

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^{1,2}, Marco Minella*³, Enzo Laurenti³, Marcello Brigante⁴, Gilles Mailhot⁴, Alessandra Bianco
4 Prevot³

5 ¹ *Department of Life Sciences and System Biology, University of Turin, via Accademia Albertina 13, 10123 Turin, Italy*

6 ² *ALPSTREAM – Alpine Stream Research Center, 102030 Ostana, Italy*

7 ³ *Department of Chemistry, University of Turin, via P. Giuria 7, 10125 Turin, Italy*

8 ⁴ *Université Clermont Auvergne, CNRS, Institut de Chimie de Clermont-Ferrand, 63000 Clermont-Ferrand, France.*

9 * marco.minella@unito.it

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15 **Supplementary Information**

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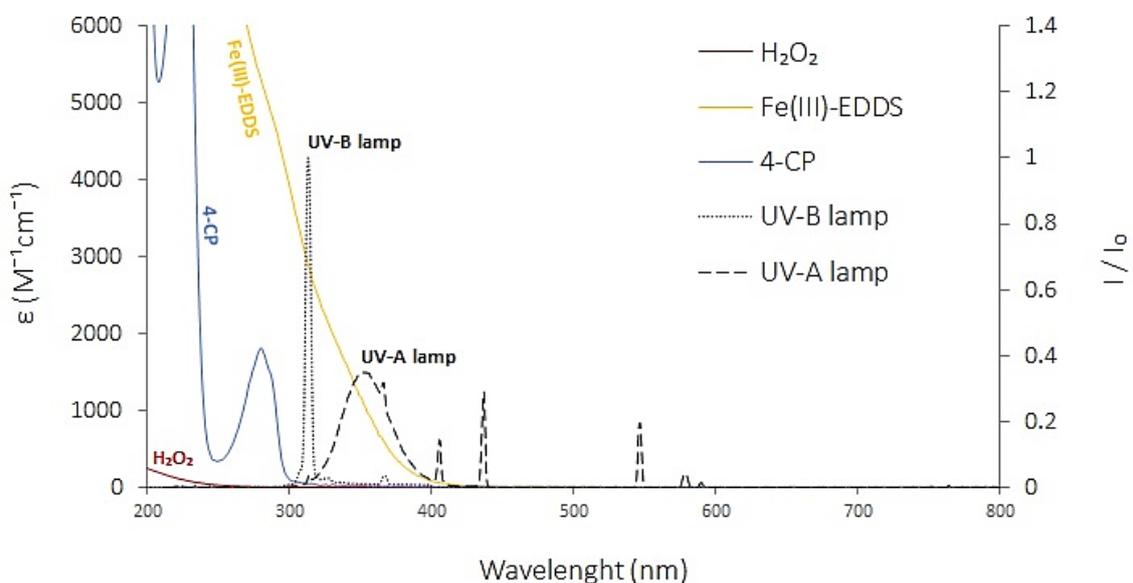
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30 **1. Material and methods**

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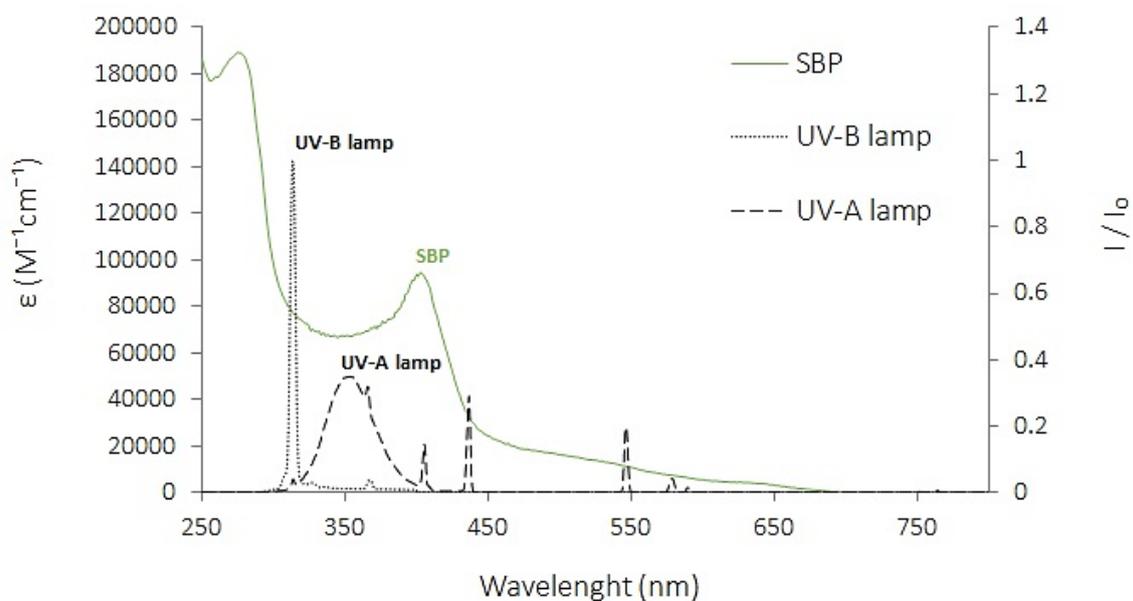
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33 **Fig. S1** Absorptivity of the reagents (*Fe(III)-EDDS 0.1 mM, H_2O_2 1.0 mM, 4-CP 0.1 mM,*) vs emission spectra of the
34 *two lamps (UV-A and UV-B) used for the irradiation.*

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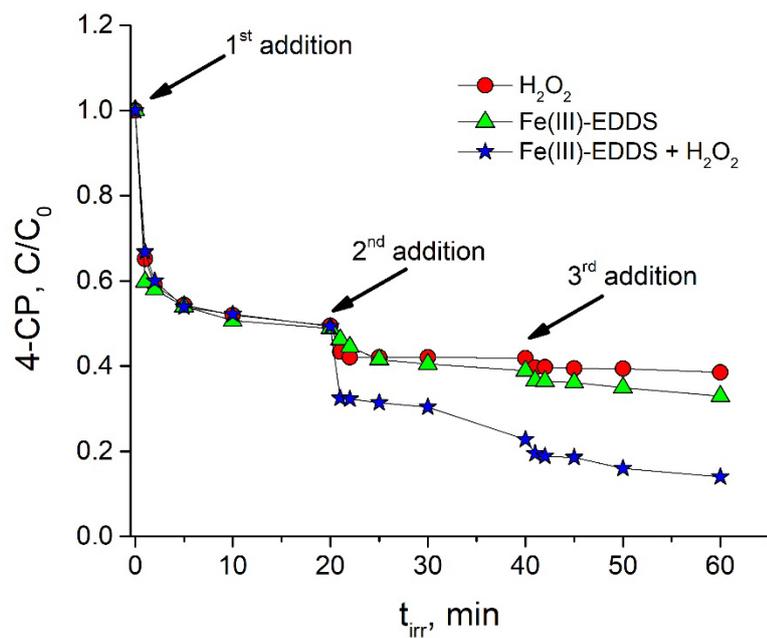


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39 **Fig. S2** Specific absorptivity of SBP and emission spectra of the two lamps (UV-A and UV-B) used for the irradiation.

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43 **Fig. S3** 4-CP degradation as a consequence of 3 consecutive additions (every 20 minutes) of (i) H_2O_2 0.1 mM, (ii)
 44 Fe(III)-EDDS 0.1 mM and (iii) H_2O_2 0.1 mM + Fe(III)-EDDS 0.1 mM

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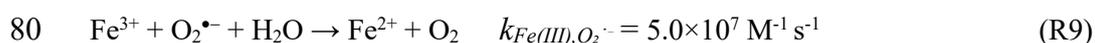
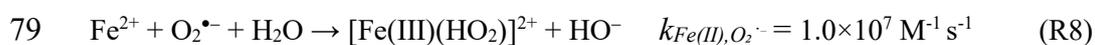
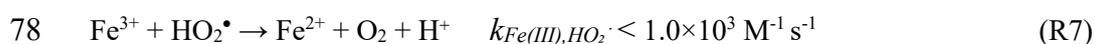
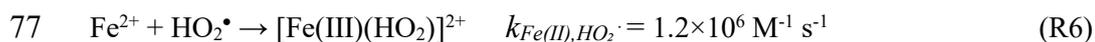
48 2. Results and discussion

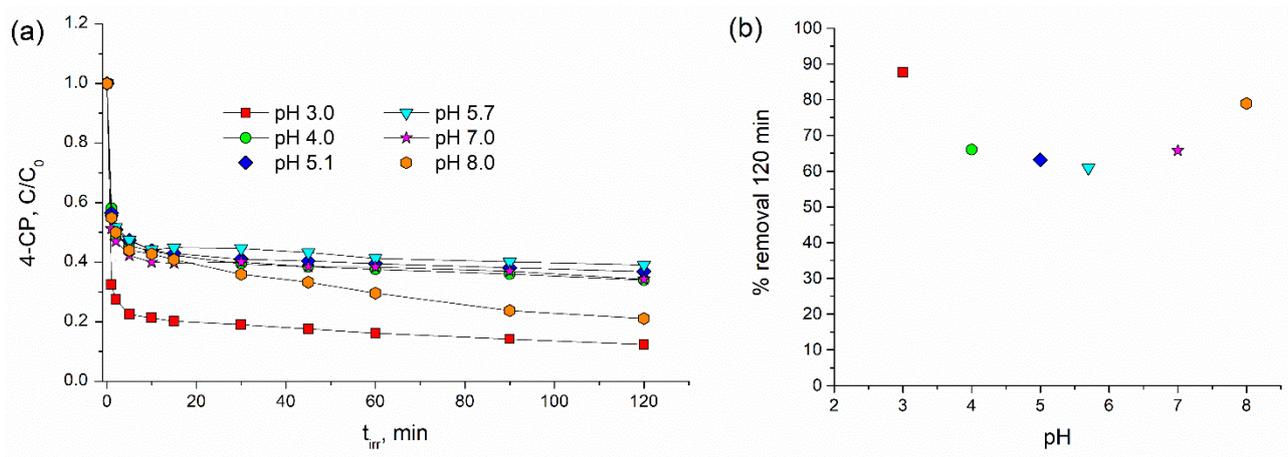
49 2.1 Effect of initial pH value

50 The pH of the solution is a key parameter for the photo-Fenton processes. At different pH the speciation in
51 solution changes and consequently not only the stability of the iron complex, but also the redox properties of
52 the transient species generated change affecting the overall efficiency of the degradation process. For this
53 reason, the 4-CP degradation was investigated (in the presence of Fe(III)-EDDS 0.1 mM and H₂O₂ 0.2 mM)
54 in the pH interval 3-8.

55 Fig. S4a shows the concentration of 4-CP as a function of the irradiation time at different pH. In all the
56 investigated pH we observed the usual behavior with an effective 4-CP degradation limited to the first part of
57 the experiments ($t_{\text{irr}} < 20$ minutes). The only difference was observed at pH 8, in this case the process had a
58 clear double kinetic regime, a rapid decrease in the first 10 minutes of treatment followed by a monotonic
59 and linear decrease of the concentration from 10 to 120 minutes. Fig. S4b shows the % removal after 120
60 minutes as a function of the pH. The highest removal was observed at the extreme investigated pH (3 and 8).
61 It is not easy to rationalize this experimental evidence due to numerous consequences that the change of the
62 pH could have on the overall processes. Three possible points to be considered and that can partially justify
63 the observed trends are:

- 64 (i) at acidic pH the concentration of the free Fe³⁺ in solution increases (i.e. the conditional constant
65 of Fe(III)-EDDS decreases as a consequence of the protonation of EDDS) and at pH 3 it has
66 been reported that the photo-Fenton process in the absence of ligands reaches its maximum of
67 efficiency;
- 68 (ii) the acid/base speciation of the hydroperoxyl radical (HO₂[•]/O₂^{•-}, pK_a = 4.88 [1]) affects
69 significantly its reductant properties toward Fe(III) (see reaction R6-9), the kinetic constant for
70 the reaction between superoxide radical anion and Fe³⁺ and hydroperoxyl radical and Fe³⁺ are
71 $5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and $< 1.0 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, respectively [2,3,4]. As a consequence, only at pH higher
72 than 4.88 Fe(III) can be easily reduced by O₂^{•-} to give Fe(II) that can react with H₂O₂ to give
73 hydroxyl radical (note that the role of this reaction in the photo-Fenton processes is mitigated by
74 the photochemical reduction of Fe(III)).
- 75 (iii) at pH > 8 is has been reported the formation of hydroxylated complex (e.g. Fe(OH)-EDDS²⁻)
76 that could have peculiar properties from the point of view of their photochemical properties [5].





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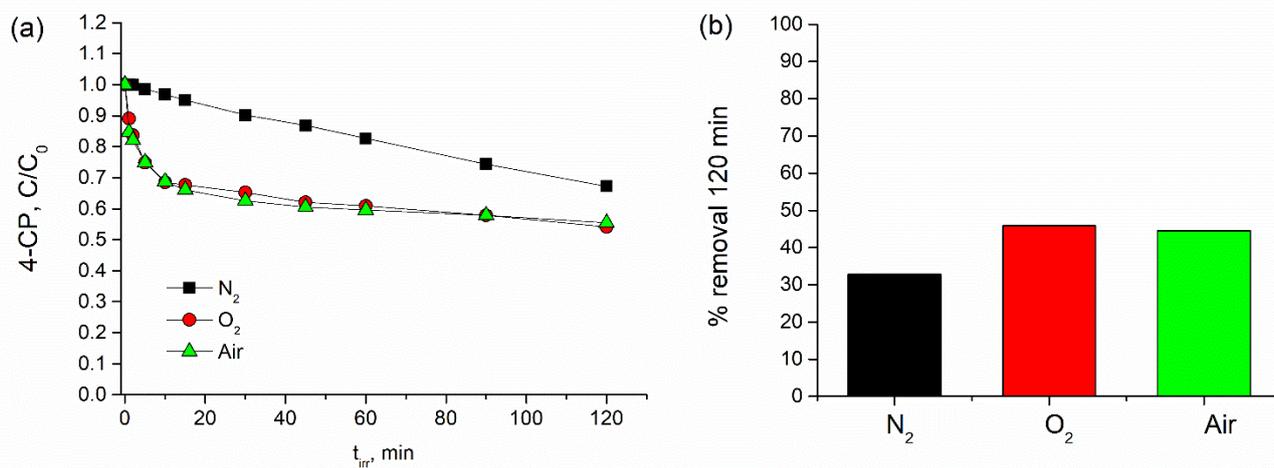
83 **Fig. S4.** (a) Photodegradation of 4-CP at different pH; (b) % removal of 4-CP after 120 min of irradiation. Conditions:
 84 $[4-CP]_0 = 0.1$ mM, $[Fe(III)-EDDS] = 0.1$ mM, $[H_2O_2] = 0.2$ mM.

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86 2.2 Effect of oxygen in the absence of H_2O_2

87 The role of the dissolved oxygen in the absence of any additional oxidants (i.e. H_2O_2) was explored. Fig. S5a
 88 shows the profiles of 4-CP concentration obtained with tested solutions equilibrated in air, saturated with
 89 oxygen and deoxygenated with nitrogen, respectively. After 120 minutes of irradiation the % removal
 90 showed the order $N_2 < air < O_2$ (see Fig. S5b). Different kinetics were observed in the presence and absence
 91 of oxygen. With oxygen saturated solutions the 4-CP concentration profile showed the usual initial step and
 92 the following plateau (the profiles in air and pure oxygen overlapped). In the absence of oxygen, the
 93 behaviour was different and a zero-order kinetics (constant rate in the whole irradiation time explored) was
 94 observed. The initial rate of 4-CP transformation was at least 6 times higher in the presence of oxygen than
 95 in anoxic conditions. Wu et al. [6,7] observed a similar decrease in the degradation of 4-tertbutylphenol in
 96 the absence of dissolved oxygen.

97 The observed experimental evidences can be interpreted considering the reaction between the radical $EDDS^{\bullet}$
 98 (formed from the photolysis of Fe(III)-EDDS complex) with oxygen that promotes the formation of
 99 $HO_2^{\bullet}/O_2^{\bullet-}$ able not only to reduce Fe(III) (especially at $pH > 4.88$, see above), but also to dismutate to give
 100 H_2O_2 . Furthermore, the dissolved oxygen reacts with the radical species generated from the oxidation of 4-
 101 CP ($4-CPOH^{\bullet}$ produced for addition of the hydroxyl radical to the 4-CP aromatic ring or $4-CP^{\bullet+}$ formed
 102 through the monoelectronic direct oxidation of the aromatic substrate) to give the related hydroquinone or
 103 benzoquinone promoting its degradation. Furthermore, the degradation of 4-CP also in anoxic condition
 104 suggests that other reactive species different from $^{\bullet}OH$ (generated through the reactions R1-R5 only in the
 105 presence of dissolved oxygen) can be operative in this conditions, as an example the production of non-
 106 radical iron-based reactive species has been often proposed in the presence of Fenton and Fenton-like
 107 processes alternatively to $^{\bullet}OH$ [8,9,10,11].



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109 **Fig. S5.** (a) Concentration of 4-CP as a function of the irradiation time in air equilibrated, oxygen saturated and N_2
 110 stripped solutions; (b) % removal of 4-CP after 120 min of irradiation in the different tested conditions. Conditions: $[4-$
 111 $CP]_0 = 0.1$ mM, $[Fe(III)-EDDS] = 0.1$ mM, no $[H_2O_2]$, initial pH = 5.5.

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