1   **Hydrogen peroxide and persulfate activation using UVA-UVB radiation:**

2   **degradation of estrogenic compounds and application in sewage treatment**

3   **plant waters**

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11

12   **Abstract**

13   In the present work, the degradation of three estrogens (17β-estradiol (E2), estrone (E1) and

14   17α-ethinylestradiol (EE2)) was investigated under photoactivation of hydrogen peroxide and

15   persulfate. Lab-scale irradiation experiments showed that both UVA and UVB radiations are

16   able to photoactivate the oxidant precursors, although UVB is more efficient to generate

17   radicals and therefore to degrade the targets. The efficiency of both oxidant precursors was

18   investigated showing higher efficiency in the system with persulfate. The pseudo-first order

19   degradation rate constants and the second order rate constants between the hydroxyl or the

20   sulfate radicals and estrogens were measured. In order to evaluate the process efficiency in

21   real treatment conditions, the degradation of the estrogens spiked into sewage treatment plant

22   effluent was studied. Measurements of second order rate constants between the radical and the

23   effluent organic matter by laser flash photolysis allowed to understand the involved

24   quenching mechanisms. A Yeast Estrogen Screen (YES) assay was used to follow the

25   decrease in estrogenic activity during the estrogen degradation. This assay permitted to ensure

26   that the studied processes are not only able to degrade the estrogens but also to remove their

27   estrogenic activity.

28

31

32   **1. Introduction**

33   Whilst estrogens are known to be hydrophobic and have significantly high partition

34   coefficients (log K_{ow} = 4) (Pal et al., 2010), their presence in surface waters and river

35   sediments has been widely reported around the world (Anderson et al., 2012; Praveena et al.,

36   2016; Valdés et al., 2015; Zuo et al., 2013). The most commonly detected estrogens are

37   estrone (E1) and 17β-estradiol (E2), which are naturally excreted by humans and animals, as

38   well as 17α-ethinylestradiol (EE2), a synthetic hormone used in contraceptive pills. It has

39   been reported that these three hormones are the main estrogenic compounds found in

40   domestic sewage treatment plant (STP) effluents (Amin et al., 2018; Desbrow et al., 1998;

41   Huang et al., 2014). Laurenson et al. (2014) reported that one human being excretes on

42   average 19.00 μg of E1, 7.70 μg of E2 and 0.41 μg of EE2 per day. As a consequence, these

43   hormones tend to reach concentrations up to tens of ng L^{-1} in domestic STP wastewaters,

44   particularly in urban areas where their impact is significant. The current treatment processes

45   are not sufficient to provide appropriate degradation rates. Therefore, STP effluents which are

46   released into the environment are likely to contain E1, E2 and EE2 at concentrations up to

47   several ng L^{-1} (Amin et al., 2018; Desbrow et al., 1998; Huang et al., 2014).

48   When entering the human or animal body via external sources, these hormones are considered

49   as endocrine disrupting chemicals because their biological activity can interfere with natural

50   hormone activities. It has been reported that these estrogens have a negative impact on river

51 wildlife, particularly downstream from STPs. Purdom et al. (1994) have highlighted that the

52 exposure of rainbow trout to EE2 concentrations of approximately 1 ng L^{-1} resulted in an

53 abnormal production of vitellogenin, a protein normally synthesized only during female

54 gestation. A study carried out on several British rivers has shown that surface water estrogens

55 can cause varying degrees of feminisation in male fish populations (Jobling et al., 1998).

56 These observations were also reported by Kidd et al. (2007) in an *in situ* study in Canada on

57 fathead minnow. They observed that concentrations around 5 ng L^{-1} of EE2 impacted the

58 gonadal development of males. Each of these reported effects have led to a disruption in

59 reproduction, and ultimately a decline in the population of wild species within impacted areas.

60 To avoid the contamination of surface waters by estrogens, the efficacy of various quaternary

61 treatments on domestic STPs is under investigation. There are two categories of treatment,

62 physical and chemical. Physical treatments include the adsorption of micropollutants on

63 activated carbon and membrane processes (microfiltration, nanofiltration, ultrafiltration or

64 reverse osmosis) whereas chemical treatments include advanced oxidation processes (AOPs)

65 such as ozonation and UV based processes (Bui et al., 2016). The former processes are costly

66 and require specialist treatment facilities. This is a barrier to their use on small and medium

67 domestic STP effluents (< 10 000 inhabitant equivalent). In order to remove estrogen from

68 these effluents, chemical treatments are necessary.

69 Cédat et al. (2016) have highlighted the efficiency of a UVC/H_2O_2 process to remove the

70 estrogenic activity of estrogen spiked water samples. Numerous recent studies have also

71 shown the ability of sulfate radicals ($SO_4^{\bullet-}$), generated through persulfate UVA or UVC

72 photo-activation, to degrade micropollutants in wastewater (Al Hakim et al., 2020; Li et al.,

73 2017; Olmez-Hanci et al., 2015; Palharim et al., 2020), including estrogens (Angkaew et al.,

74 2019). In the majority of cases, the photo-activation in AOPs is carried out with UVC

75 radiation (254 nm). However, they have several disadvantages compared to UVA and UVB

76 radiations: UVC lamps are more expensive and can be hazardous to manipulate. The use of

77 UVA or UVB radiations also aims at more sustainable processes. They are less energy-

78 consuming and could even be replaced by solar light for real-scale application. Lamps are

79 nevertheless easier to use and to control for research purposes than solar light.

80 In this work we investigated the ability of UVA and UVB activation of H_2O_2 and $S_2O_8^{2-}$

81 processes to degrade three commonly found hormones in wastewaters: 17β-estradiol, estrone

82 and 17α-ethinylestradiol. Preliminary degradation studies were carried out on 17β-estradiol in

83 milli-Q water and few parameters such as oxidant dosage were investigated. Subsequent

84 studies were conducted to establish the efficacy of the technique on STP effluent spiked with

85 the three estrogens. Moreover, estrogenic activity assays were performed during degradation

86 process to follow the harmfulness evolvement during irradiation.

87

88 **2. Materials and methods**

89 *2.1. Chemicals and reagents*

90 17β-estradiol (E2), estrone (E1) and 17α-ethinylestradiol (EE2) were purchased from Sigma-

91 Aldrich, as well as hydrogen peroxide (H_2O_2) (30% in water) and sodium persulfate

92 ($Na_2S_2O_8$). Acetonitrile was supplied by Carlo Erba Reagents. Ultrapure water was obtained

93 from a milli-Q system. Wastewaters were collected at the outlet of the treatment from the "3

94 rivières" urban STP, Clermont-Ferrand, France in September (STPW1) and December

95 (STPW2) 2019. This STP is equipped with a conventional activated sludge process. STP

96 waters were filtered on a paper filter followed by a filtration on a CHROMAFIL® Xtra RC-

97 45/25 syringe filter from Macherey-Nagel. Main physico-chemical parameters after filtration

98 are reported in Table SM1.

99

100 *2.2. Irradiation experiments*

101 UVA and UVB irradiations were carried out in a 150 mL Pyrex reactor, magnetically stirred

102 and kept at room temperature (20°C) by a cooling system. The reactor was placed in a home-

103 made rectangular box equipped on the top with four polychromatic fluorescence tubes (UVA

104 F15W/350BL, Sylvania Blacklight, Germany, or UVB G15T8E, Sanyo Denki, Japan). The

105 UVA (λ_{max} = 352 nm) and UVB (λ_{max} = 308 nm) lamp emission spectra were measured on top

106 of the reactor using an optical fibre and a charge-coupled device spectrophotometer (Ocean

107 Optics USD 2000 + UV-vis), calibrated using a DH-2000-CAL Deuterium Tungsten Halogen

108 reference lamp (Figure SM1). UV-visible spectra of the estrogens, oxidants and STPW2 were

109 carried out with a Cary 300 scan UV-visible spectrophotometer and reported in Figures SM2,

110 SM3 and SM4. H_2O_2 concentration was followed using p-hydroxyphenylacetic acid (HPAA,

111 purity > 98%) and horseradish peroxidase (POD), according to the spectrofluorimetric

112 quantification method (Miller & Kester, 1988) with a Varian Cary Eclipse fluorescence

113 spectrophotometer setting excitation wavelengths at 320 nm and emission maximum at 408

114 nm. The formation of the dimer of HPAA was correlated with the concentration of H_2O_2

115 using standard solutions.

116 Estrogen stock solutions (1 mM) were prepared in acetonitrile and stored in the dark at 4°C.

117 Solutions of 100 mL containing 5 µM of estrogens and different oxidant precursor

118 concentrations (from 0 to 5 mM) were irradiated under polychromatic wavelengths (UVA or

119 UVB). Such concentration of estrogens do not require the use of pre-concentration techniques

120 before HPLC analysis which avoids a source of errors while using relatively low

121 concentration. 1 mL of solution was withdrawn at fixed interval times for HPLC

122 quantification of estrogen concentrations. However, small volume variations did not impact

123 the irradiation efficacy.

124

125 *2.3. Sample analysis and data processing*

126 Estrogen concentrations were followed using a Waters Acquity Ultra High Performance

127 Liquid Chromatography (UPLC) system equipped with a BEH C18 column (100×2.1 mm,

128 1.7 µm), coupled to a diode array detector (200-400 nm) and a fluorescence detector ($\lambda_{ex} =$

129 280 nm, $\lambda_{em} = 305$ nm). Elution flow rate was 0.6 mL min^{-1} and eluents were a mixture of

130 milli-Q water and acetonitrile. A gradient raising the acetonitrile percentage from 30% to 70%

131 in 4 minutes and then 1 min constant at 70% was used. Injection volume was 6 µL and

132 column temperature was fixed at 40°C.

133 Concentration of estrogen during irradiation was fitted by the following first order equation:

134 $^{C_t}/_{C_0} = \exp{(-k't)}$ where C_0 and C_t are respectively the initial concentration and the

135 concentration at time t and k' is the pseudo-first order rate constant.

136 The error bars associated to the rate data represent 3σ, derived from the scattering of the

137 experimental data around the fit curves (intra-series variability).

138

139 *2.4. Laser flash photolysis*

140 A time resolved spectroscopy was used to determine the second order rate constant of

141 hydroxyl and persulfate radicals with E1, E2 and EE2 but also with the organic and inorganic

142 carbon of the STPW. The second order rate constants allowed to estimate the radicals

143 selectivity between the various species in solution.

144 All experiments were carried out using the fourth harmonic ($\lambda_{exc} = 266$ nm) of a Quanta Ray

145 GCR 130-01 Nd:YAG laser system instrument and an energy of ~45 mJ/pulse. The

146 experimental setup has been described before (Wu et al., 2015). Briefly, for hydroxyl radical

147 reactivity, a competition kinetic method using thiocyanate anion was used and reactivity was

148 determined following the absorption at 450 nm of $(SCN)_2^{\bullet-}$ transient (Huang et al., 2018). For

149 sulfate radical, the decay (k', s^{-1}) of $SO_4^{\bullet-}$ signal at 370 nm was plotted as a function of

150   quencher concentration (i.e. estrogen or carbon from STPW) concentration. The slope of the

151   linear correlation gives the value of the second order rate constant (k'', M^{-1} s^{-1}).

152

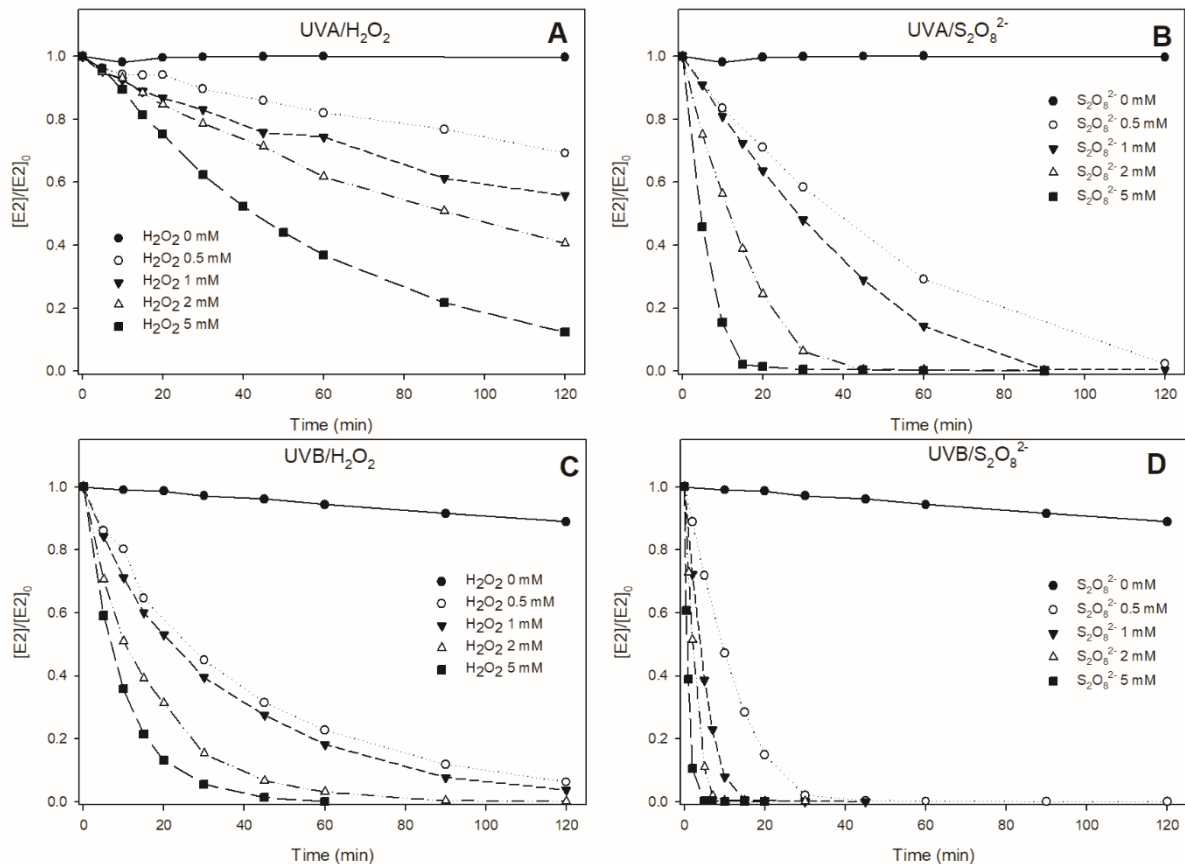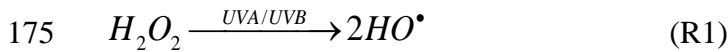153   *2.5. Estrogenic activity removal*

154   The Arxula Yeast Estrogen Screen assay (A-YES) ready-to-use test kit was provided by New-

155   diagnostics (Germany). This assay allows to quantify the estrogenic activity caused by

156   estrogen-active substances in aqueous samples. The results are obtained in E2 equivalent

157   concentration. It includes the use of genetically modified *Arxula adeninivorans* yeast cells

158   which contain the gene for human estrogenic receptor. The estrogenic activity of the aqueous

159   solution is correlated to the chromogenic activity of the final solution (Hettwer et al., 2018).

160   The assays were carried out in 96-well plates. The calibration standards were analysed in

161   duplicates, in the range of 1 to 80 ng L^{-1}, along with two blanks. The samples were diluted to

162   fit in the studied range and analysed in triplicates.

163

164   **3. Results and discussion**

165   *3.1. Effects of H_2O_2 and $S_2O_8^{2-}$ under UVA and UVB radiations on the degradation of E2*

166   In Figure 1, E2 degradations were followed under UVA and UVB radiations in the presence

167   of different H_2O_2 or $S_2O_8^{2-}$ concentrations (from 0 to 5 mM). Despite no photolysis observed

168   under UVA, about 15% degradation of E2 was observed after 4 hours under UVB. In all

169   systems, E2 undergoes faster disappearance in the presence of H_2O_2 and $S_2O_8^{2-}$. This trend

170   was attributed to the photoactivation of both radical precursors (reactions R1 and R2) leading

171   to the generation of highly oxidative species such as hydroxyl ($HO^{\bullet}$) and sulfate ($SO_4^{\bullet-}$)

172   radicals in solution. Faster degradation of E2 in the presence of UVB compared to the UVA

173   lamp was predicted considering higher absorption of both oxidant precursors at shorter

174   wavelength irradiations (Figure SM3).

175 $$H_2O_2 \xrightarrow{UVA/UVB} 2HO^\bullet \qquad\qquad \text{(R1)}$$

176 $$S_2O_8^{2-} \xrightarrow{UVA/UVB} 2SO_4^{\bullet-} \qquad\qquad \text{(R2)}$$

177

178 *Figure 1. Effect of the oxidant precursor concentration (from 0 to 5 mM) on E2 (5 µM) degradation in milli-Q water at pH 6*
179 *under various conditions: UVA/H$_2$O$_2$ (A), UVA/S$_2$O$_8^{2-}$ (B), UVB/H$_2$O$_2$ (C) and UVB/S$_2$O$_8^{2-}$ (D).*

180 As clearly depicted on Figure 1, $S_2O_8^{2-}$ allows for faster degradation than H_2O_2 under both

181 UVA and UVB radiations. Degradation of E2 up to 99% was achieved after 45 min under

182 UVB + 5 mM H_2O_2 and after 5 min under UVB + 5 mM $S_2O_8^{2-}$. In fact, E2 degradation can

183 be attributed to the higher photolysis yield of radicals in the $S_2O_8^{2-}$ system compared to H_2O_2.

184 Oxidant precursors photolysis yield under the polychromatic UVA and UVB lights were

185 determined following their degradations in solutions containing respectively H_2O_2 and $S_2O_8^{2-}$

186 as the only species. 1 mL of methanol (hydroxyl and sulfate radicals quencher) was added to

187 the solutions (100 mL) to prevent self-quenching between the radicals and the oxidant

188 precursors (i.e. radical reactivity) and ensure photolysis as the only degradation path. $S_2O_8^{2-}$

189 photolysis constant ($k'_{S_2O_8^{2-}}$) were determined to be $1.93 \pm 0.09 \times 10^{-5}$ s^{-1} under UVA radiation

190 and $2.72 \pm 0.13 \times 10^{-5}$ s^{-1} under UVB radiation while for H$_2$O$_2$ lower constants ($k'_{H_2O_2}$) of 1.48

191 $\pm 0.07 \times 10^{-6}$ s^{-1} under UVA radiation and $6.08 \pm 0.33 \times 10^{-6}$ s^{-1} under UVB radiation were

192 measured.

193 In Figure 2, the correlation between E2 pseudo-first order rate constant and oxidant precursor

194 concentrations is presented. An increase of E2 degradation was observed when the

195 concentration of radical precursors (H$_2$O$_2$ and S$_2$O$_8^{2-}$) increases. However, no linear

196 correlation can be established in the different system. This effect is mainly observed on the

197 H$_2$O$_2$ systems. It can be explained considering the competition undergone by the

198 photogenerated radical HO$^\bullet$ between E2 and H$_2$O$_2$ (R3 and R4). Hydrogen peroxide plays a

199 role of hydroxyl radical scavenger, enhanced at high concentrations (Table SM2).

200 Considering the H$_2$O$_2$ initial concentration and the second order rate constants between HO$^\bullet$

201 and E2 (Table 1) ($k''_{HO^\bullet,E2} = 2.9 \times 10^{10}$ M^{-1} s^{-1}) and between HO$^\bullet$ and H$_2$O$_2$ ($k''_{HO^\bullet,H_2O_2} = 2.7 \times$

202 10^7 M^{-1} s^{-1}) (Buxton et al., 1988), we can estimate that in the presence of H$_2$O$_2$ at 5 mM, about

203 48% of the hydroxyl radicals react through reaction R3 leading to the strong decrease of the

204 reactivity towards E2 and the formation of less reactive species i.e. HO$_2^\bullet$/O$_2^\bullet$- (pKa = 4.88).
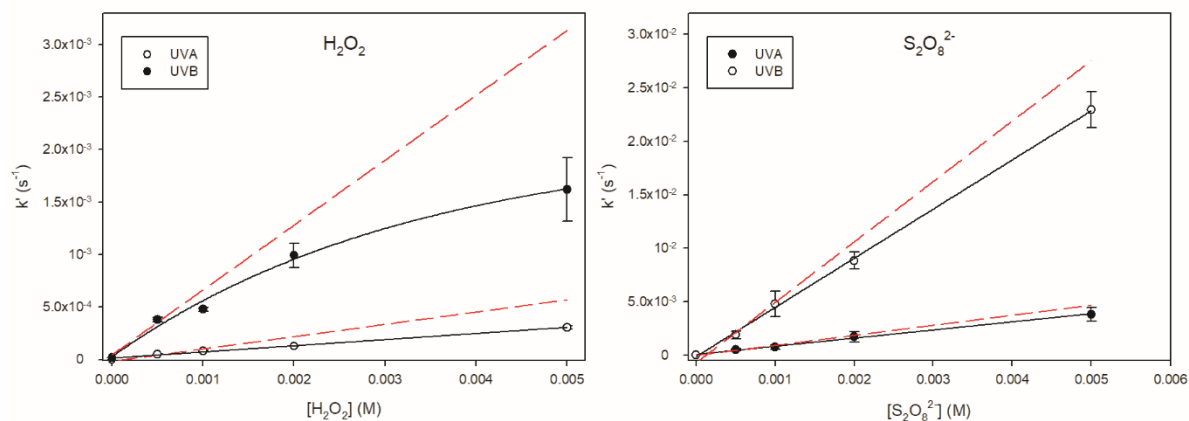
205 $HO^\bullet + H_2O_2 \rightarrow H_2O + HO_2^\bullet$ (R3)

206 $HO^\bullet + E2 \rightarrow E2ox$ (R4)
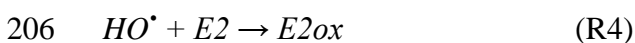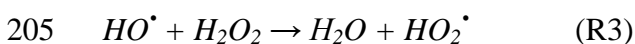
207
208
209 *Table 1. Second order rate constant of HO$^\bullet$ and SO$_4^{\bullet-}$ with estrogens or STPW2 carbon, at neutral pH. Data are in M^{-1} s^{-1} for estrogens and in M$_C^{-1}$ s^{-1} for STPW2. TOC constants were determined after acidification of STPW2 to pH 4 to remove the inorganic carbon.*

	E2	EE2	E1	STPW2 TC	STPW2 TOC
$k''_{HO^\bullet}$	$2.91 \pm 0.09 \times 10^{10}$	$1.81 \pm 0.02 \times 10^{10}$	$2.85 \pm 0.03 \times 10^{10}$	$2.8 \pm 0.1 \times 10^8$	$2.5 \pm 0.1 \times 10^8$
$k''_{SO_4^{\bullet-}}$	$2.66 \pm 0.03 \times 10^9$	$1.84 \pm 0.02 \times 10^9$	$4.11 \pm 0.04 \times 10^9$	$2.4 \pm 0.1 \times 10^8$	$2.2 \pm 0.2 \times 10^8$

210 On the contrary, S$_2$O$_8^{2-}$ scavenging effect is minor due to the lower reactivity constant

211 between SO$_4^{\bullet-}$ and S$_2$O$_8^{2-}$ ($k''_{SO_4^{\bullet-},S_2O_8^{2-}} = 6.1 \times 10^5$ M^{-1} s^{-1}) (McElroy & Waygood, 1990)

212 whereas $k''_{SO_4^{\bullet-},E2} = 2.7 \times 10^9$ M^{-1} s^{-1}. In the presence of 5 mM of S$_2$O$_8$$^{2-}$, the quenching of the

213 photogenerated sulfate radical can be estimated to only 18%. The calculations for all oxidant

214 precursors concentrations and competition reactivity are presented in the Supplementary

215 material section.

216

217 *Figure 2. E2 pseudo-first order rate constants (s^{-1}) depending on the oxidant precursor concentrations at pH 6: H$_2$O$_2$ (A) and*
218 *S$_2$O$_8$$^{2-}$ (B), under UVA and UVB radiations. Dashed red lines estimate the curves without the oxidant precursors scavenging*
219 *effects. The error is $\pm$ 3σ, obtained from the scattering of the experimental data.*
220

221 *3.2. Effect of STP effluent on the degradation of E2 in the different systems*

222 In this work, two STP effluents sampled at different times of the year were characterised.

223 Similar E2 degradation rates were obtained when spiked into STPW1 and STPW2. STPW2

224 will be considered for the rest of this study. In Table 2, E2 pseudo-first order degradation rate

225 constants in STP effluent (STPW2) are compared to the results obtained in milli-Q water. E2

226 removal inhibition was about 90% ($\pm$ 3%) in STPW using 2 mM of H$_2$O$_2$ or S$_2$O$_8$$^{2-}$ under both

227 UVA or UVB. Such effect can be attributed to the presence of naturally occurring scavengers

228 in STPW able to react with the photogenerated radicals. Chloride (Cl$^-$), bicarbonates (HCO$_3$$^-$)

229 and occasionally nitrate (NO$_3$$^-$) ions are known as possible interfering species during radical

230 based degradation processes (Tao et al., 2020; Zhang et al., 2018). The inhibition effect on E2

231 degradation was tested for each individual ion to their concentration measured in STPW2

232 (Table SM1) and no significant impact was observed (Figure SM5). Only in the presence of

233 chloride ions a slight inhibition ($< 5\%$) of E2 degradation can be observed and attributed to

234 the formation of less oxidant species such as dichloride radical ions ($Cl_2^{\bullet-}$) (Armstrong et al.,

235 2015).

236 The composition of the organic matter in STPW2 has not been determined. Several studies

237 highlighted that effluent compositions have a large variation range, depending on the influent

238 characteristics but also on the type of treatment process upstream (Imai et al., 2002; Ma et al.,

239 2001; Yu et al., 2012; Zhang et al., 2009). In this work, the organic matter reactivity has been

240 standardised on the organic carbon reactivity.

241 Considering that the reactivity of organic carbon with hydroxyl and sulfate radicals is

242 respectively $k''_{HO^\bullet,TOC} = 2.5 \times 10^8 \ M_C^{-1} \ s^{-1}$ and $k''_{SO4^{\bullet-},TOC} = 2.2 \times 10^8 \ M_C^{-1} \ s^{-1}$ (Table 1), we

243 can estimate that about 82% and 74% of $HO^\bullet$ and $SO_4^{\bullet-}$ are scavenged by organic matter of

244 STPW2 in the H_2O_2 and $S_2O_8^{2-}$ systems respectively. The inhibition effect is not far from the

245 experimental value reported in Table 2 and shows that the organic matter is mainly

246 responsible for slowing down E2 degradation in STPW2. In their study on the degradation of

247 Bisphenol A, Olmez-Hanci et al. (2015) have also determined that the natural organic matter

248 from raw freshwaters was the main $HO^\bullet$ and $SO_4^{\bullet-}$ scavengers, and Ghauch et al. (2017)

249 reported that inorganic anions had a minor implication in the degradation inhibition.

250 However, Ma et al. (2018) observed that carbonates were also significant scavengers, whereas

251 the scavenging effect of chlorides depended on the studied pollutants and their concentration.

252
253 *Table 2. E2 pseudo-first order degradation rate constants in milli-Q water or STPW2 in the presence of H_2O_2 or $S_2O_8^{2-}$ (2 mM) under UVA/UVB radiations at neutral pH.*

|	UVA/H_2O_2	UVA/$S_2O_8^{2-}$	UVB/H_2O_2	UVB/$S_2O_8^{2-}$
k' (s^{-1}) in milliQ water	1.3×10^{-4}	1.7×10^{-3}	9.9×10^{-4}	8.8×10^{-3}
k' (s^{-1}) in STPW2 effluent	1.4×10^{-5}	1.2×10^{-4}	1.2×10^{-4}	1.1×10^{-3}
Inhibition (%)	89	93	88	87

254

255 *3.3. Comparison between E1, E2 and EE2 degradation under UVB radiation*

256 Figure 3 illustrates E1, E2 and EE2 degradations under UVB radiation of a mixture (5 μM

257 each) in milliQ water and in STPW2. For the same reasons as explained about E2 in section

258 3.1., E1 and EE2 degradations are faster when using $S_2O_8^{2-}$ than in the presence of H_2O_2 due

259 to the higher sulfate radical formation rate. The study was carried out with 2 mM of oxidant

260 precursors in order to minimize the oxidant precursors quenching effect (see section 3.1.). It

261 was also seen that all the hormones degradations are inhibited in a wastewater matrix.

262 In all the studied systems, E1 degradation was faster than E2 and EE2 degradations, which are

263 similar. The three hormones are subjected to photolysis and photo-induced degradation with

264 oxidant $S_2O_8^{2-}$ or H_2O_2. As seen in Table 1, second order reaction rate constants between each

265 hormone and $HO^{\bullet}$ or $SO_4^{\bullet-}$ have the same order of magnitude. Therefore, E1 faster

266 degradation is explained by its faster photolysis. In milli-Q water only, Figure 4 shows that E1

267 reached 95% degradation after 4 hours under UVB radiation while E2 and EE2 degradations

268 were around 15%.

269 This phenomenon also advantaged E1 degradation in STP water. As seen on Figure 3, its

270 degradation was less inhibited than E2 and EE2 degradations by STP water constituents,

271 particularly when using H_2O_2 (inhibition of 33%). Because hydrogen peroxide has a slower

272 degradation effect compared to persulfate, photolysis is effective in larger proportion. It also

273 allows E1 degradation to be less impacted by the inhibition effect from the STP water
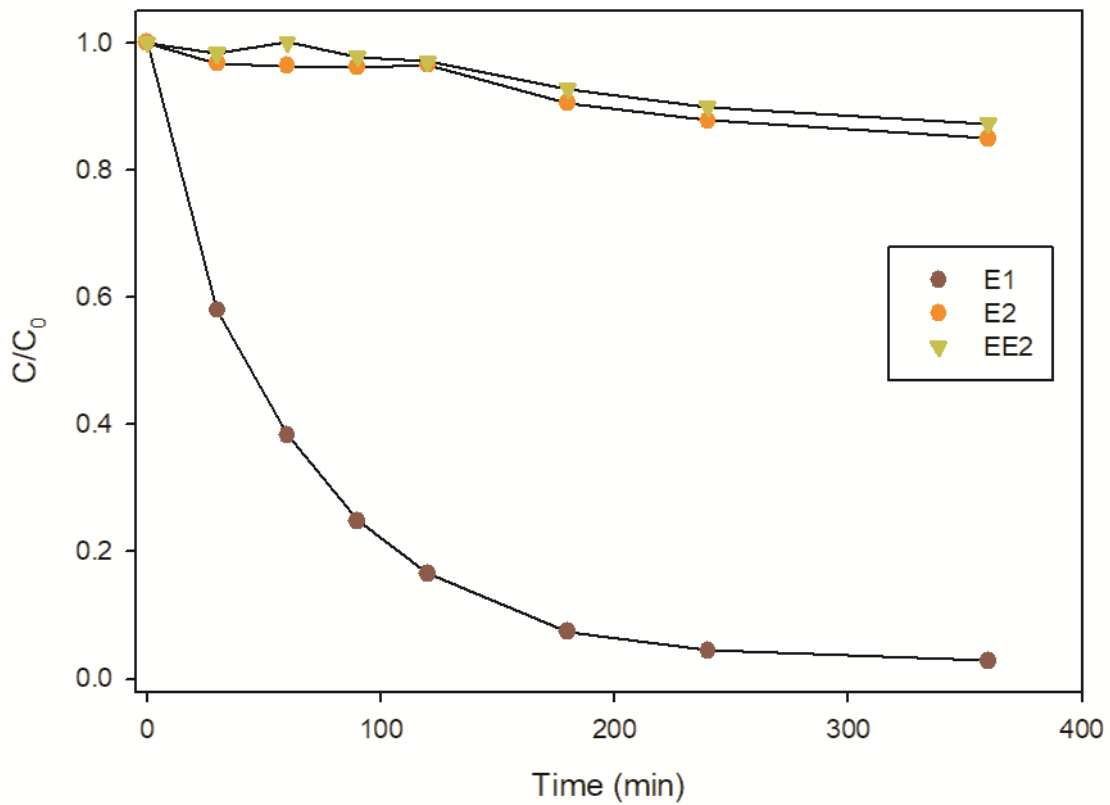
274 scavengers.

275
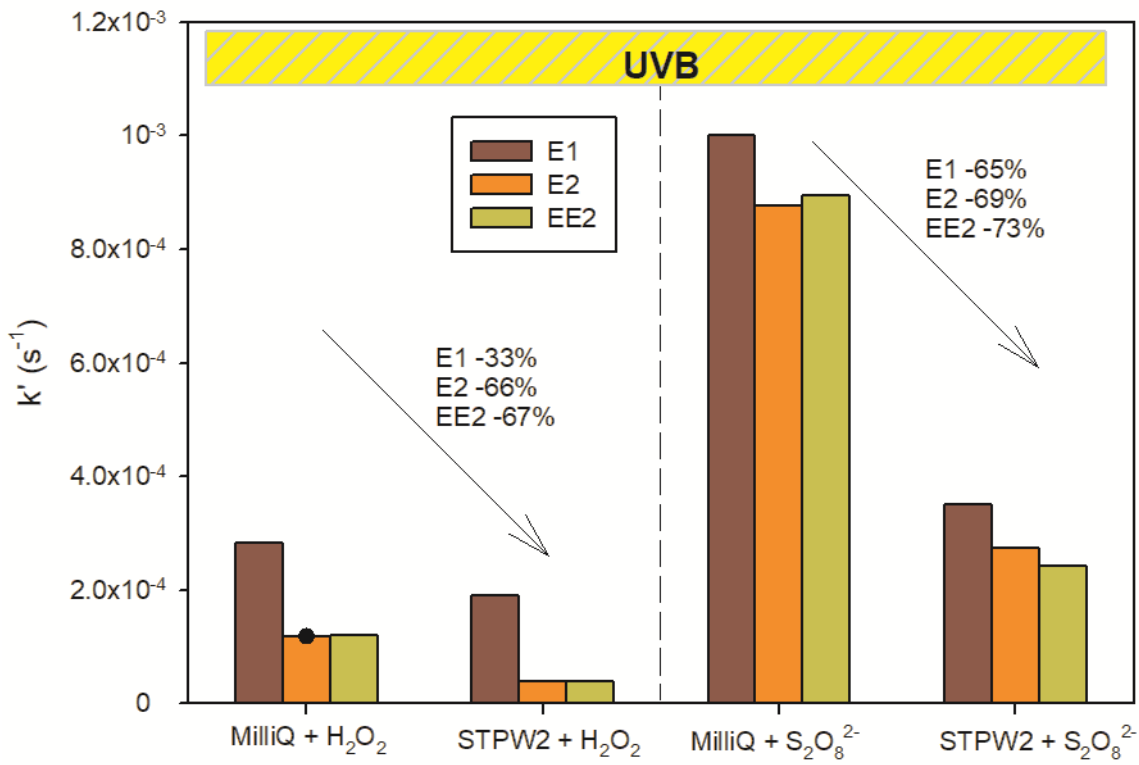
Figure 3. *E1, E2 and EE2 pseudo first-order degradation rate constants in a mixture (5 µM each) under UVB radiation.*
276
277 *Comparison between the use of H_2O_2 and $S_2O_8^{2-}$ (2 mM) as an oxidant precursor and milli-Q water (pH 6) and STP*
278 *wastewater (pH 8) as a matrix. Inhibition percentages between milli-Q water and STPW2 are mentioned.*


279

280 Figure 4. *E1, E2 and EE2 photolysis under UVB radiation in milli-Q water (pH 6.5).*
281

282 *3.4. Estrogenic activity removal and effect of hormone mixture under UVB radiation*

283 The aim of the YES assay was to ensure that the estrogen degradation goes along with a

284 decline in the estrogenic activity of the sample, responsible for its harmfulness. The

285 estrogenic activity of a sample was expressed in E2 equivalent concentration. The degradation

286 of a mixture of the three hormones (0.5 μM each) in milli-Q water under UVB radiation and

287 oxidant precursors (0.1 mM) was investigated. Concentrations of hormones were lower than

288 previous experiments in order to get closer to environmental concentrations, however the ratio

289 estrogen/oxidant precursor was similar to keep consistency. The estrogenic activity of the

290 mixture is reported in Figure 5 and compared to the degradation of each estrogen during UVB

291 irradiation. The use of both oxidant precursors shows a concordance between the decrease in

292 E2 and EE2 concentrations and the decline in estrogenic activity. E1 faster degradation seems

293 to have a minor effect on the estrogenic activity of the solution, which is mainly governed by

294 E2 and EE2 concentrations because of their higher estrogenic potencies. Considering that the

295 estrogenic potency of E2 is 1, those of E1 and EE2 are respectively 0.1 and 1.2 in agreement

296 with literature data (Murk et al., 2002). Theoretical estrogenic activity of the solution based

297 on the estrogenic potencies of each estrogen and on their concentrations have the same order

298 of magnitude than experimental values. Therefore, degradation products do not seem to have

299 a significant impact on the total estrogenic activity of the solutions. This could be due to a low

300 estrogenic activity of the by-products or to the fast disappearance of potentially high

301 estrogenic activity compounds.

302 However, while the estrogen concentration has fallen below 99.9% of the initial concentration

303 after 24 hours when using H_2O_2 and after 3 hours when using $S_2O_8^{2-}$, the estrogenic activity

304 remains around 30 nM which represents almost 3% of the initial estrogenic activity. It is

305 unknown whether this is due to the persisting estrogenic activity potentially caused by

306 degradation by-products, to the proximity with the limit of quantification (about 15 nM), or to

307 an experimental contamination. Anyway, after 24 hours of treatment using H_2O_2 and 3 hours

308 using persulfate, a strong decrease of estrogenic activity, more than 97%, is obtained.

309
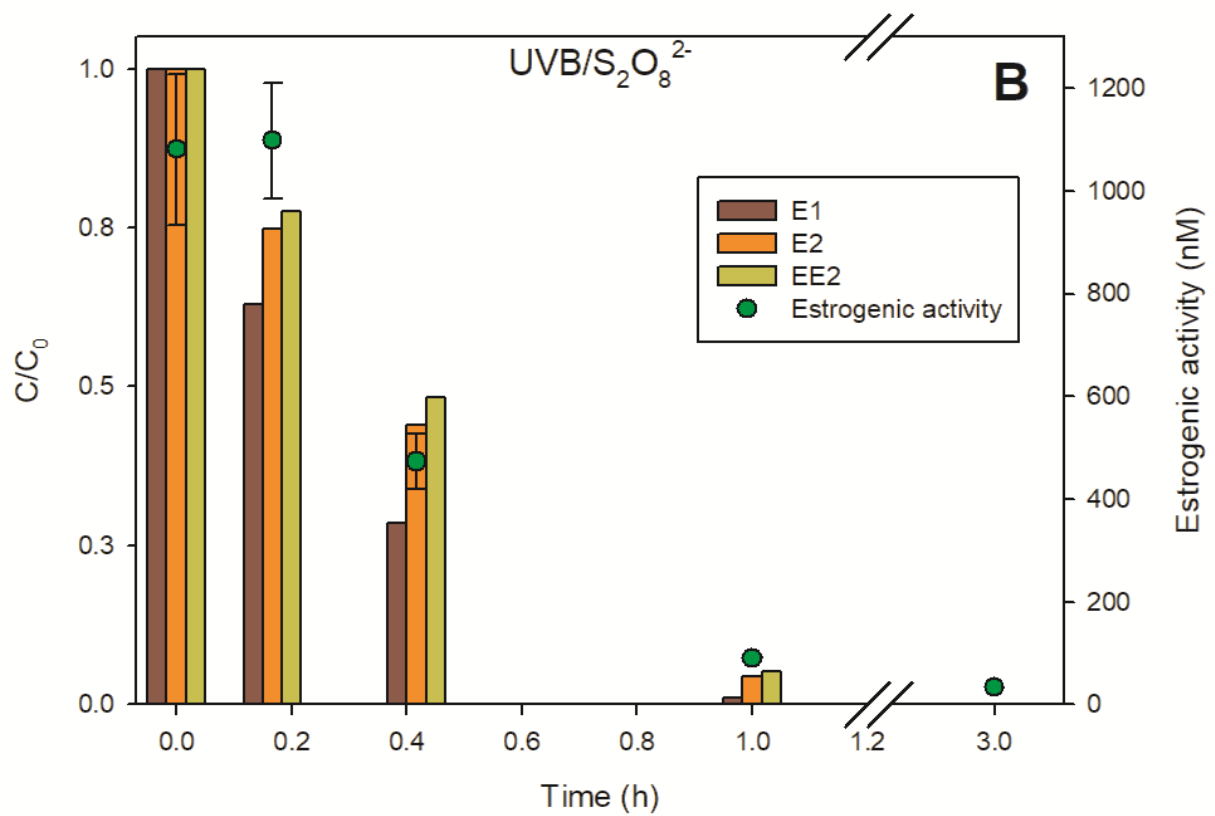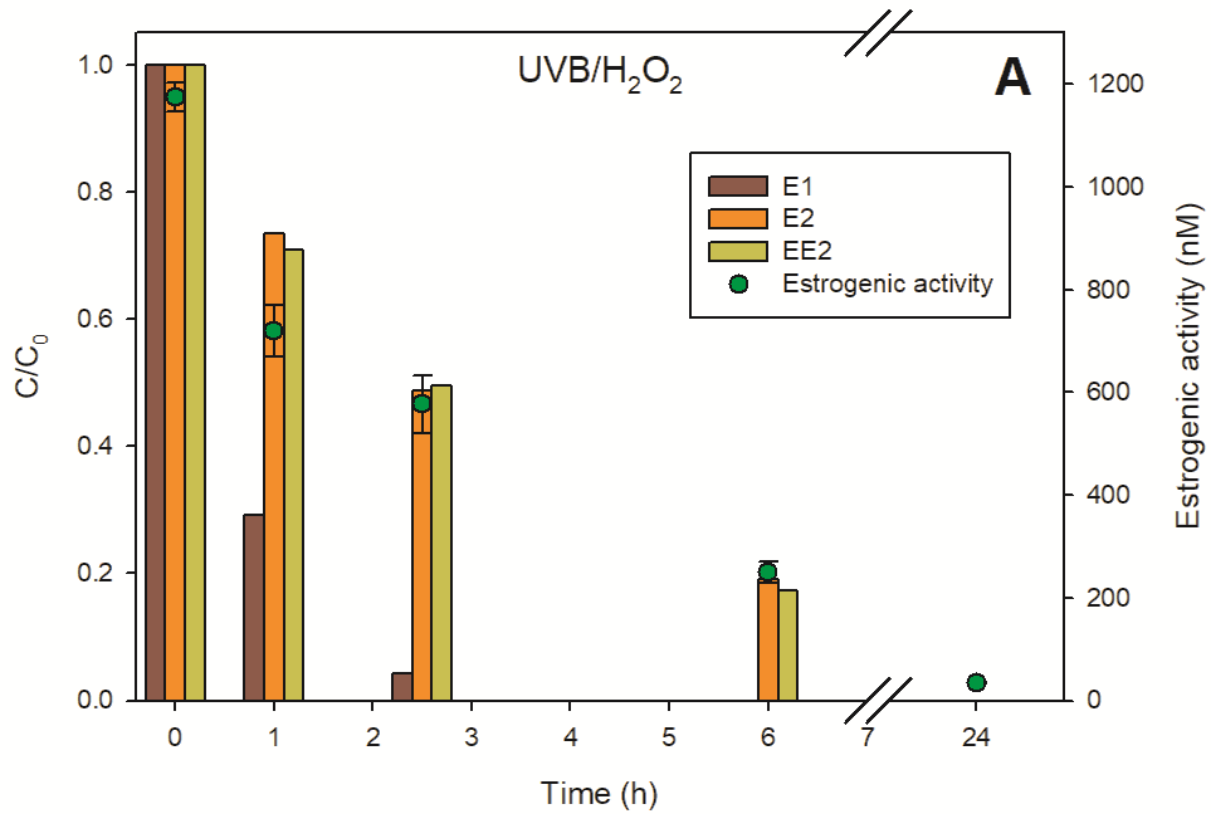
Figure 5. Estrogenic activity assessment of a mixture of E1, E2 and EE2 (0,5 μM each) depending on UVB irradiation time. H_2O_2 0,1 mM in A, $S_2O_8^{2-}$ 0,1 mM in B, pH 6.

312

4. Conclusion

In this work, UVA and UVB photoactivation of hydrogen peroxide and persulfate were tested for the degradation of three common estrogens: E2, E1 and EE2. It was seen that UVA and UVB radiations are both efficient to produce hydroxyl and sulfate radicals, although photo-induced degradation is faster under UVB radiation. However, higher efficiency of sulfate radicals formation was observed compared to the hydroxyl radicals under these irradiation wavelengths. Although an increase in the oxidant precursor concentration produced faster degradation, this phenomenon did not follow a linear trend because the radicals are quenched by the oxidant precursors, particularly with H_2O_2.

In a mixture, E2, E1 and EE2 are competing to react with the generated radicals because the different reaction constants are similar. E1 was seen to degrade faster because it undergoes higher photolysis than E2 and EE2 under UVB radiation. E2 degradation speed was slowed down by approximately 90% in a STP effluent. Experiments have shown that the dissolved carbon and particularly the organic carbon present in the matrix was the main quencher of the hydroxyl and sulfate radicals. The YES assay was seen to give very enriching data confirming that the studied processes allow to remove efficiently the estrogenic activity responsible for the estrogens harmfulness in the environment. These experiments were carried out on a lab-scale. However, further experiments on a pilot or real scale are required to fully assess the different processes efficiency on real wastewaters.

5. Declaration of Competing Interest

All authors declare no conflict of interest.

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