Research Article

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# Combined chemical oxidation and biological processes for herbicide degradation

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## Abstract

**BACKGROUND: Fenton and photo-Fenton processes were explored as photochemical pre-treatments to improve the biodegradability of wastewater containing atrazine, a commercial pesticide. The effects of $H_2O_2$, $Fe^{3+}$ and irradiation level on the degradation processes were studied and the optimal conditions determined.**

**RESULTS: Fenton and photo-Fenton oxidation systems were able to remove 85 and 100% of atrazine, respectively, in a 120 min period. These processes produced a biocompatible solution, removing 100% of the initial biorecalcitrant compound; however, cyanuric acid remained in the effluent after the chemical oxidation treatment due to the stability of the N-heterocyclic ring of this acid. Nevertheless, it was found that the cyanuric acid remaining from the photo-oxidative process can be removed by means of an anaerobic treatment.**

**CONCLUSIONS: The results obtained in this work indicate that a coupled photo-Fenton–biological treatment system is a feasible method to achieve the complete mineralization of biorecalcitrant pollutants such as atrazine.**


**Keywords:** advanced oxidation processes; photo-Fenton; biological treatment; combined process; biorecalcitrant compound; atrazine

## INTRODUCTION

The biological treatment of wastewater is often the most cost-effective alternative of all treatment options. However, some industrial wastewaters containing certain toxic and/or biorecalcitrant organic compounds cannot be treated with this conventional process.[1–3] Consequently, it is necessary to explore other alternatives to treat these wastewaters.

Advanced oxidation processes (AOPs) are widely known to be highly effective for the degradation of complex organic substances.[4–6] The Fenton process is one of the AOPs that have gained increasing attention in recent years due to its high efficiency for the remediation of contaminated water.[7–9] In some cases, the combination of Fenton reaction with radiation (called photo-Fenton reaction) has been used to enhance the efficiency of the Fenton process.

Nowadays, there is a need to consider how to provide adequate treatment efficiency at reasonable operating costs. Unfortunately, a major drawback of most AOPs is their high operating costs if high levels of mineralization, or total mineralization, have to be achieved. Nonetheless, the use of AOPs is more suitable or even indispensable when the wastewater to be treated contains a not easily biodegradable contaminant. Moreover, the use of AOPs as a pre-treatment step to enhance the biodegradability of wastewater containing persistent or inhibitory contaminants to common microorganisms can be justified if the resulting intermediates are easily degradable by a biological treatment.[1,10,11]

In this work, atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino) -striazine] was selected as a model contaminant because it is a widely used herbicide for controlling broadleaf and grassy weeds worldwide. It is quite persistent in a neutral environment and is toxic to various living organisms.[12] Atrazine is a possible carcinogen[13] and a known endocrine disruptor in amphibians of the aquatic biota.[14] Interestingly, atrazine is hardly degradable by biological treatments.[3,15,16] Some studies have focused on the biodegradation of atrazine in wastewater.[17–19] However, most of these studies employed pure culture microorganisms, and the biodegradation rate depended on (i) the types of pure culture bacteria, (ii) carbon/nitrogen ratio, (iii) pH, and (iv) moisture content.[20–22] However, degradation was partial and total mineralization was achieved only in a few cases.[23–25]

This pesticide is of increasing interest since several articles published in the literature about the photo-degradation of s-triazines[4,8,26] have reported that mineralization is not complete, due to the high stability of the triazine nucleus and the formation of stable cyanuric acid.[27–29] Cyanuric acid was reported to have lower toxicity and is more biodegradable than atrazine and its degradation products.[30–32] This compound causes slight irritation to the eyes but not to the skin.[33] The consumption of these substances combined with melanin poses a risk to both human and animal health.[34–36] In addition, it is known that cyanuric acid is generally difficult to be chemically hydrolyzed or oxidized.[37,38] In contrast, biological degradation using pure cultures has been

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successful and several species of microorganisms capable of degrading cyanuric acid have been reported: *Homodendrum sp.*,[39] *Klebsiella pneumoniae*,[40–42] *Pseudomonas sp.*,[39–41,43] *Lipomyces starkeyi*,[44] *Phanerochaete chrysosporium*, *Trametes versicolor*,[45] *Xanthobacter autotrophicus*,[46] *Acinetobacter sp.* and *Agrobacterium tumefaciens*.[47] However, very few studies have reported the use of mixed microbial cultures.[48]

The main aim of this paper was to propose a general strategy to develop a combined photo-Fenton and biological process for the treatment of atrazine-containing wastewater. For this purpose, minimum Fenton reactant doses able to convert atrazine-containing waters into biodegradable effluents were selected, and then a biological treatment was applied. The study also explored the potential of aerobic and anaerobic microbial consortia to degrade cyanuric acid (final atrazine intermediate) as a carbon and energy source.

## EXPERIMENTAL

### Chemicals

Atrazine (≥90%, commercial formulation, $C_8H_{14}ClN_5$, SYNGENTA, Daphnia magna 48 h EC50 6.9 mg $L^{-1}$,[49]) was chosen as the reference compound (Fig. 1(a)). The final intermediate of the atrazine degradation process was the cyanuric acid (2,4,6(1H,3H,5H)-Triona-1,3,5-triazina, 99 % technical grade $C_3N_3(OH)_3$, Sigma-Aldrich) (Fig. 1(b)). Ferric salt ($Fe(SO_4)_3.8.87H_2O$, Carlo Erba, RPE) and hydrogen peroxide ($H_2O_2$, 30% w/v solution, Carlo Erba, ACS) were used as Fenton and photo-Fenton reagents. Sulphuric acid (95–98% Pro-analysis, Ciccarelli p.a.) was used to adjust pH and methanol purchased from Carlo Erba (RPE) was employed as a quenching solution. Analytical standards for chromatography analyses were purchased from Sigma-Aldrich.

For the aerobic biological system, a synthetic effluent was generated with glucose as organic source ($C_6H_{12}O_6$, Cicarelli), magnesium sulphate heptahydrate ($MgSO_4.7H_20$, Cicarelli), anhydrous potassium phosphate monobasic ($KH_2PO_4$, Anedra), and salts of diverse composition (potassium 14%, nitrogen 12%, sulphur 6%, phosphate 5%, calcium 3.6% and magnesium 1.2%). Freeze-dried bacteria, taken from a commercial consortium (Bi-Chem SM 700, Sybrom Chemical), were used as inoculums of the reactor.

A sample of wastewater from a brewery plant located in Santa Fe, Argentina, containing ethanol, residual carbohydrates and cereal proteins, among other compounds, was used as a medium in the anaerobic biological assays. Sludge from the upflow anaerobic sludge blanket (UASB) reactor of the brewery wastewater treatment plant was used as inoculum. The pH was maintained neutral during the biological treatment by the addition of sodium hydroxide.

### Analytical methods

Samples were pre-filtered through 0.22 μm syringe filters (25 mm, Millipore). The concentration of atrazine was determined using an HPLC (Waters, Model Code 5CH) with a UV detector (detection wavelength of 221 nm). A reverse phase column (X-Terra® RP, C-18) was used with an isocratic mixture of acetonitrile (50%) and water (50%) as the mobile phase (flow rate 1 mL $min^{-1}$). The limit of detection (LoD) for atrazine was determined to be 2.12 mg $L^{-1}$. The hydrogen peroxide concentration was analyzed with a modified iodimetric technique[50] using a UV–VIS CARY 100 BIO at 350 nm and ferrous ion with a standard spectrophotometric technique (absorbance measurements of the $Fe^{2+}$-phenanthroline complex at 510 nm[51]). The mineralization of the commercial herbicide was monitored by measuring the total organic carbon (TOC) by direct injection of filtered samples into a Shimadzu TOC-5000A analyzer.

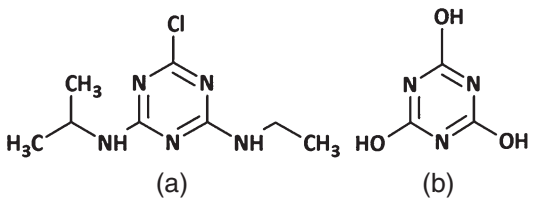


**Figure 1.** Chemical structures of atrazine (a) and cyanuric acid (b).

The performance of the biological system was evaluated in terms of chemical oxygen demand (COD) removal efficiency and cyanuric acid degradation. Determinations of COD were carried out according to Standard Methods[51] and HPLC (Waters, Model Code 5CH) with a UV detector (detection wavelength equal to 220 nm) and a C-18 column was used to determine the cyanuric acid concentrations in the sample after the photo-degradation process. The LoD for cyanuric acid was determined to be 1.44 mg $L^{-1}$. Previously, the C-18 column was loaded with di-dodecyldimethylammonium bromide (DDMAB) by fluxing 30 mL of a $1 \times 10^{-2}$ mol $L^{-1}$ DDMAB solution and acetonitrile/water (25/75) for 30 min and then washing with 30 mL of water; finally a $5 \times 10^{-3}$ mol $L^{-1}$ phosphate buffer (pH around 7.5) was used as eluent at 1 mL $min^{-1}$.[26] This phosphate buffer was used as an eluent for separating atrazine intermediates.

### Chemical experiments: device and procedures

A set of experimental runs for Fenton and photo-Fenton reactions was performed, using different values of the ferric salt initial concentration ($C^0_{Fe^{3+}}$) and of the hydrogen peroxide to the atrazine initial molar ratio ($R = C^0_{H_2O_2}/C^0_{ATZ}$). As is known, both parameters have important effects on the photo-Fenton process for the treatment of pollutants. Besides, optimum values of these parameters usually depend on the biorecalcitrant compound. The initial concentration of the pesticide was 30 mg $L^{-1}$, which is the value expected in the wastewater to be treated.

The experimental device employed for the chemical oxidation of atrazine was an isothermal, well-stirred batch recycling system. Figure 2 shows a schematic representation of the experimental setup. The reactor ($V_R = 69.94$ $cm^3$) was made of borosilicate glass and circular cross-section with two parallel flat windows. Each window was irradiated with a tubular black light UV-lamp (TLK40W/09 N, Philips, 315–400 nm range), placed at the focal axis of a cylindrical reflector of parabolic cross-section. The recycling system includes a pump with a high recirculation flow rate to provide good mixing conditions (83.3 $cm^3$ $s^{-1}$), a heat exchanger (for temperature control) and a large volume, well-stirred store tank with provisions for liquid sampling, temperature control and pH measurements ($V_T = 3000$ $cm^3$).

Experimental runs began when the previously prepared atrazine, hydrogen peroxide and ferric sulphate solutions were incorporated in the storage tank with distilled water, and the pH was adjusted to 2.8–3. Two lamp shutters interposed between the illuminating systems and the reactor windows allowed us to obtain the required operating conditions. Then, the first sample was withdrawn and the shutter removed, this operation defining the starting time of the photo-Fenton reaction. Experimental runs were

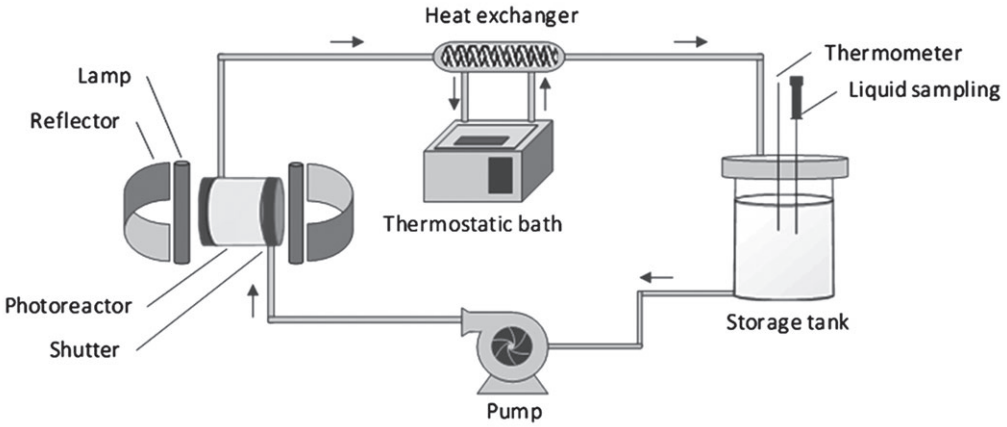


**Figure 2.** Schematic representation of the experimental set-up.

**Table 1.** Operating conditions for the experimental runs

| Fenton | | photo-Fenton | |
|---|---|---|---|
| $C^0_{Fe^{3+}}$ (mg $L^{-1}$) | $R = C^0_{H_2O_2}/C^0_{ATZ}$ | $C^0_{Fe^{3+}}$ (mg $L^{-1}$) | $R = C^0_{H_2O_2}/C^0_{ATZ}$ |
| 5 | 35 | 5 | 35 |
| 5 | 175 | 5 | 175 |
| 5 | 350 | 5 | 350 |
| 15 | 35 | 15 | 35 |
| 15 | 175 | 15 | 175 |
| 15 | 350 | 15 | 350 |
| 25 | 35 | 25 | 35 |
| 25 | 175 | 25 | 175 |
| 25 | 350 | 25 | 350 |

performed at constant temperature (298 K). Runs lasted 480 min and liquid samples were taken at equal time intervals (30 min).

The potassium ferrioxalate actinometry was used to evaluate the radiative flux at the reactor wall ($q_w$). From these experiments, it was determined that $q_w$ is equal to $4.315 \times 10^{-9}$ Einstein $cm^{-2}$ $s^{-1}$.

*Full factorial designs*

To optimize the operating conditions, the effects of ferric initial concentration ($C^0_{Fe^{3+}}$), hydrogen peroxide to atrazine initial molar ratio ($R = C^0_{H_2O_2}/C^0_{ATZ}$) and irradiation level on the degradation percentage, were investigated by two Full Factorial Experimental Designs (one for Fenton and the other one for photo-Fenton). The main objective was to identify the factors that have a significant effect on the response, as well as to investigate the influence of possible interactions. To do this, 18 experiments were carried out. Table 1 shows a summary of the operating conditions selected for the experimental runs. The atrazine to hydrogen peroxide molar ratios employed in our work ($R = 35$, 175 and 350) correspond to 4.87, 24.33 and 48.65 mmol $L^{-1}$ initial concentrations of the $H_2O_2$, respectively.

### Biological experiments: device and procedure

The biological assays under aerobic conditions were carried out in a laboratory-scale reactor (1 L) operated in batch mode. The reactor was equipped with a liquid sampling device and a thermometer. This bioreactor was mounted inside a fume hood on an orbital shaker. The system was aerated using air pumps and diffusers placed at the bottom of the reactor. The air flow was adjusted to provide a dissolved oxygen (DO) concentration in the 7–8 ppm range. It was monitored by means of a DO probe. The pH was controlled and adjusted between 6.5 and 7.5.

A solution of glucose as C source and salts as sources of N and P were used as culture media. Then, a cyanuric acid solution was added in order to achieve a concentration of 18 mg $L^{-1}$, which is the remaining concentration in the effluent after the chemical oxidation treatment of a solution containing 30 mg $L^{-1}$ of atrazine. A consortium of microorganisms (Bi-Chem SM 700 of Sybrom Chemical), previously acclimated, was used as inoculum. All experiments were carried out in duplicate and at room temperature (20–25 °C) for periods of 120 h.

Afterwards, a biological treatment under anaerobic conditions was carried out in a laboratory-scale stirred-tank reactor. A mix of 100 mL of sample of brewery wastewater and 100 mL of the photo-treated wastewater containing cyanuric acid was used as medium. The resulting mixture was inoculated with 100 mL of sludge taken from the plant UASB reactor.

A scheme of the experimental device is shown in Fig. 3. This reactor was operated in batch mode, and it was also mounted inside a fume hood on a magnetic shaker, in order to ensure a homogeneous suspension of microorganisms at all times.

During the course of the experiment, pH and temperature were measured online. The pH varied slightly around a mean of 6 and the average temperature was 25 °C. Runs lasted 6 to 7 days and liquid samples were collected at equal time intervals (every 12 h). Once collected, the samples were centrifuged at 6000 rpm (Labnet Mini 6) for 5 min and then filtered with 0.2 μm filters to remove the biomass prior to analyses (25 mm, Millipore). All samples were taken and analyzed in duplicate. Initially, a run was carried out on a herbicide-free medium. This blank run was used as a control for subsequent runs. The physical characteristics of the sludge were periodically monitored by microscopic observation.

## RESULTS

### Chemical oxidation as pre-treatment process

The feasibility of the atrazine degradation by Fenton and photo-Fenton processes was investigated. A set of experimental runs for different hydrogen peroxide to atrazine molar ratios ($R$) and constant value of ferric iron initial concentrations ($C^0_{Fe^{3+}}$) were performed, for irradiated and non-irradiated conditions. Table 2

  

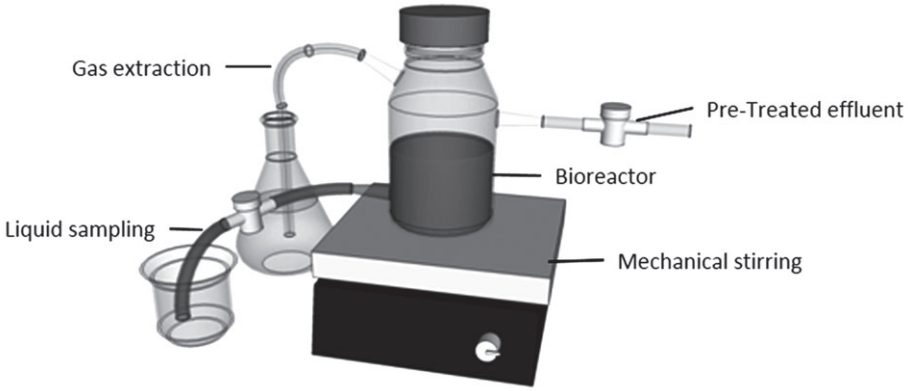


**Figure 3.** Diagram of anaerobic biological system.

illustrates atrazine conversions for Fenton and photo-Fenton processes after 120 min. Notice that three different values of R were investigated: 35 (stoichiometric requirement), 175 and 350. It should also be noted that for R = 35 the Fenton degradation rates were low. However, an important increase in atrazine conversion is reached for the Fenton process when *R* is increased from 35 to 350.

The atrazine degradation for different initial iron concentrations (between 5 mg $L^{-1}$ and 25 mg $L^{-1}$) and the same values of *R* on Fenton and photo-Fenton treatments is also shown in Table 2.

Figure 4 illustrates the experimental results in a 3D plot of the atrazine conversion as a function of *R* and $C^0_{Fe^{3+}}$ for both Fenton and photo-Fenton processes.

The R-squared ($R^2$), the standard error of the estimate (SEE) and a mean absolute error (MAE) were used to evaluate the accuracy of the statistical models. For Fenton reaction, the model explains 99.41% of the conversion variability, SEE was 3.93% and MAE was 1.98%. For photo-Fenton reaction $R^2$ was 99.25%, SEE 3.24% and MAE 1.63%.

As observed in Fig. 4 efficient pollutant degradation was achieved at the highest hydrogen peroxide concentration. However, an increase of the initial iron concentration for lower values of *R* did not introduce a significant increase on the pollutant conversion.

Conversely, an increase of the hydrogen peroxide to atrazine molar ratio from 35 to 350 introduced an important atrazine conversion enhancement. Additional runs (results not shown) demonstrated that the oxidation rate was negatively affected by the increase of $H_2O_2$ above the ratio $R = 350$. This is probably due to scavenging of the ·OH radicals in solution by the excess of $H_2O_2$, which reduces the treatment efficiency.[52,53]

Figure 4 also indicates that the pollutant conversion for the photo-Fenton reaction was always higher than that obtained with the Fenton process. An experimental pollutant conversion enhancement of 21.3% was achieved when the hydrogen peroxide to atrazine molar ratio was low ($R = 35$). However, when the hydrogen peroxide concentration increased, the pollutant conversion enhancement was reduced; for higher values of *R* (350), this conversion enhancement was practically negligible (<3%), i.e. a comparable degradation rate was achieved.

For the photo-Fenton process, it was found that the optimal *R* and $C^0_{Fe^{3+}}$ values were 175 and 15 mg $L^{-1}$, respectively, and that the atrazine mineralization was 60%. On the other hand, $R = 350$ and $C^0_{Fe^{3+}} = 25$ mg $L^{-1}$ were the optimal values for the Fenton process, with a mineralization of 48%.

It was demonstrated that both the Fenton and the photo-Fenton processes may be applied to remove atrazine from water in a simple way. Initially, it was known that the atrazine photo-oxidation generated intermediate reaction products that were equally or more toxic than the original reactant. Afterward, however, a less toxic compound than atrazine was obtained: the cyanuric acid. Residual TOC concentration evidenced the incomplete mineralization of atrazine by the Fenton or photo-Fenton processes and the formation of recalcitrant intermediates. The maximum value of mineralization that can be reached is 3/8 of the TOC corresponding to the atrazine original molecule (62.5%). By means of the photo-Fenton reaction, the maximum TOC reduction obtained in this work was approximately 60%. For a typical run, Fig. 5 shows the TOC variation and the cyanuric acid generation as a function of the reaction time.

As shown from experimental runs, this photoreactor was able to reach an almost complete degradation of the atrazine after 180 min of treatment, as well as a high mineralization of the sample after 360 min. From these results, it is concluded that it is not necessary to completely mineralize the herbicide solution by Fenton or photo-Fenton processes because the generated intermediate organic compound (cyanuric acid) after 360 min of treatment is probably easily biodegradable and presents low toxicity values (Daphnia magna 48 h EC50 1000 mg $L^{-1}$ [54]). It is important to emphasize that this is one of the very few organic molecules known to survive such treatment.[8,26,27,55]

Supplementary information about the effect of the main operating conditions on the degradation processes of this herbicide under Fenton and photo-Fenton systems can be found in Benzaquén *et al.*[56] The authors developed a kinetic model for predicting the Fenton and photo-Fenton decomposition rates of atrazine in water solution.

## Biological treatment

### *Preliminary experimental runs*

Initially, in order to establish a viable biomass, runs were carried out on a pollutant-free medium, both in the aerobic and anaerobic reactors. Both reactors were operated for 120 h and 180 h, respectively. In both cases, complete removals of substrate were achieved. These 'blank runs' were used as a control for subsequent runs.

### *Aerobic conditions*

The experimental results of relative COD and cyanuric acid concentration as a function of time can be observed in Fig. 6. It should be

**Table 2.** Experimental conversions of atrazine (ATZ) after 120 min

| | | Fenton | | | Photo-Fenton | | |
|---|---|---|---|---|---|---|---|
| | | $C^0_{Fe^{3+}}$ (mg L$^{-1}$) | | | $C^0_{Fe^{3+}}$ (mg L$^{-1}$) | | |
| $X^{120}_{ATZ}$ (%) | | 5 | 15 | 25 | 5 | 15 | 25 |
| ***R*** | 35 | 16.7 | 21.9 | 24.3 | 38.0 | 43.0 | 45.0 |
| | 175 | 33.2 | 39.9 | 44.8 | 47.9 | 59.5 | 65.0 |
| | 350 | 70.1 | 94.9 | 99.5 | 71.9 | 96.5 | 100.0 |

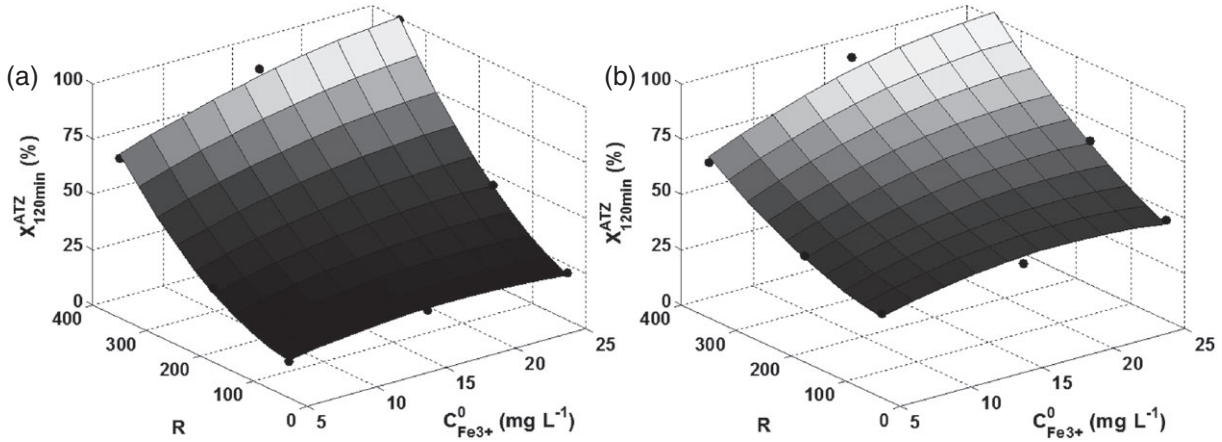


**Figure 4.** Experimental atrazine conversions after 120 min vs. hydrogen peroxide to pollutant initial molar ratios and ferric iron concentrations. (a) Fenton and (b) photo-Fenton. Keys: symbols (experimental data) and 3-D surface (estimated using second degree polynomials).

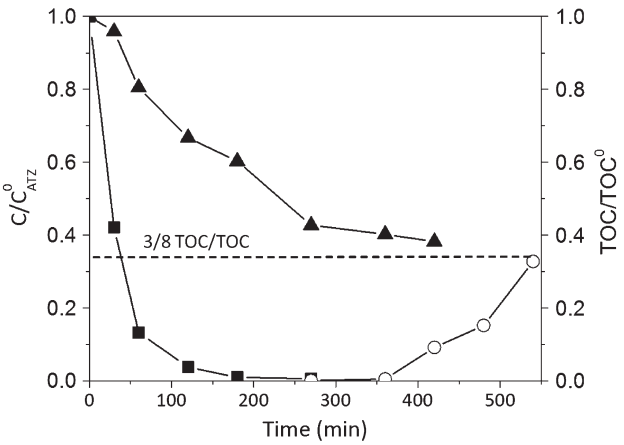


**Figure 5.** Evolution of atrazine, its main intermediate and TOC as a function of time for photo-Fenton process, for $C^0_{Fe^{3+}}$ = 15 mg L$^{-1}$ and R = 350. Relative atrazine concentration (—■—), cyanuric acid to initial atrazine concentration ratio (—○—) and relative TOC (—▲—) *3/8 TOC/TOC$^0$ dash line represents the non-degradable triazine ring [26–28].

noted that the total main carbon source is consumed in approximately 36 h, whereas the cyanuric acid concentration exhibits only a slight reduction. The addition of an extra carbon source was necessary to sustain the biological activity along the duration of the assay. The experiments show that the microbial population was not able to incorporate or use significant amounts of C or N from the cyanuric acid.

Note that cyanuric acid does not produce inhibitory effects on the biological activity, which is in agreement with the results of several contributions[47,48] The adsorption of cyanuric acid on the biomass can be disregarded, since no contaminant was detected in water when the biomass was washed at the end of the assays.

*Anaerobic conditions*

Figure 7(a) shows the results of the anaerobic treatment of a substrate (wastewater from a brewery), whereas Fig. 7(b) corresponds to the same biological assay with the addition of cyanuric acid. Both media were inoculated with the same amount of sludge withdrawn from the UASB reactor of the brewery wastewater treatment plant.

By comparing the evolution of the COD in Fig. 7(a) and (b), it can be concluded that cyanuric acid has no inhibitory effect on the biological activity of the anaerobic bacteria used as inoculum. Moreover, the cyanuric acid is degraded along the assay, being almost completely consumed after 7 days. A similar behaviour with gram-negative bacterium capable of using cyanuric acid as a C and N sources under anaerobic conditions has previously been reported.[57]

This result indicates that the Fenton and photo-Fenton processes, when applied as pre-treatment systems, can break down or rearrange molecular structures of atrazine, which results in a significant enhancement of its biodegradability under anaerobic conditions. In this sense, the photo-Fenton–biological integrated system was more convenient to achieve the total mineralization of the solution, since using the Fenton or photo-Fenton reactor alone is not economically attractive to reach satisfactory mineralization levels.

## CONCLUSION

The efficiency of Fenton and photo-Fenton processes to eliminate atrazine from a water solution was demonstrated. A parametric

  

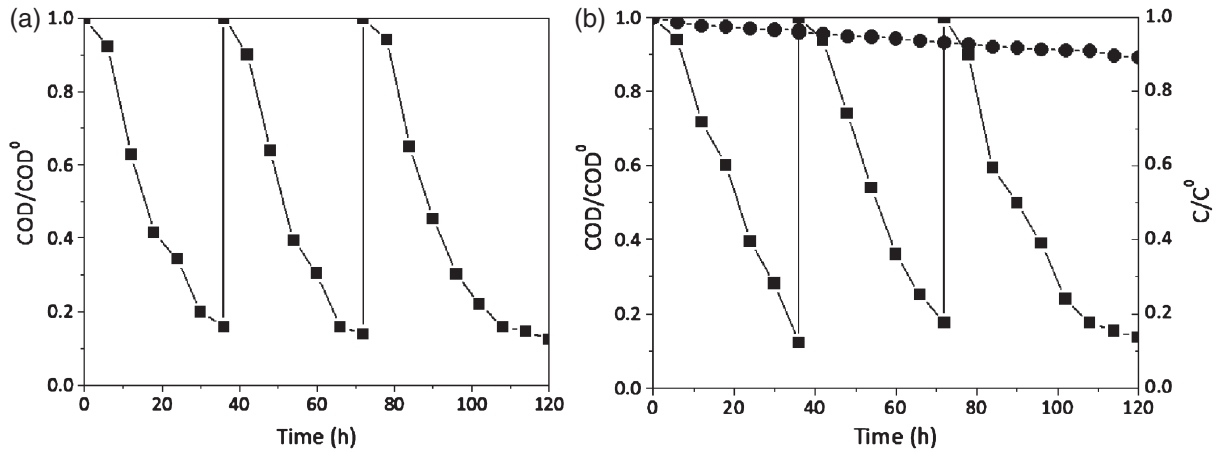


**Figure 6.** Evolution of cyanuric acid and COD as a function of time under aerobic conditions. (a) Blank run and (b) run with cyanuric acid. Cyanuric acid (—●—) and COD (—■—).

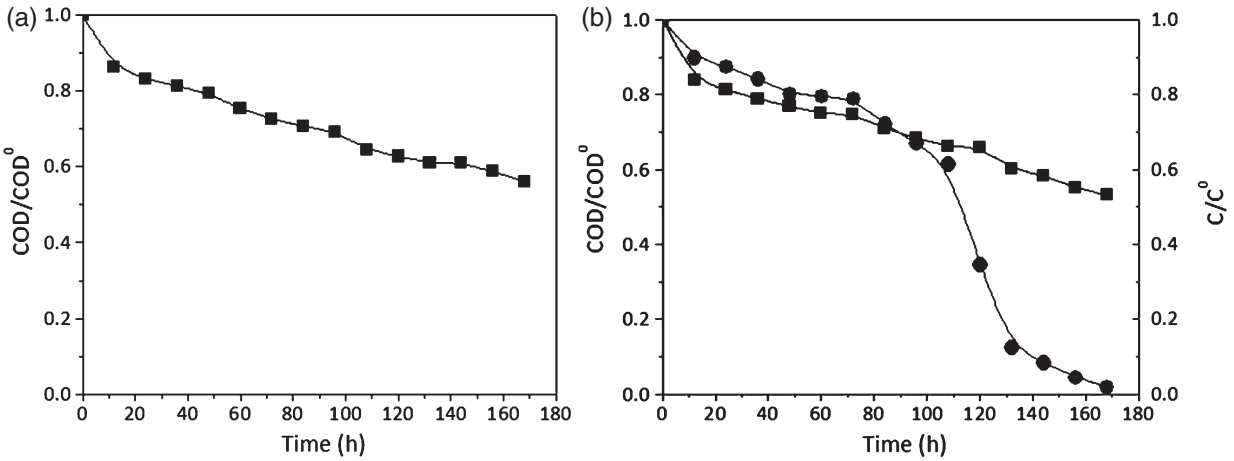


**Figure 7.** Evolution of cyanuric acid and COD as a function of time under anaerobic conditions. (a) Blank run and (b) run with cyanuric acid. Cyanuric acid (—●—) and COD (—■—).

study was performed to compare Fenton and photo-Fenton processes under different operating conditions. It was also shown that the Fenton and photo-Fenton degradation rates of atrazine increase when the hydrogen peroxide to atrazine concentration ratio and the ferric ion concentration are increased.

Atrazine was degraded by the Fenton or photo-Fenton processes to cyanuric acid but, after this step, no further degradation took place. Cyanuric acid was responsible for the residual TOC after these chemical oxidation treatments, which allowed achieving 60% of TOC reduction.

In contrast, biologically based degradations in anaerobic conditions were successful as a complementary treatment. It was found experimentally that the biomass used in the assay was able to metabolize the cyanuric acid, depleting 95% of this contaminant in 7 days. Consequently, a treatment based on Fenton or photo-Fenton processes followed by an anaerobic treatment can be considered as a suitable solution for the removal of atrazine from water.

## ACKNOWLEDGEMENT

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useful in mineralization of biorecalcitrant industrial pollutants. *Catal Today* **54**:341–352 (1999).
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