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## **Correlation between degradation pathway and toxicity of acetaminophen and its by-products by using the electro-Fenton process in aqueous media**

Thi Xuan Huong Le, Thi Van Nguyen, Zoukifli Amadou Yacouba, Laetitia Zoungrana, Florent Avril, Duy Linh Nguyen, Eddy Petit, Julie Mendret, Valerie Bonniol, Mikhael Bechelany, et al.

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1 Degradation pathway and toxicity of acetaminophen by electro-Fenton processes in  
2 aqueous media

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13  
14 **ABSTRACT**

The evolution of the degradation by-products of an acetaminophen (ACE) solution was monitored by HPLC-UV/MS and IC in parallel with its ecotoxicity (*Vibrio fischeri* 81.9%, Microtox® screening tests) during electro-Fenton (EF) oxidation performed on carbon felt. The aromatic compounds 2-hydroxy-4-(N-acetyl) aminophenol, 1,4-benzoquinone, benzaldehyde and benzoic acid were identified as toxic sub-products during the first stage of the electrochemical treatment, whereas aliphatic short-chain carboxylic acids (oxalic, maleic, oxamic, formic, acetic and fumaric acids) and inorganic ions (ammonium and nitrate) were well identified as non-toxic terminal sub-products. Electrogenenerated hydroxyl radicals then converted the eco-toxic and bio-refractory property of initial ACE molecule (500 mL, 1 mM) and subsequent aromatic sub-products into non-toxic compounds after 2 h of EF treatment. The toxicity of every intermediate produced during the mineralization of ACE was quantified, and a relationship was established

between the degradation pathway of ACE and the global toxicity evolution of the solution. After 8 h of treatment, a total organic carbon removal of 86.9% could be reached for 0.1 mM ACE at applied current of 500 mA with 0.2 mM of Fe<sup>2+</sup> used as catalyst.

Key words: Carbon felt, Advanced Oxidation Process, TOC, Mineralization, By-products, Toxicity

## 1. Introduction

Over the past few years, pharmaceutical drugs have become a severe problem for the domestic wastewater treatment (Schwarzenbach et al., 2006). Pharmaceuticals such as anti-inflammatories, analgesics, lipid regulators, antibiotics, anti-epileptics, betablockers and oestrogens are used extensively for both humans and livestock. When consumed, 10–90% of these drugs are metabolized. A significant quantity of the active ingredients is therefore excreted, mainly through urine and faeces, and collected in urban sewer systems or discharged directly into the environment by livestock. Many studies have confirmed the presence of a large number of medicinal substances in all environmental compartments: ground and surface water (Andreozzi et al., 2003; Leónidas A. Pérez-Estrada et al., 2005), drinking water (Ternes et al., 2002), tap water (Doll and Frimmel, 2003), ocean water, sediment and soil (Halling-Sørensen et al., 1998); this indicates the inefficiency of conventional water treatment processes to remove these kinds of micro-pollutants. Even if the amount of these pharmaceuticals in aquatic media is low, usually with concentrations lower than 10 µg L<sup>-1</sup> (Ternes, 1998; Huerta-Fontela et al., 2011; Huerta et al., 2016), its continuous input constitutes at long term a real risk for aquatic and terrestrial organisms (Zhou et al., 2013; García-Mateos et al., 2015). Some pharmaceuticals are indeed suspected to affect the endocrine system of fishes, and available data on antibiotics indicate that they can exert toxic effects on algae and invertebrates and may favour the development of multi-drug resistant microbial strains (Zuccato et al., 2000; Balcıoğlu and Ötker, 2003). Among pharmaceuticals,

20 acetaminophen (ACE) is an analgesic molecule consumed worldwide, and is usually selected by  
21 researchers as an emerging contaminant model (García-Mateos et al., 2015). ACE is also called  
22 paracetamol, and its chemical name is N-acetyl-p-aminophenol. This drug has been detected in  
23 European wastewater treatment plant (WWTP) with a concentration of  $6 \mu\text{g L}^{-1}$  (Ternes, 1998), up  
24 to  $10 \mu\text{g L}^{-1}$  in natural water resource in the USA (Kolpin et al., 2002) and more than  $65 \mu\text{g L}^{-1}$  in  
25 the Tyne River, UK (Kabdaşli et al., 1999; Roberts and Thomas, 2006).

26

27 Because of the toxicity and persistency of pharmaceuticals, it is necessary to develop  
28 powerful methods to ensure complete degradation of pharmaceutical pollutants as well as their  
29 potentially toxic degradation by-products. Conventional technologies that are currently used, such  
30 as activated carbon adsorption, flocculation, biological degradation, and chemical process like  
31 chlorination, have the disadvantages of being poorly efficient for non-biodegradable compounds  
32 or to transfer the pollutants into the solid phase (sludge or sorbent). Pollution is, in this case, only  
33 displaced and not solved. Advanced oxidation processes (AOPs) (Ozonation, UV/H<sub>2</sub>O<sub>2</sub>, electro-  
34 and photo-Fenton) can be easily incorporated in pre-existing treatment plants and were found to  
35 achieve efficient reduction of micropollutant discharge in the aquatic environment (Reungoat et  
36 al., 2010; Reungoat et al., 2012; Eggen et al., 2014; Sirés et al., 2014; Moreira et al., 2017).  
37 Because non-selective radicals such as hydroxyl radicals (OH) are generated, AOPs are considered  
38 a competitive water treatment technology for the degradation of these refractory organic  
39 micropollutants and the improvement of effluent quality from treatment plants (Gerrity et al.,  
40 2011; Giannakis et al., 2015). Several publications have highlighted the potentialities of AOPs  
41 such as UV/H<sub>2</sub>O<sub>2</sub> (Frontistis et al., 2012; Lee et al., 2016); ozonation (Huber et al., 2005; Gerrity  
42 et al., 2011; Zimmermann et al., 2011; Reungoat et al., 2012; Margot et al., 2013), Fenton and  
43 photo-Fenton (Tekin et al., 2006; Kulik et al., 2008), semiconductor photocatalysis (Molinari et  
44 al., 2006; Abellán et al., 2007), and electrochemical separation and degradation technologies  
45 (Sirés and Brillas, 2012) to eliminate biorefractory pharmaceuticals. Recently, textural

46 modification of carbon felt (Le et al., 2016) and graphene deposition on graphite substrates (Le et  
47 al., 2015a&b) have shown a great potential to enhance mineralization of the azo dye acid Orange 7  
48 and ACE by the electro-Fenton (EF) process. Derived Fenton processes have also already proved  
49 to be efficient methods to degrade ACE from water: combined EF and photo-Fenton using a  
50 double cathode electrochemical cell (de Luna et al., 2012), coupling adsorption and photo-Fenton  
51 using nano-zeolites and cobalt ferrite nanoparticles (Irani et al., 2015), bio-electrochemical  
52 degradation in a microbial fuel cell-Fenton system (Zhang et al., 2015), or other methods relevant  
53 to the photo-Fenton process (Trovó et al., 2008; Trovo et al., 2012; Cabrera Reina et al., 2015;  
54 Rad et al., 2015). As a more detailed example, Sirés et al. could totally mineralize ACE in CO<sub>2</sub>  
55 after 6 h of treatment by using the catalytic action of Fe<sup>2+</sup>, Cu<sup>2+</sup> and UVA light to generate  
56 hydroxyl radicals. During the electrolysis, several by-products were detected, such as  
57 hydroquinone, p-benzoquinone, or short chain carboxylic acids (Sirés et al., 2006). Using the solar  
58 photoelectro-Fenton (SPEF) method, ACE was efficiently mineralized in the study of Lucio Cesar  
59 Almeida et al. (Almeida et al., 2011). In particular, after 120 min of electrolysis, total organic  
60 carbon (TOC) removal attained was 75% with an energy cost of 93 kWhkg<sup>-1</sup> TOC (7.0 kWhm<sup>-3</sup>)  
61 and a mineralization current efficiency of 71%. In addition, many by-products including aromatic  
62 compounds as well as carboxylic acids were adequately detected, such as hydroquinone, p-  
63 benzoquinone, 1,2,4-trihydroxybenzene, 2,5-dihydroxy-p-benzoquinone and tetrahydroxy-p-  
64 benzoquinone, maleic, fumaric, succinic, lactic, oxalic, formic and oxamic acids. In addition, the  
65 combination of adsorption and photo-Fenton method by Irani et al. could lead to 99.80% of  
66 paracetamol removal in 30 min (Irani et al., 2015).

67         Depending on the AOP technique used, several degradation pathways of ACE have been  
68 identified and already published (Andreozzi et al., 2003; Brillas et al., 2005; Skoumal et al., 2006;  
69 Yang et al., 2008a). All authors observed the formation of reactive by-products due to partial  
70 oxidation of the initial molecule and reaction with matrix components (Von Gunten, 2003). It is  
71 important to track the intermediates generated through degradation reactions and to know their

72 eco-toxicity, with the view to verify the safety of the water treatment process. Unfortunately, very  
73 few works were aimed at combining analytical chemistry to individual and global toxicity  
74 measurement of both products and solution during AOP treatment (Hamdi El Najjar et al., 2014)  
75 as presented in this study.

76 Research at laboratory and pilot scale clearly demonstrated that AOPs can be applied to  
77 improve the quality of effluents from municipal and industrial WWTPs by attenuating trace  
78 organic contaminants (micropollutants) (Gerrity et al., 2011; Giannakis et al., 2015). However, a  
79 potential disadvantage of these processes is the formation of unknown reactive by-products due to  
80 partial oxidation of the targeted compounds and reaction with matrix components (von Gunten,  
81 2003). Thus, the final toxicity of treated effluent should be determined together with the quantity  
82 of targeted compounds in terms of the detection limits of the analytical protocols. In this study, the  
83 EF reaction was applied to ACE solution. ACE and its by-products generated during the  
84 degradation were monitored by HPLC-UV/MS, IC and TOC analysis. In addition, acute toxicity  
85 of the ACE solution under EF treatment as well as individual and mixed intermediate standards  
86 were assessed through Microtox® toxicity test with *Vibrio Fischeri* marine bacteria. Through this  
87 study, the relationship between the ACE degradation pathway, the individual by-product toxicity  
88 and the global toxicity of the solution could be newly and clearly established, thus providing a  
89 more comprehensive understanding and safety control of the mineralization process of ACE by the  
90 EF process.

91

## 92 **2. Experimental**

### 93 *2.1. Materials*

94 ACE (acetaminophen: paracetamol), sodium sulphate (anhydrous, 99.0–100.5%), sodium  
95 hydroxide (99%), sulphuric acid (95–97%), iron (II) sulphate hepta-hydrate (99%), benzoquinone,  
96 benzaldehyde, benzoic acid, oxalic acid, maleic acid, oxamic acid, formic acid, fumaric acid and  
97 acetic acid were obtained from Sigma-Aldrich, USA. Bacterial strain of *Vibrio fischeri* NRRL B-

98 11177 involved in toxicity tests was obtained from Hach Lange GmbH, Germany. Osmotic  
99 adjusting solution (MilliQ water with 22% NaCl) and diluent (MilliQ water with 2% NaCl) were  
100 used for the preparation of the bacterial solution. The carbon felt was purchased from A Johnson  
101 Matthey Co., Germany. TOC standard of 1000 mg L<sup>-1</sup> (Sigma-Aldrich, USA) and sodium  
102 hydrogen carbonate (≥. 99.5%, ACS, Karlsruhe, Germany) were used for TOC and inorganic  
103 carbon (IC) calibration curves assessments, respectively.

104

## 105 2.2. Electrochemical system for ACE degradation

106 EF experiments were performed at room temperature in a 500-mL single cylindrical glass  
107 cell with a two-electrode system. The applied current was controlled using a power supply  
108 (Lambda Electronic, USA) in a galvanostatic mode. The carbon felt cathode (60 cm<sup>2</sup>) was used as  
109 a working electrode and a platinum cylindrical mesh as a counter electrode. The anode was  
110 centred in the electrolytic cell, surrounded by the cathode, which covered the inner wall of the cell.  
111 The distance between the electrodes was 1.5 cm. The aqueous solution of ACE, Na<sub>2</sub>SO<sub>4</sub> (50 mM)  
112 as the supporting electrolyte and FeSO<sub>4</sub>·7H<sub>2</sub>O as the catalyst were prepared by adjusting the pH at  
113 3.0 with sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). The intermediate compound monitoring experiment was  
114 performed in 500 mL solution of ACE 1 mM and/or 200 mL solution of ACE 0.1 mM with  
115 FeSO<sub>4</sub>·7H<sub>2</sub>O (0.2 mM) at an applied current of 500 mA. Before the electrolysis, oxygen bubbling  
116 was performed for 5 min to saturate the solutions, and the solutions were kept under continuous  
117 magnetic stirring at a rate of 800 rpm during the experiment.

118 The specific energy consumption per unit TOC mass (EC<sub>TOC</sub>) was calculated by Eq. (1) (Ruiz et  
119 al., 2011):

$$120 \quad EC_{TOC} \text{ (kWh g}^{-1} \text{ TOC)} = \frac{VIt}{(\Delta TOC)_{exp} V_s} \quad (1)$$

121 where V is the average cell voltage (V), V<sub>s</sub> is the solution volume (L), Δ(TOC)<sub>exp</sub> is the  
122 experimental TOC decay (mg L<sup>-1</sup>), I is the applied current (A) and t is the electrolysis time (h).

123

### 124 2.3. Analytical procedures

125 Concentrations of ACE and their aromatic oxidation by-products were analysed by a HPLC  
126 system (Waters 717 Autosampler and Waters 616 Pump) with a Photodiode Array Detector  
127 (Waters 2996 Photodiode Array Detector) fitted with a reverse phase Thermo Scientific C18  
128 column (L=250 mm, I.D=4.6 mm, and 5- $\mu$ m particle size). The column temperature was  
129 thermostated at 30°C. The injection volumes were 20  $\mu$ L. The mobile phase was constituted of  
130 buffer A (HPLC grade water + 0.1 % (v/v) trifluoroacetic acid) and buffer B (HPLC grade  
131 acetonitrile + 0.1 % (v/v) trifluoroacetic acid). The flow rate was 1 mL.min<sup>-1</sup>. The eluent gradient  
132 started with 10% of eluent B, gradually increasing to 70% in 35 min. Eluent B was elevated to  
133 90% in 5 min, and the system was kept for 5 min, returning to 10% in 1 min and equilibrating for  
134 9 min (returning to initial condition and re-equilibrating the column). UV detection was performed  
135 at  $\lambda$ =254 nm for substituted benzene structure monitoring.

136 The degradation products of ACE were identified by an LC-MS system. LC-MS was  
137 performed with an Alliance e2695 (Waters HPLC pump and autosampler system), and a Quattro-  
138 Micro mass spectrometer was equipped with an Electrospray probe (Waters Micromass,  
139 Wythenshawe, Manchester, UK). The detection conditions were as follows: capillary potential of  
140 3.5 kV, cone voltage of 30 V, source temperature of 120°C, desolvation temperature of 450°C,  
141 cone gas flow of 50 L h<sup>-1</sup> and desolvation gas flow of 450 L.h<sup>-1</sup>. Nitrogen was used as the  
142 nebulizer gas. A Waters column HSST3 (L=100 mm, D.I= 2.1 mm, and 5- $\mu$ m particle size) was  
143 used to separate intermediates. The column was set at room temperature (22°C). The injection  
144 volumes were 20  $\mu$ L. The mobile phase comprised buffer A (HPLC grade water + 0.1% (v/v)  
145 formic acid) and buffer B (HPLC grade acetonitrile + 0.1% (v/v) formic acid). The flow rate was  
146 0.25 mL min<sup>-1</sup>. The eluent gradient started with 10% of eluent B and was kept for 1 min, gradually  
147 increasing to 90% in 2 min, and the system was then kept for 1 min, returning to 10% in 1 min and  
148 equilibrating for 2 min (returning to initial condition and re-equilibrating the column). The eluent  
149 from the chromatographic column successively entered the UV-Vis diode array detector (Waters

150 PDA996) and the electrospray ionization (ESI) interface of the mass spectrometer. Mass  
151 spectrometry (MS) analysis was performed in a positive mode. The mass range was 90–400 (m/z).

152 The eluted components having specific retention time were characterized by their UV-  
153 spectra and/or their mass. The quantification using the standard addition method (HPLC-UV) or  
154 external calibration against standard (LC-MS) was performed. Short-chain acids were identified  
155 and quantified using the HPLC-UV system already described, but fitted with a SHODEX KC 811  
156 (D.I=8 mm, L=300 mm and 7- $\mu$ m particles) column, which was thermostated at 30°C. UV  
157 detection was performed at  $\lambda=210$  nm. The mobile phase was buffer A (HPLC grade water + 0.1%  
158 (v/v) Phosphoric acid 85%). The flow rate was 0.50 mL min<sup>-1</sup>.

159 Inorganic ions (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) generated during decomposition of ACE were identified by an  
160 ion chromatography equipment. For anion, Dionex ICS-1000 system was connected to an AS19  
161 column (4 mm × 250 mm), and the eluent KOH was applied according to the elution gradient: 10  
162 mM in 10 min, then gradient in 20 min to 45 mM. Dionex ICS-900 was used to measure the cation  
163 concentration in the treated solution with the column CS12A and 20-mM methane sulphonic acid  
164 as eluent. Both columns were thermally controlled at 30°C, and DS6 conductivity detector was  
165 used.

166 The TOC and the IC of the initial and treated samples were determined using a TOC-L  
167 CSH/CSN Shimadzu (Japan) analyser. Calibration curves for TOC and IC analysis were  
168 constructed by automatic dilution on the same machine from solutions of TOC standard of 1000  
169 mg/L and sodium hydrogen carbonate.

170

171

172

#### 173 *2.4.Toxicity tests*

174 The Microtox® bacterial toxicity test is based on the general principles described by ISO  
175 (2007) (Romero et al., 2008). The toxicity of ACE and its intermediate by-products formed during

176 its degradation was determined by measuring the effect on the luminescence of marine bacteria.  
177 Bacterial luminescence was measured using a Microtox® Model 500 Analyzer (Modern Water  
178 Inc., UK). The bacterial strain used in this method was *Vibrio fischeri* NRRL B-11177. During  
179 growth, this bacterium emitted luminescence that was related to its cellular respiration and cell  
180 activity. As the activity of the bacterial cell could be reduced by the presence of toxic elements,  
181 bioluminescence is therefore a very good indicator of state of the bacterium and thus of the global  
182 toxicity of the sample.

183 This device allowed for performing the acute toxicity tests by using the software  
184 MicrotoxOmni®. To identify the relative toxicity of each samples solution, an 81.9% screening  
185 test was used to characterize the inter-sample toxicity variability: the influence of treatment  
186 processes on the effluent toxicity was monitored with a sampling method performed as a function  
187 of time. The test performed in this study is called 81.9% screening test because all samples were  
188 diluted at 81.9% of the initial sample concentration by adding a 22% NaCl solution to allow *V.*  
189 *fischeri* normal activity and thus luminescence emission. Before measuring the bacterial  
190 luminescence, pH of the samples were adjusted between 6.5 and 7.5 by sodium hydroxide or  
191 sulphuric acid, and filtration was then performed with a 0.2-µm syringe filter to eliminate any  
192 precipitate or solid matter in the solution.

193 In the absence of toxicity, the luminescence of bacteria decreases over time under the effect  
194 of environmental conditions. Thus, it is necessary to compensate the errors due to these factors by  
195 considering the variability of the luminescence  $R(t)$  of the bacteria in a control solution (MilliQ  
196 water and NaCl), which gives the LU0 values. Equation (2) gives the corrected term  $R(t)$ .

$$197 \quad R(t) = \frac{LU0(t)}{LU0(0)} \quad (2)$$

198 where LU0(t) is the intensity of luminescence emitted by bacteria after a t=5 min or  
199 t=15min of contact with the control solution (MilliQ water and NaCl) (Arbitrary Units (A.U.));  
200 and LU0(0) is the initial intensity of luminescence emitted by bacteria before the addition of the  
201 control solution (MilliQ water and NaCl) (A.U.).

202 The corrected inhibition rate at time t: I(t) intrinsically attributable to the sample toxicity is  
203 then calculated using Eq. (3). To simplify, I(t) is called “Inhibition” hereafter (Figs. 5, 6 and 7) and  
204 is expressed as percentage.:

$$205 \quad I(t) (\%) = \left(1 - \frac{LU(t)}{R(t) \times LU(0)}\right) \times 100 \quad (3)$$

206  
207 where LU(t) is the intensity of luminescence emitted by bacteria after a t=5 min or  
208 t=15min of contact with the sample (A.U.) and LU(0) is the initial intensity of luminescence  
209 emitted by bacteria before the addition of the sample (A.U.).

210 Furthermore, eco-toxicity tests were performed on the commercially available by-products  
211 to confirm the toxicity of the compounds formed during the degradation of the main molecule,  
212 ACE. These by-products and their mixtures were prepared with the maximum concentrations  
213 detected by HPLC analysis during the EF experiment.

214

### 215 **3. Results and discussion**

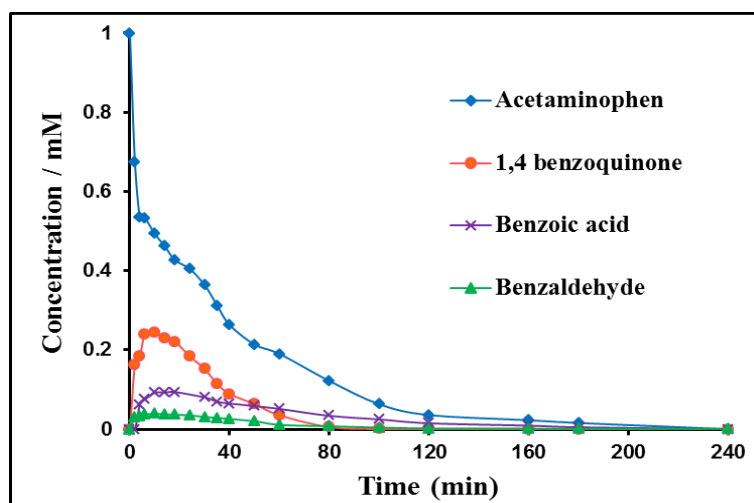
#### 216 *3.1. Degradation pathway of acetaminophen during the EF process*

##### 217 *3.1.1. Evolution of aromatic compounds*

218 The degradation of ACE by the EF process formed some aromatic compounds such as 1,4-  
219 benzoquinone, benzaldehyde and benzoic acid identified by HPLC (Fig. 1) and 2-hydroxy-4-(N-  
220 acetyl) aminophenol identified by r LC/MS (Table S1). It was noted that at the beginning of the  
221 ACE degradation, 1,4-benzoquinone was the main aromatic by-product identified. Its highest  
222 concentration reached 0.23 mM after 10 min of the degradation process; its concentration then  
223 decreased, and this product disappeared totally after 120 min. The other identified aromatic  
224 intermediates are also detected at a very short time of decomposition process with a concentration  
225 peak of 0.09 and 0.039 mM at 14 and 10 min for benzoic acid and benzaldehyde, respectively.  
226 These aromatic compounds were totally decomposed after 120 min of electrolysis and were

227 converted into other aliphatic carboxylic acids and inorganic ions with the appropriate mass  
228 balance.

229



230

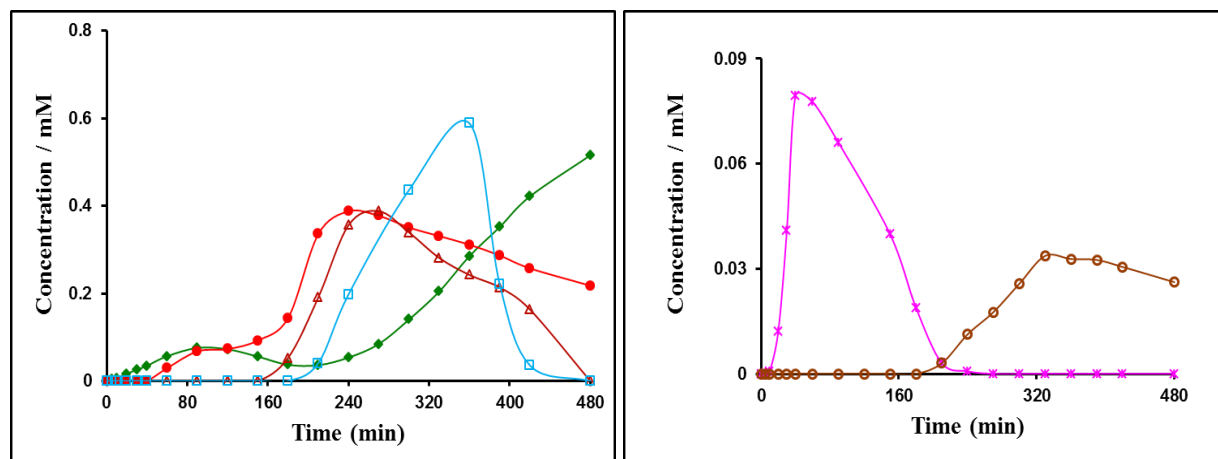
231 **Fig. 1.** Evolution of the concentration of ACE and the aromatic degradation by-products (RSD 2%  
232 n=3) during EF treatment of 500 mL ACE solution at  $C_0 = 1.0$  mM; pH =3;  $[\text{Na}_2\text{SO}_4]=50$  mM,  $I =$   
233 500 mA and  $[\text{Fe}^{2+}] = 0.2$  mM.

234

### 235 3.1.2. Evolution of short-chain carboxylic acids

236 The evolution of carboxylic acid concentration during the electrolysis treatment of a 1.0-  
237 mM ACE aqueous solution at pH 3.0 is reported in Fig. 2. These by-products are usually found in  
238 ACE degradation by AOPs such as ozonation (Skoumal et al., 2006) or electrogenerated hydrogen  
239 peroxide process (Reungoat et al., 2010). Oxalic, maleic, oxamic, formic, acetic and fumaric acids  
240 were detected at 11.6, 13.59, 15.23, 20.59, 22.25 and 22.89 min of retention time, respectively,  
241 during the degradation experiment. Figure 2 shows that fumaric and oxamic acids were generated  
242 after around 20 min of electrolysis, while the other by-products such as maleic, acetic, formic and  
243 oxalic acids were detected only after 3 h. The accumulation of oxalic acid is well known in  
244 Fenton-related processes because of the formation of iron-oxalate complexes that are slowly  
245 removed by  $\cdot\text{OH}$ . At the end of the EF treatment (8 h), fumaric, acetic and formic acids were  
246 completely removed from solution, while oxalic and maleic acid were still present in the solution.

247 Singularly, oxamic acid concentration remained high (around 0.52 mM) after 8 h of treatment,  
 248 with a TOC removal of 51%. This indicated that oxamic acid remained stable under our  
 249 experimental conditions.  
 250



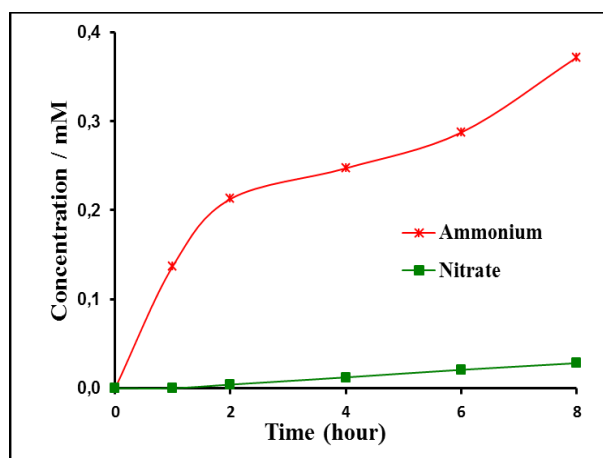
251  
 252 **Fig. 2.** Evolution of the concentration of carboxylic acids (RSD 2% n=3) during EF degradation of  
 253 ACE: Oxalic acid (●), Acetic acid (□), Formic acid (Δ), Oxamic acid (◆), Fumaric acid (\*),  
 254 Maleic x 10 (○). Conditions: 500 mL ACE solution at  $C_0 = 1.0$  mM; pH = 3;  $[\text{Na}_2\text{SO}_4] = 50$  mM,  $I$   
 255 = 500 mA and  $[\text{Fe}^{2+}] = 0.2$  mM.

256

### 257 3.1.3. Evolution of inorganic ions

258 The mineralization of ACE and their intermediates also produced inorganic ions.  
 259 Ammonium and nitrate concentrations were monitored during the 8 h of treatment (Fig. 3). Nitrate  
 260 was measured at very low concentrations compared to ammonium. As observed by E. Brillas *et al.*  
 261 (Brillas *et al.*, 2009), the first limiting step of ACE degradation is the hydroxylation reaction  
 262 leading to 2-hydroxy-4-(N-acetyl) aminophenol (Table 1), which is then converted into oxamic  
 263 acid. The presence of  $\text{Fe}^{2+}$  in the solution as catalyst could lead to the concomitant production of  
 264 persistent iron-oxamate complexes, which are very difficult to mineralize by  $\bullet\text{OH}$ , thus limiting  
 265 the oxidation ability of the EF process. Therefore, nitrate was clearly detected and quantified only  
 266 after 2 h of treatment and reached 0.02 mM in 8 h, while ammonium concentration reached up to  
 267 0.38 mM at the same time (Fig. 3). At the end of the treatment process, the initial nitrogen-

268 containing compound (1 mM) was converted into oxamic acid (0.52 mM), ammonium (0.38 mM)  
269 and trace of nitrate (0.02 mM) (Figs. 2 and 3). Therefore, a total of 0.92 mM of N in by-products  
270 was quantified, which means that the nitrogen mass balance is confirmed, considering that around  
271 8% of nitrogen lost during the EF process could be explained by the fact that nitrogen could be  
272 transferred to the volatile phase through N<sub>2</sub> and NH<sub>3</sub> apparition (Hammami et al., 2008).  
273



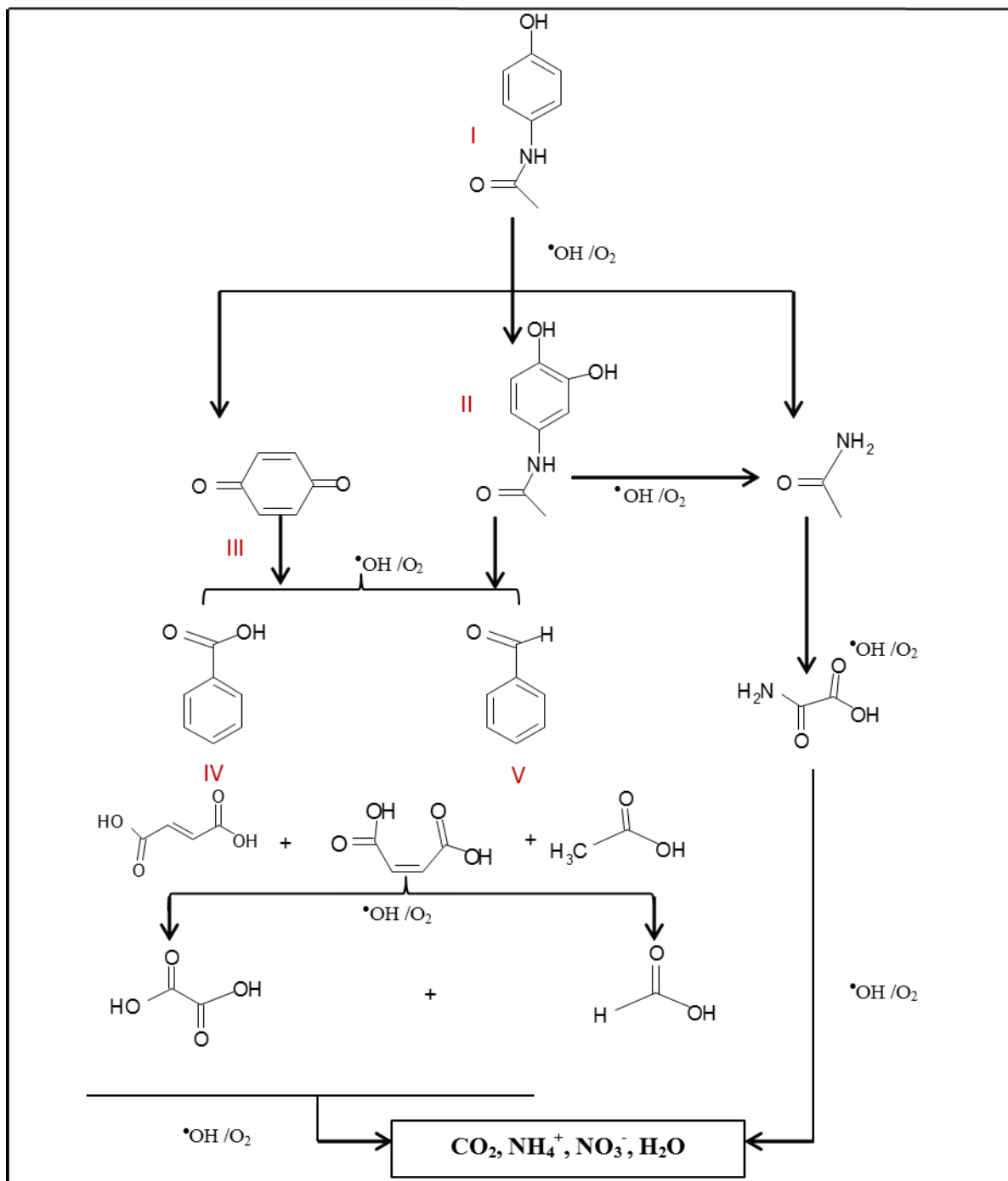
274  
275 **Fig. 3.** Evolution of the inorganic ions concentration (RSD 5% n=3) during EF degradation of  
276 ACE. Conditions: 500 mL ACE solution at C<sub>0</sub> = 1.0 mM; pH =3; [Na<sub>2</sub>SO<sub>4</sub>]=50 mM, I = 500 mA  
277 and [Fe<sup>2+</sup>] = 0.2 mM.

278  
279 *3.1.4. Degradation pathway of ACE mineralization during the EF process*

280 After 8 h of treatment, almost 90% of organic compounds in the initial ACE solution (200  
281 mL at 0.1mM) were mineralized, proving the efficiency of the EF process for removing the  
282 pharmaceutical pollutants (Fig. S1). The specific energy consumption in 120 min was calculated at  
283 3.6 kWh g<sup>-1</sup> TOC, which is a low value, proving the interest of the system, compared to the  
284 reported value of 93 kWh g<sup>-1</sup> TOC obtained by the solar SPEF method (Almeida et al., 2011).

285 A general reaction sequence for the EF degradation of ACE in acidic medium is proposed  
286 in Fig. 4. The process was initiated by •OH radical attack either on the C(2) position of ACE (I),  
287 which led to the formation of 2-hydroxy-4-(N-acetyl)aminophenol (II), or on its C(4)-position

288 which led to 1,4-benzoquinone (III). The formation of these intermediate products could also be  
289 proposed in other AOP processes during the degradation of ACE (Yang et al., 2008b). 2-Hydroxy-  
290 4-(N-acetyl) aminophenol (II) was then degraded into benzoic acid (V), benzaldehyde (IV) and  
291 acetamide. Subsequently, all aromatic by-products were further oxidized to produce aliphatic  
292 organic acids such as fumaric, oxalic, acetic, formic and maleic acids. Furthermore, oxamic acid  
293 was generated from the oxidation of acetamide (Skoumal et al., 2006). At the end of the  
294 degradation process, unlike oxamic and oxalic acids which persisted under our experimental  
295 conditions, all these last by-products were transformed into CO<sub>2</sub>, H<sub>2</sub>O and inorganic ions.  
296



297

298 **Fig. 4.** General reaction sequence proposed for acetaminophen (ACE) mineralization in an acidic  
 299 medium by hydroxyl radicals during the EF process.

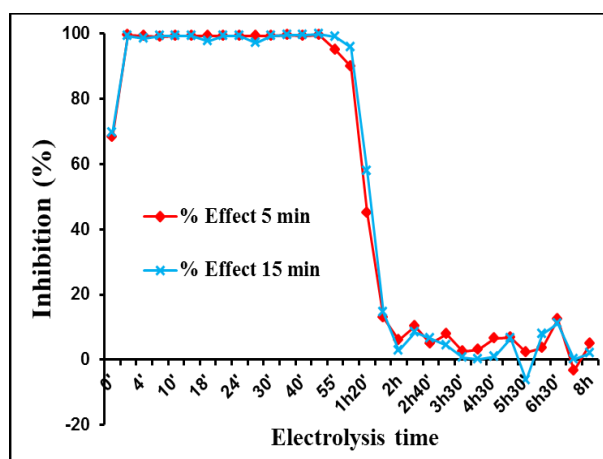
300

301 *3.2. Toxicity evolution of ACE solution during the EF process*

302 *3.2.1. Global toxicity*

303 The global toxicity evolution of 500 mL of 1.0-mM ACE solution during 8 h of EF  
304 treatment is shown in Fig. 5. The acute toxicity of the treated solution after 5- and 15-min time  
305 contact with *V. fischeri* bacterial strain increased rapidly at the early stage of the treatment and  
306 reached 100% between 2 and 55 min. This result is relevant and consistent with regard to the  
307 previously proved formation of toxic aromatic by-products such as 1,4-benzoquinone, benzoic  
308 acid and benzaldehyde (Lee and Chen, 2009; Das et al., 2010; Dirany et al., 2012). After 60 min,  
309 the toxicity markedly declined which well agreed with aromatic compounds found only at trace  
310 levels at this stage of the EF reaction (see part 2.1.1 and Fig. 1). The predominance of very low  
311 toxic short-chain carboxylic acids in this period did not significantly contribute to the inhibition of  
312 the bacterial luminescence; consequently, the solution toxicity became zero at about 120 min.  
313 Subsequently, the inhibition value was continuously maintained at around zero until 8 h where  
314 short-chain carboxylic acids were continuously transformed under  $\bullet\text{OH}$  attack during the EF  
315 process. The two exposure times measured yielded identical inhibition (%) results.

316

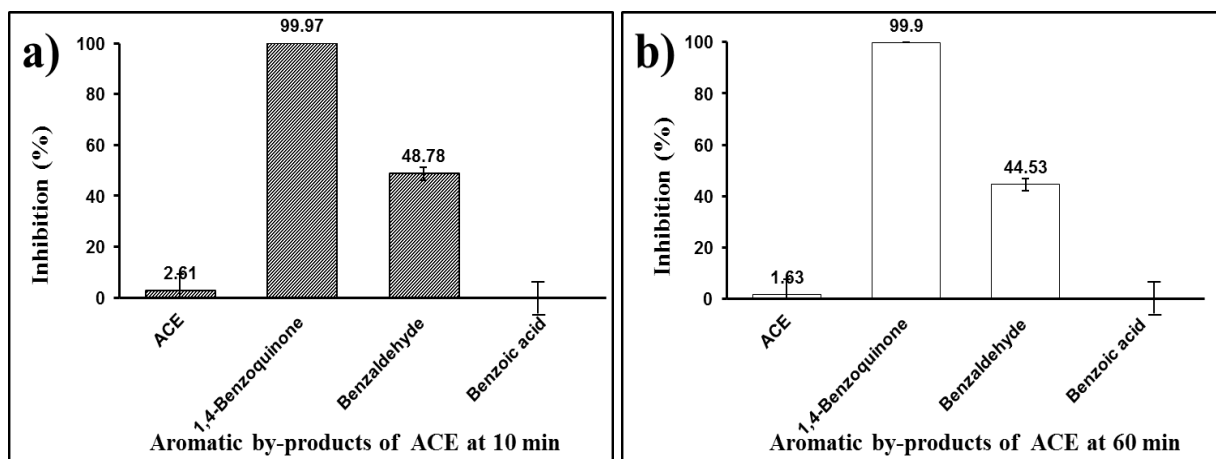


324 The toxicity of individual and mixed aromatic by-products taken as standard solutions was  
325 investigated by the ecotoxicity assays. Their concentration was selected with regard to those  
326 analysed during the ACE degradation. For this purpose and according to by-products formation  
327 shown in Fig. 2, specific times of EF treatment were considered: 10, 60, 80 and 120 min  
328 corresponding to different mixture solutions and concentrations of by-products (Table S2).

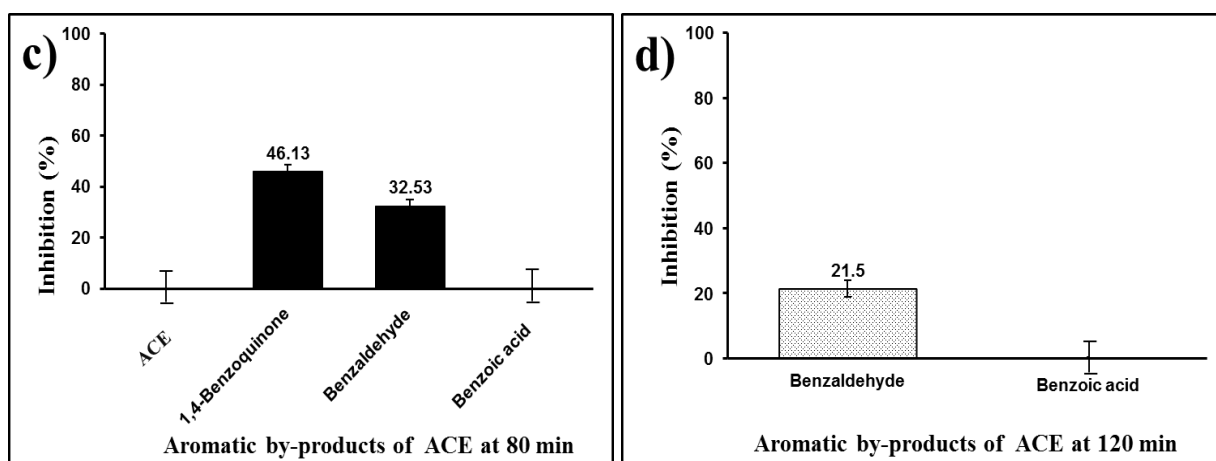
329  
330 As shown in Fig. 6a, b, c and d, ACE itself is not a hazardous pollutant for this strain of  
331 bacteria because its toxicity was very low even at the high concentration (0.5 mM) found after  
332 10 min of treatment. Similarly, benzoic acid was also a low toxic by-product because its  
333 negative toxicity values towards the bacterial strain during the degradation process signifies a  
334 positive effect on bacteria growth and activity (Velegraki et al., 2010). In contrast, 1,4-  
335 benzoquinone showed an important toxic effect as the inhibition ratio was around 100% even at  
336 very low amount, 0.04 mM (60-min degradation), compared to other formed aromatic  
337 intermediates. Although benzaldehyde was not as toxic as 1,4-benzoquinone, this compound  
338 still largely affected bacterial luminescence (Paulraj et al., 2011). It caused an acute toxicity  
339 between about 50% and 20% at the concentration of 0.039 mM (10 min) and 0.002 mM (120  
340 min), respectively. In addition, the mixtures of these aromatic compounds at four periods from  
341 10, 60, 80–120 min were ecotoxicologically assessed (Fig. 6e). The inhibition ratio reached 100% at  
342 the early stage of the treatment (10 and 60 min), again proving the high toxicity of 1,4-  
343 benzoquinone. This agrees with previously published results (Rubio et al., 2011). Subsequently,  
344 a remarkable detoxification was observed after 80 and 120 min due to the decline of the  
345 corresponding toxic aromatics.

346

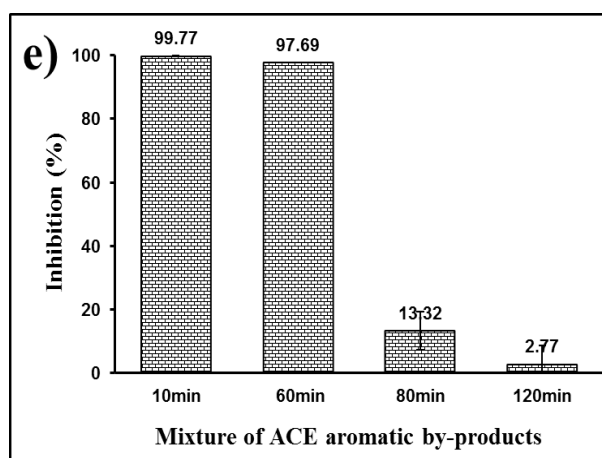
347



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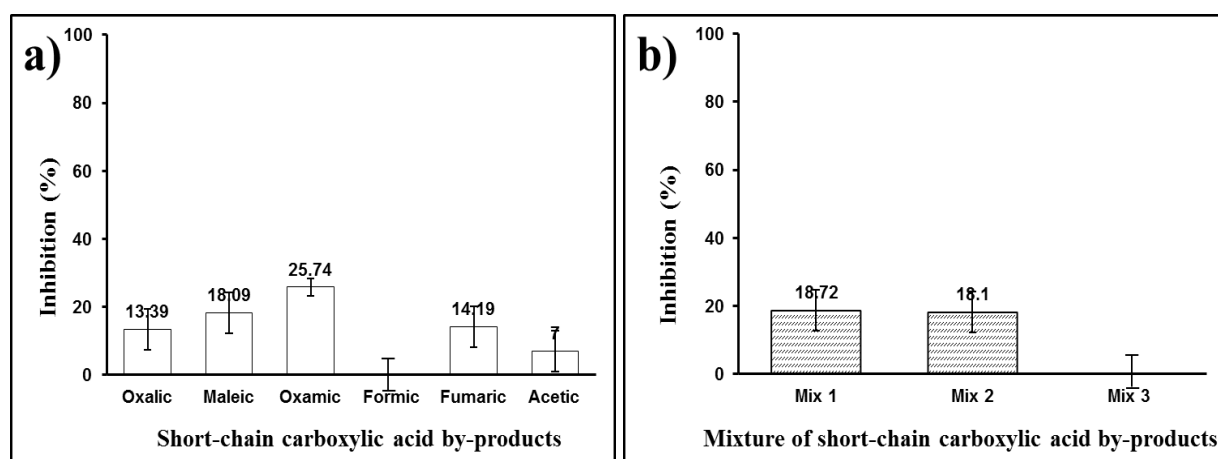
349



350 **Fig. 6.** Toxicity of standard solutions of ACE and other aromatic compounds, taken alone (a, b, c,  
351 d) or in mixture (e), under identical concentration, during the EF process on 500 mL (1-mM ACE)  
352 and for different treatment times: (a) 10 min, (b) 60 min, (c) 80 min, (d) 120 min. (e) Mixture  
353 solutions of standard ACE and aromatic compounds at different treatment times.

354

355 At the end of electrolysis, the oxidation by  $\bullet\text{OH}$  generated during the EF process in  
 356 solution led to the production of ring-opened products such as aliphatic carboxylic acids. These  
 357 acids such as formic acid or acetic acid are known to be low or non-toxic compounds (Liu et al.,  
 358 2010). This suggestion was confirmed by their toxicity measurement presented in Fig. 7 in two  
 359 ways: individual test on each single standard compound in MilliQ water at its maximum  
 360 concentration measured during the EF process (Fig. 7a) and the combination (mixture) of all  
 361 carboxylic by-products at their maximal concentrations measured during three periods of the  
 362 electrolysis (Fig. 7b): 210–270 min (Mix 1); 270–330 min (Mix 2) and 330–480 min (Mix 3)  
 363 (Table S3). Acetic and oxamic acids toxicity was measured for similar maximum concentration of  
 364 about 0.5 mM, but oxamic acid seemed to promote higher toxicity than acetic acid with individual  
 365 toxicity values of 25.7% and 7%, respectively. The toxicity of maleic, oxalic and fumaric acids  
 366 was around 15%. In comparison with other intermediates, formic acid contributed negligibly to the  
 367 toxicity of the solution. Interestingly, a cocktail effect was observed: synergistic (Mix 1) as well as  
 368 antagonist effects (Mix 3) were observed during the comparative study on single and mixture of  
 369 standard compounds. As observed in other studies with pesticides or antioxidants, the  
 370 combinations of ACE carboxylic acid by-products did not induce an additive increase in  
 371 individual toxicity (Liu et al., 2010). The toxicity of three mixtures decreased gradually as a  
 372 function of treatment time, 18.7%; 18.1% and 0%, respectively, from 210 to 480 min. These  
 373 results confirmed that the EF process is an environmentally friendly technology, which can  
 374 efficiently degrade toxic pollutants into non-toxic compounds.



375

376 **Fig. 7.** Toxicity of (a) short-chain carboxylic acid by-products at their maximum concentrations  
377 (Table 3), and (b) Mixture of the short-chain carboxylic acid by-products corresponding to three  
378 periods: 210–270 min (Mix 1); 270–330 min (Mix 2) and 330–480 min (Mix 3) during EF  
379 treatment of 500 mL (1-mM ACE) at pH = 3; [Na<sub>2</sub>SO<sub>4</sub>] = 50 mM, I = 500 mA, [Fe<sup>2+</sup>] = 0.2mM.

380

#### 381 **4. Conclusions**

382 The evolution of TOC, intermediate compound concentrations and acute toxicity of ACE  
383 solution in aqueous medium during the EF mineralization process was monitored through HPLC-  
384 UV/MS, IC, TOC analysis and Microtox® toxicity tests. During the first 60 min of treatment, the  
385 acute toxicity of the treated solution reached 100%, which was identified on the basis of the  
386 formation of toxic aromatic products such as 1,4-benzoquinone, benzoic acid and benzaldehyde. A  
387 total detoxification of the solution was then accomplished after 120 min due to the decomposition  
388 of ring-compounds into short-chain carboxylic acids. The mineralization of ACE by hydroxyl  
389 radicals ( $\bullet$ OH) formed through the EF process also produced inorganic ions such as ammonium or  
390 nitrate by bond breakage between nitrogen and carbon in the initial molecule. The mineralization  
391 of 200 mL of 0.1-mM ACE was 86.9% using an applied current of 500 mA and a catalyst  
392 concentration of 0.2 mM. The relationship between the decomposition pathway and the evolution  
393 of the ACE solution toxicity as a function of EF treatment time was clarified by the toxicity  
394 measurement of both individual and mixture of standard compounds (ACE and sub-products).  
395 This comprehensive degradation pathway of ACE can be further applied to other persistent  
396 pharmaceuticals in an aquatic environment.

397

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