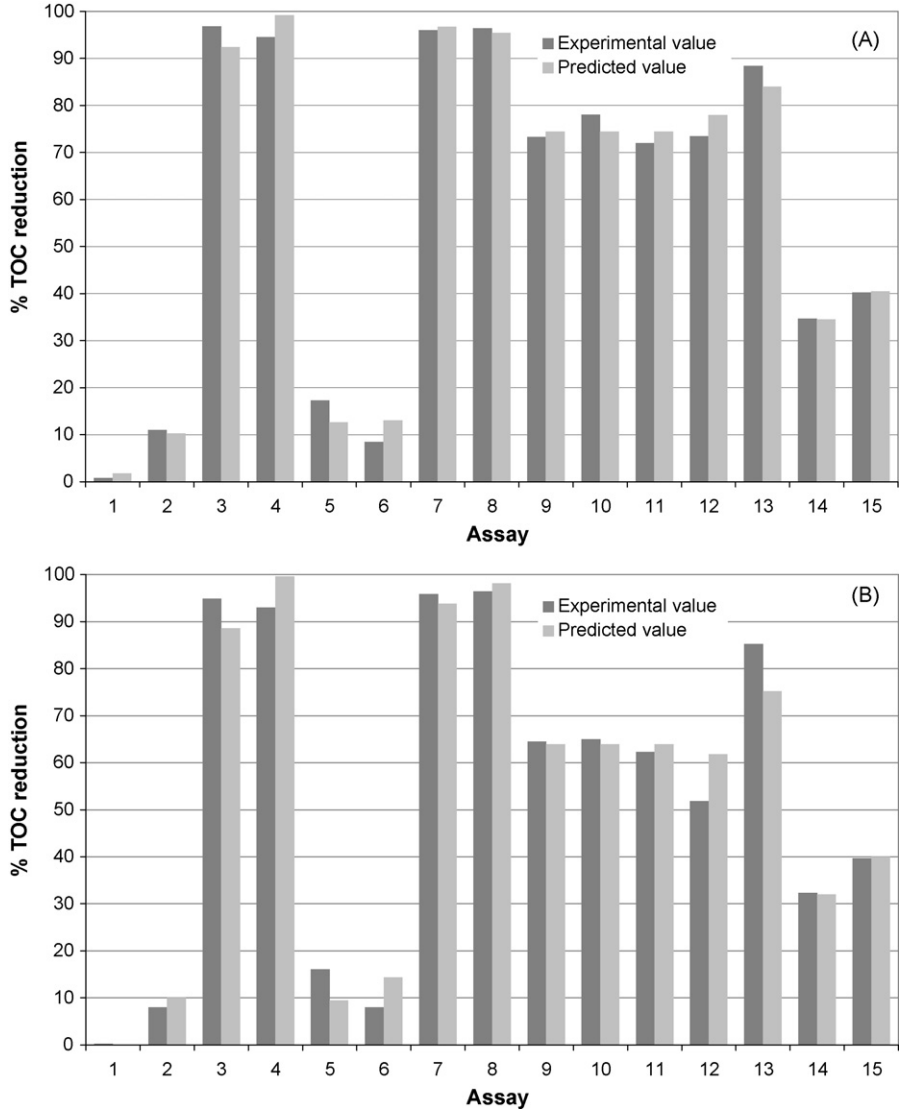


Fig. 1. Comparison between experimental value (dark column) and estimated value (light column). (A) For 15 min reaction time and (B) for 30 min reaction time.

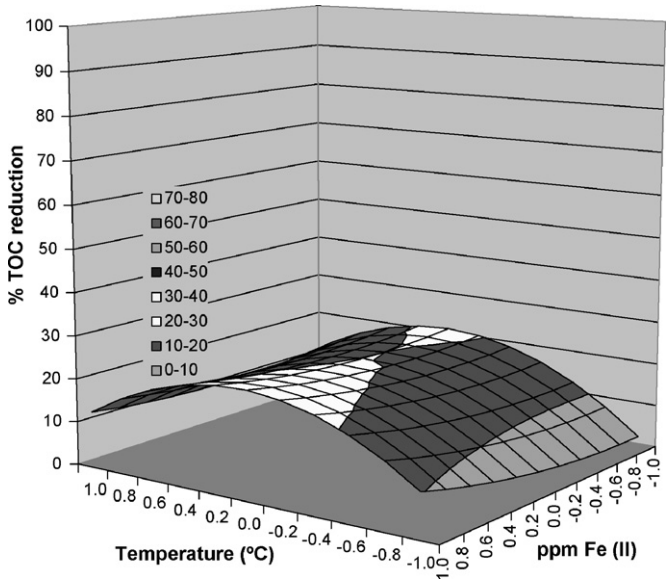


Fig. 2. Three-dimensional representation of the response surface for the TOC removal percentage after 30 min of reaction. Fe(II) and temperature are represented in coded values in the abscissa while the removal percentage is shown in the ordinate. $H_2O_2$ load is fixed at 200 ppm (the lowest value).

the fact that the error minimization problem is addressed unconstrained by the standard methods available (e.g. FATORIAL). This is a critical drawback to highlight for understanding the limits of the model proposed. Multivariate analysis leads to interesting qualitative results regarding the weight of the different variables in the system response, the trend of this response, and the interaction among the variables. However, the assumption of a polynomial model is questionable from the quantitative point of view.

For a favorable TOC reduction, the main direct effects are due to the hydrogen peroxide concentration ($X_2$), while the iron concentration ($X_1$) and temperature ($X_3$) play a less significant role. No synergistic or antagonistic effects between variables were observed. Quadratic effects of the three variables indicate that maximum values can be found for each parameter.

The polynomial equation allowed a three-dimensional representation of the phenomena when one of the studied parameters is fixed. The response surfaces were built assuming that the non-fixed parameters are represented in the abscissa in coded values while the TOC removal percentage after 30 min of reaction is shown in the ordinate.

The two different views of the response surface generated with polynomial equation (8) at different $H_2O_2$ loads (Figs. 2 and 3) show that for fixed $H_2O_2$ concentration, the TOC degradation reaches a maximum value at temperature about 45 °C. At higher or lower temperatures, the degradation rate of the contaminant load decays. This behavior may be explained by the trade-off between the opposite temperature dependence of the degradation rate and the hydrogen peroxide decomposition rate. The influence of iron loads is less than temperature as can be clearly observed in Figs. 2 and 3.

For the highest doses of hydrogen peroxide complete TOC reduction is almost achieved (see Table 1). The flat removal response in this region makes the fitting of a quadratic model inaccurate; leading to meaningless predicted values over 100% for the TOC removal.

The detrimental effect of the competitive reaction, which appears when the reagent ratio is not appropriate, is partly compensated with the positive action due to increasing temperature, as can be seen in Figs. 2 and 3. From these response surfaces, it is advisable to use the right temperature interval, especially when the hydrogen peroxide concentration is low.

For this case, low hydrogen peroxide load (Fig. 2), at 25 °C, the percentage of TOC reduction is around 5%, while TOC

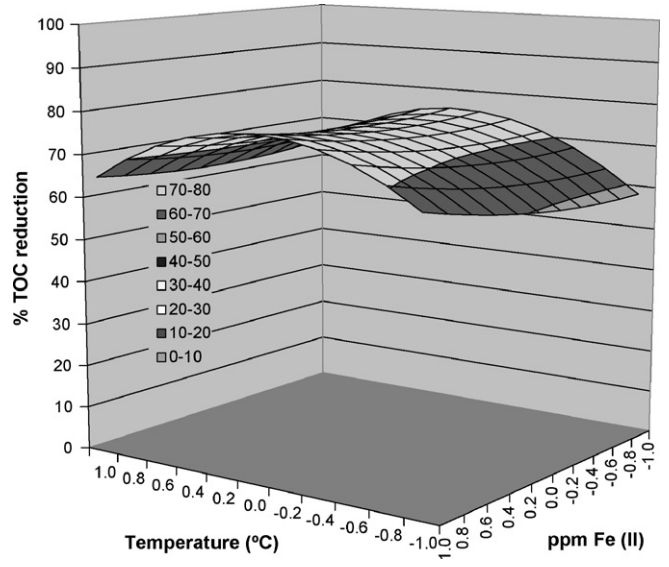


Fig. 3. Three-dimensional representation of the response surface for the TOC removal percentage after 30 min of reaction. Fe(II) and temperature are represented in coded values in the abscissa while the removal percentage is shown in the ordinate. $H_2O_2$ load was fixed at 450 ppm (the medium value).

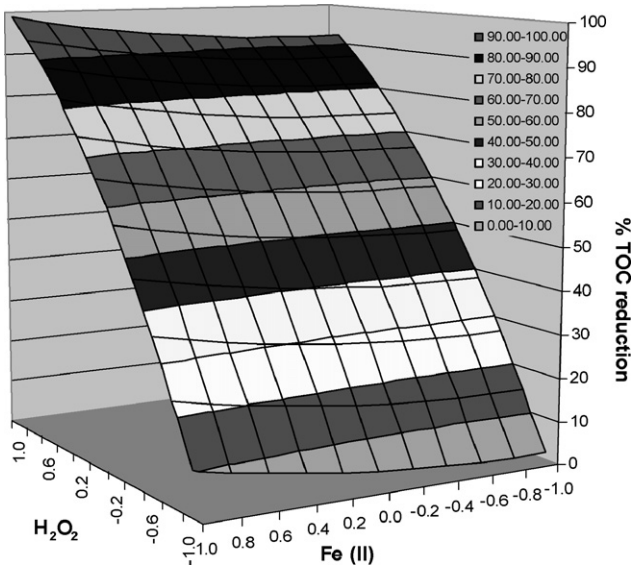


Fig. 4. Three-dimensional representation of the response surface for the TOC removal percentage after 30 min of reaction. Fe(II) and $H_2O_2$ load are represented in coded values in the abscissa while the removal percentage is shown in the ordinate. Temperature is fixed at 25 °C (the lowest value).

decay reaches a maximum near 20–25% for temperatures within the interval 40–50 °C. It is important to note that the 20% increase in the contaminant load degradation has been obtained in only 30 min of treatment. Since 200 ppm hydrogen peroxide—20 ppm iron cannot directly produce the $OH^{\bullet}$ radicals required for a 20–25% decay, the temperature influence is not only accelerating the reaction system but it also seems to alternatively assist hydrogen peroxide cleavage and $OH^{\bullet}$ formation, or Fe(II) recovery [22].

The detrimental effect of large $H_2O_2$ concentrations, based on the fact that hydrogen peroxide could react with the OH radicals (Eq. (6)), is not observed in the set of experiments performed. In contrast, as expected, the studied $H_2O_2$ range achieves higher TOC decay when a larger amount of hydrogen peroxide was used without reaching any limiting value that modifies the trend, as shown in Fig. 4.

Similar behavior is observed when the temperature is fixed at any other value within the studied interval.

A qualitative analysis of the treated solution using HPLC allows detecting the presence of intermediate compounds that appear during the reaction process. Fig. 5 shows the evolution of 2-chlorophenol and resulting intermediates at different Fenton reagent loads.

The HPLC analysis provides qualitative evidence of the presence of intermediates. However, the identification of these compounds and the investigation of the degradation pathways are beyond the scope of this work. Hence, two main retention times, different from that given by 2-chlorophenol, have been detected in the treated samples. These peaks may result from the contribution of several compounds, but for discussion purposes they will be referred as byproduct I and byproduct II from here on.

It is important to compare the Fenton, Fenton-like and Photo-Fenton reactions under different reagent loads (see Fig. 5) at 25 °C. When the reagents were Fe(II) = 20 mg $L^{-1}$ and $H_2O_2$ = 200 mg $L^{-1}$ (the lowest loads), all 2-chlorophenol was eliminated after 30 min of reaction while two intermediate products (byproduct I and byproduct II) appear from the reaction's start (Fig. 5A–C). In the reaction system with low hydrogen peroxide concentration (Fig. 5A), byproduct I increases slightly its concentration and then disappears after 20 min irradiation. Byproduct II initially increases its concentration, maintaining invariable in the course of the reaction. On the other hand, in the reaction with medium and highest peroxide loads (Fig. 5B and C), 2-chlorophenol was completely eliminated after less than 30 min of reaction and byproduct I is formed in a negligible amount. In contrast, byproduct II decreases considerably its concentration although it is not completely eliminated even at high reaction times.

Fig. 5 shows the importance of studying the toxicity evolution through the entire degradation process when characterizing the toxicity level of 2-chlorophenol and its reaction intermediates as a function of reaction time for a fixed temperature and Fenton reagent loads.

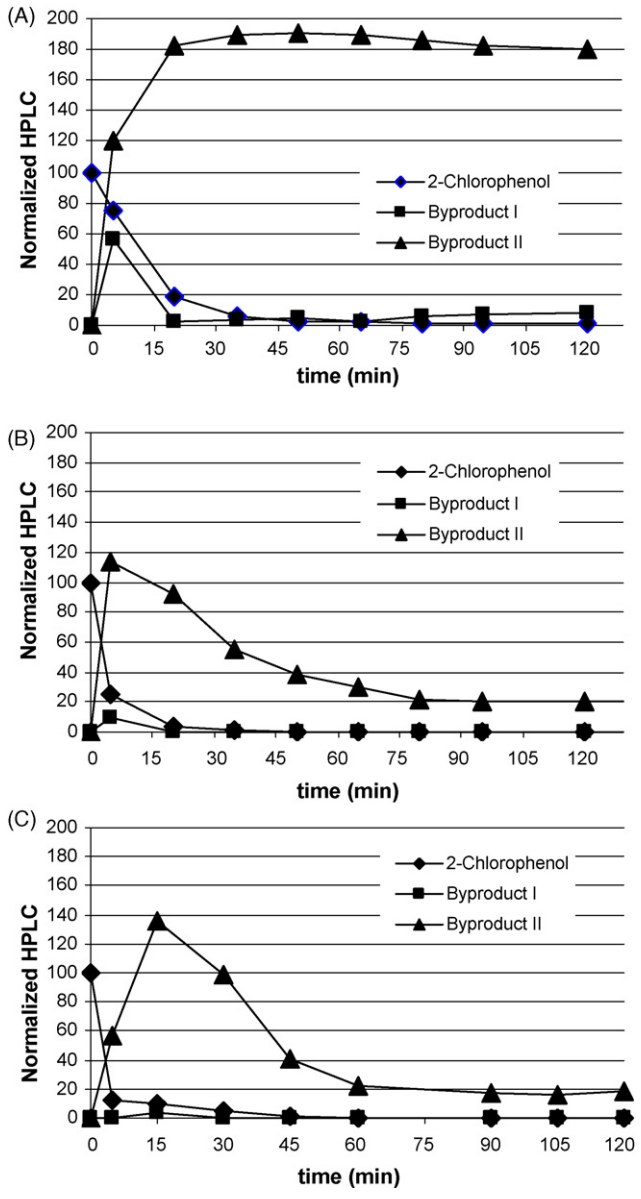


Fig. 5. Evolution of normalized areas from HPLC assay of 2-chlorophenol and its intermediate reaction compounds (2-chlorophenol retention time: 12.48 min, byproduct I: 2.06 min, byproduct II: 2.48 min) over time under Fenton at Photo-Fenton processes carried out at 25 °C. (A) Fe(II) = 20 mg $L^{-1}$ and $H_2O_2$ = 200 mg $L^{-1}$ (the lowest loads) (B) Fe(II) = 45 mg $L^{-1}$ and $H_2O_2$ = 450 mg $L^{-1}$ (medium loads) (C) Fe(II) = 70 mg $L^{-1}$ and $H_2O_2$ = 700 mg $L^{-1}$ (highest loads).

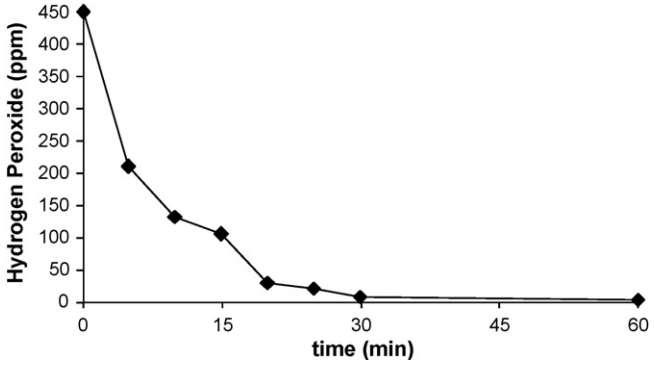


Fig. 6. Monitoring Hydrogen peroxide amount when the Fenton system mixture (Fe(II) = 45mg $L^{-1}$ and $H_2O_2$ = 450 mg $L^{-1}$, medium loads) was under the presence of the light source at 25 °C.

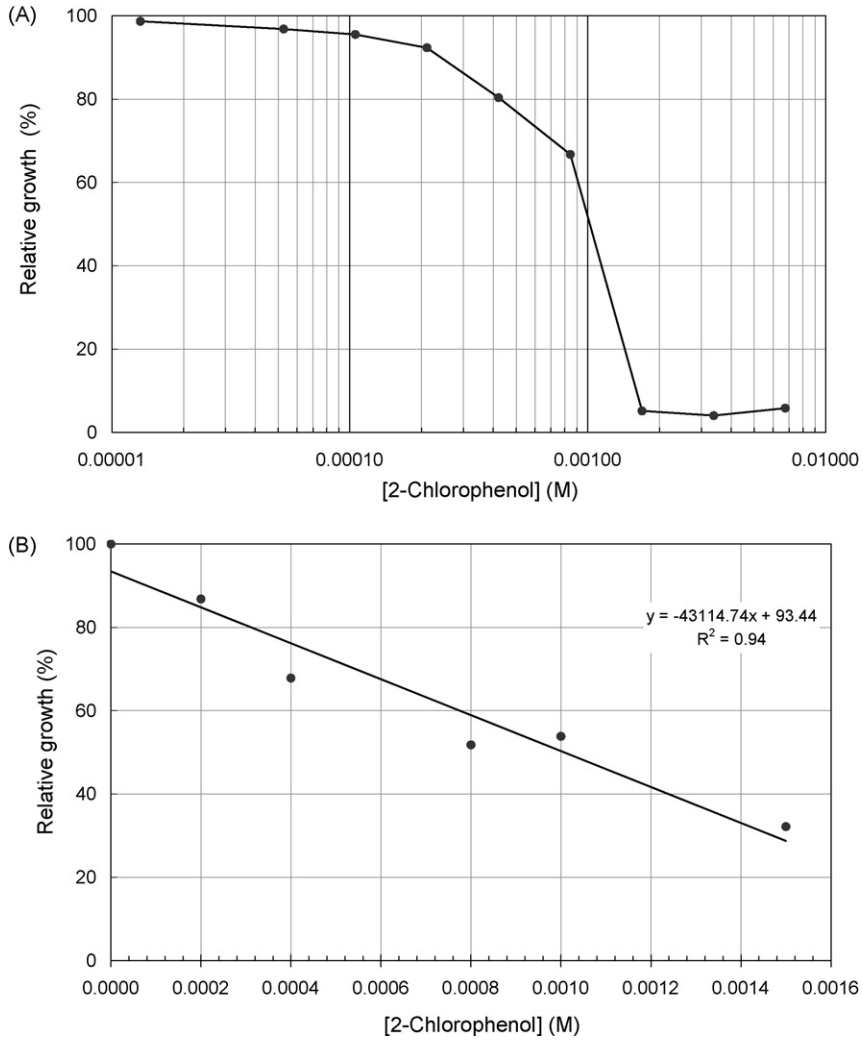


Fig. 7. (A) Toxicity curve of 2-Chlorophenol. (B) Linear model permits $LD_{50}$ determination under the following conditions: $10^4$ bacteria of *Escherichia coli* DH5α were cultured in 200 μL of LB broth containing different 2-chlorophenol concentrations in the range $2 \times 10^{-4}$ to $1.5 \times 10^{-3}$ M.

Several pathways to remove 2-chlorophenol have been proposed, but unfortunately treatments always led to persistent intermediates. Authors are concerned with 2-chlorophenol elimination and they are also apprehensive for the abatement of the intermediate products. Becker et al. [23] report that there is a potential limiting the use of anaerobic microbial communities to remove chlorinated phenols since transformation of 2-chlorophenol to other chlorinated compound is not acceptable from treatment standpoint if this chlorinated product is not further degraded.

Before performing toxicity studies on the system, the residual hydrogen peroxide concentration needs to be determined due to its well-known biocide effect. Experiments were performed to monitor the hydrogen peroxide concentration. The reagent concentration after 30 min of reaction is negligible for all experimental sets studied; Fig. 6 shows hydrogen peroxide evolution for a concrete condition, as example. The results are according the pseudo-first order constant proposed for hydrogen peroxide decomposition in the literature [24] what denotes the fast consumption of this reagent. Thus, it proves there is no need to remove residual hydrogen peroxide (e.g. adding sulfite as scavenger) when the toxicity experiments are performed for more time than this threshold.

Chemical stress produced by 2-chlorophenol reduced the *E. coli* DH5α growth rate in LB broth. The complete toxicity curve for the 2-chlorophenol is given in Fig. 7A, which reveals total inhibition growth for the high dose ($1.5 \times 10^{-3}$ M) and a very small inhibitory effect for the very low doses

Table 2
TOC evolution during the degradation process for lowest and medium reagent loads

| Time (min) | Assay 1[a] | | Assay 9[b] | |
|---|---|---|---|---|
| | TOC (ppm) | TOC reduction (%) | TOC (ppm) | TOC reduction (%) |
| 0 | 74.0 | 0.0 | 74.0 | 0.0 |
| 30 | 73.4 | 0.8 | 19.7 | 73.3 |
| 45 | 73.1 | 1.2 | 12.8 | 82.7 |
| 60 | 72.5 | 2.0 | 5.0 | 93.2 |

[a] $[H_2O_2]$: 200 ppm, [Fe(II)]: 20 ppm, temperature: 25 °C (Table 1).
[b] $[H_2O_2]$: 450 ppm, [Fe(II)]: 45 ppm, temperature: 45 °C (Table 1).

($1 \times 10^{-4}$ M). The growth rate inhibition is a direct function of the 2-chlorophenol concentration to which the bacteria were exposed. When bacteria were exposed, during 24 h, to 2-chlorophenol (in concentrations from $2 \times 10^{-4}$ M to $1.5 \times 10^{-3}$ M) the growth rate decreased from $86.8 \pm 9.6\%$ to $32.2 \pm 3.5\%$. A detail of the toxicity curve is given in Fig. 7B, which includes a lineal model established between growth rates ($Y$ in %) and 2-chlorophenol concentration ($X$ in M): $Y = 93.43–43115X$ ($r = -0.97$; ANOVA, $p = 0.00148$). From the linear model $LD_{50} = 1 \times 10^{-3}$ M, 2-chlorophenol was determined.

The study of several Fenton reagent loads has demonstrated the effect of different reagent ratios: after 30 min of reaction with Fe(II) = 70 mg $L^{-1}$ and $H_2O_2$ = 700 mg $L^{-1}$, the velocity of exponential growth after 24 h was 0.032 $h^{-1}$; while with Fe(II) = 20 mg $L^{-1}$ and $H_2O_2$ = 700 mg $L^{-1}$, the velocity of growth after 24 h was 0.026 $h^{-1}$. Under the same experimental conditions, a medium load (Fe(II) = 45 mg $L^{-1}$ and $H_2O_2$ = 450 mg $L^{-1}$) resulted in a velocity of 0.007 $h^{-1}$, while it was 0.006 $h^{-1}$ for the lowest load (Fe(II) = 20 mg $L^{-1}$ and $H_2O_2$ = 200 mg $L^{-1}$). Data suggests that the byproduct II generated in the lowest and medium Fenton reagent loads may consist of a molecular species that inhibits growth.

From these results, detailed toxicity monitoring was selected for those experiments showing the most significant inhibition effects. Table 2 shows the TOC evolution for experiments 1 (lowest load) and 9 (medium load) defined in Table 1, which are clearly different.

*E. coli* DH5α bacterial growth (after 0, 24, 48 and 72 h of incubation) was studied in presence of the products of the Fenton, Fenton-like and Photo-Fenton reactions during the entire degradation process for the lowest and medium loads where the reagents are: Fe(II) = 20 mg $L^{-1}$ and $H_2O_2$ = 200 mg $L^{-1}$ and Fe(II) = 45 mg $L^{-1}$ and $H_2O_2$ = 450 mg $L^{-1}$, respectively (Fig. 8). Tests were carried out by adding 40 μL of the reaction sample and 160 μL of LB broth (final volume of 200 μL). At initial time (when only 2-chlorophenol is present in the reaction sample), the 40 μL of reaction sample correspond to 2-chlorophenol, $2 \times 10^{-4}$ M. As Fig. 7B shows, the growth rate decreases $86.8 \pm 9.6\%$. Similar inhibition of bacteria growth for the other reaction sample, about 15%, was obtained by monitoring relative bacteria growth, Fig. 8.

Despite the different TOC reduction attained in assays 1 and 9, both experiments reached very similar toxicity levels. Fig. 8 illustrates that there is a similar reduction in bacteria growth in LB broth supplemented with either Fenton and Photo-Fenton reaction products or 2-chlorophenol.

The toxicity analysis corroborates that byproduct II and 2-chlorophenol toxicities are similar because even at low concentrations (after 60 min of reaction at Fe(II) = 45 mg $L^{-1}$ 1 and $H_2O_2$ = 450 mg $L^{-1}$, medium loads), relative growth is still distant from control growth.

Consideration of the toxicity evolution is essential. For example, medium-load Fenton reactants achieve 75% TOC reduction in 30 min reaction and about 93% TOC reduction in 60 min. However, the toxicity study clearly demonstrates that a slight amount of byproduct II is sufficient to produce a positive toxicity test. Essentially, to evaluate treatment quality, biological toxicity studies should complement the chemical ones.

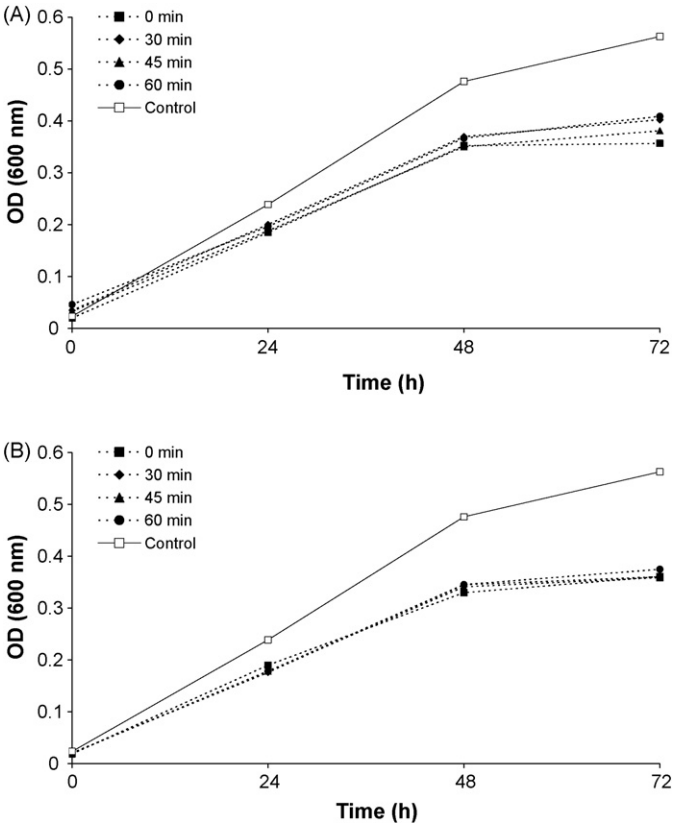


Fig. 8. Bacteria's growth relative to the control (LB broth alone) after 0, 24, 48, and 72 h incubation times. The culture medium comes from $2 \times 10^{-4}$ M of reactive Fenton system mixture after 0, 30, 45 and 60 min reactions times. (A) Fenton reactive: Fe(II) = 20 mg $L^{-1}$ and $H_2O_2$ = 200 mg $L^{-1}$ (the lowest loads). (B) Fenton reactive: Fe(II) = 45 mg $L^{-1}$ and $H_2O_2$ = 450 mg $L^{-1}$ (medium loads).

These results establish that partial contaminant degradation, monitored through a lumped parameter such as TOC, is not a guarantee for a matching hazardousness reduction. Hence, further investigation is required to identify the intermediates under Fenton and Photo-Fenton reaction conditions.

## 4. Conclusions

Fenton, Fenton-like and Photo-Fenton reactions efficiently eliminate 2-chlorophenol as shown by HPLC analysis. The combination of Fenton reagents and light for the mineralization of chlorinated compounds presents clear advantages in terms of application.

The careful experimental design was a valuable tool to identify the roles played by the different system variables studied. The selection of the correct Fenton reagent load is the most important aspect that should be considered during the treatment process. HPLC analysis also shows how the time required for reaching total 2-chlorophenol decay is highly dependent of Fenton reagent loads. Temperature is also shown to be a particularly significant factor for increasing TOC

removal when the system runs under low reagent load or with inadequate reagent ratios. Multivariate analysis allowed the characterization of this behavior.

Furthermore, due to the presence of byproducts during the reaction, a toxicity study that ensures the biological quality of the treated toxic compound is required. Byproducts obtained in the partial degradation of the samples have revealed toxicity levels similar to 2-chlorophenol.

The occurrence of 2-chlorophenol byproducts, and thus the toxicity of the system, is also reliant with the Fenton system conditions.

Even 90% TOC removal rates have also proved to be insufficient for ensuring an acceptable shortage of toxicity of the system. Consequently, an appropriate toxicity study is shown to be always required for completing the global study.

## References

[1] R. Bauer, H. Fallmann, Res. Chem. Intermed. 23 (1997) 341.
[2] M. Pérez, F. Torrades, X. Domènech, J. Peral, J. Chem. Technol. Biotechnol 77 (5) (2002) 525.
[3] J.J. Pignatello, Environ. Sci. Technol. 26 (1992) 944.
[4] Y. Sun, J.J. Pignatello, Environ. Sci. Technol. 27 (1993) 304.
[5] J.J. Pignatello, D. Liu, P. Huston, Environ. Sci. Technol. 33 (1999) 1832.
[6] A. Safarzadeh-Amiri, J.R. Bolton, S.R. Cater, J. Adv. Oxid. Technol. 1 (1996) 18.
[7] M. Pérez, F. Torrades, J.A. García Hortal, X. Domènech, J. Peral, Appl. Catal. B: Environ. 36 (2002) 63.
[8] S.H. Bossmann, E. Oliveros, S. Göb, S. Siegwart, E.P. Dahlen, L. Payawan, M. Straub, M. Wörner, A.M. Braun, J. Phys. Chem. 102 (1998) 5542.
[9] H. Fallmann, T. Krutzler, R. Bauer, S. Malato, J. Blanco, Catal. Today 54 (1999) 309.
[10] M. Pera-Titus, V. García-Molina, M.A. Baños, J. Jiménez, S. Esplugas, Appl. Catal. B: Environ. 47 (2004) 219.
[11] C.A. Mason, J. Dünner, P. Indra, T. Colangelo, Appl. Environ. Microbiol. 65 (1999) 3433.
[12] B. de Barros, I.S. Scarminio, R.E. Bruns, Planejamento e Otimização de Experimentos, Unicamp, Brasil, 1996.
[13] M. Pérez, F. Torrades, J. Peral, C. Lizama, C. Bravo, S. Casas, J. Freer, H.D. Mansilla, Appl. Catal. B: Environ. 33 (2001) 89.
[14] J. Rodríguez, S. Fuentes, J. Freer, H.D. Mansilla, A. Ferráz, J. Baeza, Environ. Technol. 19 (1998) 75.
[15] E. Oliveros, O. Legrini, M. Hohl, T. Müller, A.M. Braun, Water Sci. Technol. 35 (1997) 223.
[16] R.F.P. Nogueira, M.C. Oliveira, W.C. Paterlini, Talanta 66 (2005) 86.
[17] F.J. Benítez, J. Beltrán-Heredia, J.L. Acero, F.J. Rubio, J. Chem. Technol. Biotechnol. 76 (2001) 312.
[18] E. Chamarro, A. Marco, S. Esplugas, Water Res. 35 (4) (2001) 1047.
[19] W.Z. Tang, C.P. Huang, Environ. Technol. 17 (1996) 1371.
[20] F. Torrades, M. Pérez, H.D. Mansilla, J. Peral, Chemosphere 53 (2003) 1211.
[21] W. Gernjak, M. Fuerhacker, P. Fernández- Ibañez, J. Blanco, S. Malato, Appl. Catal. B: Environ. 64 (2006) 121.
[22] M. Pérez, F. Torrades, X. Domènech, J. Peral, Water Res. 36 (2002) 2703.
[23] J.G. Becker, D.A. Sthal, B.E. Rittmann, Appl. Environ. Microbiol. 65 (1999) 5169.
[24] R. Baciocchi, M.R. Boni, L. D'Aprile, J. Hazard. Mater. 96 (2003) 305.