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1 **Natural Iron Ligands Promote a Metal-Based Oxidation**

2 **Mechanism for the Fenton Reaction in Water Environments**

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20 **Abstract**

21 The Fenton reaction is an effective advanced oxidation process occurring in nature and  
22 applied in engineering processes toward the degradation of harmful substances, including  
23 contaminants of emerging concern. The traditional Fenton application can be remarkably  
24 improved by using iron complexes with organic ligands, which allow for the degradation of  
25 contaminants at near-neutral pH and for the reduction of sludge production. This work  
26 discusses the mechanisms involved both in the classic Fenton process and in the presence of  
27 ligands that coordinate iron. Cyclohexane was selected as mechanistic probe, by following  
28 the formation of the relevant products, namely, cyclohexanol (A) and cyclohexanone (K). As  
29 expected, the classic Fenton process was associated with an A/K ratio of approximately 1,  
30 evidence of a dominant free radical behavior. Significantly, the presence of widely common  
31 natural and synthetic carboxyl ligands selectively produced mostly the alcoholic species in  
32 the first oxidation step. A ferryl-based mechanism was thus preferred when iron complexes  
33 were formed. Common iron ligands are here proven to direct the reaction pathway towards a  
34 selective metal-based catalysis. Such a system may be more easily engineered than a free  
35 radical-based one to safely remove hazardous contaminants from water and minimize the  
36 production of harmful intermediates.

37

38

39

40 **Keywords:** advanced oxidation; Fenton mechanism; iron ligands; free radical mechanism;  
41 metal-based catalysis.

42

43 **Highlights**

- 44 • Systematic work investigating the Fenton mechanism in the presence of ligands
- 45 • The presence of ligands promotes metal-based catalysis
- 46 • The oxidation mechanism depends on the concentration of the iron ligand
- 47 • Different Fenton mechanisms occur by varying the pH
- 48 • A ferryl species is reasonably involved in the oxidation pathway with ligands

49

## 50 1. INTRODUCTION

51 The Fenton reaction has been known since over a century (Fenton 1894; Giannakis 2019) and  
52 represents an effective advanced oxidation process to remove many recalcitrant pollutants  
53 (Barbeni et al. 1987; Lipczynskakochany 1991; Maillard et al. 1992; Zhang et al. 2006; Liu et  
54 al. 2018; Ricceri et al. 2019). Nevertheless, this process has some major drawbacks when  
55 applied to contaminated waters, including the need to operate under acidic conditions, the  
56 formation of slurries of precipitated iron in the final basification step, and the competition of  
57 pollutants with other aqueous species, e.g., carbonate and dissolved organic matter, in the  
58 reaction with non-selective free radicals (Pignatello et al. 1999; Vione et al. 2014). A possible  
59 solution to tackle these limitations is the use of iron ligands, in analogy with the biological  
60 oxidations catalyzed by iron complexes, e.g., Cytochrome P450 (Cyt P450) or Taurine  
61 Dioxygenase (TauD) (Hohenberger et al. 2012). The use of similar iron(II) complexes as  
62 engineered catalysts is a promising variation of the traditional Fenton process, because it  
63 permits the degradation of persistent contaminants at near-neutral pH, while reducing the  
64 sludge production by keeping iron in solution (dos Santos et al. 2011; Song et al. 2015;  
65 Giannakis et al. 2016; Clarizia et al. 2017; Messele et al. 2019; Das et al. 2020; Pan et al.  
66 2020). Moreover, the use of an iron ligand can promote a more selective oxidative action,  
67 which increases the efficiency of the treatment and allows control over by-product formation.

68 Such improvements in the selectivity of the reaction depend on the reaction mechanism, more  
69 specifically on the active species, but little is known regarding the reaction pathway in these  
70 systems. The reaction mechanism is widely debated both for the classic Fenton process and  
71 for the modified Fenton process involving the chelation of iron (Barbusinski 2009; Zhang and  
72 Zhou 2019). In this work, we evaluate and discuss the application of a simple method to  
73 investigate the reaction pathway when iron ligands are present in water.

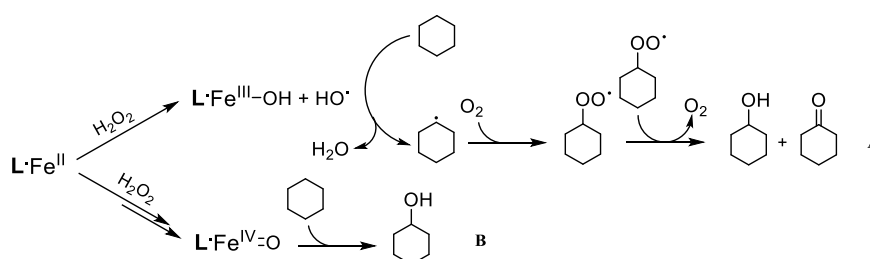
74 The classic description of the traditional Fenton process is based on the first reaction of the  
75 Haber-Weiss mechanism proposal (eq. 1) (Haber and Weiss 1932):



77 More recent investigations also proposed a mechanism that includes the formation of a  
78 transient species where iron has a formal redox state of IV (Goldstein et al. 1993; Bossmann  
79 et al. 1998). The presence of other superoxidized iron species (not only  $\text{Fe}^{\text{IV}}_{(\text{aq})}$ , but also  
80  $\text{Fe}^{\text{V}}_{(\text{aq})}$  and  $\text{Fe}^{\text{VI}}_{(\text{aq})}$ ) has been additionally proven (Wink et al. 1994). However, the reactivity,  
81 role, and the stability of such species is only partially known. Essential contributions were  
82 provided in the works by Bossmann et al. (1998) and Pignatello et al. (1999), who  
83 highlighted the different reactivity of ferryl and hydroxyl radicals and provided evidence of  
84 the concurrent presence of different reactive species. More recently, Minero and co-workers  
85 corroborated that  $\bullet\text{OH}$  (60% yield) and other species (e.g.,  $\text{FeO}^{2+}$ ) (40% yield) are formed  
86 simultaneously (Minero et al. 2013). As mentioned above, the oxidation mechanism is amply  
87 debated also when the system comprises iron complexes, e.g., in the presence of iron ligands.  
88 Rush and Koppenol investigated a variety of chelated iron complexes, concluding that a  
89 metal-oxo species was generated in neutral solutions, while  $\bullet\text{OH}$  species dominated the  
90 process in acidic solutions of non-chelated iron (Rush and Koppenol 1988). Sutton et al.  
91 (1987) reached a different conclusion, proposing that free iron generates a metal-oxo species  
92 as the primary oxidant while  $\bullet\text{OH}$  is dominant when chelated iron is present. A reasonable  
93 rationalization of this apparent discrepancy is that metal-oxo species and  $\bullet\text{OH}$  can both be  
94 generated concurrently in Fenton systems. Indeed, Yamazaki and Piette (1990) suggested that  
95 more than one type of oxidizing intermediate is present, and that the stoichiometry  
96  $\bullet\text{OH}:\text{Fe(II)}$  is also a function of the nature of the prevailing iron chelators. Different chelating  
97 agents for Fe(II) have been reported to promote the formation of oxoiron (ferryl) species in

98 addition to, or instead of,  $\cdot\text{OH}$ , thus accelerating (e.g., with fulvic acid (Southworth and  
99 Voelker 2003), oxalate (Park et al. 1997), and EDTA (Rush and Koppenol 1986)) or  
100 suppressing (e.g., with phosphates) the Fenton reaction (Iwahashi et al. 1990).

101 Because the direct experimental observation of the key intermediates involved in the  
102 oxidation pathways is challenging, indirect probes were developed (Klopstra et al. 2004;  
103 England et al. 2008; Oloo and Que 2013; Dong et al. 2018). Cyclohexane (Cy) was used in  
104 previous studies as an advantageous tool to discriminate between the different pathways of  
105 the Fenton reaction in organic solvents, by following the selective production of two  
106 products, namely cyclohexanol and cyclohexanone in different ratios (Oloo and Que 2013).  
107 Reactions initiated by hydroxyl radicals produce long-lived alkyl radical intermediates. These  
108 intermediates may react with dissolved molecular oxygen at diffusion-controlled rates to  
109 produce alkylperoxyl radicals, whose subsequent reaction is a Russell-type termination that  
110 gives equimolar quantities of alcohol (A) and ketone (K) (**Scheme 1a**) (Russell 1957;  
111 Meslennikov et al. 1979). Therefore,  $A/K \sim 1$  suggests the occurrence of hydroxyl radical-  
112 based reaction pathways. In contrast, an A/K ratio different than 1 is indicative of a non-free  
113 radical mechanism of oxidation, i.e., the presence of metal-based oxidant species (**Scheme**  
114 **1b**). However, to our knowledge, Cy oxidation has never been used as a probe to clarify in a  
115 systematic way the mechanism of the Fenton reactions in water.



116

117 **Scheme 1.** Proposed mechanism for cyclohexane oxidation. **A:** free radical path with Russel  
118 termination type. **B:** a metal-based path.

119 By using this mechanistic tool based on Cy oxidation, in this work we develop a facile  
120 method to provide evidence of the nature of Fenton reactive species in water in the presence  
121 of several common Fe(II) ligands. Specifically, the A/K ratio is used as a selectivity proxy to  
122 relate a structural parameter of the iron ligands (binding constants) with its influence on the  
123 reaction. Furthermore, we demonstrate the possibility to control the reaction pathway by  
124 adjusting the ligand concentration and other environmental conditions. One of the hypotheses  
125 of this work is that numerous iron ligands can promote a metal-based reaction process. As  
126 such, eight ligands are tested, namely, citric acid, tartaric acid, malic acid, quinic acid,  
127 EDTA, EDDS, and NTA, as well as Fe-TAML<sup>®</sup> (see Figure S1 in the Supplementary  
128 Material for its molecular structure). The first seven ligands are well-known iron chelators,  
129 applied to perform oxidation reactions in water at near-neutral pH. Fe-TAML<sup>®</sup> and the classic  
130 Fenton reagents (pH 3) are studied as standard controls for a metal-based and (supposed) free  
131 radical process, respectively (Collins 2002; de Oliveira et al. 2007; Ghosh et al. 2008).  
132 Through an active species linked to the iron-ligand complex, a metal-based mechanism can  
133 modulate the path of the reaction and generate fewer and more predictable by-products.  
134 Therefore, by verifying the involvement of a metal-based mechanism during a classic or  
135 modified Fenton process in water, one can open the route toward a safer oxidation of  
136 hazardous substances, e.g., phenols, pharmaceuticals, and pesticides.

137

## 138 **2. EXPERIMENTAL**

### 139 **2.1 Chemicals**

140 Fe(III)-TAML<sup>®</sup> was purchased from GreenOx Catalysts Inc. (Pittsburgh, PA, U.S.A.).

141 Sodium phosphate tribasic was obtained from Carlo Erba (Italy). All the other reagents,



142 buffer solutions, and solvents were purchased from Sigma-Aldrich. Water was of Milli-Q  
143 quality (TOC 2 ppb, resistivity  $\geq 18.2 \text{ M}\Omega \text{ cm}$ ).

## 144 **2.2 Reaction conditions**

145 The reaction experiments were carried out at room temperature in a 20 mL solution for 10  
146 min under continuous stirring, and were performed within 40 mL vials equipped by caps  
147 provided with septum. The concentrations of the catalyst (computed in terms of iron  
148 concentration), reagent (hydrogen peroxide), and substrate (cyclohexane) were  $10^{-7}$ ,  $10^{-6}$ ,  
149 and  $10^{-4}$  mol/L, respectively, resulting in a relative ratio of 1:10:1000. While a 1:10  
150 catalyst:oxidant ratio is typical of engineered applications, an excess of substrate was used  
151 here to avoid the subsequent oxidation of one of the major by-products, namely,  
152 cyclohexanol. Phosphate buffer (10 mM) or perchloric acid were used to fix the pH. Because  
153 the value of the solubility constant of phosphate with iron is approximately  $10^{-16}$ , significant  
154 formation of iron phosphate can be ruled out in favor of the formation of iron-ligand  
155 complexes. All the reactions were quenched by using *tert*-butyl alcohol (t-BuOH) as  
156 scavenger of reactive species (excess concentration of 30 mM, thus 300:1 compared to  
157 cyclohexane) for subsequent analysis (Rahhal and Richter 1988; Farinelli et al. 2019). The  
158 iron-ligand complexes were prepared in equimolar ratio in a concentrated stock solution (0.01  
159 mM) by stirring the mixture of the iron and ligand for 5 min, and were then diluted to 0.1  
160  $\mu\text{M}$ .

## 161 **2.3 Analytical conditions**

162 The headspace, solid phase microextraction technique (HS-SPME) was chosen as extraction  
163 method before carrying out GC-MS analysis. This technique does not require solvents and  
164 allows for highly sensitive analyses. Following each reaction experiment, the vials were left  
165 in a thermostatic bath at 50 °C for 10 min to promote the transfer of all the relevant

166 compounds into the gas-phase headspace. Then, a SPME fiber (df 75  $\mu\text{m}$ , fiber assembly  
167 carboxen/polydimethylsiloxane) was injected through the septum of the cap and was left in  
168 the headspace for 10 min, before withdrawing it for the subsequent GC-MS analysis. Samples  
169 were analyzed on an Agilent 6890 GC system coupled with an Agilent 5973N mass selective  
170 detector (MSD). For the chromatographic separation, a Zebron-5MS capillary column (30  
171  $\text{m} \cdot \times 250 \text{ mm} \cdot \times 0.25 \mu\text{m}$ ) was used. The injection port temperature was 280  $^{\circ}\text{C}$ , and the oven  
172 temperature program was set as follows: 40 $^{\circ}\text{C}$  for 5 min, then an increase to 310 $^{\circ}\text{C}$  at a rate  
173 of 15  $^{\circ}\text{C}/\text{min}$  (total run time 28.00 min). Helium was used as carrier gas at a constant flow of  
174 1.2 mL/min, and the injector was held in splitless mode. The interface temperature was  
175 280 $^{\circ}\text{C}$ , the ionization energy was 70 eV, and the mass spectrometer operated in SIM mode  
176 acquiring the following fragments: 84, 56, 41 (cyclohexane); 82, 67, 57 (cyclohexanol); 98,  
177 55, 42 (cyclohexanone). Duplicate experiments for some of the tests discussed in this study  
178 indicated high repeatability of the results; the error associated to the data and presented below  
179 is related to the intrinsic uncertainty of the SPME technique, computed as the average among  
180 the standard deviations reported in the 525.2 method provided by the EPA.

181 A few control experiments were carried out to study the Fenton degradation of phenol (by  
182 both  $\text{Fe}^{2+} + \text{H}_2\text{O}_2$  and  $\text{Fe}^{\text{III}}\text{-TAML}^{\text{®}} + \text{H}_2\text{O}_2$ ) using t-BuOH as scavenger, to take advantage of  
183 the ability of this compound to react with  $\bullet\text{OH}$  faster than with electron-capture oxidants,  
184 such as ferryl (Buxton et al. 1988; Rahhal and Richter 1988). The time evolution of phenol  
185 was monitored by liquid chromatography (see the Supplementary Material for additional  
186 details).

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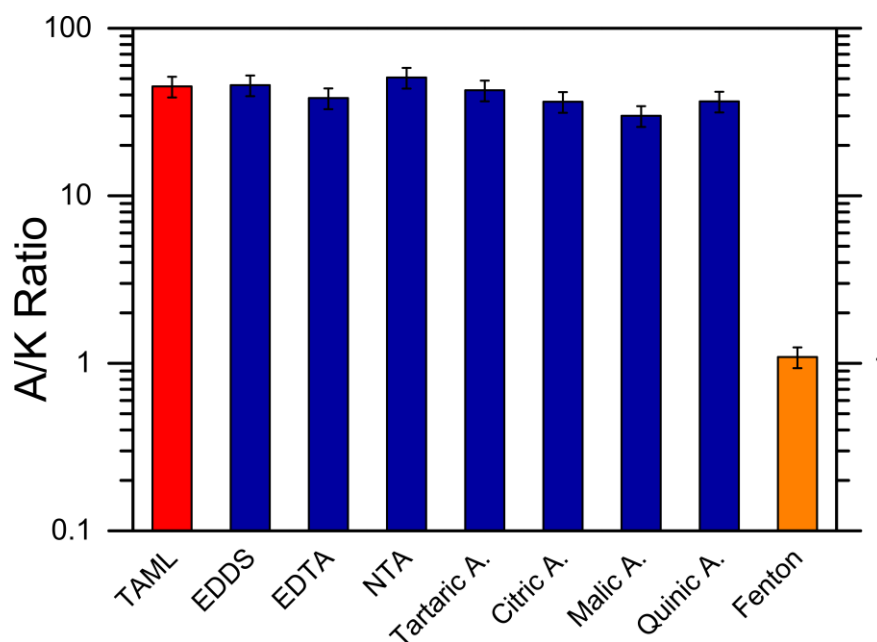
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### 191 3. RESULTS AND DISCUSSION

#### 192 3.1 Iron ligands promote the preferential formation of cyclohexanol

193 Eight widely available ligands able to complex iron in a stable fashion were tested, namely:  
194 citric acid, tartaric acid, malic acid, quinic acid, EDTA (ethylenediaminetetraacetic acid),  
195 EDDS (ethylenediamine-N,N'-disuccinic acid), NTA (nitrilotriacetate), and TAML  
196 (tetraamidomacrocyclic ligand). These ligands belong to two macro-categories: natural  
197 (citric, tartaric, malic, quinic acid) and artificial ligands (EDTA, EDDS, NTA, TAML). This  
198 choice was provisionally made to gain insight into any possible correlation between the two  
199 categories, or among ligands in the same category. The Fe-TAML<sup>®</sup> system is well-known to  
200 induce a metal-based oxidation process via a ferryl species, thus we expected an  
201 alcohol/ketone (A/K) product ratio different from 1 upon oxidation of cyclohexane (Collins  
202 2002; de Oliveira et al. 2007). Conversely, the Fenton process at pH 3 generates mostly  
203 hydroxyl radicals, or at least the hydroxyl radical is the most reactive (although not the only  
204 one) species in the system. Therefore, the reaction should proceed mostly via a free radical  
205 mechanism, with an A/K ratio around 1 (Minero et al. 2013).

206 **Figure 1** shows the A/K ratio values obtained with all the investigated ligands and in the  
207 absence of ligands, i.e., classic Fenton. The results obtained from oxidation tests are in line  
208 with expectations, thus attesting to the validity of the method. The classic Fenton process at  
209 pH 3 showed an A/K ratio around 1, while an A/K ratio significantly higher than 1 was  
210 obtained with all the other investigated ligands. Therefore, it is reasonable to hypothesize that  
211 the presence of an iron ligand in water promotes a metal-based oxidation.



212  
213

214 **Figure 1.** A/K ratio observed in the oxidation of cyclohexane with different iron ligands at  
215 pH 7 (phosphate buffer) and with the classic Fenton process at pH 3 (perchloric acid) after 30  
216 min of reaction.

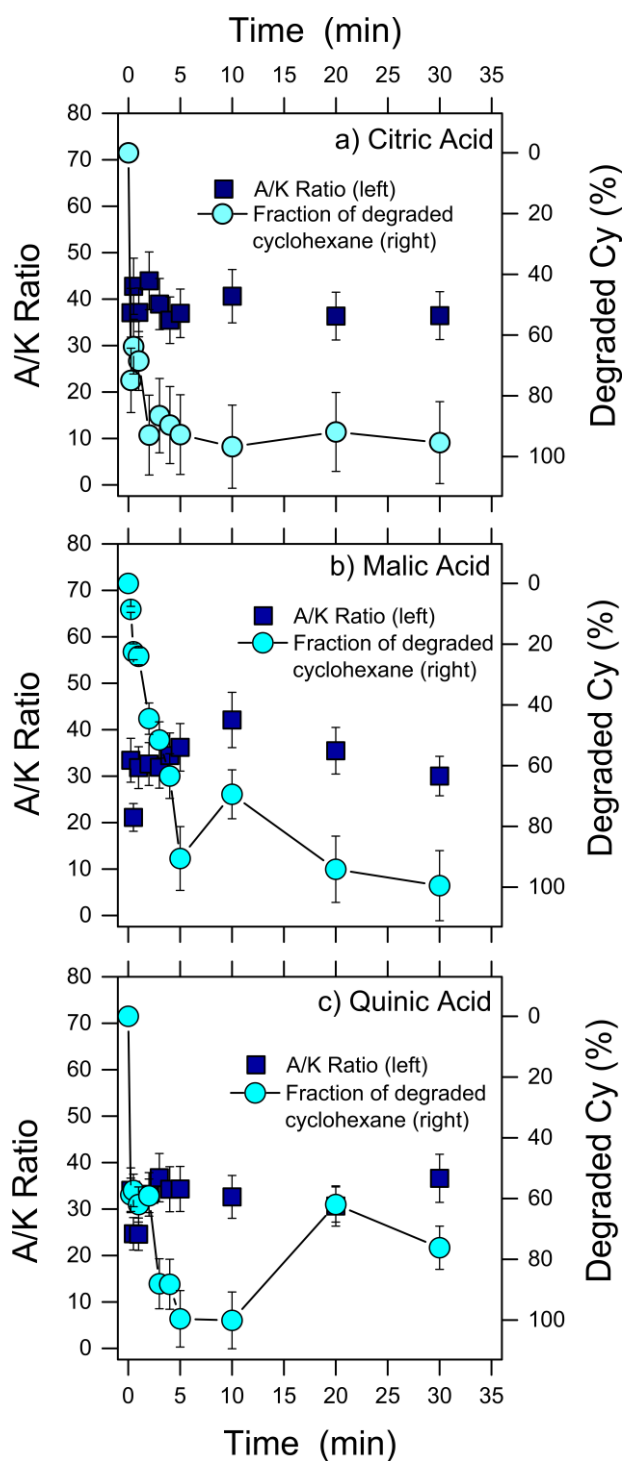
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218 These conclusions are confirmed by the results of the degradation of phenol, with  $\text{Fe}^{2+} +$   
219  $\text{H}_2\text{O}_2$  at pH 3 and with  $\text{Fe-TAML}^{\text{®}} + \text{H}_2\text{O}_2$  (see Figure S2 in Supplementary Material, as well  
220 as the related text). We carried out these experiments both in the absence and in the presence  
221 of t-BuOH, which reacts with  $\bullet\text{OH}$  faster than with ferryl (Buxton et al. 1988; Rahhal and  
222 Richter 1988). However, to avoid total quenching of the system by t-BuOH, the t-  
223 BuOH:phenol ratio was 40:1 and not 300:1 as per the Cy experiments. In the case of  $\text{Fe}^{2+} +$   
224  $\text{H}_2\text{O}_2$ , t-BuOH strongly inhibited phenol degradation, while in the case of  $\text{Fe-TAML}^{\text{®}}$  the  
225 effect of the alcohol was practically negligible. These findings are consistent with  $\bullet\text{OH}$  being  
226 involved in phenol degradation by  $\text{Fe}^{2+} + \text{H}_2\text{O}_2$  at pH 3, and with ferryl playing the same role  
227 with  $\text{Fe-TAML}^{\text{®}} + \text{H}_2\text{O}_2$ . Indeed, the t-BuOH scavenging experiments agree with the A/K  
228 ratios derived from the Cy degradation experiments (**Figure 1**).

229 It is important to check for possible variations of the A/K ratio with reaction time, to ensure  
230 that unbiased conclusions are obtained. **Figure 2** shows the kinetics of Cy degradation with  
231 citric acid (**Figure 2a**), malic acid (**Figure 2b**), and quinic acid (**Figure 2c**) as iron ligands  
232 (left Y-axis: A/K ratio, right Y-axis: Cy degradation). Cy degradation with citric and quinic  
233 acids was very fast and the process reached completion after roughly 2 min of reaction. The  
234 corresponding A/K ratio remained stable and significantly larger than 1 during the entire  
235 duration of the test (30 min). On the other hand, **Figure 2b** (malic acid) shows slower  
236 kinetics of degradation, with an A/K ratio reaching a peak value after 10 min of reaction. This  
237 slower degradation allowed for an easier monitoring of the initial preferred formation of the  
238 alcohol species ( $A/K > 1$ ) and the subsequent oxidation of the alcohol into the ketone, which  
239 caused a slight A/K reduction following the peak. The large excess of the initial Cy  
240 consumed almost all the reactive species, thereby limiting their availability for alcohol  
241 oxidation. By monitoring the A/K time evolution, one can thus be confident that there is  
242 negligible bias linked to the further evolution of the system ( $A \rightarrow K$  oxidation, or further  
243 Fenton processes involving, e.g., Fe(III) after total Fe(II) consumption) after the initial  
244 reaction step (Russell 1957).

245 The fact that the A/K ratio did not change much after the initial step suggests that the  
246 mechanistic conditions reflect those of the initial reaction between Fe(II) and  $H_2O_2$ . Under  
247 our experimental conditions and based on the stoichiometry of reaction (1), this process  
248 would entail total consumption of Fe(II) that would be oxidized to Fe(III), and 10%  
249 degradation of  $H_2O_2$ . Afterwards, Fe(III) would be recycled to Fe(II) at the expense of the  
250 remaining  $H_2O_2$ .

251



252

253 **Figure 2.** Fraction of degraded cyclohexane with respect to the total degraded amount  
 254 (circles, right axis) and trend of selectivity, i.e., A/K ratio (squares, left axis), as a function of  
 255 time in a system containing hydrogen peroxide as reactant and a) citric acid, b) malic acid, or  
 256 c) quinic acid as iron ligands. The pH of the aqueous system was buffered at 7 (phosphate  
 257 buffer). The solid lines connecting the circles are only intended as a guide for the eye.

258 The influence of the reaction environment was also studied by following the A/K ratio in the  
259 oxidation of Cy at different pH values (3-7) (**Table 1**). All the individual concentrations of  
260 alcohol and ketone products obtained during the experiments are presented in the  
261 Supplementary Material (Tables S1-S3). Citric, malic, and quinic acid were chosen as iron  
262 ligands because the iron binding constant of these compounds would not change significantly  
263 within the explored pH range, thereby allowing for the pH value to solely affect the Fenton  
264 process (Supplementary Material, Figure S3). Phosphate (10 mM) was used to buffer the pH  
265 at values of 5, 6, and 7, while perchloric acid (1.16 M stock solution) was employed in tests  
266 performed at pH 3. In all these cases, the A/K ratios were higher than 1, independently of the  
267 pH values. This finding suggests that a metal-based mechanism in the presence of the three  
268 ligands was observed regardless of the acidity of the solution. Although acidity has no direct  
269 effect on the mechanistic path, the conditional binding constant may be function of the pH  
270 and this indirect effect of the solution acidity is discussed below.

271

272 **Table 1.** A/K ratio for the oxidation of cyclohexane at different pH values with citric, malic  
273 and quinic acid as iron ligands. The pH was fixed with phosphate buffer except for pH 3  
274 ( $\text{HClO}_4$ ). The reaction time was 30 min.

275

	pH 3	pH 5	pH 6	pH 7
<b>Citric acid</b>	10±1	7.4±1.0	8.5±1.2	8.6±1.2
<b>Malic acid</b>	18±3	15±2	16±2	15±2
<b>Quinic acid</b>	28±4	31±4	23±3	28±4

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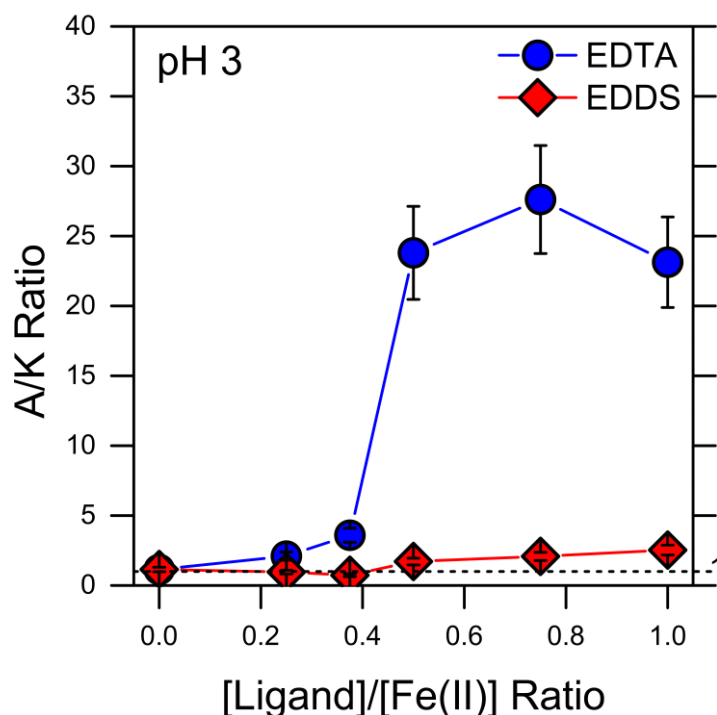
## 280 3.2 Switching the mechanism from free radical to metal-based catalysis

281 In the previous section, we hypothesized that the mechanistic degradation path depends on  
282 the presence of the ligand, and possibly on its concentration and conditional binding constant.  
283 In order to investigate the influence of the ligand concentration and of its conditional binding  
284 constant with the metal (i.e., the value of the binding constant that takes into account the  
285 protonation of the ligand at the given pH value), EDTA and EDDS were chosen as iron  
286 ligands and applied at acidic pH. Despite their structural similarity, these two ligands behave  
287 differently in terms of their conditional binding constant as a function of pH. Specifically,  
288 while the conditional binding constant at pH 7 is high for both EDTA and EDDS ( $\sim 10^{11}$  and  
289  $10^6$ , respectively), at pH 3 EDDS features a low conditional binding constant ( $10^{-3}$ ), while  
290 that of EDTA is still relatively high ( $\sim 10^4$ ) (see Supplementary Material, Figure S3).  
291 Therefore, when using EDDS as iron ligand at pH 3, one expects a high amount of free iron  
292 to occur in solution, which could reasonably induce a classic Fenton process (free radical  
293 mechanism). In this series of experiments, the pH value was fixed at a value of 3 by addition  
294 of perchloric acid.

295 **Figure 3** reports the A/K ratios observed when EDDS and EDTA were used as ligands at pH  
296 3, at various [Ligand]:[Fe(II)] ratios. All the individual concentrations of alcohol and ketone  
297 products obtained during the experiments are presented in the Supplementary Material  
298 (Tables S4-S5). Please note that **Figure 1** summarized instead the A/K ratios measured at 1:1  
299 [Ligand]:[Fe(II)] ratio and at pH 7. Consistently with the hypotheses, at 0:1 ligand:iron ratio  
300 (i.e., with no ligand in solution) we observed the classic Fenton process and the A/K ratio  
301 was close to 1. The A/K ratio remained always close to 1 with EDDS, regardless of its  
302 concentration. Based on the conditional binding constants, when using EDDS as iron ligand  
303 at pH 3, one expects free iron to occur in solution, which could reasonably promote a process  
304 similar to a classic  $\bullet\text{OH}$ -based Fenton reaction. In contrast, with EDTA the reaction clearly



305 switched from a free radical mechanism to a metal-based one when the ligand concentration  
306 increased (Bernasconi and Baerends 2009; Mang et al. 2016). These results strongly suggest  
307 that the presence of a bonded Fe(II)-ligand complex plays a crucial role in the direction of the  
308 mechanistic path. This parameter is a strong function of the concentration of the ligand as  
309 well as of the conditional binding constant of the complex metal/ligand. Therefore, one can  
310 generalize that the mechanistic path may be mostly imputable to the concentration and to the  
311 conditional binding constant of the ligand, and that the environmental conditions have  
312 importance only if they affect complex formation.

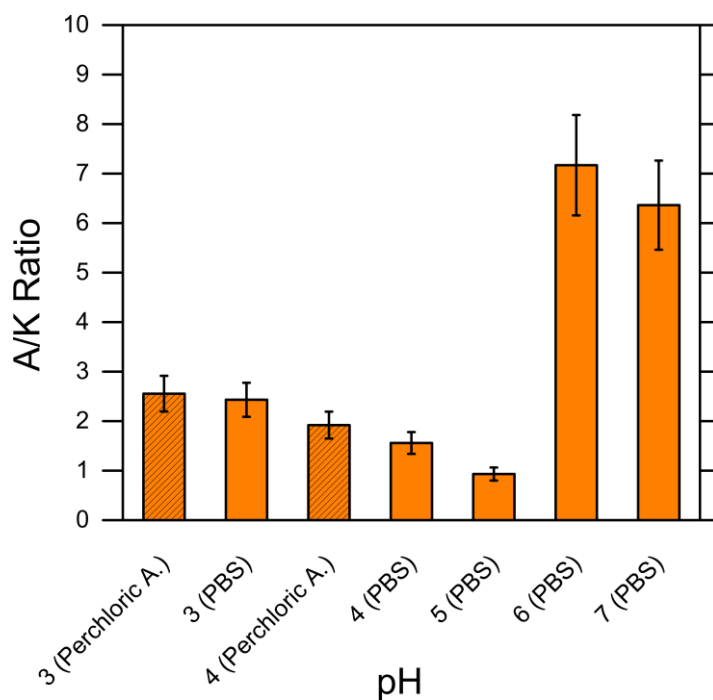


313  
314 **Figure 3.** A/K ratio observed after 30 min of reaction in the oxidation of cyclohexane with  
315 EDTA and EDDS as iron ligands, added at different concentrations. The runs were carried  
316 out at pH 3. The condition of no added Ligand ( $[Ligand]/[Fe(II)] = 0$ ) corresponds to the  
317 classic Fenton process. The solid lines are only intended as a guide for the eye. The dashed  
318 line depicts the expected A/K ratio for a pure free radicals-based catalysis.

319

### 320 3.3 Evidence of the effect of pH on the traditional Fenton process

321 Finally, we provide some insight into the Fenton mechanism at different pH values. **Figure 4**  
322 shows the A/K ratio obtained with the classic Fenton process in the 3-7 pH range, fixed by  
323 use of phosphate buffer (PBS). Additional experiments were also conducted by fixing the pH  
324 at 3 and 4 using perchloric acid instead of phosphate buffer. Please note that Fe(II) was dosed  
325 at low concentration (0.1  $\mu\text{M}$ ) to avoid its precipitation as hydroxide, which would otherwise  
326 take place at near-neutral pH ( $\text{pKs}^{\text{Fe(OH)}_2} = 15.1$  (Harris 2006)). The results obtained in the  
327 absence of ligands imply that the classic Fenton reaction proceeds through a free radical or  
328 mixed mechanism up to pH 5. Above this value, the mechanism switched to a preferential  
329 metal-based one. This result is supported by previous reports proposing that a ferryl species is  
330 involved in the Fenton reaction at near-neutral pH (Rush et al. 1990; Bossmann et al. 2004;  
331 Bataineh et al. 2012). The presence of a ferryl species at pH 6-7 means that the classic Fenton  
332 reaction will be less reactive under near-neutral conditions, since the non-coordinated ferryl  
333 species is considered less reactive than the hydroxyl radical (Bataineh et al. 2012).



334

335 **Figure 4** A/K ratio observed in the classic Fenton process performed at different pH values.

#### 336 4. CONCLUSIONS

337 The oxidation mechanism of the Fenton reaction was here investigated with and without iron  
338 ligands in solution. Eight widely common ligands that are able to complex iron(II) were  
339 studied, namely, citric acid, tartaric acid, malic acid, quinic acid, EDTA, EDDS, and NTA,  
340 plus the Fe-TAML<sup>®</sup> system. The ligand performance was tested toward the oxidation of  
341 cyclohexane by following the formation of the main products, namely, cyclohexanol (A) and  
342 cyclohexanone (K). Measurement of the concentration ratio between these two species (A/K  
343 ratio parameter) during the reaction provides evidence of the mechanism involved in the  
344 oxidation of the substrate. This simple method was proven effective to discriminate in water  
345 between the predominance of a non-selective active species, free radicals, or of a more  
346 selective ferryl species.

347 All the tested ligands showed high A/K ratios, which is a proper index of a metal-based  
348 behavior, including the well-known Fe-TAML<sup>®</sup> system that was expected to behave in this  
349 fashion. Also expected was the fact that the classic Fenton process was associated with an  
350 A/K ratio of approximately 1, evidence of a free radical process, which adds further evidence  
351 in favor of cyclohexane as suitable probe when coupled with the A/K ratio. The results  
352 suggested that the ligand/iron concentration ratio plays a role in the mechanism of reaction  
353 and this parameter may be thus exploited to control the pathways of contaminant degradation  
354 in water. Also, if the ligand-specific binding constant changes with environmental conditions,  
355 e.g., the pH, the reaction mechanism may be controlled by adjusting such conditions.

356 This work proves the ability of simple iron ligands to drive the reaction pathway towards  
357 selective metal-based catalysis. Selective catalysis allows for better control of the degradation  
358 pathway of harmful contaminants, to avoid the formation of toxic by-products. The  
359 ubiquitous character of the Fenton process in nature and of iron complexes formed in water

360 (e.g., citrate), alongside the generation of H<sub>2</sub>O<sub>2</sub> in illuminated NOM-containing  
361 environments, lead to (photo)Fenton reactions during the diurnal cycles. As such, the present  
362 study holds important implications in the elucidation of the Fenton process that occurs both in  
363 nature and in engineering applications, and represents a step further in understanding the  
364 effectiveness of ligand-mediated oxidation of natural and anthropogenic contaminants.

365

## 366 **ASSOCIATED CONTENT**

367 **Supporting Information.** The Supporting Information is available free of charge on the ACS  
368 Publications website

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### 373 **Notes**

374 The authors declare no competing financial interest.

375

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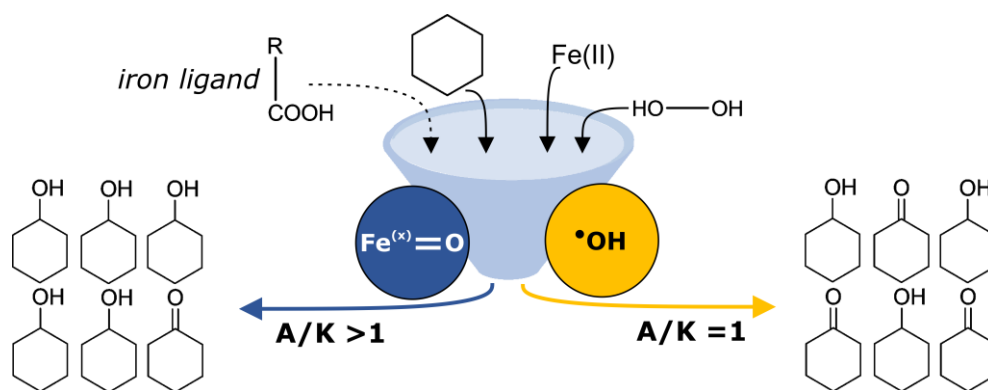
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543 **Graphical Abstract**



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