Highlights

- ➤ Solar photo-Fenton has been able to remove a mixture of six emerging pollutants.
- ➤ The reaction was performed at acidic and circumneutral media.
- ➤ Reaction rate was highly influenced by the sample matrix.
- ➤ Intermediates were detected and related with changes in toxicity.

1 # Solar photo-Fenton at mild conditions to treat a mixture of six

2 # emerging pollutants

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9 **Abstract**

10 The applicability of photo-Fenton to degrade a mixture of emerging pollutants (EPs)

11 namely amoxycillin, acetaminophen, acetemiprid, caffeine, clofibric acid and

12 carbamazepine has been studied at different scenarios. At high concentrations, acidic

13 photo-Fenton was able to achieve a fast removal of the EPs. Although, complete

14 mineralization was not reached, the toxicity of the solution was decreased according to

15 the respiration of activated sludge and luminescence of *V.fischeri* assays, although

16 according to this last assay a transitory enhancement of the toxicity was found,

17 attributable to the formation of toxic by-products such as phenols, chlorophenols and

18 chlorinated pyrydines. Experiments carried out with 5 mg/l ofeach EP showed that at

19 neutral media the process was two orders of magnitude less efficient than at acidic pH,

20 although it was still able to remove the EPs. The aqueous matrix has a remarkable effect

21 on the process as the presence of humic acids increased the reaction rate and inorganic

22 salts played an inhibitory role. Finally, experiments performed with 10 μg/l of each EP

23 showed that under those experimental conditions nearly complete removal of the EPs

was reached with neutral photo-Fenton after 60 min of irradiation; in this case, humic substances played a disfavourable role.

1. Introduction

In recent years, an important number of synthetic compounds have been found in surface waters at trace amounts, as a consequence of human activity. They are commonly referred to as emerging pollutants (EPs), because they have been recently detected and hence their potential effects on humans and natural ecosystems have not yet been elucidated [1]. Among the substances that can be considered within this group are perfluorinated compounds, pharmaceuticals, hormones, endocrine disruptors, sunscreens, algal toxins, dioxane or nanomaterials [2,3].

Emerging pollutants have been found in the effluent of wastewater treatment plants, which constitutes a major limitation for their potential reuse. Hence, development of new strategies to deal with this corncern are needed. In particular, coagulation-floculation, adsorption onto activated carbon, biological processes or nanofiltration have been employed, as recently reviewed [4,5]. Oxidative processes are promising methods to deal with this environmental concern [6]: electrochemical methods have been demonstrated to be efficient to remove pharmaceuticals and personal care products, as reviewed by Brillas et al. [7] and, on the other hand, photochemical methods employing

sunlight as irradiation source might also be of special interest because of the enhanced sustainability they involve [8]. In this context, the Fenton reagent could be useful. It consists in a mixture of iron salts and hydrogen peroxide at acidic pH; under these conditions, iron catalyzes decomposition of hydrogen peroxide into highly oxidizing spicies, such as hydroxyl radicals. Although this process occurs in the dark, it is greatly enhanced upon UV-vis irradiation (λ < 500 nm) and hence, sunlight can be used as irradiation source [9].

There is some information on the treatment of emerging pollutants by the photo-Fenton process [10]; however, the use of highly acidic pH might be an important drawback for the real applicability of this method. Processes based in the Fenton reagent, including photo-Fenton, are less efficient at circumneutral media than at the optimum acidic pH because of the low solubility of iron at neutral media, but also to a modified mechanism which favours formation of highly oxidized iron species (e.g. ferryl ions) instead of the more reactive hydroxyl radical [11-14]. However this process might be a useful alternative to remove chemicals at μg-ng L^{-1}, as it is the case of EPs in effluent of wastewater treatment plants. In fact, some recent work has been devoted to explore the applicability of this methodology for this purpose [15, 16].

However, further research on this topic is meaningful. As EPs show diverse chemical structures, their behaviour vs. photo-Fenton might be different. In addition, primary removal of the pollutant might not represent detoxification of the effluent and a careful identification of major intermediates and assessment of the toxicity of treated solutions

70   is requiered. Finally, the presence of natural organic matter (e.g. humic acids) can play

71   an important role in the process that deserves to be investigated.

72

73   With this background, the aim of this work is to gain further insight into the use of

74   circumneutral photo-Fenton as a tertiary process to remove EPs at the effluent of a

75   wastewater treatment plant. Experiments have been performed with a mixture of six

76   EPs, namely amoxycillin (antibiotic), acetaminophen (analgesic), acetemiprid

77   (insecticide), caffeine (stimulating agent), clofibric acid (metabolite of clofibrate, also

78   employed as herbicide) and carbamazepine (psychiatric drug); chemical structures can

79   be found at Scheme 1. Preliminary experiments have been performed at high initial

80   concentrations of the EPs in order to allow a reliable monitoring of the process, then

81   concentrations have been decreased down to 10 μg/l to better reproduce EPs in the

82   effluents of wastewater treatment plants. Changes in the toxicity, major by products

83   formed in the process and the role of humic acids have been studied

84

85   **2. Experimental**

86   **2.1. Reagents**

87   Acetaminophen, caffeine, amoxicillin, clofibric acid, carbamazepine, acetamiprid and

88   humic acid sodium salt (AHA) were purchased from Sigma-Aldrich. Hydrogen

89   peroxide (30% v/v) and ferrous sulphate, used in the photo-Fenton reactions, were

90   obtained from Panreac.

91

92   Water employed in most reactions was Milli-Q grade. In other experiments, EPs were

93   spiked in samples taken from the effluent of the secondary settling tank of a wastewater

94   treatment plant, which consisted in a physical-chemical primary treatment followed by

95   an aerobic activated sludge biological reactor. The main parameters of these samples

96   were systematically inside the following ranges: pH, 7.5; conductivity, 2.4-2.8 mS/cm;

97   dissolved organic carbon (DOC), 15-50 mg/l; inorganic carbon, IC, 70-90 mg/l (which

98   results in a HCO_3^- concentration of ca. 350-450 mg/l) and chemical oxygen demand

99   (COD) 60-120 mg/l.

100

2.2. Photo-Fenton experiments

102   Photo-Fenton processes were carried out with the mixture of six EPs; the initial

103   concentration of each EP was between 10 μg/l and 50 mg/l. The amount of iron was 5

104   mg/l, added as iron(II) sulphate. The stoichiometric amount of hydrogen peroxide

105   required to mineralize all the organics was added at the beginning of the experiment.

106   The reaction was carried out at the optimal pH value (2.8), and at pH = 7; sulphuric acid

107   or sodium hydroxide were employed to adjust the pH. Eventually, humic acids (10

108   mg/l) were added to the reaction mixture.

109

110   Samples were irradiated with a solar simulator (Oriel Instruments, Model 81160

111   equipped with a 300 W xenon lamp) in cylindrical open glass vessels. Magnetic stirring

112   was kept all along the reaction time and water was periodically added in order to

113   compensate for the evaporation loss. Temperature was ca. 30ºC throughout the

114   experiment. Before analysis all samples were filtered through polypropylene (0.45μm)

115   and, eventually, preconcentrated following the procedure described below, or

116   neutralized when submitted to bioassays. Those samples used for biological analysis or

117   for COD measurements were free of hydrogen peroxide.

118

119   **2.3. Analysis**

120   The concentration of each EP was determined by UHPLC (Perkin Elmer model Flexar

121   UPLC FX-10) equipped with a Brownlee Analytical column (DB-C18). A mixture of

122   acetonitrile (A) and a 0.1% formic acid aqueous solution (B) was used as eluent. The

123   relative amount of each solvent was changed in a linear gradient, from 3% A to 70% A

124   in 8 min; the flow rate was 0.3 mL/min. Retention times were 1.7 for acetaminophen 2.1

125   for amoxicillin, 3.3 for caffeine, 4.9 for acetamiprid, 5.6 for carbamazepine and 6.2 for

126   clofibric acid. The wavelengths used for the EPs quantitations were: 205 nm

127   (acetaminophen, amoxicillin, caffeine and carbamazepine), 225 nm (clofibric acid) and

128   245 nm (acetamiprid). For the experiments containing 10 μg/L of each EP, the samples

129   were preconcentrated by solid phase extraction (SPE); briefly, 250 ml of sample were

130   flown through a LiChrolut EN 200 mg (Merck) cartridge; then EPs were recovered with

131   3 mL of methanol.

132

133   Eventually, the reaction products were analyzed by GC-MS; a gas chromatograph

134   GCMS-QP2010S (Shimadzu) equipped with a quadrupole mass analyzer was

135   employed. 10 μl of sample obtained after SPE concentration (see above) were injected;

136   the temperature of the injector was 250ºC and the split ratio was 1:30. A Meta X5

137   Teknokroma column was used and helium was employed as carrier gas, with a flow rate

138   of 35 cm/s. The column temperature was kept at 60ºC for 3 min, then it was increased

139   with a 8 ºC/min rate from 60 ºC to 280 ºC and finally it was kept at 280 ºC for 3 min.

140

141 DOC was determined with a Shimadzu model TOC-V CSH apparatus. COD was

142 determined photometrically according to the dichromate method [17]; digestions were

143 performed at 148ºC in a Thermoreaktor TR300 (Merck) and a Spectroquant NOVA 60

144 (Merck) was used for the photometric determination. The concentration of iron was

145 monitored according to the o-phenantroline standardized procedure [16]. Briefly, iron

146 is reduced to iron (II) by addition of hydroquinone at a pH = 3.5; then o-phenantroline is

147 added and the red complex formed is determined photometrically at 508 nm. Hydrogen

148 peroxide was estimated by a colorimetric method with test stripes (Merckoquant),

149 purchased to Merck.

150

151 Inhibition of the luminescence of *V. fischeri* assays were performed according to the

152 standardized ISO 11348-3 norm, using lyophilized bacteria (*V. fischeri*, NRRL B-

153 11177, Macherey-Nagel). Reconstitution of the bacteria was performed according to the

154 standard procedure, using a salty solution. The luminescence was determined with a

155 Luminometer Lumifix-Bio-10 (Macherey-Nagel). Toxicity was determined after 15 min

156 incubation. Distilled water and zinc sulphate were used in control experiments. Toxicity

157 of slightly coloured samples, due to the presence of humic substances, was corrected by

158 subtracting their absorbances at 490 nm. Inhibition of the respiration of activated sludge

159 respiration was performed according to an adaptation of the OECD 209 test, as

160 described in detail elsewhere [18], using a BM3-LAB (Neurtek) respirometer equipped

161 with an oxygen sensor (WTW-Cell Ox); Briefly, activated sludge was brought to its

162 maximum oxygen uptake rate (OUR) by addition of highly biodegradable sodium

3. Results and discussion

3.1. Photo-Fenton process at acidic pH

The effect of a photo-Fenton process was first checked at acidic medium (pH = 2.8) using an initial high concentration of the target pollutants (50 mg/l). Figure 1 shows that complete abatement of the parent pollutants was achieved after 50 min of irradiation. In fact, more than 90% removal of the pollutants was reached after 15 min, except for acetamiprid, which was more reluctant to this treatment.

However, the elimination of the pollutants did not result in a complete mineralization of the sample, due to the formation of organic intermediates during the process. Figure 2 shows that the elimination of dissolved organic carbon after 60 minutes of irradiation was only moderate (ca. 30%). A more remarkable decrease of chemical oxygen demand was observed during the reaction (ca. 50% in 60 min) indicating that noticeable oxidation occurred. This is more easily appreciated by the carbon oxidation state (COS), which is an intensive parameter that can be considered as a good indicator of the efficiency of the oxidative process [19]. COS can be calculated by equation 1,

$$COS = 4 - 1.5\frac{COD}{DOC_0} \qquad \text{Equation 1}$$

185   where DOC_o is the initial dissolved organic carbon of the solution and COD is the

186   chemical oxygen demand at the sampling time (both parameters expressed as mg/l).

187   Before t = 30 min, there was a fast increase in this parameter, while it remained more

188   stable beyond this point, showing that the photo-Fenton was more efficient at the early

189   stages of the process.

190

191   Another parameter relating DOC and COD is average oxidation state (AOS). It can be

192   calculated by equation 2, which is similar to that employed for COS, but with DOC

193   determined at the sampling time.

194

195   $$AOS = 4 - 1.5\frac{COD}{DOC}$$
      Equation 2

196

197   Also in this case, a noticeable variation in this parameter was observed until t = 30 min

198   (see Figure 2), which can be associated to changes in the composition of dissolved

199   organics, being more oxidized as the process proceeded [20] (note that AOS only refers

200   to dissolved organics, as DOC at the sampling time is used for calculations, while CO_2

201   formed by mineralization is also included with an oxidation state +4 in the COS, as

202   initial DOC is used in this case).

203

204   Changes in the dissolved organic matter might result in a concomitant variation in the

205   toxicity of the sample. This was studied using two different methods, namely inhibition

206   of the respiration of activated sludge and inhibition of the luminescence of *V. fischeri*.

207   Results in Figure 3 show that according to the activated sludge assay, the initial toxicity

208   was ca. 50% and the sample was detoxified after 10 min of irradiation. In sharp contrast

209   with this, luminescence assays showed an increase in the toxicity in the first stages of

210   the process, reaching a maximum after 10 minutes of reaction. Then, toxicity decreased

211   to reach values below 10% after 40 min. This quantitative divergence in the toxicity

212   values is not surprising, as this parameter strongly depends on the bioassay that has

213   been employed [21]. In particular, differences between activated sludge and *V. fischeri*

214   assays have been previously observed [22] and they are attributable to the sensibility of

215   the microorganisms that are employed: activated sludge consists in a consortium of

216   microorganisms present in biological reactors of wastewater treatments plants and

217   hence, they have developed some adaptation and resistance to chemical contaminants;

218   on the other hand, *V. fischeri* bacteria show high sensibility towards pollutants [23].

219   Using 50 mg/l as the initial concentration of each EP is far above those typically found

220   in the effluent of wastewater treatment plants; hence, detoxification observed at these

221   high concentrations might indicate that applying photo-Fenton at µg/L level to this

222   mixture of EPs will not produce any worrying effect on toxicity.

223

224   It seems interesting to gain further insight into the changes in toxicity determined

225   according to the *V.fischeri* assay. First, the EC_{50} was calculated for each pollutant, as

226   well as their individual toxicity at the concentration employed in the experiment (see

227   Table 1). It is interesting to note that the toxicity of the untreated sample (ca. 60%) was

228   well above the cumulative effect of the individual contaminants (below 25%), indicating

229   that there is some synergetic effect due to the mixture of all six emerging pollutants.

230   The trends observed in the *V. fischeri* experiments along the photo-Fenton process can

231   be explained by the formation of toxic by-products in the oxidation of the parent EPs.

232 This behaviour has been already observed by other authors in advanced oxidation

233 processes; for instance, Oturan et al. associated an initial increase in the toxicity of a

234 solution containing diuron submitted to an electro-Fenton treatment to the formation of

235 more toxic oxidation by-products [24]. In order to investigate this point, GC-MS

236 analyses were performed with samples taken after different periods of reaction (see

237 table 2). Although it is difficult to associate the enhancement of the toxicity in such a

238 complex mixture to the formation of a single compound, it is remarkable that significant

239 amounts of phenols, chlorophenols and chlorinated pyridines, were detected in those

240 samples showing higher toxicity (t = 5-15 min of treatment). In fact, the toxicity of all

241 six EPs used in the work (EC$_{50}$ of hundreds or even thousands mg/l, see Table 1) is

242 well below that found in literature for chlorinated aromatics, whose EC$_{50}$ are typically

243 below some tens of mg/l [25, 26]. Formation of these chlorinated by-products can be

244 attributed to the photo-oxidation of clofibric acid and acetamiprid. In this context, it has

245 been reported that no significant detoxification or even some increase in the toxicity has

246 been observed along the reaction of acetamiprid with $^{\bullet}$OH radicals or singlet oxygen

247 [27, 28].

248

249 3.2. Photo-Fenton at neutral media

250

251 Although using 50 mg/l as the initial concentration of each EP is useful to obtain a

252 better monitoring of the reaction, these values are far above those typically found in the

253 effluents of wastewater treatment plants. Lower EP concentrations might allow using

254 milder reaction conditions and hence, testing other strategies might be meaningful,

255 namely, the use of the Fenton reagent at circumneutral media. In a first step, a more

256 diluted mixture of the six emerging pollutants (5 mg/l) was treated at the pH value of

257 2.8 and at circumneutral pH (7.2). Figures 4A and 4H show that at the optimal acidic

258 pH all the pollutants were removed after 15 min of treatment, while under neutral

259 conditions irradiation times were noticeably higher, as some EPs remained in the

260 solution after 6 hours. In order to obtain more quantitative data, the kinetic profiles

261 obtained for each pollutant were fitted to a semilogarithmic plot and the pseudo-first

262 order rate constants were calculated from the slop of this plot. Rate constants obtained

263 at neutral pH (ranging from 0.002 min^{-1} for acetamiprid to 0.015 min^{-1} for clofibric

264 acid) were two orders of magnitude lower than at the optimal pH (which were between

265 0.17 min^{-1} for acetamiprid and 0.72 for amoxicillin); the worse performance of the

266 photochemical process at neutral medium is attributable to the low solubility of iron

267 salts at this pH, changes in the speciation of dissolved iron and to the existence of an

268 alternative reaction mechanism, which favors formation of less reactive iron(IV) species

269 instead of hydroxyl radicals [11]. However, the photochemical elimination of the EPs in

270 the neutral media in the presence of the Fenton reagent is faster than in the case of

271 photolysis (Figure 4F). Only in the case of clofibric acid, which suffers very fast

272 photolysis under the studied conditions, and acetamiprid, similar reaction rates were

273 measured in neutral photo-Fenton and photolysis.

274

275 In order to gain further insight into the mechanism at neutral medium, experiments were

276 carried in the presence of t-butanol, a well known hydroxyl radical scavenger. In order

277 to simplify the system, experiments were carried out only with caffeine; this compound

278 was chosen because suffers negligible photolysis and reaction is slow enough to allow

279 the measurement of accurate kinetic data. Pseudo first order rate constants were

280 determined with and without t-butanol at neutral pH; for the sake of comparison these

281　experiments were also driven at the optimal pH = 2.8 (Figure 5). In both cases, a

282　decrease in the reaction rate was observed in the presence of t-butanol, however, it was

283　more remarkable at acidic medium as k was 0.45 min^{-1} without t-butanol and 0.018 min^{-}

284　^{1} in the presence of the radical scavenger, what means a 96% decrease, in sharp contrast,

285　only 59% decrease was measured at neutral medium (0.027 min^{-1} and 0.011 min^{-1}

286　respectively). This is in agreement with a higher formation rate of ·OH at acidic pH

287　according to a classical photo-Fenton process, while a modified mechanism occurs at

288　circumneutral pH favoring the formation of other oxidizing agents, such as ferryl

289　species.

290

291　Other species found in the aqueous matrix (e.g. inorganic ions or dissolved organic

292　matter) might show some influence on neutral photo-Fenton. Regarding to the effect of

293　organic matter, studying the effect of humic substances appeared interesting, as this

294　group of compounds are the most widely distributed organic materials [29] and they are

295　photochemically active; for this purpose, humic acids (10 mg/l) were added to mixture

296　of the EPs. Comparison between Figures 4 A and B indicates that faster removal of the

297　pollutants was reached in the presence of the humic substances. This effect has been

298　previously observed in the removal of other chemicals by Fenton process [30, 31] and a

299　complex formed between iron and the humic substances was proposed as the key

300　species in the process. Recently, a significant increase in the photo-Fenton reaction rate

301　has been observed in the presence of humic acids [32]; however, according to literature,

302　·OH radical have been found not to play a major role in the process and hence, other

303　oxidizing species might be responsible for the pollutants oxidation [12]. In fact,

304　experiments carried out in the presence of t-butanol with caffeine as model compound

305　showed that in the presence of humic acids, the decrease in the rate constant was only

306   37% (data not shown), even lower than the 59% recorded at neutral media without

307   humic acids; this is in agreement with an alternative mechanism to ·OH playing a more

308   relevant role under these conditions. Despite the possible modification of the

309   mechanism at neutral pH and in the presence of humic substances, the Fenton reagent

310   plays the major role in the process and hydrogen peroxide is the actual oxidizing agent,

311   as irradiation of a solution containing humic acids and iron (but without H_2O_2) gave

312   similar reaction profiles than photolysis of the EPs (compare Figures 4F and 4G).

313

314   Experiments were carried out using tap water, with and without humic substances

315   (Figure 4C and 4D, respectively). In this case, lower reaction rates were obtained,

316   showing that the sample matrix played an inhibitory role. This can be attributed to the

317   presence of inorganic salts in the medium, which have been described to inhibit the

318   (photo)-Fenton reaction, probably because of their scavenging role towards the reactive

319   species involved in the process. In this context, the presence of carbonates (mainly

320   HCO_3^-) at neutral pH, have been reported to inhibit the photo-Fenton process [33] as it

321   is well known that CO_3^{2-} and HCO_3^- compete with organic contaminants for hydroxyl

322   radical reactions, and significantly decrease the pollutants degradation [34]; hence, the

323   remaining reactivity detected under these conditions might be attributed to the existence

324   of alternative oxidizing species, as indicated by the t-butanol experiments. In addition to

325   this, carbonates have been reported to change iron speciation, which might also have a

326   remarkable influence on the performance of the Fenton system [35].

327

328   Finally, an effluent from a wastewater treatment plant (see experimental section for

329   main parameters) was spiked with 5 mg/l of each EP and submitted to a neutral photo-

330   Fenton process (Figure 4E). In this complex sample, different effects are expected to

331   occur: a) the presence of inorganic salts might inhibit the photo-Fenton process, b)

332   organics able to form photo-active complexes with iron might increase the reaction rate

333   and c) other organics could compete with the EPs for the reactive species resulting in a

334   lower reaction rate. Comparison of Figures 4A and 4E indicates that the removal of the

335   EPs was slower than in distilled water, meaning that the inhibitory effects are

336   predominating. In fact, results are only slightly better than those achieved with tap

337   water. However, the EPs removal is still faster than for photolysis, showing that reactive

338   species generated by neutral photo-Fenton are responsible for some EPs oxidation.

339

340   Toxicity was measured in all cases according to the *V fischeri* assay. The initial values

341   were in this case ca. 5%, and some increase was determined at the beginning of the

342   process, attributable to the formed by-products; nonetheless toxicities were

343   systematically below 20 %. As the concentration of EPs found in the effluents of

344   wastewater treatment plants are several orders of magnitude below those employed in

345   these experiments, a hypothetical increase in the toxicity along the photo-Fenton

346   process should not be considered a major threat.

347

348   3.3. Neutral photo-Fenton at low EPs concentrations

349

350   Experiments above reported have been conducted at concentrations well above those

351   commonly found at the effluents of wastewater treatment plants. Hence, it is convenient

352   to explore the behavior of those compounds at lower concentrations. For this purpose, a

353   series of experiments were performed ==in distilled water== with the mixture of EPs, at an

354   initial concentration of 10 μg/l of each contaminant, in the presence and absence of

355   humic acids. The percentages of removal for the reaction with and without humic acids,

356   together with the photolysis of the EPs after 30 and 120 min of irradiation are given in

357   Figure 6. Neutral photo-Fenton enhanced significantly the elimination of

358   acetaminophen, carbamazepine and caffeine; this was not the behavior of clofibric acid,

359   which suffered the fastest photolysis among the studied EPs, and acetamiprid, which

360   was the most reluctant to photo-Fenton. Finally, removal of amoxicillin was very fast in

361   all cases, as its concentration was below the quantitation limit after 30 min.

362

363   Regarding to humic substances, the presence of these organics did not result in an

364   improved performance of the photo-Fenton, in sharp contrast with observations with the

365   mixture containing 5 mg/l of each EP. This could be due to a competitive role of humic

366   acids for the reactive species formed in the process which compensates for the potential

367   enhanced formation of reactive species: as the employed amount of humics was the

368   same in both situations (10 mg/l), in the more diluted solution, the ratio EP/humic

369   substances decreases and hence, reaction of the generated oxidative species becomes

370   more likely with the humic substances than with the EPs.

371

372   4. Conclusions

373   Photo-Fenton process at mild conditions has been able to treat a mixture of six

374   emerging pollutants at moderate reaction times when they are at low concentrations.

375   The aqueous matrix had a remarkable influence in the process; in particular, humic

376   substances played a favorable role in the process at high EPs concentrations, although

the opposite behavior was observed as the EPs/humics ratio decreased, most probably due to a scavenging effect of these substances. Despite some toxic by-products have been detected when experiments were conducted at high concentration of the EPs, this is not a major drawback, as they are eliminated after longer reaction times and, more important, toxicities are very low at the concentration ranges commonly reported for EPs at the effluents of wastewater treatment plants.

Acknowledgements

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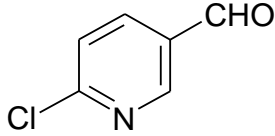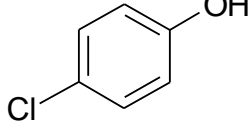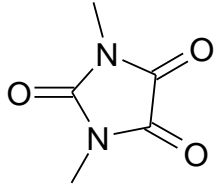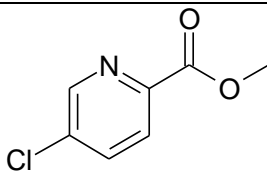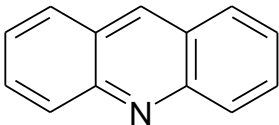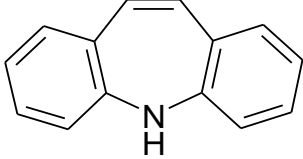
490   Table 1. EC_{50} and inhibition caused by 50 mg/l of each EP, determined according to the

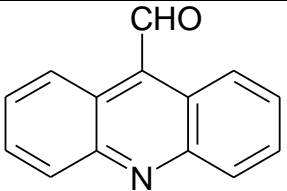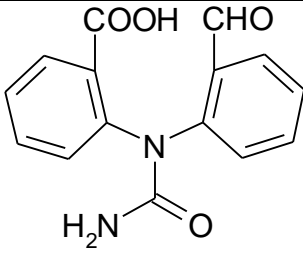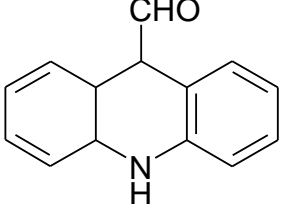491   inhibition of luminescence of *V. fischeri* bacteria.

Compound	EC_{50} (mg/l)	% Inhibition caused by 50 mg/l
Acetominophene	2600	>1
caffeine	3700	>1
Acetamiprid	110	20
Amoxycillin	1350	>1
Carbamazepine	280	>1
Clofibric acid	280	>1

492

493

494 Table 2. Organic by products detected in sampes taken at diferent periods of a photo-

495 Fenton process at acidic pH under simulated sunlight (initial concentration of each EP =

496 50 mg/l. Compounds are qualitatively devided into major (M), noticeable or tare (T)

497 amounts depending on the peak area. When possible, a parent EC has been assigned to

498 each intermediate.

tr	Compound	t= 5min	t= 10 min	t=15 min	t=30 min	Parent compound
11.4	(structure: 2-chloro-5-pyridinecarboxaldehyde)	T	T	T	T	Acetamiprid
12.0	(structure: 4-chlorophenol)	M	M	N	-	Clofibric acid
12.1	(structure: 1,3-dimethyl imidazolidine-2,4,5-trione)	T	N	T	N	Caffeine
13.5	Unidentified chlorophenol	-	T	-	-	
13.8	(structure: methyl 5-chloropyridine-2-carboxylate)	-	-	-	T	
16.5	Unidentified chlorophenol	N	N	N	-	Acetamiprid
19.0	Clofibric acid	T	-	T	-	-
20.8	Acetominophene	M	M	M	-	
21.6	(structure: acridine)	T	T	-	-	Carbamazepine
22.3	Caffeine	M	M	N	-	-
24.0	(structure: iminodibenzyl / dibenzazepine)	N	N	T	-	Carbamazepine

25.0		N	N	T	-	Carbamazepine
27.2		T	-	-	-	Carbamazepine
27.6		N	N	T	-	Carbamazepine
28.4	Carbamazepine	M	N	N	-	-
29.6	Acetamiprid	M	M	M	N	-

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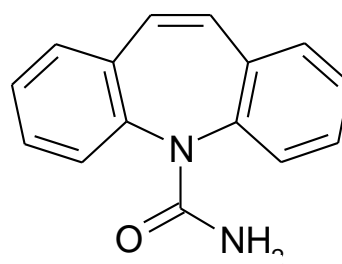
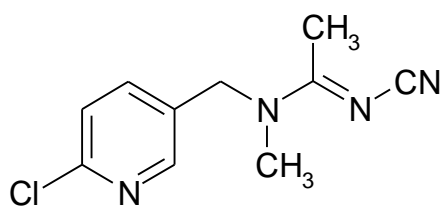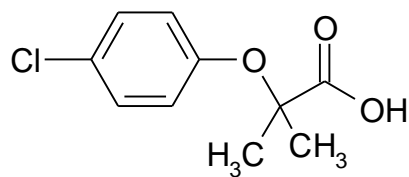Scheme 1: Chemical structures of EPs employed in this work
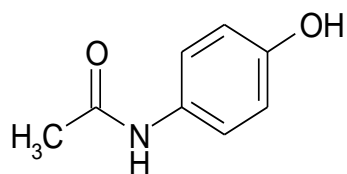
Acetominophen
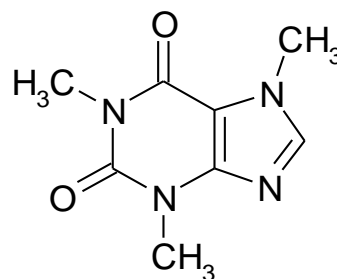
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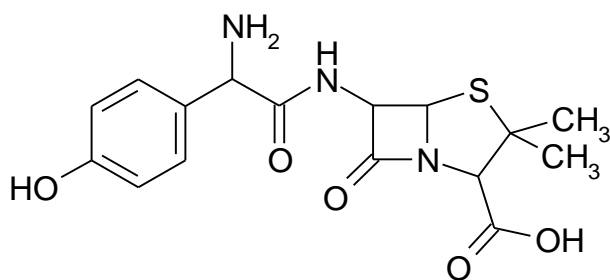
Amoxicillin

Carbamazepine

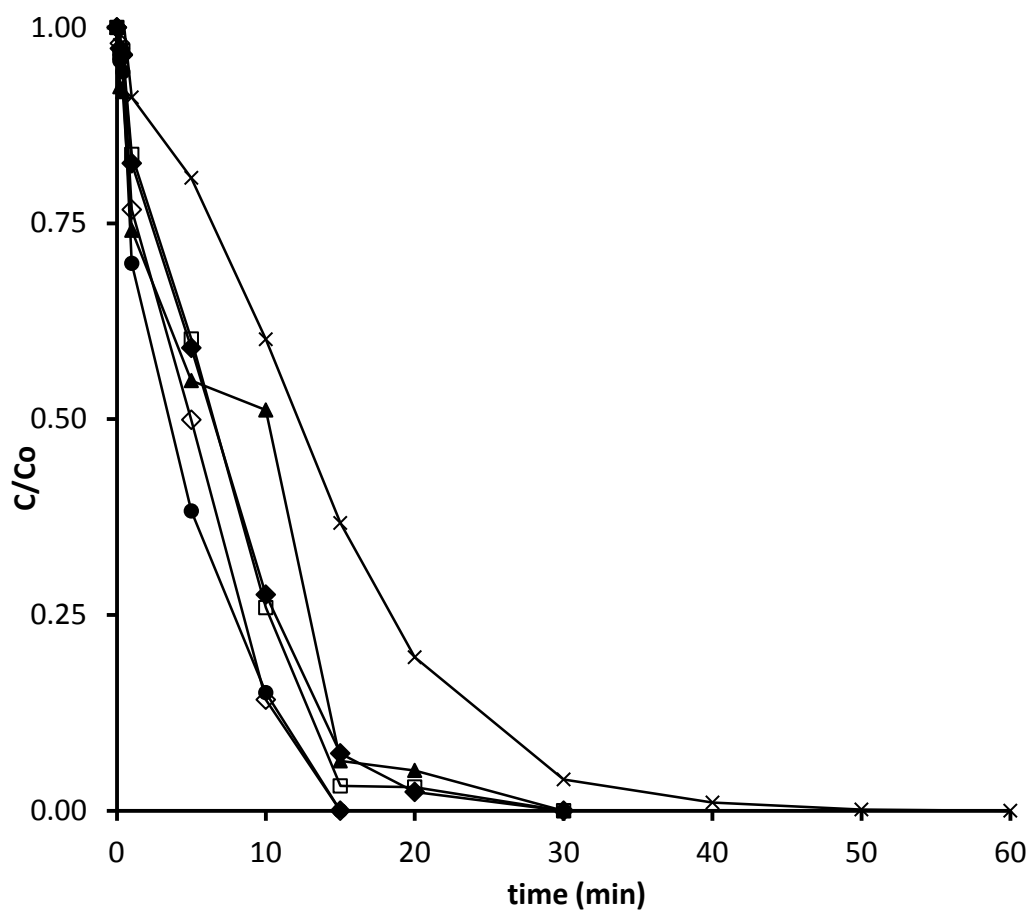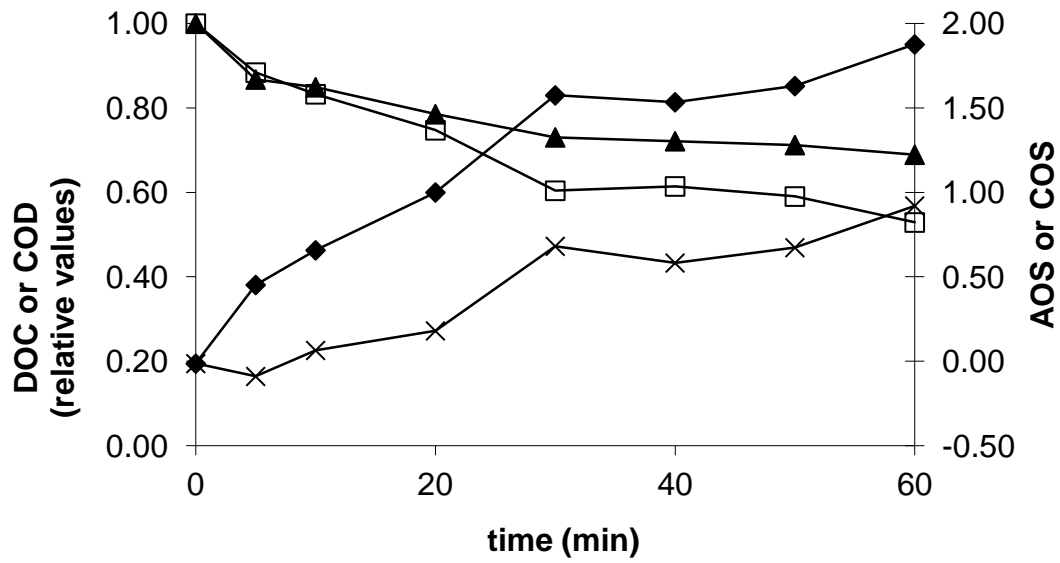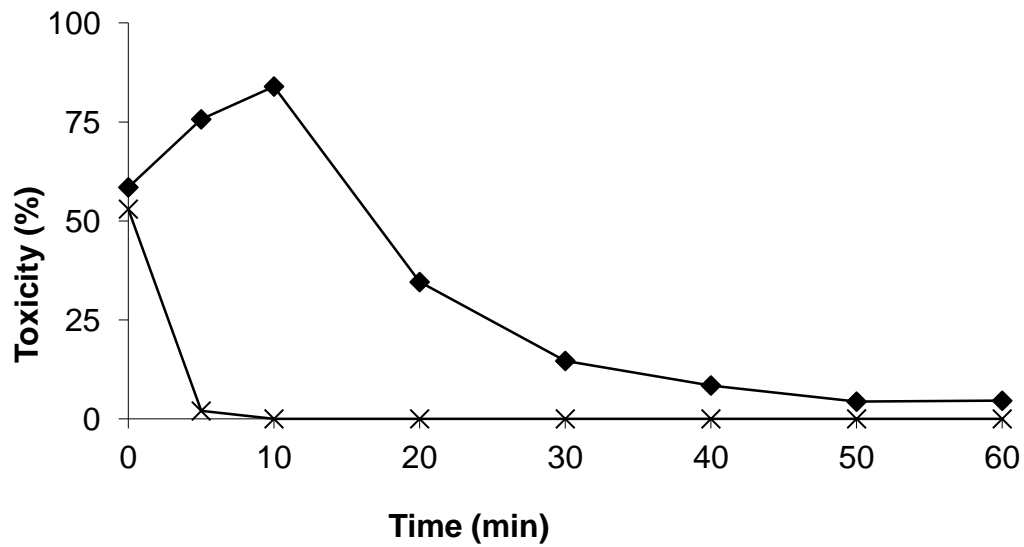Acetamiprid

Clofibric acid

502

503

504 Figure 1. Photodegradation of a mixture of 6 EPs with an initial concentration of 50

505 mg/l by means of a photo-Fenton process at acidic medium under simulated sunlight.

506 Plot of the relative concentration vs time: amoxycillin (▲), acetaminophen (●),

507 acetamiprid (✕), caffeine (□), clofibric acid (♦) and carbamazepine (◊).

508

509

510

Figure 2. Photodegradation of a mixture of 6 EPs with an initial concentration of 50

mg/l by means of a photo-Fenton process at acidic medium under simulated sunlight.

Plot of changes in COD (□), DOC (▲), AOS (×) and COS (♦) vs. irradiation time.

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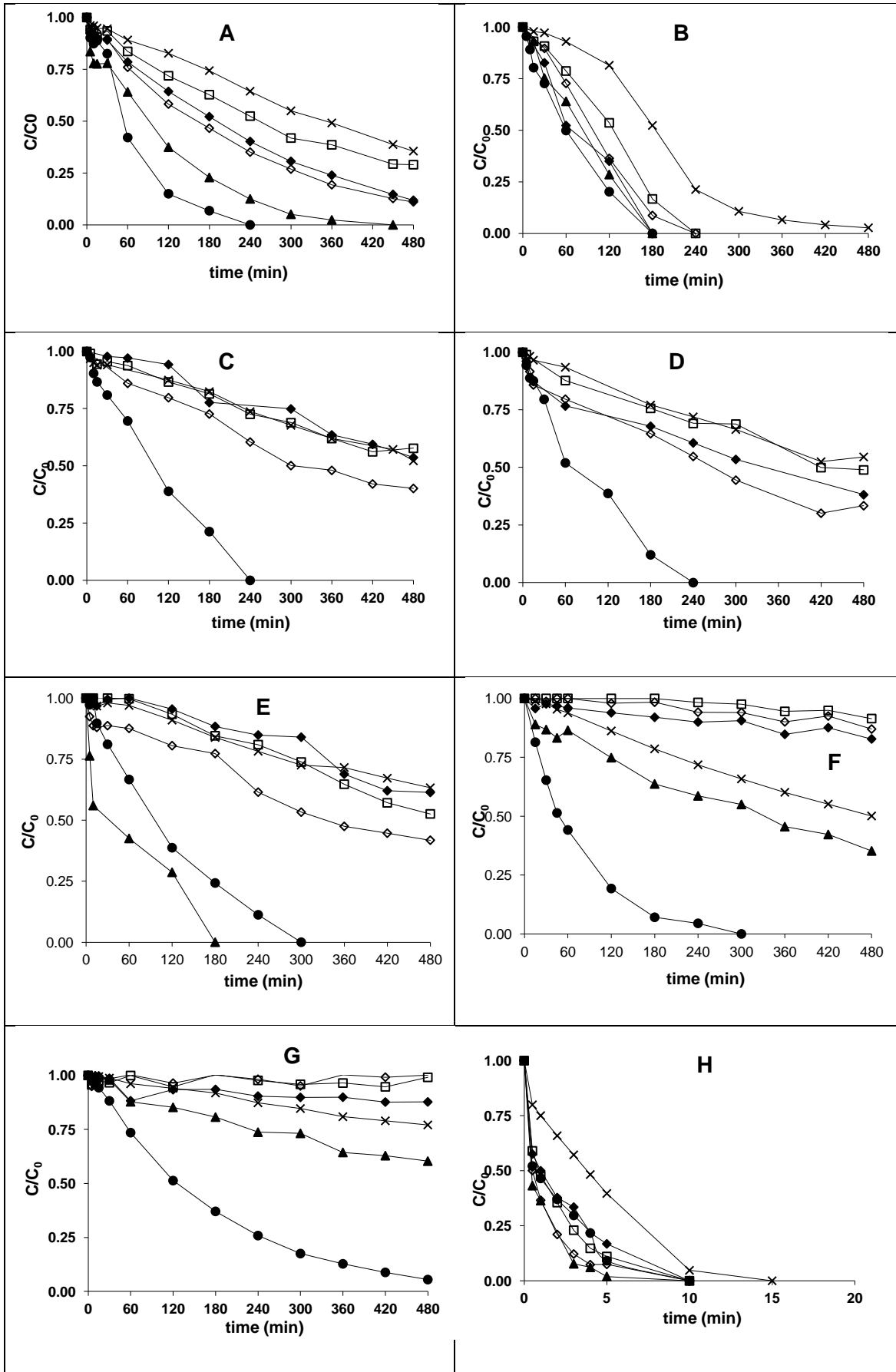
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Figure 3. Changes in the toxicity according to the *V. fischeri* assay (♦) and inhibition of

the respitation of activated sludge (×) of a mixture of 6 EPs with an initial

concentration of 50 mg/l of each EP submitted to a photo-Fenton process at acidic
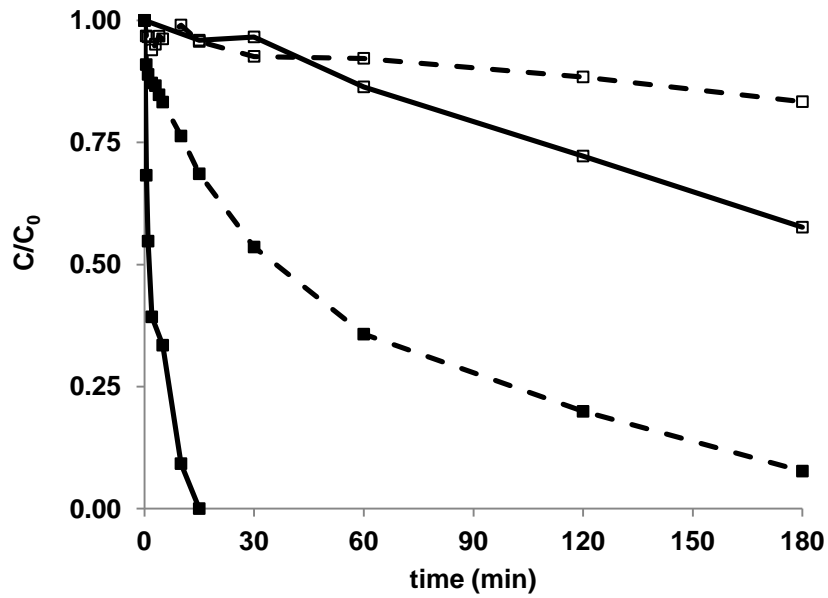
medium under simulated sunlight.

520

521   Figure 4: Photodegradation of a mixture of 6 EPs with an initial concentration of 5 mg/l

522   under simulated sunlight. Plot of the relative amounts of amoxycillin (▲),

523   acetaminophen (♦), acetemiprid (✕), caffeine (□), clofibric acid (●) and carbamazepine

524   (◊). Experimental condition were: neutral photo-Fenton in distilled water (A), neutral

525   photo-Fenton in distilled water and humic acids (B), neutral photo-Fenton in tap water

526   (C), neutral photo-Fenton in tap water and humic acids (D), neutral photo-Fenton

527   spiking the EPs in a wastewater treatment plant effluent (E), photolysis in distilled

528   water (F), photolysis in the presence of humic acids and iron (G), and photo-Fenton at

529   pH = 2.8 in distilled water (H, note the different scale).
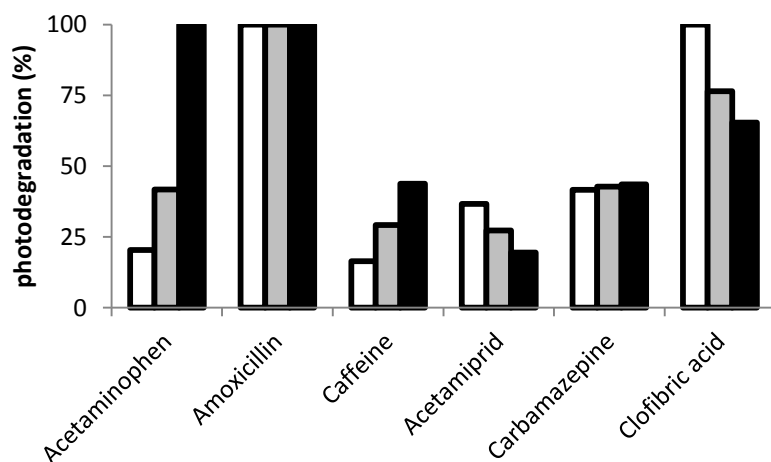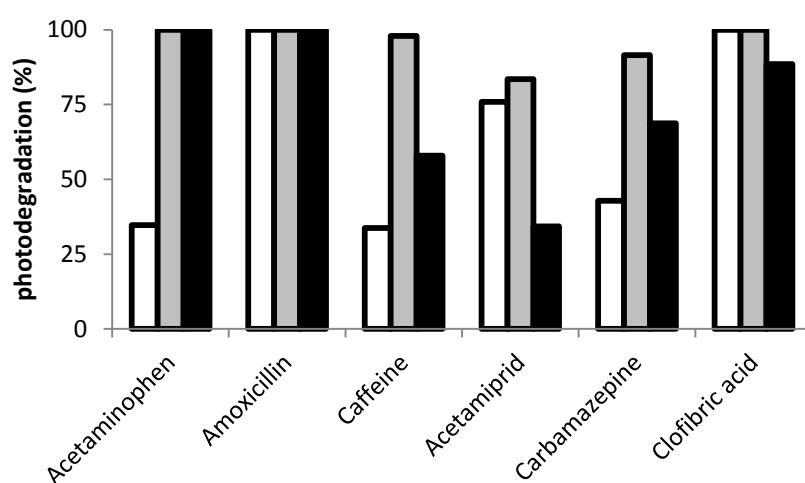
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533   Figure 5: plots of the concentration of caffeine during a photo-Fenton process with

534   (discontinuous line) and without (continuous line) t-butanol at acidic (black symbols) or

535   neutral (empty symbols) media.

536

537

538

539   Figure 6: Photodegradation of a mixture of 6 EPs with an initial concentration of 10 μg/l

540   by means of a photo-Fenton process at neutral pH under simulated sunlight. Percentage

541   of photodegradation achieved after 30 min (above) and 120 min (below) of irradiation.

542   Reactions were carried out without (grey bars) and with 10 mg/l of humic acids, (black

543   bars). Photolysis of the EPs is also shown (white bars)

544