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

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## Abstract

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

The application of the Fe(III)-EDDS complex in Fenton and photo-Fenton (mainly under UV-B) processes, using 4-chlorophenol (4-CP) as a model pollutant was explored to give insights into the operative mechanism. Furthermore, the potential synergistic contribution of soybean peroxidase (SBP) in the process was investigated, since it has been reported that upon irradiation of Fe(III)-EDDS the generation of H<sub>2</sub>O<sub>2</sub> can occur. SBP did 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) 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 solvent cage of the complex). So, a concerted mechanism in which the photochemically produced H<sub>2</sub>O<sub>2</sub> and Fe(II) react inside the hydration sphere of the Fe(III)-EDDS complex is proposed.

## Keywords

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

## 1. Introduction

The water resources on which human life unavoidably depends are facing an increasing stress: while growing urbanization and climate change affect water quantity and availability, the presence of toxic recalcitrant compounds represent a major threat to the quality. Among these, Contaminants of Emerging Concern (CECs), spanning from pharmaceuticals to pesticides and microplastics, are increasingly being detected in surface water, groundwater, wastewater and drinking water in amounts ranging from ng/L to few ug/L [1]. The secondary treatments adopted in most wastewater treatment plants (WWTPs) normally rely on the biological degradation of the organic substances that cannot ensure their complete removal, even leading to transformation into toxic intermediates [2]. Thus, WWTPs should be equipped with additional advanced tertiary treatments allowing a complete mineralization of the compounds, or at least their degradation into less harmful and biodegradable by-products [3].

Advanced Oxidation Processes (AOPs) generate highly reactive radicals (e.g. hydroxyl radicals that are among the strongest known oxidants,  $E_0 = 2.8 \text{ V vs NHE}$ ) that would attack with scarce selectivity the pollutants [4].

Fenton and photo-Fenton processes produce  $\bullet\text{OH}$  and/or superoxidised iron species by addition of  $\text{H}_2\text{O}_2$  to Fe(II) and by irradiation of Fe(III). The addition of iron chelating agents as ethylenediamine-N,N'-disuccinic acid (EDDS) allows to widen the range of pH at which the two processes work [5]. EDDS is a structural isomer of ethylenediaminetetraacetic acid (EDTA) and, thanks to its high biodegradability, represents its safer and eco-friendly alternative [6]. EDDS can bind Fe(III) with a 1:1 ratio resulting in a highly reactive Fe(III)-EDDS complex [7,8,9,10]. Fe(III)-EDDS is easily photolyzed under UV radiation and generates  $\bullet\text{OH}$  radicals in a pH range between 3 and 9, but the mechanism of photo-activation of  $\text{H}_2\text{O}_2$  has not been fully clarified yet.

This work aims to provide further knowledge on the role of pH, concentration of reagents and  $\text{O}_2$  in photo-Fenton and Fenton processes employing Fe(III)-EDDS as homogeneous photo-catalyst and 4-chlorophenol (4-CP) as target compound. So, a set of degradation tests has been carried out varying the abovementioned parameters and evaluating the kinetics of 4-CP degradation case by case.

Chemical processes are not the only valuable method to reach a successful removal of CECs: biological processes with microorganisms and enzymes have been studied for the same purpose, and the most recent approaches rely on their coupling with AOPs [11].

Soybean peroxidase (SBP: EC 1.11.1.7) is isolated from soybean (*Glycine max*, L.) seed hulls. The great chemical and thermal stability and its ability to activate  $\text{H}_2\text{O}_2$  producing reactive species make this enzyme interesting for possible application in wastewater treatment on a larger scale. Previous studies have demonstrated the advantages of coupling SBP with oxidant systems as titanium dioxide ( $\text{TiO}_2$ ) to obtain an enhanced degradation of the target substrates, with the process undergoing up to three cycles thanks to the regeneration of the photo-produced reactive species [12,13].

It is noteworthy that soybean is one of the major industrial and food crops grown in every continent, with a global production of 368.12 million metric tons in 2020-2021 [15]: thereby SBP represents a widely available and relatively cheap biocatalyst whose exploitation in wastewater treatment could represent both an economical advantage, both a valuable example of waste recovery and reuse.

Though the interaction between SBP and Fe(II) salts has been already tested [14], as far as we know the effects of the co-presence of SBP and Fe(III)-EDDS have never been investigated before.

This study explored for the first time the possible synergistic effect between the two systems. The objective is twofold: *i*) the investigation of the photo-reactivity of Fe(III)-EDDS in presence of SBP may offer further information on the degradation mechanistic activated by the irradiation of Fe(III)-EDDS, especially on the availability and reactivity of H<sub>2</sub>O<sub>2</sub>, hypothetically photo-produced during the photo-Fenton process but never been measured in an effective way and *ii*) the use of chelated iron may overcome the main drawback of Fe(II)/SBP system: the precipitation of Fe(II) at neutral-basic pH values, which makes the application of Fe(II)/SBP poorly efficient on natural waters.

## 2. Material and methods

All chemicals were used as received without further purification. The full list is reported as Supplementary Information (hereinafter SI), together with the irradiation, degradation and analytical procedures.

Briefly, irradiation was carried out in closed Pyrex glass cells or tubes placed in two different photoreactors (low volume, LVR, and high volume, HVR) and irradiated for 0 to 120 minutes. The 4-CP solution was kept under stirring at 293±2 K and pH ≈ 5.5 and was sampled at fixed time intervals.

The residual concentration of 4-CP was measured through a HPLC system in isocratic mode. The decomposition of H<sub>2</sub>O<sub>2</sub> was monitored using the method outlined by Klassen et al. [16].

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

Spectrophotometric analysis was performed using a Varian CARY 100 Scan double-beam UV-vis spectrophotometer.

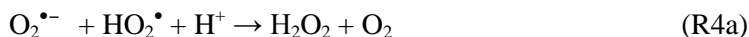
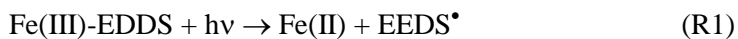
## 3. Results and discussion

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 agreement with previous work [8,17]. The choice of an UV-B irradiation, despite of the previously investigated UV-A, was carried out not only to test different conditions respect to the previous works, but

also to maximize the overlap between the emission spectrum of the used lamp(s) and the absorption spectrum of the Fe(III)-EDDS complex (see Fig. S1 and S2 of the SI).

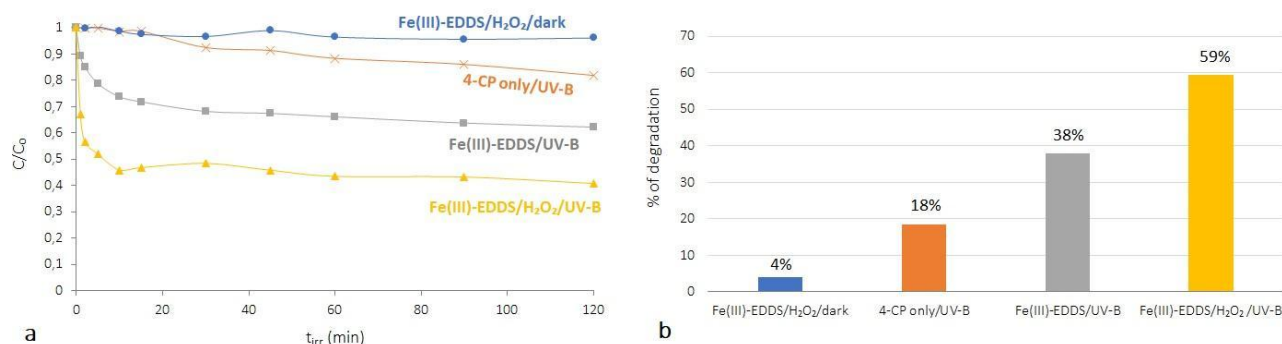
### 3.1 Degradation kinetics of 4-CP in photo-Fenton processes

Figure 1 shows the effects of the presence of Fe(III)-EDDS, H<sub>2</sub>O<sub>2</sub> and the role of the UV-B irradiation on the concentration of 4-CP. The direct photolysis of 4-CP under irradiation was evaluated too and, as expected from the absence of significant absorption of this molecule at  $\lambda > 300$  nm, a limited degradation of 4-CP was observed ( $\approx 18\%$  after 120 minutes of irradiation). Furthermore, the complex Fe(III)-EDDS was not able in the dark to activate H<sub>2</sub>O<sub>2</sub> promoting the removal of the substrate, as manifested from the stability of 4-CP in the presence of both Fe(III)-EDDS and H<sub>2</sub>O<sub>2</sub> in the dark. On the contrary, the UV-B irradiation of Fe(III)-EDDS alone is able to activate the production of reactive species (e.g.  $\bullet\text{OH}$  and  $\text{HO}_2\bullet/\text{O}_2^{\bullet-}$ ) also in the absence of additional oxidant (i.e. H<sub>2</sub>O<sub>2</sub>), in agreement with the following reactions (R1-R5 [8, 17,18]):



The addition of H<sub>2</sub>O<sub>2</sub> increased the degradation of 4-CP up to 59 % after 120 minutes in agreement with the fast reaction between the added H<sub>2</sub>O<sub>2</sub> and the Fe(II) photochemical generated from the photolysis of the Fe(III)-EDDS (R5).

The profiles of 4-CP concentration under irradiation gave some additional insights. The UV-B irradiation in the tested experimental conditions activated a fast initial degradation ( $t_{\text{irr}} < 15$  min) and a following step in which a slower (at least null) degradation was observed. The reasons of this behavior deserved to be investigated, for this reason experiments at different dosages of both H<sub>2</sub>O<sub>2</sub> and Fe(III)-EDDS were carried out (*vide infra*).



**Figure 1** (a) Degradation of 4-CP 0.1 mM in presence and absence of Fe(III)-EDDS, H<sub>2</sub>O<sub>2</sub> and irradiation.; (b). % removal of 4-CP after 120 min of irradiation. Conditions: [4-CP]<sub>0</sub> = 0.1 mM, [Fe(III)-EDDS] = 0.1 mM, [H<sub>2</sub>O<sub>2</sub>] = 0.2 mM, initial pH = 5.5

### 3.2 Effect of H<sub>2</sub>O<sub>2</sub> concentration under irradiated Fe(III)-EDDS

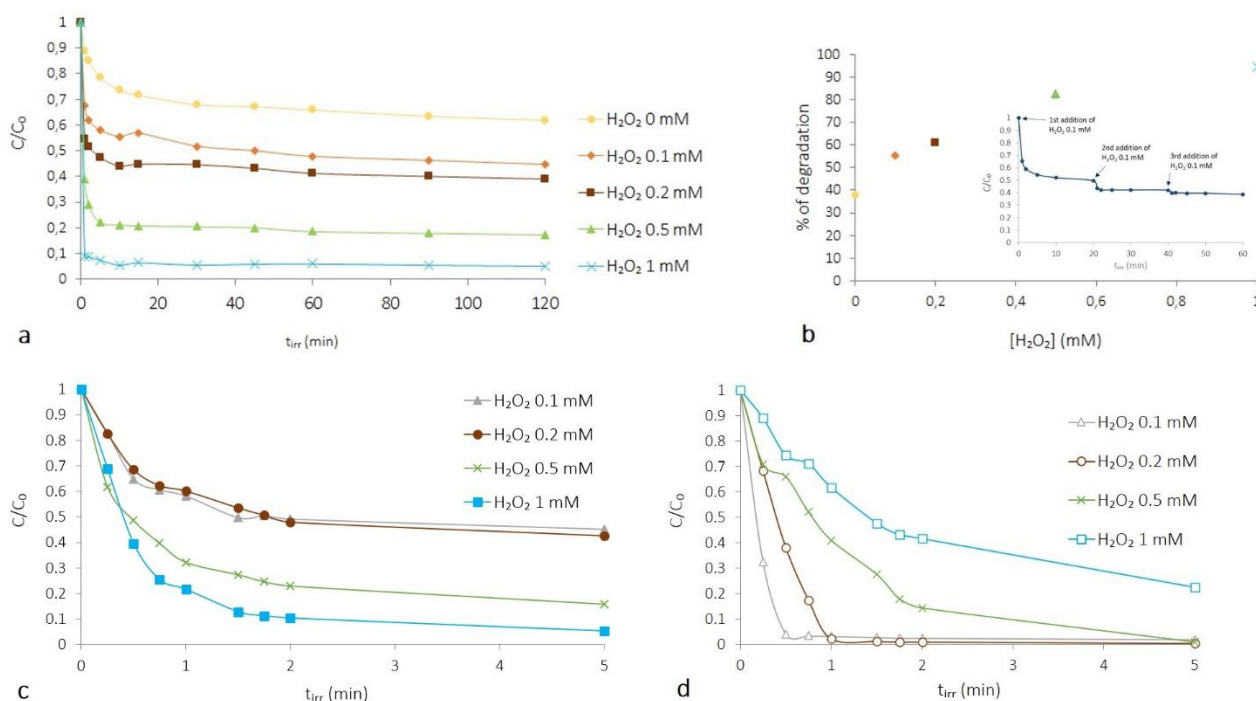
Figure 2a shows the degradation profiles of 4-CP under irradiation and in the presence of different concentrations of hydrogen peroxide (from 0 to 1 mM). Figure 2b summarized the % removal of 4-CP after 120 min as a function of the initial [H<sub>2</sub>O<sub>2</sub>]. The same concentration profiles previously commented were observed, with the major part of the degradation happening in the first part of the irradiation time (<10-15 minutes). The addition of an increasing amount of H<sub>2</sub>O<sub>2</sub> gave an increasing % of 4-CP removal with saturative behaviour (see Figure 2b) indicating that in the explored conditions H<sub>2</sub>O<sub>2</sub> could be the limiting reagent for the process. In this light, the effect of sequential additions of H<sub>2</sub>O<sub>2</sub> was studied (see inset of Figure 2b) and a profile with 3 sequential drops of the 4-CP concentration was observed. Note that during the addition of H<sub>2</sub>O<sub>2</sub> no other Fe(III)-EDDS was added. The effect of the H<sub>2</sub>O<sub>2</sub> addition was less effective after the first step, with a decrease of the degradation in absolute value during the successive additions. This highlighted that (i) H<sub>2</sub>O<sub>2</sub> played as a limiting reagent for the process and (ii) that the catalytic properties of Fe(III)-EDDS faded as a consequence of the prolonged treatment (in agreement with a limited catalytic role for Fe(III)-EDDS in the explored experimental conditions).

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

To give insight into the role of H<sub>2</sub>O<sub>2</sub>, the concentration of 4-CP and H<sub>2</sub>O<sub>2</sub> was monitored at short irradiation time ( $\leq 5$  minutes) in experiments at the same concentration of 4-CP (0.1 mM) and increasing amount of H<sub>2</sub>O<sub>2</sub> (from 0.1 to 1 mM). Figure 2c and d show the 4-CP and H<sub>2</sub>O<sub>2</sub> concentration profiles, respectively. At

the lowest concentration of oxidant (0.1 and 0.2 mM),  $\text{H}_2\text{O}_2$  was totally consumed within 1 minute of irradiation, while at the highest concentration (1 mM) a residual concentration of  $\text{H}_2\text{O}_2$  was observed even after 5 minutes of irradiation.

From the comparison of the  $\text{H}_2\text{O}_2$  and 4-CP profiles, it is manifest that (i) the complete disappearance of  $\text{H}_2\text{O}_2$  did not 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 concentration of  $\text{H}_2\text{O}_2$ , 4-CP reached its plateau value even in the presence of a residual  $\text{H}_2\text{O}_2$  that was not able to further promote the degradation of the organic substrate. These experimental evidences highlighted the essential role of  $\text{H}_2\text{O}_2$  to activate the first and fast step of the 4-CP degradation (and that higher is the concentration of oxidant, higher is the % 4-CP removal reached). However, it is also true that the decline of the degradation ability of the system might be ascribed also to the faded catalytic properties of the Fe(III)-EDDS complex (which was not recycled or was only partially recycled after the activation of  $\text{H}_2\text{O}_2$ ).

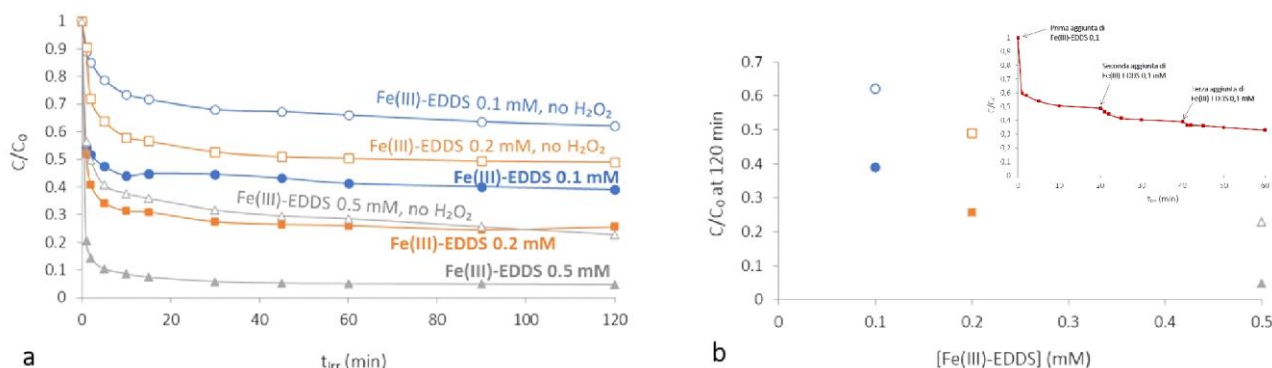


**Figure 2** (a) Photodegradation of 4-CP at different concentrations of  $\text{H}_2\text{O}_2$ ; (b) % removal of 4-CP after 120 min of irradiation. Insert 4-CP degradation after 3 consecutive additions (every 20 minutes) of  $\text{H}_2\text{O}_2$  0.1 mM. Phototransformation of 4-CP (c) and  $\text{H}_2\text{O}_2$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

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

Figure 3 shows the degradation profiles and the residual fraction of 4-CP after 120 minutes of irradiation in the presence of increasing concentration of Fe(III)-EDDS without and with  $\text{H}_2\text{O}_2$  0.1 mM. With the increase of the concentration of the iron complex - both in the presence and in absence of  $\text{H}_2\text{O}_2$  - an increase of the 4-CP degradation was observed. At higher complex concentration, an increase of photo-formed Fe(II) and

consequently production of  $\bullet\text{OH}$  (or alternative reactive species) was observed.. In all the cases, after the first step of degradation, the process slowed down and the concentration of 4-CP reached a plateau. In the absence of  $\text{H}_2\text{O}_2$  (i) the 4-CP residual 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) the degradation rate of the initial step was significantly lower in absence of hydrogen peroxide than in presence - being  $\text{H}_2\text{O}_2$  not immediately at disposal of the photo-produced Fe(II).



**Figure 3** (a) Photodegradation of 4-CP at different concentrations of Fe(III)-EDDS; (b) % removal of 4-CP after 120 min of irradiation. Inset: 4-CP degradation after 3 consecutive additions (every 20 minutes) of Fe(III)-EDDS 0.1 mM. Conditions:  $[\text{4-CP}]_0 = 0.1$  mM,  $[\text{Fe(III)-EDDS}] = 0.1\text{-}0.5$  mM,  $[\text{H}_2\text{O}_2] = 0.1$  mM, initial pH = 5.5.

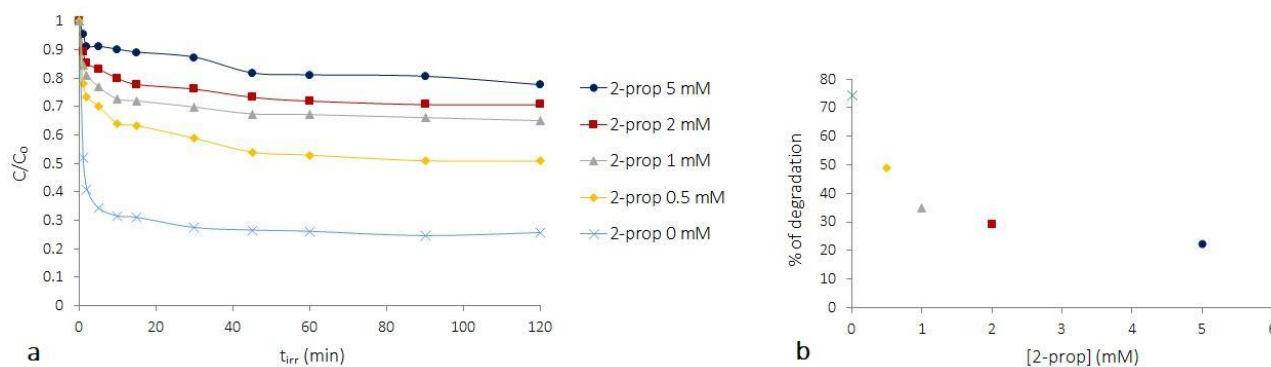
The inset of Figure 3b shows the effect of successive addition of Fe(III)-EDDS 0.1 mM (every 20 minutes). Note that the second addition was carried out in a solution in which the  $\text{H}_2\text{O}_2$  concentration was null (see Figure 2c). The addition of the sole Fe(III)-EDDS gave similar effects of those observed with the successive addition of  $\text{H}_2\text{O}_2$  alone, that was a further 4-CP degradation less manifest after each addition. Lastly, a test in which the successive additions were composed by both  $\text{H}_2\text{O}_2$  0.1 mM and Fe(III)-EDDS 0.1 mM was carried out and compared with the profiles measured with the successive additions of  $\text{H}_2\text{O}_2$  and Fe(III)-EDDS alone (see Fig. S3). The simultaneous addition of both reactants gave the best results, suggesting that the real active reagents in the UV-B activated photo-Fenton process (with EDDS as ligand for  $\text{Fe}^{+3}$ ) is the couple iron-complex +  $\text{H}_2\text{O}_2$  and that catalytic cycles able to regenerate a photoactive catalyst are scarcely operative. The limited (but not null) effect of the third addition of  $\text{H}_2\text{O}_2$  + Fe(III)-EDDS can be explained with the progressive accumulation of organic by-products (e.g. product of the Fe(III)-EDDS UV-B photolysis) that competed with 4-CP for the same oxidative reactive species.

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

With the aim to investigate the role of the hydroxyl radical, the degradation of 4-CP in the presence of Fe(III)-EDDS and  $\text{H}_2\text{O}_2$  was investigated in the presence of increasing concentration of propan-2-ol. This compound is an efficient scavenger for  $\bullet\text{OH}$  ( $k_{2\text{-prop}, \text{HO}\bullet} = 1.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  [19]).



Figure 4 shows the effect of the addition of propan-2-ol on the 4-CP degradation profiles as a function of irradiation time (Figure 4a) and the % removal ( $t_{irr} = 120$  min) as a function of the propan-2-ol concentration. The addition of 2-prop had a clear inhibitory effect on the maximum % removal reached at the end of the irradiation and on the initial rate of transformation of 4-CP in the first minutes of irradiation. This is a clear evidence of the predominant role of the  $\cdot\text{OH}$  radicals in the 4-CP degradation under UV-B and in the presence of Fe(III)-EDDS and  $\text{H}_2\text{O}_2$ . Furthermore, Figure 4 shows that the reactivity with  $\cdot\text{OH}$  cannot explain totally the substrate removal: with the increase of the scavenger concentration the % removal decreased monotonically down to a plateau value equal to  $\approx 15\%$ . This is in agreement with an operative role of alternative (even if minority) reactive species (e.g. ferryl ion), that were not totally scavenged by 2-prop. Huang et al. [8] demonstrated in similar experimental conditions, but in the presence of chloroform, an important role of  $\text{HO}_2\cdot/\text{O}_2^{\cdot-}$ , not as reactive species involved in the removal of the organic substrate, but as reductant for Fe(III)-EDDS and so as species involved in the indirect generation of  $\cdot\text{OH}$ .



**Figure 4** (a) Photodegradation of 4-CP ( $C_0 = 0.1$  mM) at increasing concentration of 2-prop (0 - 5 mM). (b) % removal of 4-CP after 120 min of irradiation. Conditions:  $[\text{4-CP}]_0 = 0.1$  mM,  $[\text{Fe(III)-EDDS}] = 0.3$  mM,  $[\text{H}_2\text{O}_2] = 0.2$  mM, initial pH = 5.5.

The degradation of 4-CP with Fe(III)-EDDS/ $\text{H}_2\text{O}_2$  under irradiation at different pH (from 3 to 8) and the role of the dissolved oxygen were also investigated. The obtained results (reported in paragraphs 2.1 and 2.2 of SI) were in agreement with the previous reports about the photo-Fenton process with Fe(III)-EDDS.[17,18]

### 3.5 Effect of SBP addition

The catalytic activity of SBP for the oxidation of phenolic compounds with  $\text{H}_2\text{O}_2$  has been documented [20] together with its potential application for the removal of organic pollutants both in homogeneous conditions [21] and as supported catalyst [22]. Furthermore, synergistic effects between advanced oxidation processes where  $\text{H}_2\text{O}_2$  is produced (e.g. heterogeneous photocatalysis under irradiated semiconductors) and the presence of SBP has been previously reported [13]. In this light, the effect of SBP on the removal of 4-CP in the presence of Fe(III)-EDDS (both in dark and under irradiation) has been investigated with the aim not only to understand if the  $\text{H}_2\text{O}_2$  eventually produced under the irradiation of the Fe(III)-EDDS complex can be efficiently used by SBP to boost the transformation rate of 4-CP, but also to give insights into the mechanism of transformation of organic compounds in the photo-Fenton process activated by Fe(III)-EDDS.

#### 3.5.1 Activity of SBP in dark

In dark 4-CP was efficiently removed through the SBP catalyzed reaction with  $\text{H}_2\text{O}_2$ : after 120 minutes of treatment in the presence of SBP 0.01  $\mu\text{M}$  and  $\text{H}_2\text{O}_2$  0.1 mM (ratio  $\text{H}_2\text{O}_2$ :SBP = 1,000) the remaining concentration of 4-CP in solution is  $\approx 10\%$  of its initial concentration (Figure 5a). The same experiments were carried out adding Fe(III)-EDDS 0.1 mM observing a significant decrease in the overall removal of 4-CP. After 120 minutes a degradation efficiency of  $\approx 50\%$  was reached. This effect can be explained with either a poisoning of the SBP active catalytic sites by Fe(III)-EDDS, or a strong competition between 4-CP and Fe(III)-EDDS for the reactive species generated during the SBP-catalyzed  $\text{H}_2\text{O}_2$  activation.

Figure 5a compares also the 4-CP degradation with  $\text{H}_2\text{O}_2$  0.1 mM in the presence of different concentration of SBP ( $[\text{H}_2\text{O}_2]/[\text{SBP}]$  ratio from 10,000 to 100). Increasing the relative amount of SBP with respect to  $\text{H}_2\text{O}_2$ , a manifest increase of both the initial degradation rate and the removal reached at the end of the experiments was observed. With the highest concentration of SBP an almost quantitative removal of 4-CP was reached since after five minutes from the  $\text{H}_2\text{O}_2$  addition, also in the presence of the Fe(III)-EDDS complex. With  $[\text{H}_2\text{O}_2]/[\text{SBP}] = 100$  the effect of either the potential poisoning of the enzymatic active site or of the competition for the same reactive species between Fe(III)-EDDS and 4-CP was not manifest.

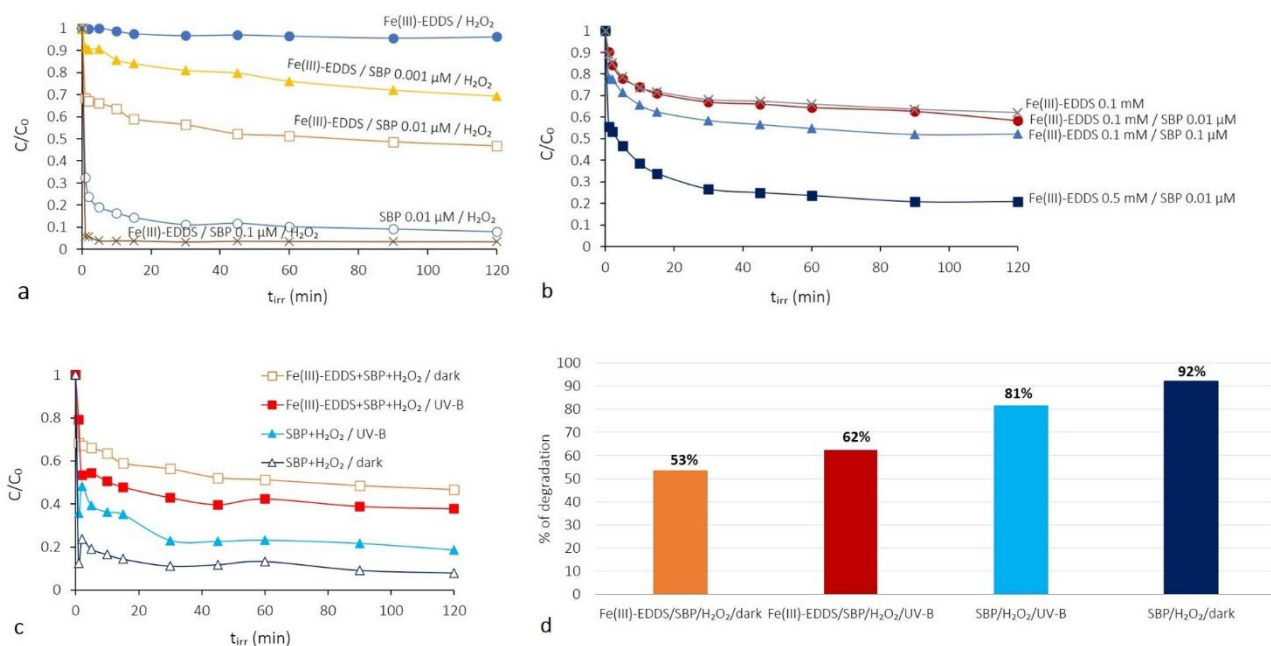
#### 3.5.2 Activity of SBP under irradiation

The experiments under UV-BUV-B irradiation reported in Figure 5b were carried out to investigate if the  $\text{H}_2\text{O}_2$  potentially formed under irradiation of Fe(III)-EDDS could be effectively used by SBP for boosting the 4-CP degradation. For this reason, in the experiments reported in Figure 5b no  $\text{H}_2\text{O}_2$  was initially added to the solution. Furthermore, the concentration of  $\text{H}_2\text{O}_2$  was also monitored since the first minutes of irradiation through the fluorimetric method reported by Lazrus et al. [23]. The measured concentrations were always lower than the LOD of the applied analytical method.

Figure 5b shows the 4-CP concentration as a function of the treatment time under UV-B irradiation in presence of different concentration of Fe(III)-EDDS and SBP. Comparing the degradation profile in the presence of Fe(III)-EDDS 0.1 mM alone and in the presence of SBP (0.01  $\mu$ M), no significant difference was manifest with an overall final degradation of  $\approx$  40%. This can be explained considering that the Fe(III)-EDDS photolysis generated Fe(II) immediately used by the photoproducted  $H_2O_2$  giving hydroxyl radicals. In this light, there is a competition between Fe(II) and SBP for the photoproducted  $H_2O_2$  that, on the basis of this experimental evidence, is totally shifted toward the Fe(II) ion. Increasing 10-times the SBP concentration, the overall degradation of 4-CP slightly increased (from 35 to 40 %), on the contrary a clear increment in the degradation of 4-CP was observed by increasing the Fe(III)-EDDS concentration (from 0.1 to 0.5 mM) at the same SBP concentration (0.01  $\mu$ M), giving a clear indication that the role of Fe(III)-EEDS on the overall degradation mechanism was higher - in the tested experimental conditions - with respect to that of SBP.

Further experiments with SBP were carried out under irradiation, but in the presence of a fixed initial concentration of  $H_2O_2$ .

The system Fe(III)-EDDS/SBP/ $H_2O_2$  under irradiation gave an overall degradation higher than in the dark (removal equal to 62 and 52 %, respectively), but not so high to be explained as the sum of the two distinguished contributions (reactivity of SBP in dark + photoreactivity of Fe(III)-EDDS). Furthermore, removing the Fe(III)-EDDS it was observed a higher 4-CP degradation, even if slightly lower than the degradation observed in the same condition but in the dark (removal equal to 81 and 92 %, respectively). So, the detrimental effect of Fe(III)-EDDS on the SBP activity was also operative under irradiation, furthermore a partial photo-deactivation of SBP under UV-B was observed. To limit the photo-deactivation of the SBP, the 4-CP degradation in presence of Fe(III)-EDDS alone and with Fe(III)-EDDS and SBP was monitored also under UV-A irradiation (data not shown). No significant difference was observed (as above reported under UV-B), so the absence of a synergistic effect between Fe(III)-EDDS and SBP could not be attributed to a detrimental effect of the UV-B on the structure of the enzyme.



**Figure 5** (a) Concentration of 4-CP ( $C_0 = 0.1$  mM) as a function of the treatment time in dark conditions in presence of Fe(III)-EDDS (0.1 mM, if present),  $H_2O_2$  (0.1 mM) and SBP (0.001, 0.01 and 0.1  $\mu M$ ); (b) Concentration of 4-CP ( $C_0 = 0.1$  mM) as a function of the treatment time under UV-B irradiation in presence of different concentration of Fe(III)-EDDS and SBP All the experiments were carried out without  $H_2O_2$ ; (c) Concentration of 4-CP ( $C_0 = 0.1$  mM) as a function of the treatment time under UV-B and in the dark with 0.1 mM initial  $H_2O_2$  concentration; (d) % removal of 4-CP after 120 minutes of reaction. Composition of each solution reported in the figure's legend

The experimental evidence reported above gives some important mechanistic information. Considering that *i*) the second order kinetic constant for the reaction between SBP and  $H_2O_2$  is quite high ( $k_{SBP,H_2O_2} = 2 \cdot 10^7$   $M^{-1} s^{-1}$  [24]) compared to the constant between Fe(II) and  $H_2O_2$  ( $k_{Fe(II),H_2O_2} = 76$   $M^{-1} s^{-1}$  [25]), *ii*) SBP is an effective catalyst for the oxidation with  $H_2O_2$  of diverse organic substrates and *iii*) no synergistic effect were observed between Fe(III)-EDDS complex and SBP under UV-B irradiation, it is possible to conclude that it is quite unlikely that the photolysis of Fe(III)-EDDS releases significant amounts of  $H_2O_2$  in the bulk of the solution. This could be not in contrast with the reported hypothesis that the Fe(II) ion, photochemical generated from the photolysis of Fe(III)-EDDS, generates hydroxyl radical (or other species with similar reactivity, e.g. ferryl ion [26]) reacting with  $H_2O_2$  (photochemically produced from the reduction of the dissolved oxygen under the photolysis of the iron complex). However, all these reactive steps (reactions R1-R4) are concerted and the photochemically generated species (i.e. Fe(II) and  $H_2O_2$ ) react immediately after their production, as an example inside the solvent cage, and they cannot diffuse in the bulk of the solution (where they could react with SBP). The experimental data suggest that Fe(III)-EDDS irradiation produces a quantity of hydrogen peroxide in the bulk of the solution too low to be effectively used by the peroxidase.

## 4. Conclusions

The photolysis of the Fe(III)-EDDS complex is an efficient tool for the removal of 4-CP under UV-B, especially in the presence of  $H_2O_2$ . Increasing the concentration of both  $H_2O_2$  and Fe(III)-EDDS, we

observed an increase of 4-CP degradation due to the increment in the production of hydroxyl radicals, the main reactive species in this process (as proved by the experiments with propan-2-ol as selective  $\bullet\text{OH}$  radical scavenger). Despite of the classic Fenton process, the UV-B activated Fe(III)-EDDS photo-Fenton is operative even at neutral or slightly basic pH. The presence of dissolved oxygen is essential to allow significant 4-CP degradation. From the photoreduction of  $\text{O}_2$  the superoxide radical anion ( $\text{O}_2^{\bullet-}$ ) is produced and this has a paramount role in the process not only to promote the production of  $\text{H}_2\text{O}_2$  (likely blocked in the solvent cage and consequently immediately used for the production of reactive species before its diffusion in the bulk), but also to reduce Fe(III) to Fe(II).

The addition of SBP did not show the desired synergistic effect with the photo-Fenton system based on the UV-B irradiation of the Fe(III)-EDDS complex, but give us some clear indication of a concerted mechanism where the possibly produced  $\text{H}_2\text{O}_2$  reacts immediately with the Fe(II) ion with so fast kinetics that the diffusion of hydrogen peroxide into the bulk of the solution (i.e. outside of the solvent cage of the complex) cannot compete with the reaction inside the hydration sphere of the photo-reactive complex.

## 5. Acknowledgments

To be written

## 6. References

- [1] L. Rizzo *et al.*, “Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater,” *Science of the Total Environment*, vol. 655. Elsevier B.V., pp. 986–1008, Mar. 10, 2019. doi: 10.1016/j.scitotenv.2018.11.265.
- [2] L. Schlüter-Vorberg, C. Prasse, T. A. Ternes, H. Mückter, and A. Coors, “Toxification by Transformation in Conventional and Advanced Wastewater Treatment: The Antiviral Drug Acyclovir,” *Environmental Science & Technology Letters*, vol. 2, no. 12, pp. 342–346, Dec. 2015, doi: 10.1021/acs.estlett.5b00291.
- [3] “Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013,” *Official Journal of the European Union*. 2013.
- [4] W. H. Glaze, J.-W. Kang, and D. H. Chapin, “The Chemistry of Water Treatment Processes Involving Ozone, Hydrogen Peroxide and Ultraviolet Radiation,” *Ozone: Science & Engineering*, vol. 9, no. 4, pp. 335–352, Sep. 1987, doi: 10.1080/01919518708552148.
- [5] J. J. Pignatello, E. Oliveros, and A. MacKay, “Advanced Oxidation Processes for Organic Contaminant Destruction Based on the Fenton Reaction and Related Chemistry,” *Critical Reviews in Environmental Science and Technology*, vol. 36, no. 1, pp. 1–84, Jan. 2006, doi: 10.1080/10643380500326564.

- [6] D. Schowanek, T. C. J. Feijtel, C. M. Perkins, F. A. Hartman, T. W. Federle, and R. J. Larson, "Biodegradation of [S,S], [R,R] and mixed stereoisomers of Ethylene Diamine Disuccinic Acid (EDDS), a transition metal chelator," *Chemosphere*, vol. 34, no. 11, pp. 2375–2391, Jun. 1997, doi: 10.1016/S0045-6535(97)00082-9.
- [7] Y. Wu, M. Brigante, W. Dong, P. de Sainte-Claire, and G. Mailhot, "Toward a Better Understanding of Fe(III)–EDDS Photochemistry: Theoretical Stability Calculation and Experimental Investigation of 4- *tert* -Butylphenol Degradation," *The Journal of Physical Chemistry A*, vol. 118, no. 2, pp. 396–403, Jan. 2014, doi: 10.1021/jp409043e.
- [8] W. Huang, M. Brigante, F. Wu, C. Mousty, K. Hanna, and G. Mailhot, "Assessment of the Fe(III)–EDDS Complex in Fenton-Like Processes: From the Radical Formation to the Degradation of Bisphenol A," *Environmental Science & Technology*, vol. 47, no. 4, pp. 1952–1959, Feb. 2013, doi: 10.1021/es304502y.
- [9] W. Huang, M. Brigante, F. Wu, K. Hanna, and G. Mailhot, "Development of a new homogenous photo-Fenton process using Fe(III)-EDDS complexes," *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 239, pp. 17–23, Jul. 2012, doi: 10.1016/j.jphotochem.2012.04.018.
- [10] J. Li, G. Mailhot, F. Wu, and N. Deng, "Photochemical efficiency of Fe(III)-EDDS complex: OH radical production and 17 $\beta$ -estradiol degradation," *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 212, no. 1, pp. 1–7, Apr. 2010, doi: 10.1016/j.jphotochem.2010.03.001.
- [11] E. Castro, A. Avellaneda, and P. Marco, "Combination of advanced oxidation processes and biological treatment for the removal of benzidine-derived dyes," *Environmental Progress & Sustainable Energy*, vol. 33, no. 3, pp. 873–885, Oct. 2014, doi: 10.1002/ep.11865.
- [12] T. Marchis, P. Avetta, A. Bianco-Prevot, D. Fabbri, G. Viscardi, and E. Laurenti, "Oxidative degradation of Remazol Turquoise Blue G 133 by soybean peroxidase," *Journal of Inorganic Biochemistry*, vol. 105, no. 2, pp. 321–327, Feb. 2011, doi: 10.1016/j.jinorgbio.2010.11.009.
- [13] P. Calza, P. Avetta, G. Rubulotta, M. Sangermano, and E. Laurenti, "TiO<sub>2</sub>-soybean peroxidase composite materials as a new photocatalytic system," *Chemical Engineering Journal*, vol. 239, pp. 87–92, Mar. 2014, doi: 10.1016/j.cej.2013.10.098.
- [14] V. Tolardo *et al.*, "Pentachlorophenol Removal from Water by Soybean Peroxidase and Iron(II) Salts Concerted Action," *Water, Air, & Soil Pollution*, vol. 230, no. 6, p. 140, Jun. 2019, doi: 10.1007/s11270-019-4189-7.
- [15] "https://ipad.fas.usda.gov/," Jun. 06, 2022.
- [16] N. V. , M. D. , M. H. C. E. Klassen, "H<sub>2</sub>O<sub>2</sub> Determination by the I<sub>3</sub>– Method and by KMnO<sub>4</sub> Titration," *Analytical Chemistry*, vol. 66, pp. 2921–2925, 1994.
- [17] Y. Wu, M. Passananti, M. Brigante, W. Dong, and G. Mailhot, "Fe(III)–EDDS complex in Fenton and photo-Fenton processes: from the radical formation to the degradation of a target compound," *Environmental Science and Pollution Research*, vol. 21, no. 21, pp. 12154–12162, Nov. 2014, doi: 10.1007/s11356-014-2945-1.
- [18] Y. Wu, M. Passananti, M. Brigante, W. Dong, and G. Mailhot, "Fe(III)–EDDS complex in Fenton and photo-Fenton processes: from the radical formation to the degradation of a target

compound,” *Environmental Science and Pollution Research*, vol. 21, no. 21, pp. 12154–12162, Nov. 2014, doi: 10.1007/s11356-014-2945-1.

- [19] G. v. Buxton, C. L. Greenstock, W. P. Helman, and A. B. Ross, “Critical Review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ( $\cdot\text{OH}/\cdot\text{O}^-$  in Aqueous Solution,” *Journal of Physical and Chemical Reference Data*, vol. 17, no. 2, pp. 513–886, Apr. 1988, doi: 10.1063/1.555805.
- [20] J. P. McEldoon, A. R. Pokora, and J. S. Dordick, “Lignin peroxidase-type activity of soybean peroxidase,” *Enzyme and Microbial Technology*, vol. 17, no. 4, pp. 359–365, Apr. 1995, doi: 10.1016/0141-0229(94)00060-3.
- [21] N. Caza, J. K. Bewtra, N. Biswas, and K. E. Taylor, “Removal of phenolic compounds from synthetic wastewater using soybean peroxidase,” *Water Research*, vol. 33, no. 13, pp. 3012–3018, Sep. 1999, doi: 10.1016/S0043-1354(98)00525-9.
- [22] P. Calza, D. Zacchigna, and E. Laurenti, “Degradation of orange dyes and carbamazepine by soybean peroxidase immobilized on silica monoliths and titanium dioxide,” *Environmental Science and Pollution Research*, vol. 23, no. 23, pp. 23742–23749, Dec. 2016, doi: 10.1007/s11356-016-7399-1.
- [23] A. L. Lazrus, G. L. Kok, S. N. Gitlin, J. A. Lind, and S. E. McLaren, “Automated fluorimetric method for hydrogen peroxide in atmospheric precipitation,” *Analytical Chemistry*, vol. 57, no. 4, pp. 917–922, Apr. 1985, doi: 10.1021/ac00281a031.
- [24] M. Nissum, C. B. Schiødt, and K. G. Welinder, “Reactions of soybean peroxidase and hydrogen peroxide pH 2.4–12.0, and veratryl alcohol at pH 2.4,” *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, vol. 1545, no. 1–2, pp. 339–348, Feb. 2001, doi: 10.1016/S0167-4838(00)00295-8.
- [25] W. G. Barb, J. H. Baxendale, P. George, and K. R. Hargrave, “Reactions of ferrous and ferric ions with hydrogen peroxide. Part II.—The ferric ion reaction,” *Trans. Faraday Soc.*, vol. 47, no. 0, pp. 591–616, 1951, doi: 10.1039/TF9514700591.
- [26] G. Farinelli *et al.*, “Natural iron ligands promote a metal-based oxidation mechanism for the Fenton reaction in water environments,” *Journal of Hazardous Materials*, vol. 393, p. 122413, Jul. 2020, doi: 10.1016/j.jhazmat.2020.122413.