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# 1 Application of Fe(III)-EDDS Complexes and Soybean Peroxidase in Photo-Fenton 2 Processes for Organic Pollutant Removal: Insights into Possible Synergistic Effects

3 Silvia Bertolotti<sup>1,2</sup>, Marco Minella\*<sup>3</sup>, Enzo Laurenti<sup>3</sup>, Marcello Brigante<sup>4</sup>, Gilles Mailhot<sup>4</sup>, Alessandra Bianco  
4 Prevot<sup>3</sup>

5 <sup>1</sup> Department of Life Sciences and System Biology, University of Turin, via Accademia Albertina 13, 10123 Turin, Italy

6 <sup>2</sup>ALPSTREAM – Alpine Stream Research Center, 102030 Ostana, Italy

7 <sup>3</sup>Department of Chemistry, University of Turin, via P. Giuria 7, 10125 Turin, Italy

8 <sup>4</sup> Université Clermont Auvergne, CNRS, Institut de Chimie de Clermont-Ferrand, 63000 Clermont-Ferrand, France.

9 \* marco.minella@unito.it

10

## 11 Abstract

12 Photo-Fenton processes activated by biodegradable Fe(III)-EDDS complexes have attracted huge attention  
13 from the scientific community, but the operative mechanism of the photo-activation of H<sub>2</sub>O<sub>2</sub> in the presence  
14 of Fe(III)-EDDS has not been fully clarified yet.

15 The application of the Fe(III)-EDDS complex in Fenton and photo-Fenton (mainly under UV-B light)  
16 processes, using 4-chlorophenol (4-CP) as a model pollutant was explored to give insights into the operative  
17 mechanism. Furthermore, the potential synergistic contribution of soybean peroxidase (SBP) was investigated,  
18 since it has been reported that upon irradiation of Fe(III)-EDDS the production of H<sub>2</sub>O<sub>2</sub> can occur. SBP did  
19 not boost the 4-CP degradation, suggesting that the possibly produced H<sub>2</sub>O<sub>2</sub> reacts immediately with the Fe(II)  
20 ion with a quick kinetics that does not allow the diffusion of H<sub>2</sub>O<sub>2</sub> into the bulk of the solution (i.e. outside the  
21 solvent cage of the complex). So, a concerted mechanism in which the photochemically produced H<sub>2</sub>O<sub>2</sub> and  
22 Fe(II) react inside the hydration sphere of the Fe(III)-EDDS complex is proposed.

23

24

## 25 Keywords

26 Fe(III)-EDDS, Contaminants of Emerging Concern, 4-chlorophenol, Fenton and photo-Fenton processes,  
27 soybean peroxidase.

28

29

## 30 **1. Introduction**

31 The more and more intensive exploitation of hydric resources due to global population, urbanization  
32 and industrialization increase, is causing a depletion of both their quantity and quality [1]. Moreover,  
33 climate change is worsening the shortage of water due to the altered precipitations and the droughts.  
34 Beside this, water quality has been deteriorating due to the presence of toxic and bio-refractory  
35 compounds not efficiently removed by the traditional wastewater treatment plants. Among these,  
36 Contaminants of Emerging Concern (CECs) are increasingly being detected in surface water,  
37 groundwater, wastewater and drinking water in quantities ranging from ng/L to few  $\mu\text{g/L}$  and are  
38 raising great concern due to their high biopersistence [2-4]. Not strictly regulated nor routinely  
39 monitored, CECs are a heterogeneous group of substances which comprises newly developed  
40 chemicals as well as known compounds whose toxicity has been discovered or re-evaluated (e.g.:  
41 pharmaceuticals, personal care products, pesticides, microplastics and antibiotic resistant bacteria and  
42 genes).

43 As CECs can threaten aquatic and terrestrial organisms and humans [5,6], their abatement has become  
44 a crucial issue in preserving water quality, not only to meet the increasing worldwide water demand,  
45 but also to implement the reuse of reclaimed water thus contributing to tackle water scarcity.  
46 Unfortunately, the biological degradation, adopted as secondary treatments in most wastewater  
47 treatment plants (WWTPs), cannot always ensure the efficient complete removal of any organic  
48 substrate, rather depending on its biorecalcitrant feature; in some cases, even CECs transformation  
49 products with an enhanced eco-toxicity have been detected [7].

50 The Directive 2013/39/EU promotes a preventive action towards the emission of CECs in the  
51 environment, upgrades the watch list of substances that must be monitored as potential sources of  
52 contamination and encourages the development of innovative water treatment technologies [8] by  
53 WWTPs. The goal is to attain the complete removal of the toxic compounds, or at least their  
54 degradation into less harmful by-products, thus minimizing the discharge of pollutants into the  
55 receiving water body.

56 When considering innovative wastewater treatments for CECs removal, in the last decades  
57 increasingly attention has been devoted to the potentiality of Advanced Oxidation Processes (AOP),  
58 previously proposed by Glaze et al. as effective tools for the degradation of organic compounds from  
59 water [9] through the generation of highly reactive radical species. Among these, hydroxyl radical is  
60 a very strong oxidant ( $E_0 = 2.8 \text{ V vs NHE}$ ) and acts unselectively against a wide variety of substances  
61 [10]. The generation of radical species can occur through different pathways, which allows to  
62 distinguish AOPs in a variety of subcategories [11] among them, Fenton and photo-Fenton processes,

63 based on the activation of H<sub>2</sub>O<sub>2</sub> by means of Fe(II) (and eventually light) to generate •OH radicals  
64 and/or other reactive species (e.g. ferryl ion), are receiving an increasing interest also for CECs  
65 removal [12,13,14].

66 One of the main drawbacks of (photo) Fenton processes is the optimum working pH around ≈ 2.8. In  
67 more recent times numerous studies have assessed the ability of Fe(III) complexes to widen the range  
68 of the operating pH, maintaining low costs and even fastening the degradative reactions [15].

69 Ethylenediamine-N,N'-disuccinic acid (EDDS) is a structural isomer of ethylenediaminetetraacetic  
70 acid (EDTA), highly biodegradable in different environmental compartments [16] and a strong iron  
71 chelating agent [17]. Several studies have demonstrated its efficiency in the photo Fenton degradation  
72 of some CECs including endocrine disruptors [18], plastics precursors [19], pathogens [20] and  
73 microcontaminants [21]: Fe(III) is complexed by EDDS with a 1:1 ratio and, under UV irradiation,  
74 is easily photolyzed and generates •OH radicals in a pH range between 3 and 9. However, the  
75 mechanism of photo-activation of H<sub>2</sub>O<sub>2</sub> during the process has not been fully clarified yet.

76 On the other hand, chemical processes are not the only valuable methods for the removal of emerging  
77 contaminants: biological processes employing microorganisms and enzymes have been studied for  
78 the same objective and one recent approach for wastewater treatment relies on the coupling of AOPs  
79 and biological methods [22].

80 Soybean peroxidase (SBP: EC 1.11.1.7) is an oxidoreductase that can be isolated from soybean  
81 (*Glycine max*, L.) seed hulls; it has shown a good ability to degrade compounds like phenols and  
82 chlorophenols [23] and synthetic dyes [24]. Moreover, the great chemical and thermal stability make  
83 this enzyme interesting for possible application in wastewater treatment on a larger scale.

84 Calza et al. made the first attempt to couple SBP with an AOP (i.e.: photocatalysis with TiO<sub>2</sub>) and  
85 observed an enhanced degradation of the target substrates compared to the use of SBP and TiO<sub>2</sub> alone  
86 [25].

87 It is noteworthy that soybean today represents one of the major industrial and food crops grown in  
88 every continent, with a global production of 368.12 million metric tons in 2020-2021 [26]: SBP from  
89 soybean seed hulls is then widely available and relatively cheap and its exploitation in wastewater  
90 treatment could represent both an economical advantage, and a valuable example of waste recovery  
91 and reuse.

92 Though a synergy between SBP and Fe(II) salts has been tested already [27], as far as we know the  
93 effects of the co-presence of the enzyme and Fe(III)-EDDS have never been investigated before.

94 Based on the above premises, the present work aims *i*) to give insights on the Fe(EDDS) working  
95 mechanism in photo-Fenton reaction, by considering in details the role of pH, concentration of  
96 reagents and presence of O<sub>2</sub> and *ii*) to investigate the effect of the contemporary presence of SBP and  
97 Fe(III)-EDDS on the removal of 4-chlorophenol (4-CP) from water solution, exploring for the first  
98 time the possibility of a synergy between the two systems. Furthermore, the photo-reactivity of  
99 Fe(III)-EDDS in the presence of SBP may offer additional information on the mechanistic functioning  
100 of the complex, especially on the availability and reactivity of H<sub>2</sub>O<sub>2</sub>, which is hypothesized to be  
101 photo-produced during the photo-Fenton process but has never been measured in an effective way.

102

## 103 **2. Material and methods**

### 104 **2.1 Chemicals**

105 Iron (III) chloride hexahydrate (FeCl<sub>3</sub>·6H<sub>2</sub>O), ferric perchlorate (Fe(ClO<sub>4</sub>)<sub>3</sub>·9H<sub>2</sub>O), S,S'-ethylenediamine-  
106 N,N'-disuccinic acid trisodium salt (EDDS, 35% in water), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), 4-chlorophenol (4-CP),  
107 2-propanol (2-prop), acetonitrile, phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), methanol and potassium iodide (KI) were obtained  
108 from Sigma Aldrich. Ammonium heptamolybdate ((NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·H<sub>2</sub>O) and potassium hydrogen phthalate  
109 (C<sub>8</sub>H<sub>5</sub>KO<sub>5</sub>) were from Merck. Sodium hydroxide (NaOH) was from Alfa Aesar. Soybean peroxidase (SBP)  
110 was from BioResearch Products. All the chemical reagents were used as received. Suspensions and standard  
111 solutions were prepared in Milli-Q<sup>®</sup> water.

112 The Fe(III)-EDDS complex solution was prepared by mixing appropriate amounts of FeCl<sub>3</sub> and EDDS salt  
113 solution with 1:1 stoichiometry. The complex was freshly prepared before each experiment.

### 114 **2.2 Irradiation setup and degradation procedure**

115 For the degradation of 4-CP via photo-Fenton process a fluorescent lamp (TL 20W/01, Philips, Netherlands)  
116 with a total flux of 7.54 W m<sup>-2</sup> in the 295-400 nm range was employed. Experiments were performed in closed  
117 Pyrex glass cells filled with 5 mL of solution under magnetic stirring and irradiated for different times (0 to  
118 120 min). After every sampling, made at fixed intervals of time, 1.0 mL of methanol was added to the 5 mL  
119 of the suspension to quench the Fenton reaction. The initial concentration of 4-CP was 0.1 mM. All the  
120 experiments were carried out at room temperature (293±2 K) and, except for the experiments at varying pH  
121 values, the pH was adjusted at a value of ≈ 5.5 using H<sub>3</sub>PO<sub>4</sub> and NaOH. pH values of the solutions were  
122 measured using a 913 Metrohm pH meter.

### 123 **2.3 Analysis**

124 The concentration of 4-chlorophenol remaining in the aqueous solution was measured through a YL9300  
125 HPLC system equipped with a YL9330 Column Compartment and a YL9150 autosampler. The column was a

126 RP C18 column (LiChroCART®, Merck, 12.5 cm × 0.4 cm; 5 μm packing). 4-chlorophenol was analyzed in  
127 isocratic mode, using a 30:70% v/v acetonitrile: phosphoric acid solution (1×10<sup>-2</sup> M) as mobile phase. The  
128 experiments were performed by UV detection at 220 nm, the flow rate was of 1 mL min<sup>-1</sup>. In these conditions,  
129 the retention time of 4-CP was 8.8 min.

130 The decomposition of H<sub>2</sub>O<sub>2</sub> was monitored using the method outlined by Klassen et al. [28], which is accurate  
131 to H<sub>2</sub>O<sub>2</sub> concentrations as low as 1 μM. This method employs an ammonium molybdate catalyzed reaction  
132 between H<sub>2</sub>O<sub>2</sub> and I<sup>-</sup> to form I<sub>2</sub> (iodine). I<sub>2</sub> then reacts with free I<sup>-</sup> ions in solution to form the I<sub>3</sub><sup>-</sup> ion which can  
133 be measured using optical absorption at λ = 350 nm. The spectrophotometric analyses were performed using a  
134 Varian CARY 100 Scan double-beam UV–vis spectrophotometer, using glass cuvettes with 1 cm path length.

135 The repetition of degradation experiments in the same conditions gave an overall relative standard deviation  
136 (RSD) for the concentration of 4-CP and H<sub>2</sub>O<sub>2</sub> of roughly 10%.

137

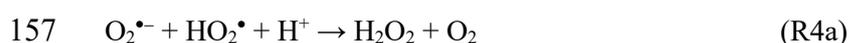
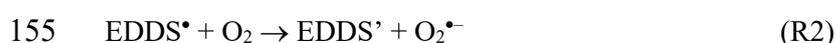
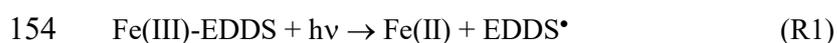
### 138 3. Results and discussion

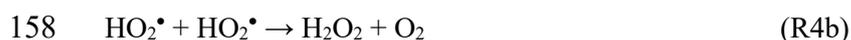
139 The initial concentrations of 4-CP (0.1 mM), Fe(III)-EDDS (0.1 mM) and H<sub>2</sub>O<sub>2</sub> (0.1-0.2 mM) were chosen in  
140 agreement with previous works [29,30]. The choice of an UV-B irradiation, despite of the previously  
141 investigated UV-A, was carried out not only to test different conditions, but also to maximize the overlap  
142 between the emission spectrum of the used lamp(s) and the absorption spectrum of Fe(III)-EDDS (Fig. S1 of  
143 the SI).

144

#### 145 3.1 Degradation kinetics of 4-CP in photo-Fenton processes

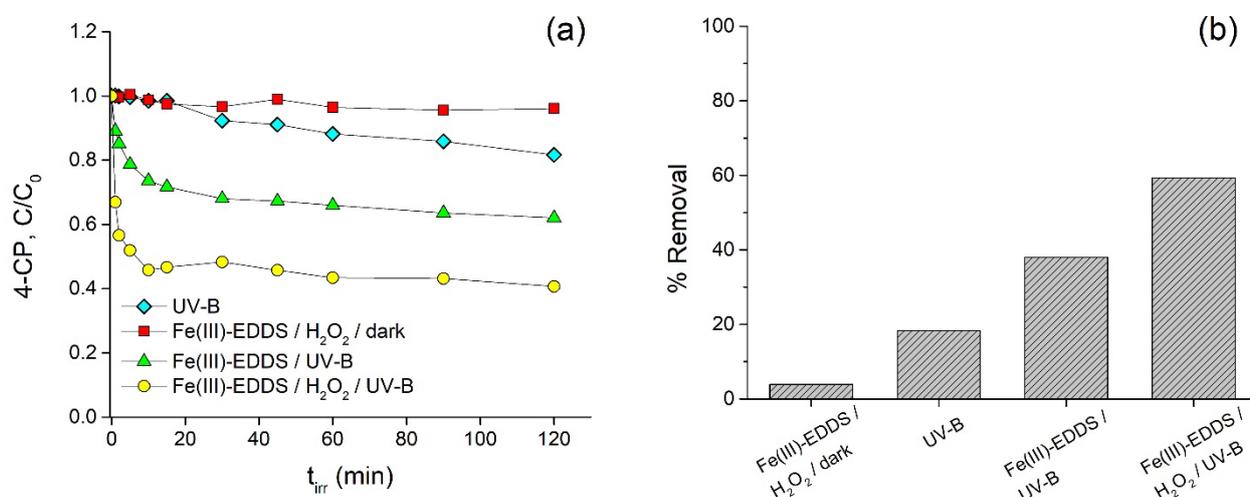
146 Fig. 1 shows the effects of the presence of Fe(III)-EDDS, H<sub>2</sub>O<sub>2</sub> and the role of UV-B irradiation on the  
147 concentration of 4-CP. The direct photolysis of 4-CP under irradiation was also evaluated and, as expected  
148 from the absence of significant absorption at λ > 300 nm, a limited degradation of 4-CP was observed (≈18%  
149 after 120 minutes of irradiation). Furthermore, in the dark the complex Fe(III)-EDDS was not able to activate  
150 H<sub>2</sub>O<sub>2</sub> and to promote the removal of the substrate, as manifested from the stability of 4-CP in the presence of  
151 both Fe(III)-EDDS and H<sub>2</sub>O<sub>2</sub>. On the contrary, the UV-B irradiation of Fe(III)-EDDS activated the production  
152 of reactive species (e.g. •OH and HO<sub>2</sub>•/O<sub>2</sub>•<sup>-</sup>) also in the absence of additional oxidants (i.e. H<sub>2</sub>O<sub>2</sub>), in agreement  
153 with the reactions (R1-R5 [29,30]).





160 The addition of  $\text{H}_2\text{O}_2$  increased the degradation of 4-CP up to 59% after 120 minutes, in agreement with the  
 161 fast reaction between the added  $\text{H}_2\text{O}_2$  and the Fe(II) photochemically generated from Fe(III)-EDDS photolysis  
 162 (R1).

163 The profiles of 4-CP concentration under irradiation gave some additional insights. The UV-B irradiation  
 164 activated a fast initial degradation ( $t_{\text{irr}} < 15$  min) and a following step in which a slower (at least null) degradation  
 165 was observed. This behaviour deserved to be investigated, so experiments at different doses of both  $\text{H}_2\text{O}_2$  and  
 166 Fe(III)-EDDS were carried out (*vide infra*).



167  
 168 **Fig. 1** (a) Degradation of 4-CP in presence and absence of Fe(III)-EDDS,  $\text{H}_2\text{O}_2$  and irradiation; (b) % removal of 4-CP  
 169 after 120 min of irradiation. Conditions:  $[\text{4-CP}]_0 = 0.1$  mM,  $[\text{Fe(III)-EDDS}] = 0.1$  mM,  $[\text{H}_2\text{O}_2] = 0.2$  mM, initial pH = 5.5  
 170

171 **3.2 Effect of  $\text{H}_2\text{O}_2$  concentration under irradiated Fe(III)-EDDS**

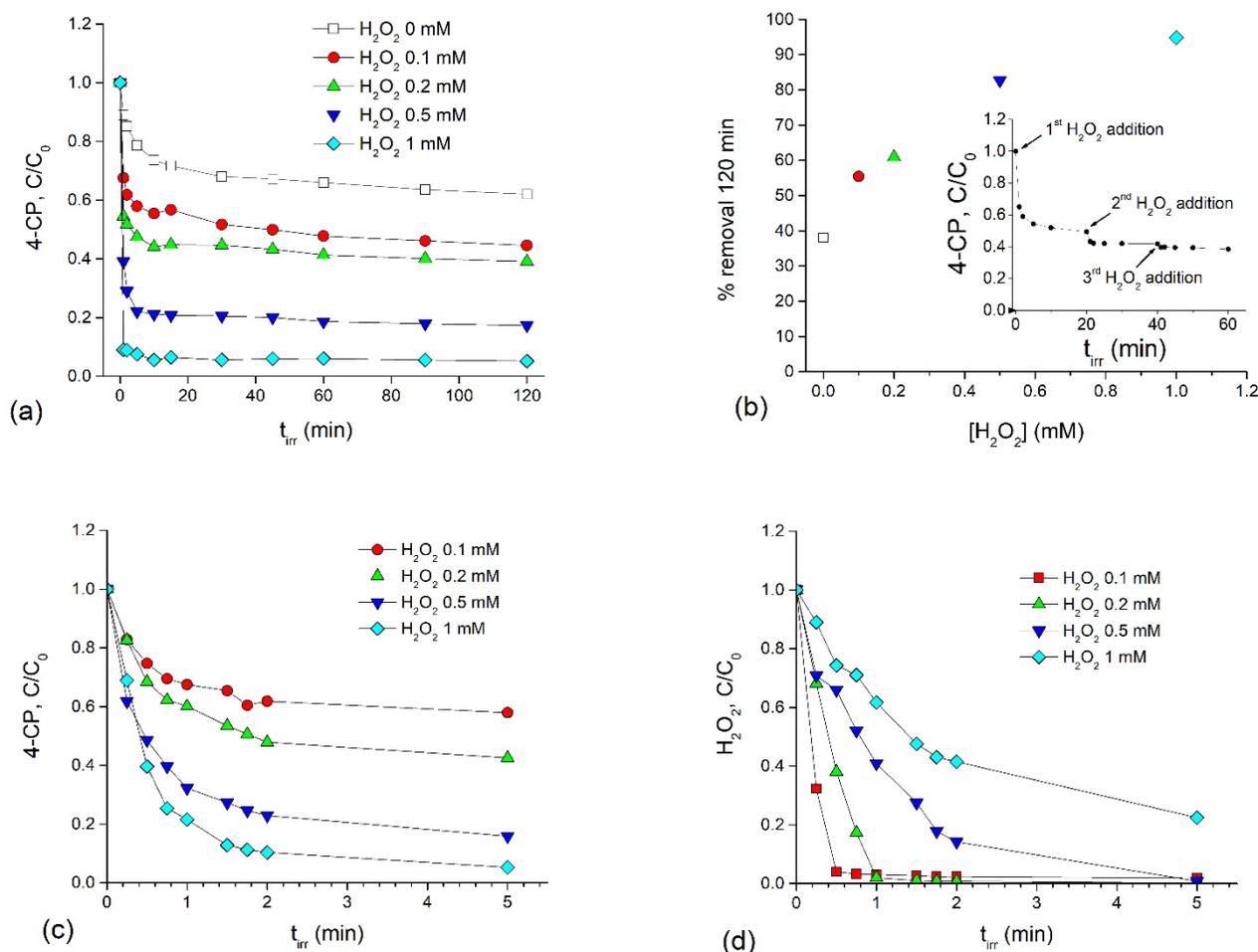
172 Fig. 2a shows the degradation of 4-CP under irradiation and in the presence of different concentrations of  
 173 hydrogen peroxide (from 0 to 1 mM). Fig. 2b summarises the % removal after 120 min as a function of the  
 174 initial  $[\text{H}_2\text{O}_2]$ . The same concentration profiles previously commented were observed, with most of the  
 175 degradation taking place in the first minutes of the irradiation time (<10-15 minutes). The addition of an  
 176 increasing amount of  $\text{H}_2\text{O}_2$  resulted in a rising % of 4-CP removal with saturating behaviour (see Fig. 2b),  
 177 indicating that  $\text{H}_2\text{O}_2$  could be the limiting reagent for the process in the explored conditions. In this light, the  
 178 effect of sequential additions of  $\text{H}_2\text{O}_2$  was studied (see Fig. 2b inset) and a profile with 3 sequential drops of  
 179 the 4-CP was observed. Note that during the addition of  $\text{H}_2\text{O}_2$  no further Fe(III)-EDDS was added. The effect  
 180 of  $\text{H}_2\text{O}_2$  addition was less effective after the first step, with a decrease of the degradation in absolute value  
 181 after the following additions. This highlighted that (i)  $\text{H}_2\text{O}_2$  played as a limiting reagent for the process and  
 182 (ii) the catalytic properties of Fe(III)-EDDS faded as a consequence of the prolonged treatment (in agreement  
 183 with a limited catalytic role for Fe(III)-EDDS in the explored experimental conditions).

184 Note that under UV-B  $\text{H}_2\text{O}_2$  can undergo direct photolysis ( $\text{H}_2\text{O}_2 + h\nu \rightarrow 2 \cdot\text{OH}$ ) as a consequence of its small  
185 absorption tail at  $\lambda > 300$  nm. The 4-CP degradation in the presence of  $\text{H}_2\text{O}_2$  0.1 mM and in the absence of the  
186 iron complex was investigated. After 120 minutes, a 40% removal of 4-CP was reached (data not reported). In  
187 the same conditions but with the addition of Fe(III)-EDDS 0.1 mM the removal was 55%. In the presence of  
188 the iron complex, this latter was the major photon absorber as the molar absorption coefficient of Fe(III)-  
189 EDDS at 313 nm is at least 3 orders of magnitude higher than that of  $\text{H}_2\text{O}_2$ . As a consequence, the role of the  
190 direct photolysis of  $\text{H}_2\text{O}_2$  in the presence of Fe(III)-EDDS was negligible.

191 To give insights into the role of  $\text{H}_2\text{O}_2$ , the concentration of 4-CP and  $\text{H}_2\text{O}_2$  was monitored at short irradiation  
192 time ( $\leq 5$  minutes) in experiments at the same concentration of 4-CP (0.1 mM) and increasing amounts of  $\text{H}_2\text{O}_2$   
193 (from 0.1 to 1 mM). Fig. 2c and 2d show the 4-CP and  $\text{H}_2\text{O}_2$  concentration profiles, respectively. At the lowest  
194 concentration of oxidant (0.1 and 0.2 mM)  $\text{H}_2\text{O}_2$  was consumed within 1 minute of irradiation, while at the  
195 highest concentration (1 mM) a residual quantity of  $\text{H}_2\text{O}_2$  was observed even after 5 minutes of irradiation.

196 From the comparison of the two profiles, it is manifest that (i) the complete disappearance of  $\text{H}_2\text{O}_2$  did not  
197 coincide with the stop of the 4-CP degradation, that continued also in the absence of  $\text{H}_2\text{O}_2$ , (ii) at the maximum  
198 concentration of  $\text{H}_2\text{O}_2$ , 4-CP reached its plateau value in the presence of a residual amount of  $\text{H}_2\text{O}_2$  that was  
199 not able to further promote its degradation. These experimental results highlighted the essential role of  $\text{H}_2\text{O}_2$   
200 in activating the first and fast step of 4-CP degradation (and that higher is the concentration of oxidant, higher  
201 is the % 4-CP removal). However, the decline of the degradation ability of the system might be ascribed also  
202 to the faded catalytic properties of the Fe(III)-EDDS complex (which was not recycled or was only partially  
203 recycled after the activation of  $\text{H}_2\text{O}_2$ ).

204



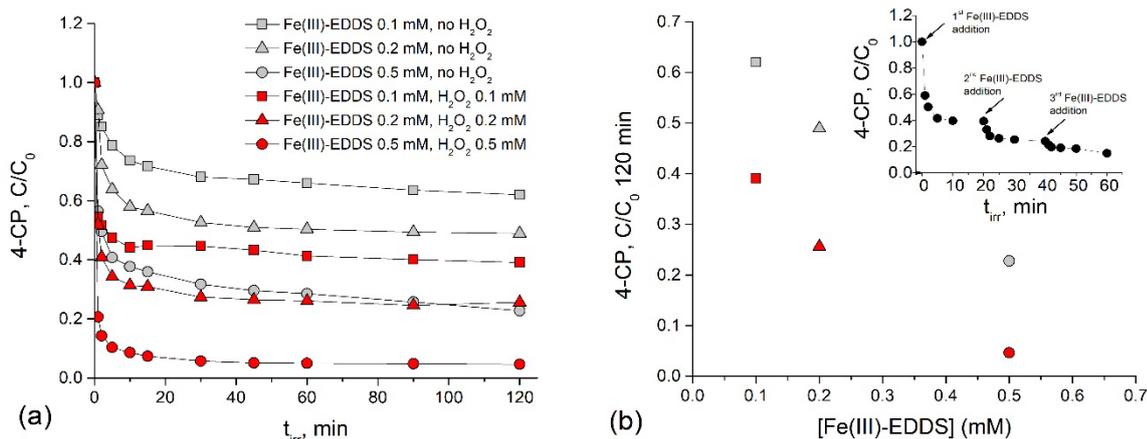
205

206 **Fig. 2** (a) Photodegradation of 4-CP at different concentrations of  $\text{H}_2\text{O}_2$ ; (b) % removal of 4-CP after 120 min of  
 207 irradiation. Inset: 4-CP degradation after 3 consecutive additions (every 20 minutes) of  $\text{H}_2\text{O}_2$  0.1 mM.  
 208 Phototransformation of 4-CP (c) and  $\text{H}_2\text{O}_2$  (d) at different concentrations of  $\text{H}_2\text{O}_2$  ( $t_{\text{irr}} \leq 5$  minutes). Conditions:  $[\text{4-CP}]_0$   
 209 = 0.1 mM,  $[\text{Fe(III)-EDDS}] = 0.1$  mM,  $[\text{H}_2\text{O}_2] = 0$ -1 mM, initial pH = 5.5.

210

### 211 3.3 Effect of Fe(III)-EDDS concentration

212 Fig. 3 shows the degradation profiles and the residual fraction of 4-CP after 120 minutes of irradiation in  
 213 presence of increasing concentrations of Fe(III)-EDDS without and with  $\text{H}_2\text{O}_2$  0.1 mM. With the rising  
 214 concentration of the iron complex - both with and without  $\text{H}_2\text{O}_2$  - an increase of the 4-CP degradation was  
 215 observed. At higher complex concentration, an increase of the photo-formed Fe(II) and consequently of  $\bullet\text{OH}$   
 216 (or alternative reactive species) production was observed. In all the cases the process slowed down after the  
 217 first step of degradation and the 4-CP concentration reached a plateau. Without  $\text{H}_2\text{O}_2$  (i) the 4-CP residual  
 218 fraction was - at all the explored Fe(III)-EDDS concentrations -  $\approx 30\%$  lower than in the presence of  $\text{H}_2\text{O}_2$ ; (ii)  
 219 the degradation rate of the initial step was significantly lower in absence of hydrogen peroxide than in presence  
 220 - being  $\text{H}_2\text{O}_2$  not immediately at disposal of the photo-produced Fe(II).



221

222 **Fig. 3** (a) Photodegradation of 4-CP at different concentrations of Fe(III)-EDDS; (b) % removal of 4-CP after 120 min of  
 223 irradiation. Inset: 4-CP degradation after 3 consecutive additions (every 20 minutes) of Fe(III)-EDDS 0.1 mM.  
 224 Conditions:  $[4-CP]_0 = 0.1$  mM,  $[Fe(III)-EDDS] = 0.1-0.5$  mM,  $[H_2O_2] = 0.1$  mM, initial pH = 5.5.

225

226 The inset of Fig. 3b shows the effect of successive addition of Fe(III)-EDDS 0.1 mM (every 20 minutes). Note  
 227 that the second addition was carried out in a solution in which  $H_2O_2$  concentration was null (see Fig. 2d). The  
 228 addition of the sole Fe(III)-EDDS gave effects similar to those observed with the additions of  $H_2O_2$  alone: a  
 229 further, but less evident, 4-CP degradation after each addition. Lastly, a test with sequential additions of both  
 230  $H_2O_2$  0.1 mM and Fe(III)-EDDS 0.1 mM was carried out and compared with the profiles measured with  
 231 sequential additions of  $H_2O_2$  and Fe(III)-EDDS alone (see Fig. S3). The simultaneous addition of both reactants  
 232 gave the best results, suggesting that the real active reagent in the UV-B activated photo-Fenton process (with  
 233 ED DS as ligand for  $Fe^{+3}$ ) is the couple iron-complex +  $H_2O_2$ , and that catalytic cycles able to regenerate a  
 234 photoactive catalyst are scarcely operative. The limited (but not null) effect of the third addition of  $H_2O_2$  +  
 235 Fe(III)-EDDS can be explained with the progressive accumulation of organic by-products (e.g. products of  
 236 Fe(III)-EDDS UV-B photolysis and 4-CP degradation) competing with 4-CP for the same oxidative reactive  
 237 species.

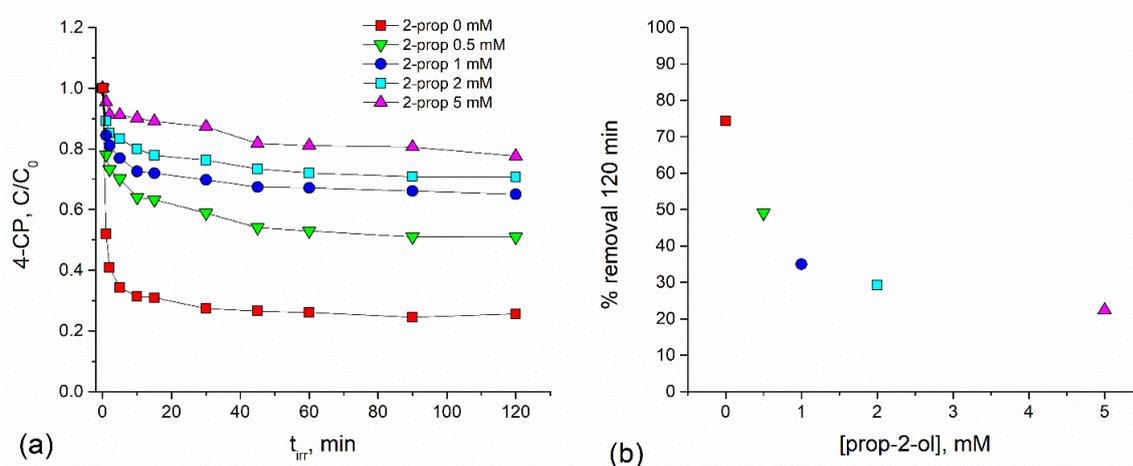
238 To summarise the results of the experiments of Paragraph 3.2 and 3.3, we argue that the initial sharp  
 239 degradation of 4-CP has to be attributed to radical species as  $\bullet OH$ ,  $HO_2\bullet$  or  $O_2^{\bullet -}$  resulting from the photolysis  
 240 of Fe(III)-EDDS, rather than to the direct photolysis of  $H_2O_2$ . These species can be formed even in the absence  
 241 of  $H_2O_2$ , as previously reported in other studies [19,30]. The main role of Fe(III)-EDDS in the process is  
 242 supported by its higher molar absorption coefficient in the UV-B ( $\lambda_{max} = 313$  nm). Nevertheless, the  $H_2O_2$  that  
 243 rapidly reacts with the Fe(II) is crucial in increasing the % of 4-CP degradation.

244

### 245 3.4 Reactive species involved in the photo-Fenton process: role of hydroxyl radicals

246 With the aim to investigate the role of  $\bullet OH$ , the degradation of 4-CP in the presence of Fe(III)-EDDS and  $H_2O_2$   
 247 was investigated with increasing concentrations of propan-2-ol. This compound is an efficient scavenger for  
 248  $\bullet OH$  ( $k_{2-prop, HO\bullet} = 1.9 \times 10^9$  M $^{-1}$  s $^{-1}$  [31]).

249 Fig. 4 shows the effect of propan-2-ol addition on the 4-CP degradation profiles (Fig. 4a) and the % removal  
 250 ( $t_{irr}=120$  min) as a function of the propan-2-ol concentration (Fig. 4b). Propan-2-ol had a clear inhibitory effect  
 251 on the maximum % removal reached at the end of the irradiation and on the initial rate of 4-CP transformation  
 252 in the first minutes of irradiation. This is a clear evidence of the predominant role of the  $\bullet$ OH radicals in the 4-  
 253 CP degradation under UV-B and in the presence of Fe(III)-EDDS and  $H_2O_2$ . Furthermore, Fig. 4 shows that  
 254 the reactivity with  $\bullet$ OH cannot totally explain the substrate removal: with the increase of the scavenger  
 255 concentration, the % removal decreased monotonically down to a plateau value equal to  $\approx 15\%$ . This is in  
 256 agreement with an operative role of alternative (even if minor) reactive species (e.g. ferryl ion) that were not  
 257 totally scavenged by propan-2-ol. In similar experimental conditions but in the presence of chloroform, Huang  
 258 et al. [29] demonstrated an important role of  $HO_2\bullet/O_2^{\bullet-}$  not as reactive species involved in the removal of the  
 259 organic substrate, but as reductants for Fe(III)-EDDS responsible for the indirect generation of  $\bullet$ OH.



260 (a) Photodegradation of 4-CP at increasing concentration of propan-2-ol (0-5 mM). (b) % removal of 4-CP after  
 261 120 min of irradiation. Conditions:  $[4-CP]_0 = 0.1$  mM,  $[Fe(III)-EDDS] = 0.3$  mM,  $[H_2O_2] = 0.2$  mM, initial pH = 5.5.  
 262  
 263

264 The degradation of 4-CP with Fe(III)-EDDS/ $H_2O_2$ /UV-B at different pH values (from 3 to 8) and the role of  
 265 the dissolved oxygen were also investigated. The results (reported in paragraphs 2.1 and 2.2 of SI) were in  
 266 agreement with the previous reports about the photo-Fenton process with Fe(III)-EDDS [30].

267

### 268 3.5 Effect of SBP addition

269 The catalytic activity of SBP for the oxidation of phenolic compounds with  $H_2O_2$  has been documented [32],  
 270 together with its potential application for the removal of organic pollutants, both in homogeneous conditions  
 271 [33] and as supported catalyst [34]. Furthermore, synergistic effects between advanced oxidation processes  
 272 where  $H_2O_2$  is produced (e.g. heterogeneous photocatalysis under irradiated semiconductors) and the presence  
 273 of SBP has been previously reported [25]. As mentioned in Paragraph 3.1, from the existing literature on  
 274 Fenton processes it is hypothesised that  $H_2O_2$  could be photoproducted during the photo Fenton reaction (R1-  
 275 R4): the radical EDDS $\bullet$  generated by the photolysis of Fe(III)-EDDS complex would react with the dissolved  
 276 oxygen and generate  $O_2^{\bullet-}$ , which is able to disproportionate (R4a-R4b) and to produce further  $H_2O_2$  and  $O_2$ .

277 The H<sub>2</sub>O<sub>2</sub> can then react with the Fe(II) resulting from the same photolysis of Fe(III)-EDDS and start a Fenton  
278 reaction that would enhance the final total degradation of the substrate. In this light, the effect of SBP on the  
279 removal of 4-CP in the presence of Fe(III)-EDDS (both in dark and under irradiation) has been investigated  
280 with the aim to understand if the H<sub>2</sub>O<sub>2</sub> possibly produced under the irradiation of the Fe(III)-EDDS complex  
281 can be efficiently used by SBP to boost the transformation rate of 4-CP. Further goal of the work was to give  
282 insights into the mechanism of transformation of organic compounds in photo-Fenton processes activated by  
283 Fe(III)-EDDS.

### 284 3.5.1 Activity of SBP in dark

285 In the dark, 4-CP was efficiently removed through the SBP catalysed reaction with H<sub>2</sub>O<sub>2</sub>: after 120 minutes of  
286 treatment with SBP 0.01 μM and H<sub>2</sub>O<sub>2</sub> 0.1 mM ([H<sub>2</sub>O<sub>2</sub>]/[SBP] ratio = 10,000) the remaining concentration of  
287 4-CP in solution was ≈10% of its initial concentration (Fig. 5a). When the same experiment was carried out  
288 adding Fe(III)-EDDS 0.1 mM, a significant decrease in the overall removal of 4-CP was observed. Indeed,  
289 after 120 minutes, only a degradation efficiency of ≈50% was reached. On the other hand, changing the  
290 concentration of SBP ([H<sub>2</sub>O<sub>2</sub>]/[SBP] ratio from 100,000 to 1000), both the initial degradation rate and the C/C<sub>0</sub>  
291 value at the end of the reaction changed as a function of SBP concentration (Fig. 5a). Moreover, at the highest  
292 concentration of SBP an almost quantitative removal of 4-CP was reached in 5 minutes from the addition of  
293 H<sub>2</sub>O<sub>2</sub>, also in the presence of the Fe(III)-EDDS complex.

294 These results did not indicate a cooperative effect between SBP and the Fe(III)-EDDS complex in the dark,  
295 but rather a possible inhibition of SBP by Fe(III)-EDDS, or a competition between SBP and Fe(III)-EDDS for  
296 H<sub>2</sub>O<sub>2</sub>.

297

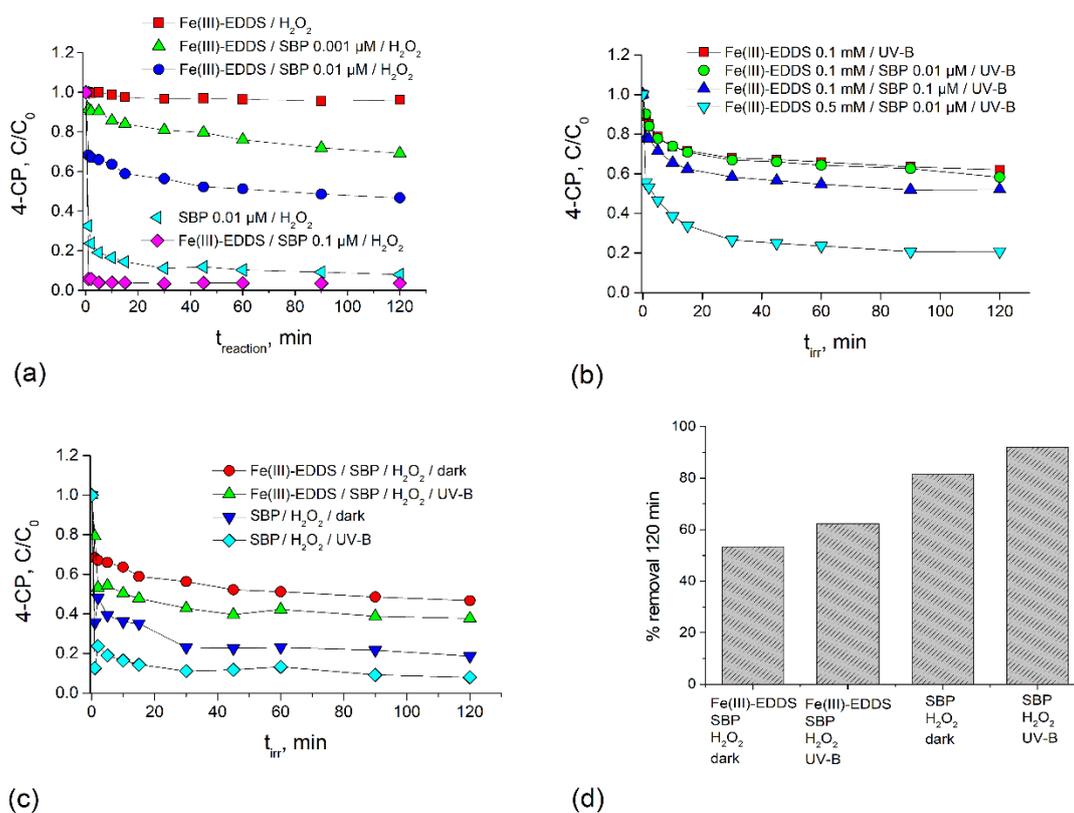
### 298 3.5.2 Activity of SBP under irradiation

299 The experiments under UV-B irradiation reported in Fig. 5b were carried out to better clarify the results  
300 obtained in the dark and investigate if the H<sub>2</sub>O<sub>2</sub> potentially formed under irradiation of Fe(III)-EDDS could be  
301 effectively used by SBP for boosting the 4-CP degradation. For this reason, no H<sub>2</sub>O<sub>2</sub> was initially added to the  
302 solution. Furthermore, the concentration of H<sub>2</sub>O<sub>2</sub> was also monitored since the first minutes of irradiation  
303 through the fluorimetric method reported by Lazrus et al. [35]. The measured concentrations were always  
304 lower than the LOD of the applied analytical method (1.2×10<sup>-8</sup> M [35]).

305 Fig. 5b shows the 4-CP concentration as a function of the treatment time under UV-B in the presence of  
306 different concentrations of Fe(III)-EDDS and SBP. Comparing the degradation profiles in the presence of  
307 Fe(III)-EDDS 0.1 mM alone and in the presence of SBP (0.01 μM), no significant difference was manifest and  
308 an overall final degradation of ≈40% was observed. This can be explained considering that the Fe(II) generated  
309 by the photolysis of Fe(III)-EDDS is immediately used by the photoproduct H<sub>2</sub>O<sub>2</sub>, giving hydroxyl radicals.  
310 In this light, there is a competition between Fe(II) and SBP for the photoproduct H<sub>2</sub>O<sub>2</sub> that, on the basis of  
311 this experimental evidence, is almost totally shifted towards the Fe(II) ion, thus limiting the SBP action. When

312 SBP concentration was increased (0.1  $\mu\text{M}$ ), the overall degradation of 4-CP slightly increased from 35 to 40%,  
 313 whereas a more evident increment in the degradation of 4-CP was observed by increasing the Fe(III)-EDDS  
 314 concentration from 0.1 to 0.5 mM at the same SBP concentration (0.01  $\mu\text{M}$ ), giving a clear indication that the  
 315 role of Fe(III)-EDDS on the overall degradation mechanism was higher - in the tested experimental conditions  
 316 - than that of SBP.

317 Further experiments with SBP were carried out under irradiation with a fixed initial concentration of  $\text{H}_2\text{O}_2$   
 318 (Fig. 5c). In this case, the degradation performed by the system Fe(III)-EDDS/SBP/ $\text{H}_2\text{O}_2$  under UV-B was  
 319 higher than in the dark (removal equal to 62 and 52%, respectively), but not so high to be explained as the sum  
 320 of the two distinguished contributions (reactivity of SBP in dark and photoreactivity of Fe(III)-EDDS).  
 321 Furthermore, removing the Fe(III)-EDDS a higher 4-CP degradation was observed, even if slightly lower than  
 322 the degradation observed in the correspondent dark conditions (removal equal to 81 and 92%, respectively).  
 323 So, the detrimental effect of Fe(III)-EDDS on the SBP activity was effective under irradiation too and a partial  
 324 photo-deactivation of SBP under UV-B was observed. To limit the photo-deactivation of the SBP, the  
 325 degradation of 4-CP in the presence of Fe(III)-EDDS alone and with Fe(III)-EDDS + SBP was also monitored  
 326 under UV-A irradiation (data not shown). As with UV-B, no significant difference was observed: the absence  
 327 of a synergistic effect between Fe(III)-EDDS and SBP cannot therefore be attributed to a detrimental effect of  
 328 the UV-B on the structure of the enzyme.



329 (c) 330 **Fig. 5** (a) Concentration of 4-CP as a function of the treatment time in dark conditions in presence of Fe(III)-EDDS (0.1  
 331 mM, if present),  $\text{H}_2\text{O}_2$  (0.1 mM) and SBP (0.001, 0.01 and 0.1  $\mu\text{M}$ ); (b) Concentration of 4-CP as a function of the  
 332 treatment time under UV-B irradiation in presence of different concentrations of Fe(III)-EDDS and SBP, all the  
 333 experiments were carried out without  $\text{H}_2\text{O}_2$ ; (c) Concentration of 4-CP as a function of the treatment time under UV-B  
 334 and in the dark with 0.1 mM initial  $\text{H}_2\text{O}_2$  concentration; (d) % removal of 4-CP after 120 minutes of reaction. Composition  
 335 of each solution reported in the figure's legend

336 The experimental evidence reported above gives some important mechanistic information. Considering that *i*)  
337 the second order kinetic constant for the reaction between SBP and H<sub>2</sub>O<sub>2</sub> is quite high ( $k_{SBP,H_2O_2} = 2 \cdot 10^7 \text{ M}^{-1} \text{ s}^{-1}$   
338 [36]) compared to the constant between Fe(II) and H<sub>2</sub>O<sub>2</sub> ( $k_{Fe(II),H_2O_2} = 76 \text{ M}^{-1} \text{ s}^{-1}$  [37]), *ii*) SBP is an effective  
339 catalyst for H<sub>2</sub>O<sub>2</sub> in the oxidation of diverse organic substrates and *iii*) no synergistic effect was observed  
340 between Fe(III)-EDDS complex and SBP under UV-B irradiation, it is possible to conclude that the photolysis  
341 of Fe(III)-EDDS is unlikely to release significant amounts of H<sub>2</sub>O<sub>2</sub> in the bulk of the solution. This could be  
342 not in contrast with the hypothesis that the Fe(II) ion produced from the photolysis of Fe(III)-EDDS would  
343 react with H<sub>2</sub>O<sub>2</sub> (photochemically produced from the reduction of the dissolved oxygen under the photolysis  
344 of the iron complex) and generate hydroxyl radicals (or other species with similar reactivity, e.g. ferryl ion  
345 [38]). However, all these reactive steps (reactions R1-R4) are concerted and the photochemically generated  
346 species (i.e. Fe(II) and H<sub>2</sub>O<sub>2</sub>) react immediately after their production, as an example inside the solvent cage.  
347 This prevents their diffusion in the bulk of the solution, where they could react with SBP. The experimental  
348 data suggest that the quantity of hydrogen peroxide produced by Fe(III)-EDDS irradiation in the bulk of the  
349 solution is too low to be effectively used by the peroxidase.

350

#### 351 **4. Conclusions**

352 The photolysis of the Fe(III)-EDDS complex is an efficient tool for the removal of 4-CP under UV-B,  
353 especially in the presence of H<sub>2</sub>O<sub>2</sub>. Increasing the concentration of both H<sub>2</sub>O<sub>2</sub> and Fe(III)-EDDS, we observed  
354 an increase of 4-CP degradation due to the increment in the production of hydroxyl radicals, the main reactive  
355 species in this process (as proved by the experiments with propan-2-ol as selective •OH radical scavenger).  
356 Despite of the classic Fenton process, the UV-B activated Fe(III)-EDDS photo-Fenton is operative even at  
357 neutral or slightly basic pH. The presence of dissolved oxygen is essential to allow significant 4-CP  
358 degradation. From the photoreduction of O<sub>2</sub> the superoxide radical anion (O<sub>2</sub><sup>•-</sup>) is produced and this has a  
359 paramount role in the process not only to promote the production of H<sub>2</sub>O<sub>2</sub> (likely blocked in the solvent cage  
360 and consequently immediately used for the production of reactive species before its diffusion in the bulk), but  
361 also to reduce Fe(III) to Fe(II).

362 The addition of SBP did not show the desired synergistic effect with the photo-Fenton system based on the  
363 UV-B irradiation of the Fe(III)-EDDS complex, but give us some clear indication of a concerted mechanism  
364 where the possibly produced H<sub>2</sub>O<sub>2</sub> reacts immediately with the Fe(II) ion with so fast kinetics that the diffusion  
365 of hydrogen peroxide into the bulk of the solution (i.e. outside of the solvent cage of the complex) cannot  
366 compete with the reaction inside the hydration sphere of the photo-reactive complex.

367 The experiments with SBP and H<sub>2</sub>O<sub>2</sub> only shown a significant effect on 4-CP degradation, both under  
368 irradiation and in dark, reaching up to the 80%-90% of removal. In general, this is consistent with the results  
369 of previous studies elucidating the efficacy of the two reagents against different compounds [34,39].  
370 Furthermore, a recent study reports a degradation of approximatively 40% of pentachlorophenol in similar  
371 conditions [27], while higher degradations were obtained when the enzyme was associated to other materials

372 such as TiO<sub>2</sub> or ZnO [40]. These promising results suggest that the efficiency of peroxidases and H<sub>2</sub>O<sub>2</sub> in  
373 degrading organic compounds may be case-specific and encourages further investigations on their functioning  
374 and effectiveness in water treatment.

375

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378

## 379 **6. Competing Interests**

380 The authors have no competing interests to declare that are relevant to the content of this article.

381

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