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# Thiamethoxam removal by Fenton and biological oxidation

E. Gomez-Herrero,<sup>a</sup> H. Lebib-ElHadi,<sup>b,c</sup> H. Ait-Amar,<sup>b</sup> M. Tobajas,<sup>a</sup> J.J.

Rodriguez,<sup>a</sup> A. F. Mohedano<sup>a</sup>

<sup>a</sup>Department of Chemical Engineering, Faculty of Science, Universidad Autónoma de Madrid, Campus de Cantoblanco, 28049 Madrid, Spain.

<sup>b</sup>Laboratoire des Sciences du Génie des Procédés Industriels (LSGPI), Faculté de Génie Mécanique et de Génie des Procédés, Université des Sciences et de la Technologie Houari Boumediene (USTHB), BP 32, El-Alia 16112 Bab-Ezzouar, Algeria

<sup>c</sup>Unité de Développement des Equipements Solaires, UDES, Centre de Développement des Energies Renouvelables, CDER, 42004, Tipaza, Algeria

Corresponding author: Esther Gomez-Herrero

Tel.: +34 914 973525

*E-mail address: [esther.gomez@uam.es](mailto:esther.gomez@uam.es)*

## Abstract

**Background:** Thiamethoxam (TMX) is a potential insecticide pollutant of hydric resources that must be removed. Advanced Oxidation Processes (AOPs) are usually effective, but expensive to implement. Combining Fenton's reagent with biological oxidation circumvents the shortcomings of Fenton technology, facilitating the biodegradation of the resulting effluents in a Sequencing Batch Reactor (SBR).

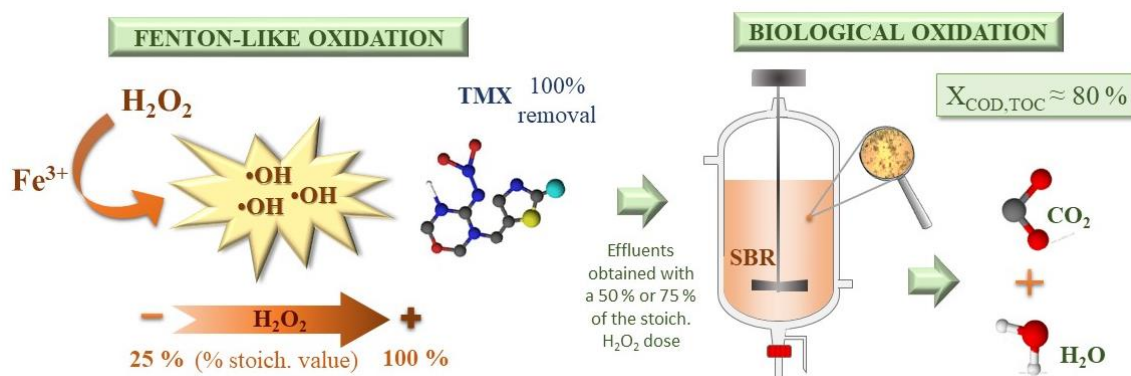
**Results:** Fenton-like oxidation of thiamethoxam with variable hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) doses afforded Total Organic Carbon (TOC) conversion and Chemical Oxygen

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Demand (COD) removal by 40% and 80% from pure TMX, and 40% and 60%, respectively, from commercial TMX. The effluents from Fenton oxidation using substoichiometric  $\text{H}_2\text{O}_2$  doses were less ecotoxic and more biodegradable than the initial solution and, therefore, susceptible to a biological treatment. Biological oxidation of effluents obtained from 50% and 75%  $\text{H}_2\text{O}_2$  doses was accomplished for different organic loads in 6-h cycles. Coupling Fenton and biological oxidation of TMX afforded TOC conversion and COD removal around 80% for the effluent obtained with a 75%  $\text{H}_2\text{O}_2$  dose.

**Conclusion:** Coupling Fenton-biological oxidation efficiently reduces the amounts of  $\text{H}_2\text{O}_2$  needed and produces biodegradable effluents. In addition, the biological treatment increases COD and TOC removal up to 80% for the overall treatment.

## Graphical abstract



## Keywords

Ecotoxicity, Fenton's reagent, Pesticide, Sequencing Batch Reactor, Thiamethoxam.

## 1. Introduction

Neonicotinoids, which were developed in the 1980s and 1990s, have been deemed the most emergent class of insecticides since the advent of pyrethroids.<sup>1</sup> Their acting as agonists of the insect nicotinic acetylcholine receptor (nAChR) in the central nervous system<sup>2</sup> makes them effective for controlling sucking and chewing pests.<sup>3</sup>

Thiamethoxam (3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-*N*-nitro-1,3,5-oxadiazinan-4-imine, TMX) is the first pesticide of the second generation of neonicotinoids. TMX has broad-spectrum insecticidal activity and exhibits excellent systemic properties and good stability, which make it suitable for leaf, grain and seed treatments.<sup>4</sup> However, the combination of such properties and its widespread use have been connected to a decline in bee populations.<sup>5</sup> Also, TMX is perceived as a potential source of water contamination owing to its high water solubility (4.1 g L<sup>-1</sup> at 20 °C) and low soil organic carbon–water partitioning coefficient (log  $K_{oc}$  = 1.75).<sup>6–8</sup> As a result, TMX has been included on the European Watch List for water emerging pollutants.<sup>9</sup>

A number of technologies have been developed in recent decades to improve water quality in order to maintain healthy aquatic environments. Although toxic compounds present in natural waters are mainly removed by photodegradation and biodegradation,<sup>10</sup> TMX is poorly degraded by hydrolysis.<sup>11</sup> In fact, TMX biodegradation with pure *Rhizobacterium*, *Pseudomonas* and *Bacillus* strains proved largely inefficient (TMX conversion after 15 days was lower than 45%).<sup>12,13</sup>

Advanced oxidation processes (AOPs), which provide highly competitive water treatments for removing pesticides refractory to conventional methods, appear to be a good choice for TMX removal. In recent years, a variety of AOPs, but particularly photocatalysis with TiO<sub>2</sub> and photo-Fenton treatments, have been successfully used to degrade neonicotinoids.<sup>14–18</sup> Other, less explored treatments for degrading TMX use

UV, O<sub>3</sub> and their combination,<sup>19</sup> or zero-valent metals under ultrasonic irradiation.<sup>20</sup> Electro-Fenton oxidation has proved more efficient than Fenton's reagent in removing TMX,<sup>21</sup> but anodic oxidation with a boron doped diamond (BDD) anode at 40 mA cm<sup>-2</sup> in 0.1 M Na<sub>2</sub>SO<sub>4</sub> was even more effective in oxidizing TMX (TOC conversion exceeded 90% after only 2 h).<sup>22</sup>

Removing recalcitrant organics with an AOP as sole oxidation treatment can be uneconomical. On the other hand, biotreatments provide an environmentally friendly choice for pollutant management but are largely inefficient as the likely result of the limited resistance of microorganisms to toxic substances.<sup>23</sup> This has led to the use of AOPs as pretreatments to increase biodegradability and reduce toxicity prior to biological degradation arousing increasing interest recently.<sup>24-26</sup> A combination of Fenton and biological oxidation allows the shortcomings of Fenton's reagent (e.g., high reactant consumption) and the restrictions of biotreatments (e.g., slowness and the need for strictly controlled conditions) to be circumvented. Several works attended a feasible combination among chemical and biological oxidation for the removal of recalcitrant pollutants, most of them focused on Fenton-based processes including Catalytic Wet Peroxide Oxidation (CWPO) and photo-Fenton processes.<sup>27-29</sup> Indeed, coupling Fenton and biological oxidation could be an efficient<sup>30,31</sup> and cost-effective<sup>32</sup> solution for the removal of Thiamethoxam, avoiding the drawbacks of Fenton oxidation alone. Biological treatments based on the use of activated sludge in sequencing batch reactors (SBR) are widely used to detoxify industrial wastewater on the grounds of their feeding flexibility, versatility and cost-effectiveness relative to other biological technologies. SBR afford using microorganisms tolerant of toxic compounds and of different types of environments (oxic, anoxic) to facilitate nutrient removal.<sup>33,34</sup>

The aim of this work was to assess the performance of a combined Fenton–biological treatment for the removal of pure TMX and a commercial formulation of the pesticide from aqueous solutions. The process was optimized in terms of TMX degradation rate, biodegradability, ecotoxicity and byproduct formation by adjusting the hydrogen peroxide dose used for Fenton-like oxidation. The efficiency of the biodegradation process was examined in long-term experiments in a sequencing batch reactor. Reactor performance was assessed in terms of TOC and COD removal with a view to identifying the point in the process where the two treatments should ideally be coupled.

## 2. Materials and Methods

### 2.1. Chemicals

Two different formulations of TMX were used. The pure formulation (PTMX, 99% pure, analytical-grade) was obtained from Sigma–Aldrich<sup>®</sup> and the commercial formulation (CTMX, ACTARA 25 WG, 25 wt. % thiamethoxam) from Syngenta AG. A general characterization of both solutions is shown in **Table 1**. Analytical-grade hydrogen peroxide (33 w/v %), hydrochloric acid (37 wt. %), iron (III) nitrate nonahydrate (98 wt. %) and sodium hydroxide (98 wt. %) were purchased from Panreac.

### 2.2. Fenton-like oxidation

Fenton-like runs were performed in a 1 L glass reactor at 50 °C, using a stirring rate of 200 rpm for 5 h. An initial TMX concentration of 0.1 g L<sup>-1</sup> was used for pure and commercial formulation in order to evaluate the potential effect of the unknown

adjuvants present in the latter. Fenton-like oxidation was done with a variable  $\text{H}_2\text{O}_2$  dose ranging from the stoichiometric concentration as calculated from COD in the starting solution as the theoretical value needed for complete mineralization of TMX ( $0.38 \text{ g L}^{-1}$  for the pure form and  $0.66 \text{ g L}^{-1}$  for the commercial formulation) to 25% that value. A fixed  $\text{Fe}^{3+}/\text{H}_2\text{O}_2$  ratio of 1/10 (M/M)<sup>35</sup> and a temperature of 50 °C were used, as well as a pH value around 3 that was adjusted with  $\text{H}_2\text{SO}_4$ . Each sample was neutralized with sodium hydroxide and passed through a glass microfiber filter (Albert FV-C). The concentrations of TMX,  $\text{H}_2\text{O}_2$ , TOC and COD were monitored at each  $\text{H}_2\text{O}_2$  dose used, and biological oxygen demand ( $\text{BOD}_5$ ) and reaction byproducts in the final effluents were analyzed.

### 2.3. *Inoculum source*

The activated sludge used as inoculum in the SBR and in the  $\text{BOD}_5$  assays was collected from a municipal sewage plant. Microorganisms were grown on sodium acetate and glucose as carbon sources in an SBR operated at 25 °C. The medium was supplemented with ammonium sulfate, phosphoric acid and mineral salts as a nitrogen, phosphorus and micronutrient source, respectively. The COD:N:D:micronutrient ratio used was 100:5:1:0.05 (w/w).

### 2.4. *Ecotoxicity tests*

The ecotoxicity of the starting TMX formulations and the effluents from Fenton-like oxidation was determined with the standard toxicity test in ISO 11348-3 1998, using the luminescent bacterium *Vibrio fischeri* and a Microtox<sup>®</sup> analyzer from SDI. Ecotoxic effects were examined through the decrease in light emission by the bacterium as a



result of exposure to the samples after incubation at 15 °C for 15 min. Ecotoxicity was assessed in terms of EC<sub>50</sub> (PTMX) or IC<sub>50</sub> (CTMX and effluents from Fenton-like oxidation), defined as the effective concentration or dilution ratio decreasing the bioluminescence by 50%.<sup>36</sup>

## 2.6. Biological treatment

The biological treatment of the selected effluent from Fenton-like oxidation was carried out in a 3 L SBR at 30 °C at 200 rpm. The reactor was equipped with an air diffuser, peristaltic pumps to feed and discharge the reactor, and a dissolved oxygen probe. Six-hour sequences were performed, the length of each stage in the cycle being as follows: 0.5 h for filling and development under anoxic conditions, 4.75 h for aeration, 0.5 h for settling and 0.25 h for withdrawal. The biomass concentration (volatile suspended solids, VSS) was maintained at 1.5 g L<sup>-1</sup> and the initial TMX commercial concentration for Fenton-like experiments was raised to 1 g L<sup>-1</sup> in order to ensure an organic load rate of 0.15 kg COD kg<sup>-1</sup> VSS d<sup>-1</sup>. Effluents from Fenton-like oxidation were used as the sole carbon sources, albeit supplemented with ammonium sulfate and phosphoric acid as a nitrogen and phosphorous source, respectively, in addition to mineral salts for microbial growth. TOC, COD, organic byproducts, and inorganic nitrogen and chloride species, were quantified in samples withdrawn at the end of the aerated biological process and throughout an aeration stage of the biological oxidation process.<sup>37</sup>

## 2.7. Analytical methods

TOC and total nitrogen (TN) were measured with a Shimadzu TOC-VCSH analyzer. COD and biomass concentrations were measured according to APHA procedure 5220A

and 2540E,<sup>38</sup> respectively. Residual hydrogen peroxide was determined by colorimetric titration with titanium sulphate<sup>39</sup> on a Shimadzu UV-1603 spectrophotometer. TMX was quantified by HPLC on a Varian Prostar 325 spectrophotometer equipped with a UV–Vis detector. A Synergy 4 mm Polar-RP 80 A column from Phenomenex (15 cm long × 4.6 mm i.d.) was used as stationary phase and phosphate buffer with an acetonitrile gradient as mobile phase. The instrument was operated at 218 nm and a constant flow rate of 0.75 mL min<sup>-1</sup>. The TMX detection limit was 0.01 mg L<sup>-1</sup>. BOD<sub>5</sub> was measured by using the APHA 5210 standard procedure (APHA, 1992) on Velp Scientifica equipment, a sample volume of 500 mL and a biomass concentration of 135 mg VSS L<sup>-1</sup>. A Metrohm 790 ion chromatograph with equipped with chemical suppression and a conductivity detector was used to detect short-chain organic acids, ammonium, nitrate, nitrite and chloride species. The instrument was used in combination with a Metrosep A supp 2-250 column (25 cm long, 4 mm i.d.) as stationary phase and an aqueous solution containing 1 mM NaHCO<sub>3</sub> and 3.2 mM Na<sub>2</sub>CO<sub>3</sub> that was circulated at a constant flow rate of 0.7 mL min<sup>-1</sup> as mobile phase. Reaction byproducts were detected by HPLC/MS, using a Quadrupole LC/MS instrument from Agilent, an ACE Excel 3 C-18-amide column (15 cm length, 4.6 mm diameter) as stationary phase and an aqueous solution of formic acid (0.1%) at a constant flow rate of 0.5 mL min<sup>-1</sup> as mobile phase. Mass spectrometry was performed by ESI + ionization, using a drying gas flow rate of 9 L min<sup>-1</sup>.

### 3. Results and discussion

#### 3.1. Fenton-like process

**Figure 1** shows the time course of the pesticide concentration, TOC and H<sub>2</sub>O<sub>2</sub> conversion, as well as normalized COD removal upon Fenton-like oxidation with the

stoichiometric  $\text{H}_2\text{O}_2$  dose. Fenton-like tests were done with both pure thiamethoxam (PTMX) and a commercial formulation (CTMX) to examine the effect of unknown adjuvants potentially interfering with oxidation of the active compound. Complete removal of TMX and  $\text{H}_2\text{O}_2$  depletion were achieved with both forms. TOC mineralization reached conversions around 40% and 55% for the pure and commercial formulation, respectively, despite the increased extent of COD removal (80% from the pure form and 60% from the commercial formulation). Therefore, the adjuvants in CTMX slightly delayed degradation of the pesticide. TMX uptake decayed rapidly early in the oxidation process and then levelled off with no significant decline in TOC for the pure and commercial compound, coinciding with the total TMX removal. Similar results were previously obtained by Gomez-Herrero et al.<sup>24</sup> for the removal of ionic liquids by Fenton oxidation, with final TOC conversion and COD removal values of about 40% and 60%, respectively, at a stoichiometric  $\text{H}_2\text{O}_2$  dose. To our knowledge, few studies have examined the removal of TMX upon Fenton oxidation. In one, Meijide et al.<sup>21</sup> obtained complete TMX conversion and 35% TOC mineralization. Combinations of chemical and biological oxidation have also been used with other pesticides such as alachlor, atrazine and diuron. All were fully depleted by Fenton oxidation, the resulting effluents being more biodegradable in the subsequent biological treatment.<sup>40</sup>

The optimum operating conditions in terms of TMX and COD removal, and TOC conversion, were established by Fenton-like oxidation with different initial  $\text{H}_2\text{O}_2$  doses ranging from 25 to 100% the stoichiometric value.  $\text{H}_2\text{O}_2$  doses higher than 25% of stoichiometric led to nearly complete TMX degradation, whereas low  $\text{H}_2\text{O}_2$  doses conducted to degradation of 81 % and 76% of PTMX and CTMX respectively, most likely due to the lack of the necessary dose of hydrogen peroxide to oxidize the target

compound present in the solution.<sup>41</sup> On the other hand, H<sub>2</sub>O<sub>2</sub> doses of 50–100 % the stoichiometric level failed to increase TOC and COD conversion, probably because H<sub>2</sub>O<sub>2</sub> was depleted and refractory byproducts such as stable ferric complexes diminished the amount of free iron available, thus inhibiting the production of hydroxyl radicals.<sup>42,21</sup>

**Table 2** and **Figure 1S** shows the kinetic constants for TMX degradation in pure form and the commercial formulation, and for TOC and COD removal. TMX removal was accurately described by a pseudo first-order kinetic model with high determination coefficients ( $R^2 \approx 0.98$ ). Rate constants increased with increasing H<sub>2</sub>O<sub>2</sub> dose and were higher for PTMX than for CTMX, which suggests that adjuvants in the latter may have hindered decomposition of the pesticide. TOC and COD removal closely fitted a second-order kinetic model. As can be seen in **Figure 1**, TOC mineralization increased faster than did the decay of COD removal. Gomez-Herrero et al.<sup>24</sup> reported pseudo first-order kinetics for the removal of ionic liquids upon Fenton oxidation, and TOC decay to fit a second-order model, which suggested that Fenton oxidation followed similar kinetics irrespective of the particular pollutant.<sup>43</sup> TMX removal by acid hydrolysis and electro-Fenton oxidation also fitted a pseudo first-order kinetic model.<sup>11,21</sup>

**Figure 2** shows the concentrations of short-chain organic acids (acetic, formic and oxalic acid) in the effluents from Fenton-like oxidation of TMX at different hydrogen peroxide doses. Formic acid and acetic acid were the main degradation byproducts from both pure TMX and its commercial formulation. The ratios of short-chain organic acids were similar for both forms; however, the total acid concentration was lower for the pure pesticide than for the commercial product, probably by effect of unknown adjuvants present in the latter being oxidized to the organic acids. The formation of acetic acid increased with increasing H<sub>2</sub>O<sub>2</sub> dose up to 75%, but decreased

at the stoichiometric dose, in both formulations. This effect of the H<sub>2</sub>O<sub>2</sub> dose was also observed in oxalic acid, although the acid concentration with CTMX was lower (0.88 mg L<sup>-1</sup> at most with the 75% dose). Overall, the concentration of formic acid in the effluent increased with increasing H<sub>2</sub>O<sub>2</sub> dose and peaked with the stoichiometric amount (4.8 and 8.4 mg L<sup>-1</sup> for PTMX and CTMX, respectively). This was the likely result of acetic acid being converted into formic acid at high oxidant doses. Formic acid was previously found to accumulate alongside oxalic acid in the oxidation of acetic acid in H<sub>2</sub>O<sub>2</sub>/UV<sup>44</sup> and electrochemical Fenton treatments.<sup>45</sup>

The oxidation byproducts formed in the Fenton-like process were identified chromatographically. **Table 3** lists the intermediates identified from their *m/z* values. Most of them (**1**, **2**, **4**, **5**, **6**, **7**, **10** and **12**) exhibited an [*M*+2] isotopic pattern suggesting that they contained chlorine. The byproducts detected at long retention times had high molecular weights and formed mostly by hydroxylation of the starting compound. Some intermediates were previously found in TMX acid hydrolysis,<sup>11</sup> electro-Fenton with a BDD anode and a carbon-felt cathode,<sup>21</sup> and photocatalysis with immobilized TiO<sub>2</sub>.<sup>46</sup> On the other hand, the compounds detected at short retention times were small and consisted largely of individual aromatic rings and their broken forms. Although no clear-cut reaction mechanism can be proposed, some byproducts may have been attacked by hydroxyl radicals and formed simpler intermediates; thus, products **13** and **14** in **Table 3** probably formed through decomposition of **8**. Further oxidation and oxidative ring opening reactions of these aromatic compounds may have yielded simple carboxylic acids that were quantified as the above-mentioned short-chain organic acids. Finally, the carboxylic acids may have been converted into carbon dioxide, water and inorganic ions such as NO<sub>x</sub> species.<sup>47</sup>

**Figure 3** shows the TOC, TN and chlorine balance closure at different  $\text{H}_2\text{O}_2$  doses. Whereas the 25% dose led to incomplete removal of CTMX (76%), increasing the dose from 50 to 100% reduced the formation of unknown intermediates, from 78 to 43 % for organic species (TOC), 75 to 65 % for nitrogen-containing species (TN) and 71 % to 32 % for chlorinated species. This was the likely result of further oxidation of the intermediates by  $\text{H}_2\text{O}_2$  remaining in the medium. Indeed, increased doses of  $\text{H}_2\text{O}_2$  favoured the formation of carboxylic acids, and also of nitrogen containing byproducts that were recovered as nitrite and nitrate. The chlorine balance revealed that increasing the  $\text{H}_2\text{O}_2$  concentration led to the presence of compounds with less chlorine atoms that were quantified as chloride ions in the effluents. However, TOC was inconsistent with the estimated value from short-chain organic acids even with the stoichiometric dose, which resulted in 43 % less conversion than expected owing to the formation of unknown byproducts that were refractory to Fenton oxidation. Similarly, the stoichiometric  $\text{H}_2\text{O}_2$  dose led to 35% TN recovery in the form of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . The nitrogen mass balance was also incomplete, probably because of the release of nitrogen-containing by-products such as chloramines<sup>48</sup> or gaseous nitrogen oxides ( $\text{N}_x\text{O}_y$ ).<sup>47,49</sup>

### 3.2. Ecotoxicity and biodegradability

Fenton-like oxidation was used here to increase the biodegradability of the effluent prior to its biological treatment. This required using biodegradability and toxicity tests to ensure biocompatibility of the Fenton effluents. The neutralization of the effluents from Fenton-like oxidation at a pH close to 7 caused the formation of iron flocs, which were removed by filtration. This withdrawal of the precipitate could decrease the organic matter content by coagulation/adsorption processes. A slight COD removal, lower than 3% of the initial COD value was observed.

Because it is not influenced by the amount of organic matter present or its oxidative status,<sup>50</sup> the BOD<sub>5</sub>/COD ratio is an accurate parameter for assessing biodegradability. Indeed, the ratio is commonly used to assess the biodegradability of pollutants in wastewater treatment effluents; thus, an increased BOD<sub>5</sub>/COD ratio is associated with a high removal efficiency.<sup>51,52</sup> In general, effluents are deemed biodegradable if their BOD<sub>5</sub>/COD ratio exceeds a threshold value of 0.3–0.4.<sup>53–55</sup> As can be seen in **Fig. 3**, biodegradability increased markedly with increasing H<sub>2</sub>O<sub>2</sub> dose above 25% the stoichiometric value. This dose failed to increase the BOD<sub>5</sub>/COD ratio as the likely result of the presence of residual TMX in the Fenton-like effluent. On the other hand, Fenton oxidation with the stoichiometric H<sub>2</sub>O<sub>2</sub> dose increased biodegradability by a factor of 6. With the intermediate doses, the BOD<sub>5</sub>/COD ratio ranged from 0.09 (poorly biodegradable TMX solution) to 0.43 (biodegradable solution). These values testify to the effectiveness of the Fenton process for increasing the biodegradability of recalcitrant wastewater.

The toxicity of the Fenton effluent was assessed through the Microtox<sup>®</sup> assay, which was previously used with various pesticides.<sup>36</sup> **Figure 3** also showed the IC<sub>50</sub> values (TU) of the effluents from Fenton-like oxidation at different H<sub>2</sub>O<sub>2</sub> doses. The assay revealed that a 25% H<sub>2</sub>O<sub>2</sub> dose dramatically decreased the luminescence of the effluent from Fenton oxidation. This was probably the result of the formation of byproducts that were more toxic than the parent compound (e.g., **1**, **2**, **3** and **5** in **Table 3**, which were all identified in the Fenton-like oxidation effluents obtained with the 25% dose). This result is consistent with previous findings in the Fenton oxidation of pesticides<sup>40,56,57</sup> including increased formation of carboxylic acids with increase in the H<sub>2</sub>O<sub>2</sub> dose and a gradual reduction of ecotoxicity to non-ecotoxic levels in the effluent treated with the stoichiometric dose. Based on these results, the Fenton-like effluents

obtained with the 50 and 75 % doses were subjected to further biological treatment in order to completely remove TMX, reduce ecotoxicity and increase biodegradability to an acceptable level. To this end, the initial concentration of TMX was increased to 1 g L<sup>-1</sup> in order to ensure an adequate organic load in the SBR. The TOC and COD values obtained upon Fenton oxidation were 153 mg L<sup>-1</sup> and 1380 mgO<sub>2</sub> L<sup>-1</sup>, respectively, with the 75% dose; and 182 mg L<sup>-1</sup> and 1616 mg O<sub>2</sub> L<sup>-1</sup>, respectively, with the 50% dose.

### 3.3. Biological oxidation

The effluents from Fenton oxidation of the commercial formulation obtained with the 75% and 50% H<sub>2</sub>O<sub>2</sub> doses were fed to an SBR for biological treatment in the absence of additional carbon sources. **Figure 4** shows TOC and COD at the end of the SBR cycles. The Fenton effluent was initially allowed to acclimate to the sewage sludge in the first cycles. Subsequently, TOC and COD decreased with time by effect of biological degradation of the effluent. Once TOC and COD leveled off, the organic load was increased from 0.15 to 0.3 kg COD kg<sup>-1</sup> VSS d<sup>-1</sup> in order to improve the efficiency of the process while maintaining a cycle length of 6 h. In increasing the organic load failed to boost TOC conversion in the effluent treated with the 50% dose but further reduced TOC (from 60 to ca. 15 mg L<sup>-1</sup>) in the effluent receiving the 75% dose. TOC and COD conversion in the effluent treated with the 75% dose reached levels as high as 80% upon biological treatment, which testifies to the efficiency of the combined chemical–biological treatment. Removal was lower in the effluent receiving the 50% dose. Residual TOC and COD at the end of each cycle were ascribed to the presence of non biodegradable intermediates and refractory microbial products resulting from cell disruption in activated sludge.<sup>58</sup> Coupling Fenton oxidation and a biological treatment of the effluent in an SBR led to overall TOC and COD conversion values above 80%.



A typical 6 h cycle was carefully examined in order to gain further insight into the operational strategy and the variation of the main overall parameters. **Figure 5** shows the profiles for TOC, COD and specific oxygen uptake rate (SOUR), as well as the concentrations of nitrogen-containing species and short-chain organic acids in the reactor over one cycle of biological oxidation where COD and TOC conversion leveled off. A significant decay in TOC and COD was observed during the first hour of the cycle, which comprised the anoxic stage, filling and the beginning of the aerobic stage. The SOUR profile changed similarly and reflected an increase in oxygen uptake by the sludge that was related to TOC and COD decay during the aeration stage. The concentration of short-chain organic acids decreased drastically at the beginning of the cycle and led to substantial biomass activity, which suggests that the reduction in TOC and COD was largely due to their uptake—22.1% of the reduction in TOC was due to carboxylic acids—, residual TOC corresponding to the depletion of unknown intermediates present in the effluent. Biological oxidation removed nitrogen-containing species in the influent, even  $\text{N-NH}_4^+$ , which came only from the sources used to maintain the sludge, included. Ammonium ion was completely removed during the aerobic stage, and nitrite and nitrate ions were reduced by more than 80% under the action of denitrifying bacteria present in the sludge in the SBR anoxic stage.<sup>31</sup> During the aerobic reaction, these compounds formed as a result of oxygen uptake by nitrifying bacteria, which can oxidize ammonium ion to nitrite and nitrate. Hence, the alternate anaerobic and aerobic stages in an SBR provide a major advantage over conventional biological systems<sup>59</sup> and can be especially useful with samples containing oxalic acid, a recalcitrant byproduct formed in the Fenton oxidation reaction.<sup>60</sup> In addition, the joint presence of carbon and nitrate sources under anoxic conditions can facilitate denitrification and increase the substrate uptake rate in the subsequent aerobic period.<sup>61</sup>

The active sludge used in the biological oxidation acclimated during the SBR cycles and facilitated removal of some byproducts present in the effluents from the Fenton oxidation of TMX.

#### **4. Conclusions**

Coupling Fenton's reagent as a chemical pretreatment with biological oxidation boosted removal of TMX. The Fenton-like process can be used as pretreatment, to produce effluents containing a substantial concentration of short-chain organic acids in addition to aromatic and other, unknown compounds that are less ecotoxic and more easily biodegraded. Biodegradability and toxicity tests allowed the optimum reagent dose for the chemical treatment to be selected. Using an  $\text{H}_2\text{O}_2$  dose 50–75 % of the stoichiometric value in the Fenton-like oxidation reaction provided suitable effluents for the combined treatment; thus, low concentrations of Fenton reagent gave rise to more ecotoxic compounds, whereas high doses resulted in no further improvement in biodegradability. Using an SBR for the biological treatment of effluents from Fenton-like oxidation boosted COD and TOC removal (ca. 80% with the combined treatment); also, it reduced the formation of unknown by-products and short-chain organic acids.

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**Table 1.** Characterization of the pure and commercial solutions of TMX.

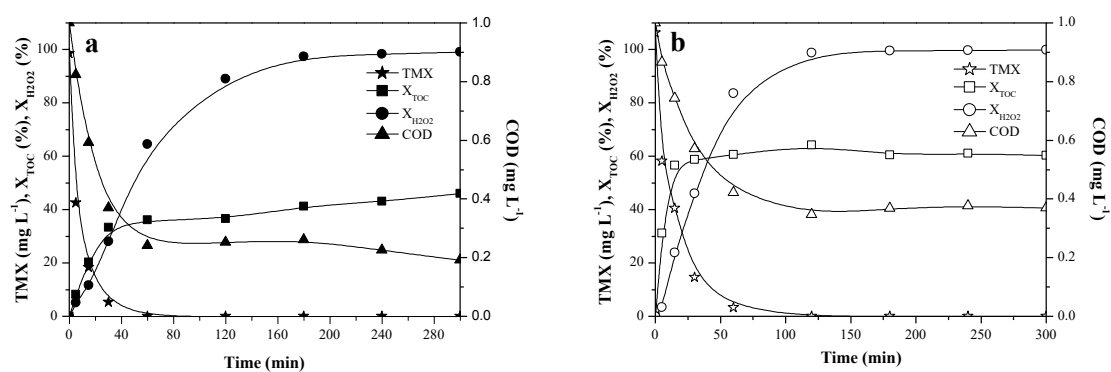
	PTMX	CTMC
Commercial name	Pure analytical grade	ACTARA
TMX content (%w)	99.99	25
TMX concentration (mg L <sup>-1</sup> )	100	100
COD (mg L <sup>-1</sup> )	405	539
TOC (mg L <sup>-1</sup> )	35.0	51.3
TN (mg L <sup>-1</sup> )	19.0	24.5
BOD <sub>5</sub> (mg L <sup>-1</sup> )	-	48.5
EC <sub>50</sub> (mg L <sup>-1</sup> )	2.5	-
IC <sub>50</sub> (TU)	-	41.2

**Table 2.** Kinetic constants for TMX, TOC and COD removal, and TOC identified as short-chain organic acids upon Fenton oxidation of pure TMX (P) and its commercial formulation (C).

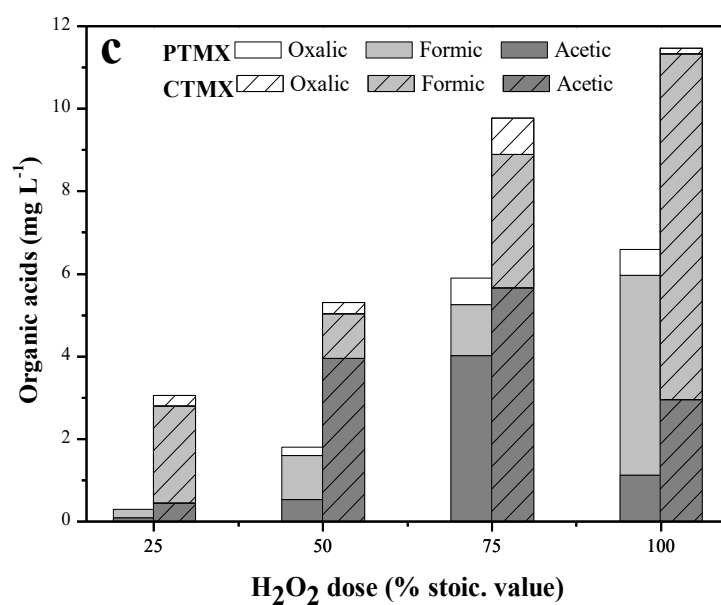
H <sub>2</sub> O <sub>2</sub> dose (% stoich. value)	$k_{\text{PTMX}}$ (min <sup>-1</sup> )	$k_{\text{PTOC}}$ (L mg <sup>-1</sup> min <sup>-1</sup> )	$k_{\text{PCOD}}$ (L mg <sup>-1</sup> min <sup>-1</sup> )	$\Sigma C_{\text{acids}}$ (mg L <sup>-1</sup> )	C. identified (%)
25	$1.2 \cdot 10^{-2}$	$0.8 \cdot 10^{-4}$	$1.5 \cdot 10^{-4}$	0.9	3.6
50	$3.9 \cdot 10^{-2}$	$1.0 \cdot 10^{-4}$	$3.6 \cdot 10^{-4}$	1.8	9.0
75	$6.5 \cdot 10^{-2}$	$1.7 \cdot 10^{-4}$	$5.4 \cdot 10^{-4}$	5.9	31.0
100	$9.4 \cdot 10^{-2}$	$1.6 \cdot 10^{-4}$	$5.7 \cdot 10^{-4}$	6.6	37.0
H <sub>2</sub> O <sub>2</sub> dose (% stoich. value)	$k_{\text{CTMX}}$ (min <sup>-1</sup> )	$k_{\text{CTOC}}$ (L mg <sup>-1</sup> min <sup>-1</sup> )	$k_{\text{CCOD}}$ (L mg <sup>-1</sup> min <sup>-1</sup> )	$\Sigma C_{\text{acids}}$ (mg L <sup>-1</sup> )	C. identified (%)
25	$1.5 \cdot 10^{-2}$	$0.7 \cdot 10^{-4}$	$0.4 \cdot 10^{-4}$	5.1	19.8
50	$2.2 \cdot 10^{-2}$	$2.3 \cdot 10^{-4}$	$0.5 \cdot 10^{-4}$	5.3	22.0
75	$3.5 \cdot 10^{-2}$	$7.9 \cdot 10^{-4}$	$1.6 \cdot 10^{-4}$	9.8	48.0
100	$5.7 \cdot 10^{-2}$	$8.8 \cdot 10^{-4}$	$1.1 \cdot 10^{-4}$	11.5	57.0

**Table 3.** Byproducts identified in the oxidation of PTMX with Fenton's reagent.

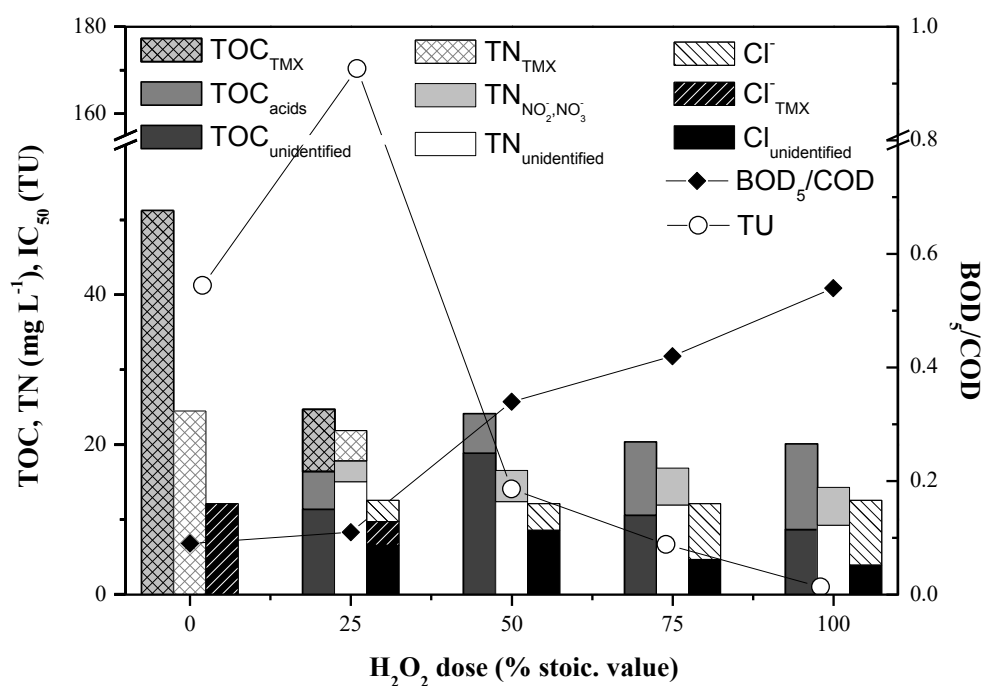
No.	Molecular structure	<i>m/z</i>	References
TMX		292	
1		278	—
2		265	—
3		274	Karmakar et al. (2009)
4		232	Meijide et al. (2016)
5		247	Zabar et al. (2012)
6		205	Zabar et al. (2012)
7		205	Zabar et al. (2012)
8		177	Karmakar et al. (2009)
9		116	—
10		173	Meijide et al. (2016)
11		115	Meijide et al. (2016)
12		149	Karmakar et al. (2009)
13		74	—
14		75	—



**Fig 1.** Time course of TMX concentration, COD removal, H<sub>2</sub>O<sub>2</sub> and TOC conversion in the Fenton-like oxidation of pure TMX (a) and its commercial formulation (b) with a stoichiometric H<sub>2</sub>O<sub>2</sub> dose.

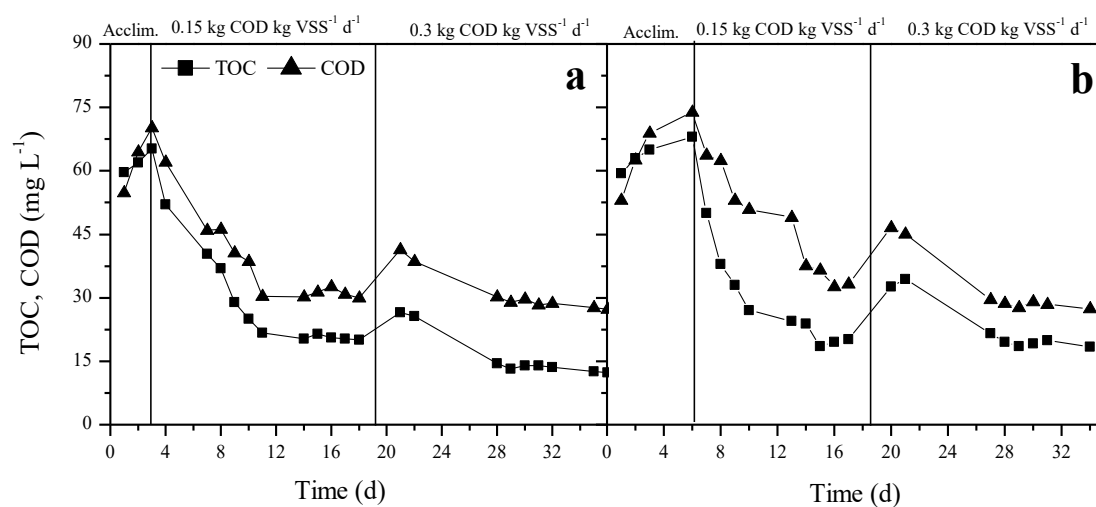


**Fig 2.** Concentrations of short-chain organic acids in the effluents from Fenton-like oxidation of the pure and commercial formulation of TMX.

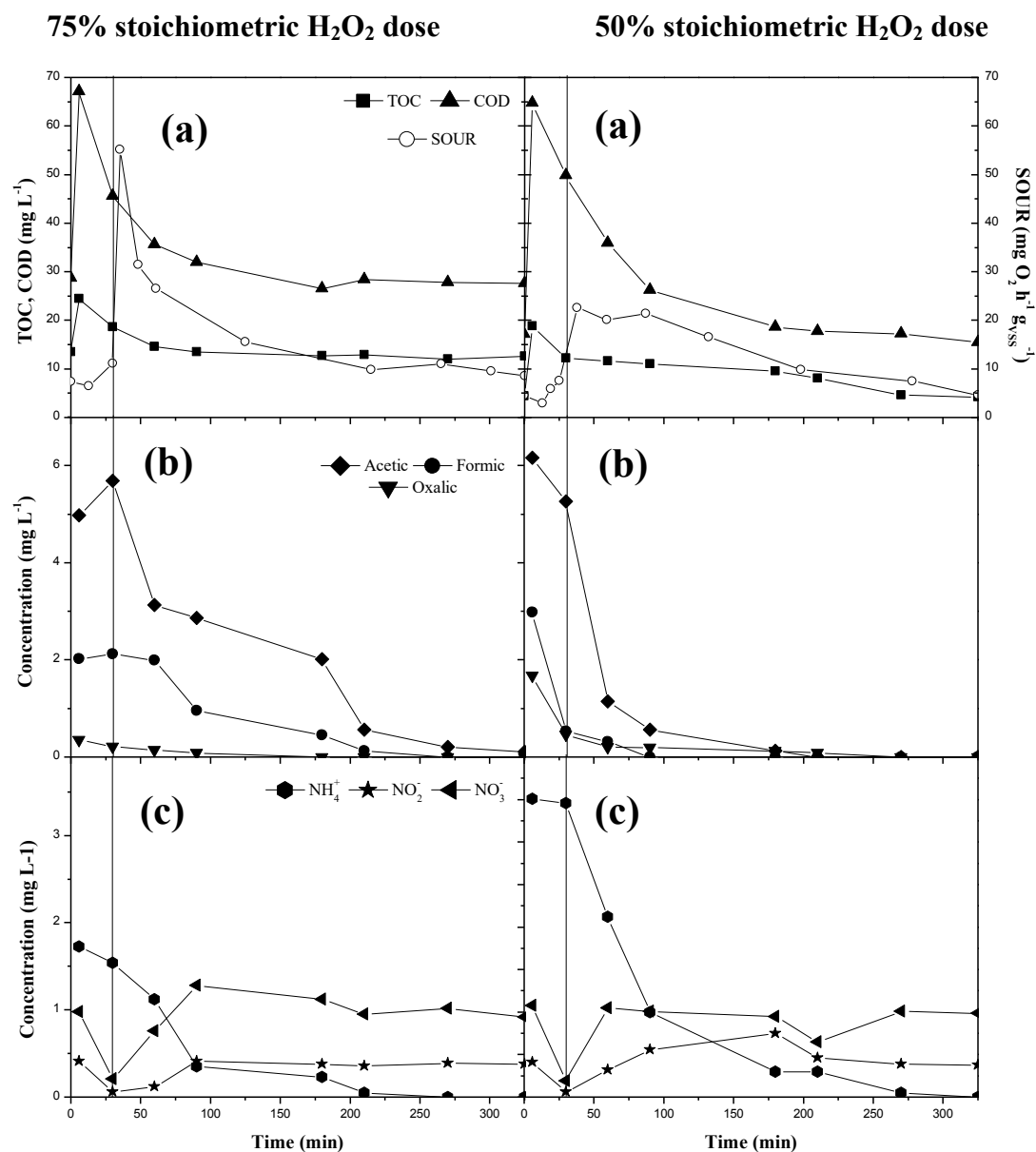


**Fig 3.** Biodegradability index, ecotoxicity, measured and unidentified TOC, TN and chloride balance closure for the Fenton-like oxidation of CTMX with different  $\text{H}_2\text{O}_2$  doses.





**Fig 4.** Time course of TOC and COD over a 6-h SBR cycle with an organic load of 0.15 and 0.3  $kgCOD\ kg^{-1}\ VVS\ d^{-1}$  in the effluents from Fenton-like oxidation of CTMX with an  $H_2O_2$  dose of 75% (a) and 50% (b).



**Fig 5.** Time course of TOC, COD and SOUR (a), short-chain organic acids (b) and nitrogen inorganic species (c) over a 6-h SBR cycle in the effluents from Fenton-like oxidation of CTMX with an  $\text{H}_2\text{O}_2$  dose of 75 and 50%.