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(Article begins on next page)

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

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

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19

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.

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

42

43 **Highlights**

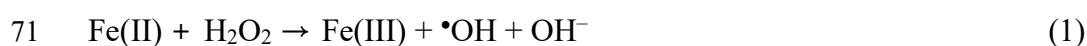
- 44 • Systematic work investigating the Fenton mechanism in the presence of ligands
- 45 • The presence of ligands promotes metal-based catalysis
- 46 • The oxidation mechanism depends on the concentration of the iron ligand
- 47 • Different Fenton mechanisms occur by varying the pH
- 48 • A ferryl 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). Nevertheless, this
54 process has some major drawbacks when applied to contaminated waters, including the need
55 to operate under acidic conditions, the formation of large slurries of precipitated iron in the
56 final basification step, and the competition of pollutants with other aqueous species, e.g.,
57 carbonate and dissolved organic matter, in the reaction with non-selective free radicals
58 (Pignatello et al. 1999; Vione et al. 2014). The reliance upon non-selective radicals decreases
59 the efficiency of the process in the presence of interfering species, and prevents control over
60 the formation of potentially hazardous reaction intermediates. A possible solution to tackle
61 these limitations is the use of iron ligands, in analogy with the biological oxidations catalyzed
62 by iron complexes, e.g., Cytochrome P450 (Cyt P450) or Taurine Dioxygenase (TauD)
63 (Hohenberger et al. 2012). The use of similar iron(II) complexes as engineered catalysts is a
64 promising variation of the traditional Fenton process, because it permits the degradation of
65 persistent contaminants at near-neutral pH, while reducing the sludge production by keeping
66 iron in solution (dos Santos et al. 2011; Song et al. 2015; Giannakis et al. 2016; Clarizia et al.
67 2017; Messele et al. 2019; Das et al. 2020). Nevertheless, little is known regarding the
68 reaction pathway in these systems.

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



72 More recent investigations also proposed a mechanism that includes the formation of a
73 transient species where iron has a formal redox state of IV (Goldstein et al. 1993; Bossmann
74 et al. 1998). The presence of other superoxidized iron species (not only $\text{Fe}^{\text{IV}}_{(\text{aq})}$, but also
75 $\text{Fe}^{\text{V}}_{(\text{aq})}$ and $\text{Fe}^{\text{VI}}_{(\text{aq})}$) has been additionally proven by stopped-flow experiments and UV-Vis
76 spectroscopy (Wink et al. 1994). However, the reactivity, role, and the stability of such
77 species is only partially known. Essential contributions were provided in the works by
78 Bossmann et al. (1998) and Pignatello et al. (1999), who highlighted the different reactivity
79 of ferryl and hydroxyl radicals. In particular, Bossmann and co-workers compared the
80 products of the reaction of 2,4-xylydine in genuine $\bullet\text{OH}$ generation processes (H_2O_2
81 photolysis) with those obtained from Fenton reactions, showing the formation of different
82 reactive species (Bossmann et al. 1998). Pignatello et al. (1999) provided evidence of
83 alternative oxidants to $\bullet\text{OH}$. More recently, Minero and co-workers concluded that $\bullet\text{OH}$ (60%
84 yield) and other species (e.g., FeO^{2+}) (40% yield) are formed simultaneously (Minero et al.
85 2013).

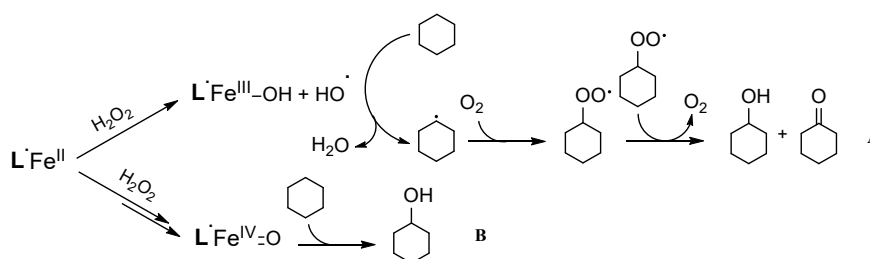
86 The oxidation mechanism is amply debated also when the system comprises iron complexes,
87 e.g., in the presence of iron ligands. Rush and Koppenol investigated a variety of chelated
88 iron complexes, concluding that a metal-oxo species was generated in neutral solutions, while
89 $\bullet\text{OH}$ species dominated the process in acidic solutions of non-chelated iron (Rush and
90 Koppenol 1988). Sutton et al. (1987) reached a different conclusion, proposing that free iron
91 generates a metal-oxo species as the primary oxidant while $\bullet\text{OH}$ is dominant when chelated
92 iron is present. A reasonable rationalization of this apparent discrepancy is that metal-oxo
93 species and $\bullet\text{OH}$ can both be generated concurrently in Fenton systems. Indeed, Yamazaki
94 and Piette (1990) suggested that more than one type of oxidizing intermediate is present, and
95 that the stoichiometry $\bullet\text{OH}:\text{Fe}(\text{II})$ is also a function of the nature of the prevailing iron

96 chelators. Different chelating agents for Fe(II) have been reported to promote the formation
97 of oxoiron (ferryl) species in addition to, or instead of, •OH, thus accelerating (e.g., with
98 fulvic acid (Southworth and Voelker 2003), oxalate (Park et al. 1997), and EDTA (Rush and
99 Koppenol 1986)) or suppressing (e.g., with phosphates) the Fenton reaction (Iwahashi et al.
100 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 ~ 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 By using this mechanistic tool based on Cy oxidation, in this work we (*i*) support previous
117 findings that (*a*) the classic $\text{Fe}^{2+} + \text{H}_2\text{O}_2$ process yields •OH in acidic conditions, but the
118 reactant shifts from •OH to ferryl at neutral pH values, and (*b*) the process combining the

119 commercial iron tetra-amido macrocyclic ligand compound (Fe-TAML[®]) and H₂O₂ operates
120 through a ferryl species.



121
122 **Scheme 1.** Proposed mechanism for cyclohexane oxidation. **A:** free radical path with Russel
123 termination type. **B:** a metal-based path.

124
125 One of the hypotheses of this work is that many iron ligands can promote a metal-based
126 reaction process. As such, eight ligands were tested, namely, citric acid, tartaric acid, malic
127 acid, quinic acid, EDTA, EDDS, and NTA, as well as Fe-TAML[®] (see Figure S1 in the
128 Supplementary Material for its molecular structure). Therefore, we (ii) provide evidence of
129 the nature of Fenton reactive species in the presence of several common Fe(II) ligands, also
130 as a function of ligand concentration to control the reaction pathway. The first seven ligands
131 are well-known iron chelators, applied to perform oxidation reactions in water at near-neutral
132 pH. Fe-TAML[®] and the classic Fenton reagents at pH 3 are studied as standard controls for a
133 metal-based and (supposed) free radical process, respectively (Collins 2002; de Oliveira et al.
134 2007; Ghosh et al. 2008). Through an active species linked to the iron-ligand complex, a
135 metal-based mechanism can modulate the path of the reaction and generate fewer and more
136 predictable by-products. Therefore, by verifying the involvement of a metal-based
137 mechanism during a classic or modified Fenton process in water, one can open the route
138 toward a safer oxidation of hazardous substances, e.g., phenols, pharmaceuticals, and
139 pesticides.

140 2. EXPERIMENTAL

141 2.1 Chemicals

142 Fe(III)-TAML[®] was purchased from GreenOx Catalysts Inc. (Pittsburgh, PA, U.S.A.).
143 Sodium phosphate tribasic was obtained from Carlo Erba (Italy). All the other reagents,
144 buffer solutions, and solvents were purchased from Sigma-Aldrich. Water was of Milli-Q
145 quality (TOC 2 ppb, resistivity ≥ 18.2 M Ω cm).

146 2.2 Reaction conditions

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

163

164 2.3 Analytical conditions

165 The headspace, solid phase microextraction technique (HS-SPME) was chosen as extraction
166 method before carrying out GC-MS analysis. This technique does not require solvents and
167 allows for highly sensitive analyses. Following each reaction experiment, the vials were left
168 in a thermostatic bath at 50 °C for 10 min to promote the transfer of all the relevant
169 compounds into the gas-phase headspace. Then, a SPME fiber (df 75 µm, fiber assembly
170 carboxen/polydimethylsiloxane) was injected through the septum of the cap and was left in
171 the headspace for 10 min, before withdrawing it for the subsequent GC-MS analysis. Samples
172 were analyzed on an Agilent 6890 GC system coupled with an Agilent 5973N mass selective
173 detector (MSD). For the chromatographic separation, a Zebron-5MS capillary column (30
174 m × 250 mm × 0.25 µm) was used. The injection port temperature was 280 °C, and the oven
175 temperature program was set as follows: 40°C for 5 min, then an increase to 310°C at a rate
176 of 15 °C min⁻¹ (total run time 28.00 min). Helium was used as carrier gas at a constant flow
177 of 1.2 mL min⁻¹, and the injector was held in splitless mode. The interface temperature was
178 280°C, the ionization energy was 70 eV, and the mass spectrometer operated in SIM mode
179 acquiring the following fragments: 84, 56, 41 (cyclohexane); 82, 67, 57 (cyclohexanol); 98,
180 55, 42 (cyclohexanone). Duplicate experiments for some of the tests discussed in this study
181 indicated high reproducibility of the results; the error associated to the data and presented
182 below is related to the intrinsic uncertainty of the SPME technique, computed as the average
183 among the standard deviations reported in the 525.2 method provided by the EPA.

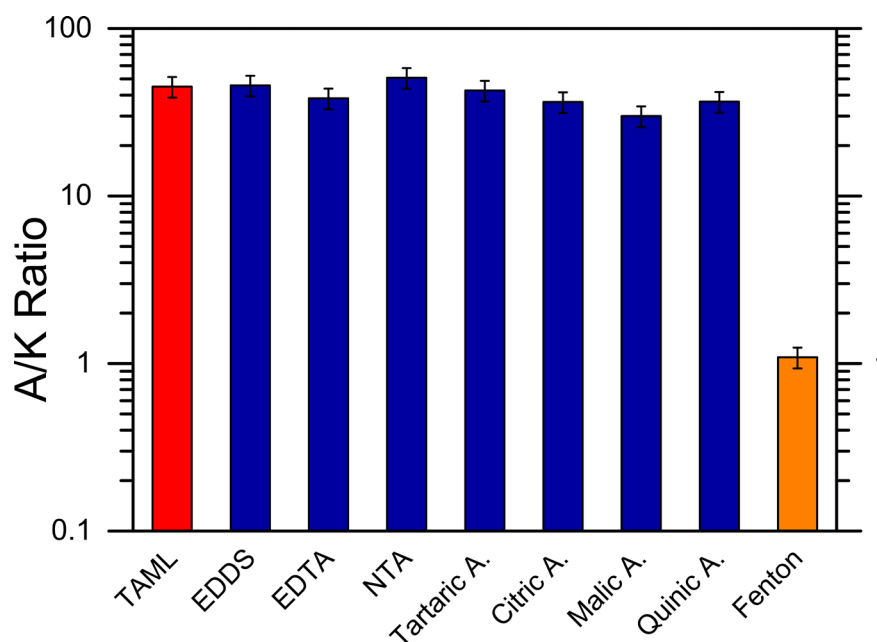
184 A few control experiments were carried out to study the Fenton degradation of phenol (by
185 both Fe²⁺ + H₂O₂ and Fe^{III}-TAML[®] + H₂O₂) using t-BuOH as scavenger, to take advantage of
186 the ability of this compound to react with •OH faster than with electron-capture oxidants such
187 as ferryl (Buxton et al., 1988; Rahhal and Richter 1988). The time evolution of phenol was
188 monitored by liquid chromatography (see the Supplementary Material for additional details).

189 3. RESULTS AND DISCUSSION

190 3.1 Iron ligands promote the preferential formation of cyclohexanol

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

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



210
211

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

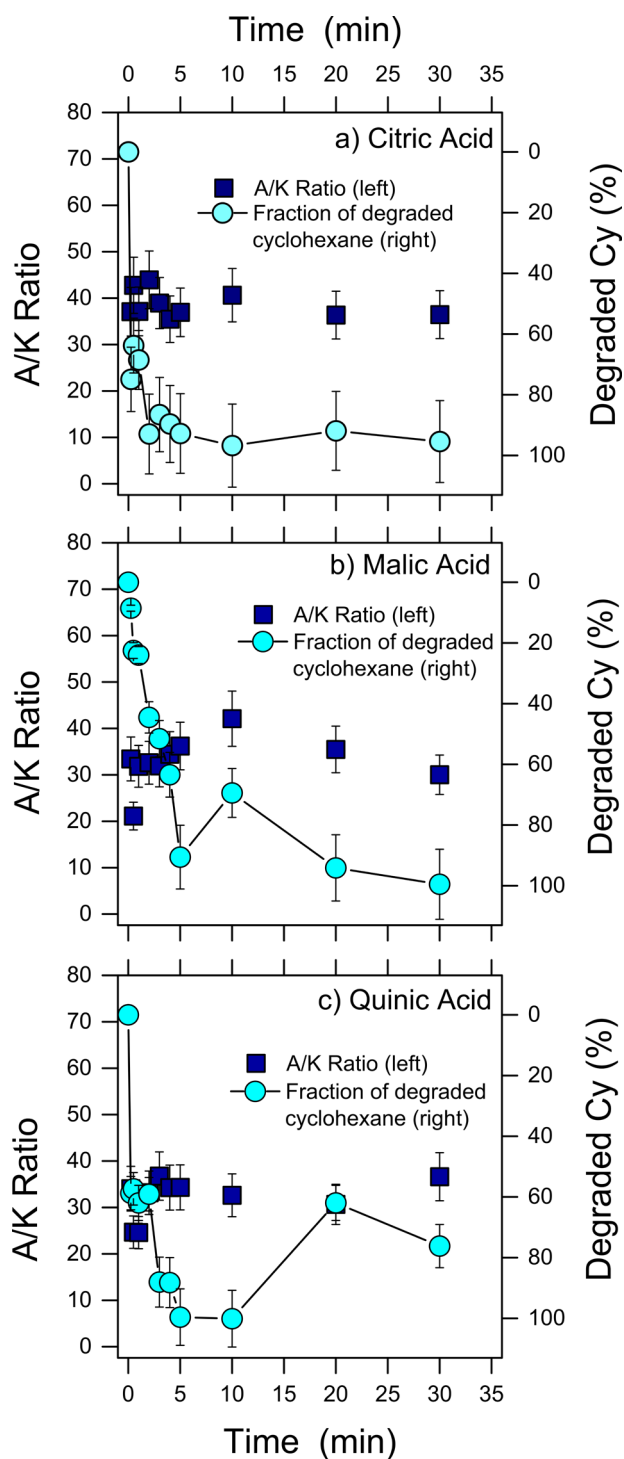
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216 These conclusions are confirmed by the results of the degradation of phenol, with $\text{Fe}^{2+} +$
217 H_2O_2 at pH 3 and with $\text{Fe-TAML}^{\text{®}} + \text{H}_2\text{O}_2$ (see Figure S2 in Supplementary Material, as well
218 as the related text). We carried out these experiments both in the absence and in the presence
219 of t-BuOH, which reacts with $\bullet\text{OH}$ faster than with ferryl (Buxton et al., 1988; Rahhal and
220 Richter 1988). However, to avoid total quenching of the system by t-BuOH, the t-
221 BuOH:phenol ratio was 40:1 and not 300:1 as per the Cy experiments. In the case of $\text{Fe}^{2+} +$
222 H_2O_2 t-BuOH strongly inhibited phenol degradation, while in the case of $\text{Fe-TAML}^{\text{®}}$ the
223 effect of the alcohol was practically negligible. These findings are consistent with $\bullet\text{OH}$ being
224 involved in phenol degradation by $\text{Fe}^{2+} + \text{H}_2\text{O}_2$ at pH 3, and with ferryl playing the same role
225 with $\text{Fe-TAML}^{\text{®}} + \text{H}_2\text{O}_2$. Indeed, the t-BuOH scavenging experiments agree with the A/K
226 ratios derived from the Cy degradation experiments (**Figure 1**).

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

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

249



250

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

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

269

270

271 **Table 1.** A/K ratio for the oxidation of cyclohexane at different pH values with citric, malic
272 and quinic acid as iron ligands. The pH was fixed with phosphate buffer except for pH 3
273 (HClO₄). The reaction time was 30 min.

274

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

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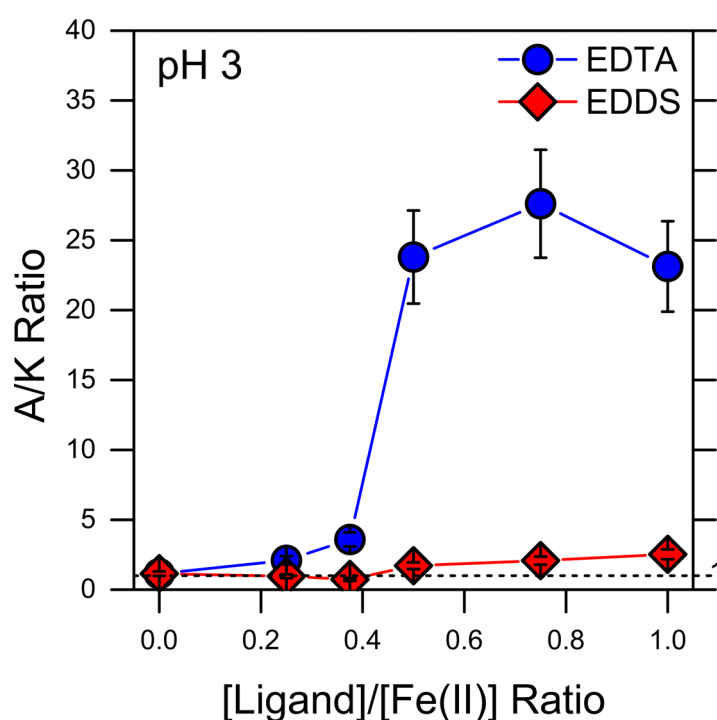
279 3.2 Switching the mechanism from free radical to metal-based catalysis

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

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

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

311



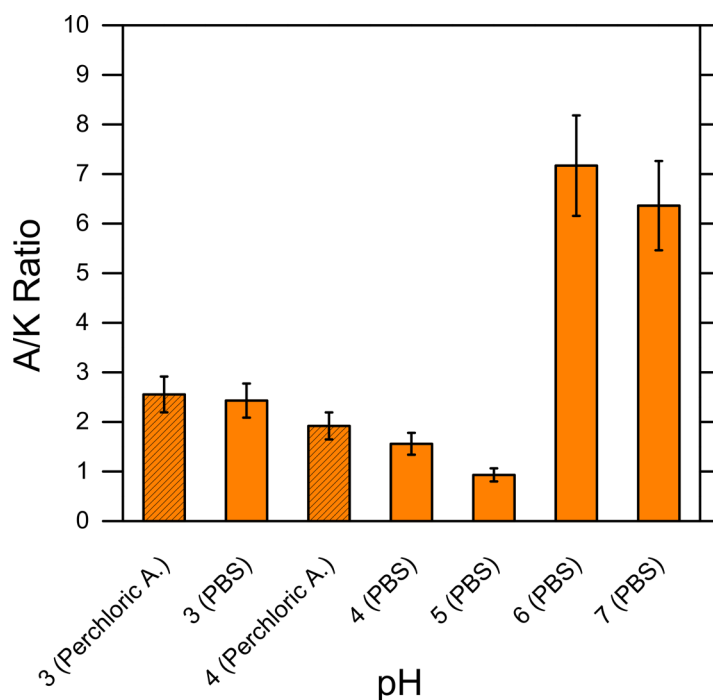
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313 **Figure 3.** A/K ratio observed after 30 min of reaction in the oxidation of cyclohexane with
314 EDTA and EDDS as iron ligands, added at different concentrations. The runs were carried
315 out at pH 3. The condition of no added Ligand ($[Ligand]/[Fe(II)]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

318

319 3.3 Evidence of the effect of pH on the traditional Fenton process

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



333 **Figure 4** A/K ratio observed in the classic Fenton process performed at different pH values.
334

335 4. CONCLUSIONS

336 The oxidation mechanism of the Fenton reaction was here investigated with and without iron
337 ligands in solution. Eight widely common ligands that are able to complex iron(II) were
338 studied, namely, citric acid, tartaric acid, malic acid, quinic acid, EDTA, EDDS, and NTA,
339 plus the Fe-TAML[®] system. The ligand performance was tested toward the oxidation of
340 cyclohexane by following the formation of the main products, namely, cyclohexanol (A) and
341 cyclohexanone (K). Measurement of the concentration ratio between these two species (A/K
342 ratio parameter) during the reaction was used to provide evidence of the mechanisms
343 involved in the oxidation of the substrate.

344 All the tested ligands showed high A/K ratios, which is a proper index of a metal-based
345 behavior, including the well-known Fe-TAML[®] system that was expected to behave in this
346 fashion. Also expected was the fact that the classic Fenton process was associated with an
347 A/K ratio of approximately 1, evidence of a free radical process, which adds further evidence
348 in favor of cyclohexane as suitable probe when coupled with the A/K ratio.

349 This work proves the ability of simple iron ligands to drive the reaction pathway towards
350 selective metal-based catalysis, as opposed to the activity of free radicals that promote
351 undifferentiated oxidation of substrates in solution. Selective catalysis allows for better
352 control of the degradation pathway of harmful contaminants, to avoid the formation of toxic
353 by-products. This study also provides a further insight into the role of pH in the classic
354 Fenton process. The ubiquitous character of the Fenton process in nature and of iron
355 complexes formed in water (e.g., citrate), alongside the generation of H₂O₂ in illuminated
356 NOM-containing environments, lead to (photo)Fenton reactions during the diurnal cycles. As
357 such, the present study holds important implications also in the elucidation of the Fenton
358 process that occurs both in nature and in engineering applications, and sets the basis for

359 further investigation concerning the effectiveness of ligand-mediated oxidation of natural and
360 anthropogenic contaminants.

361

362 **ASSOCIATED CONTENT**

363 **Supporting Information.** The Supporting Information is available free of charge on the ACS
364 Publications website

365

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371 **Notes**

372 The authors declare no competing financial interest.

373

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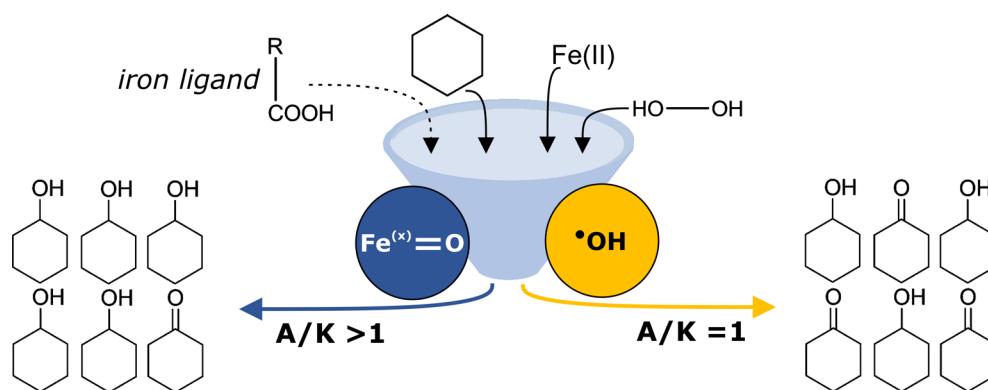
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509 **Graphical Abstract**



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