



Short communication

Selective oxidation of glycerol to formic acid catalyzed by iron salts

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ABSTRACT

Glycerol is oxidized by hydrogen peroxide to formic acid with excellent selectivity in the presence of iron salts. The oxidation takes place at room temperature in water; at the end of the reaction the catalytic system is still active and available to restart the oxidation.

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1. Introduction

In recent years the blooming business of biodiesel fuels has led to an increasing surplus of glycerol as the main by-product of transesterification of biomass-derived triglycerides with methanol. As a consequence, studies regarding the development of feasible and convenient ways to transform glycerol in higher value products have proportionally increased in number. Such valorization would make the biofuels production significantly cheaper and therefore affordable in comparison to traditional fossil fuels.

From several points of view glycerol is a versatile molecule, however its high number of functional groups - which is a valuable characteristic for its use as chemical building block - causes at the same time the hardest difficulties in the set-up of selective reactions. Among the possible glycerol valorization routes oxidation is one of the most studied, since the potentially obtainable products are all commercially relevant (Scheme 1) [1,2]. However, some of the most appealing products obtainable by glycerol oxidation, e.g. dihydroxyacetone (DHA), are themselves rather reactive compounds: therefore, selective oxidation of glycerol often leads to poor yields due to subsequent decomposition of the oxidation products [3].

The difficulties experienced in developing selective routes for a partial oxidation of glycerol would be overcome by pushing the oxidation process to its end, for example to formic acid (FA) as final product. Few studies report glycerol oxidation to FA in the past literature [4], but their number has been growing in very recent years [5–8]. Most of

the reported systems are based on heterogeneous catalysts and the oxidant agent can be hydrogen peroxide as well as molecular oxygen.

The rising interest in efficient FA production lies in its use as hydrogen carrier. With its 4.4% hydrogen content and several well-known systems able to decompose it in mild conditions to hydrogen and carbon dioxide [9–12], FA might provide a supply for the hydrogen fuel cells [13], as well as for the hydrogenation process of biomasses [14] and even directly as automotive alternative fuel [15] which would perfectly fit with the biofuel production above cited. On the other hand, FA is also a convenient source of C1 raw material for the chemical industry, as it can be easily transformed into carbon monoxide [16].

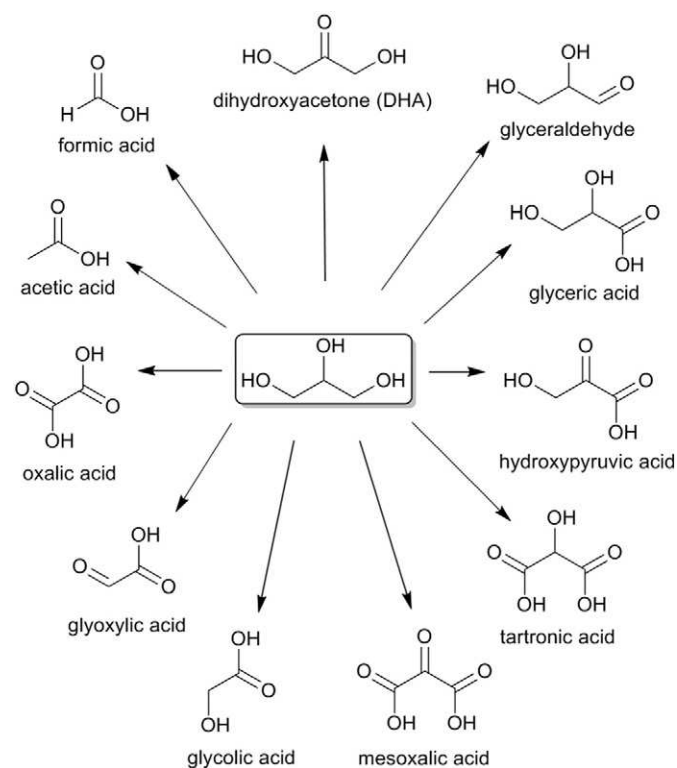
The pushing requirements of the twelve Green Chemistry principles [17] urge to shift towards more sustainable reactions and the use of iron catalysts in place of rarer and more expensive transition metals [18] meets such expectations, as demonstrated by the growing interest in their applications [19]. Iron compounds have been known to promote alcohols and diols oxidation by hydrogen peroxide since nineteenth century and even glycerol oxidation was briefly described in 1899 [20], but also in the last years both iron salts [21–24] and iron complexes [25–28] were reported to catalyze alcohol oxidation by either molecular oxygen or hydrogen peroxide.

Our group has recently described glycerol oxidation by hydrogen peroxide catalyzed by iron complexes with the tridentate ligand bis(2-pyridinylmethyl)amine (BPA) [29]; these studies have shown that by careful tuning of the experimental setup a remarkably selective formation of DHA can be obtained: on the other hand, in some cases we also detected a significative amount of FA as by-product.

Here we present our results in the oxidation of glycerol by hydrogen peroxide catalyzed by iron salts to give FA with high selectivities and in very mild conditions.

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Scheme 1. Possible products of glycerol oxidation.

2. Results and discussion

The oxidation of glycerol by hydrogen peroxide catalyzed by $\text{Fe}(\text{OTf})_2$ (Tf = trifluoromethanesulfonate) and BPA in acetonitrile/water 2:1 gave a 25% selectivity of FA besides the main product DHA (Table 1 entry 1). The reaction was carried out at r.t. with a 2.8 hydrogen

peroxide/glycerol ratio and three equivalents of BPA with respect to the iron salt. Similar results were obtained using the preformed complex $[\text{Fe}(\text{BPA})_2(\text{OTf})_2]$ in the presence of added BPA as catalytic system (entry 2): all these findings are consistent with those reported in our previous paper [29].

In order to push the oxidation further, we increased the total amount of H_2O_2 to 11 equivalents, which were added in 1.1 eq portions at intervals of 30 min (entry 3 and Fig. 1). GC analysis of the reaction mixture after each addition showed that the first two equivalents of hydrogen peroxide caused partial oxidation of glycerol to a mixture of DHA and glyceraldehyde; upon addition of further amounts of hydrogen peroxide we detected the formation of FA which rapidly became the main product of the reaction. In the course of the addition of H_2O_2 , initially formed glyceraldehyde disappeared and besides FA only traces of DHA were detected in solution, together with minor amounts of a new product which was identified as glycolic acid (GA) (see Scheme 2). After ten additions (11 equivalents of hydrogen peroxide), the oxidation of glycerol was almost complete to produce FA and GA; no other products were detected. Further addition of H_2O_2 caused no modifications to the reaction mixture. On the other hand, no significative differences were observed when the same overall amount of hydrogen peroxide was added in a single step (entry 4).

When a catalytic test was performed replacing glycerol with FA as substrate no reaction was observed, as opposed to the results reported by Walton [4] on the iron catalyzed oxidation of FA to carbon dioxide and water.

The use of $\text{Fe}(\text{OTf})_2$ as catalyst in the absence of BPA gave even better results, with an almost complete glycerol conversion to FA and only traces of DHA and glycolic acid as by-products (entry 5). We repeated several times the catalytic cycle by adding new loads of fresh glycerol and hydrogen peroxide, obtaining comparable yields of FA: thus, at the end of the reaction the catalytic system was still active and available to restart the oxidation without significative loss of activity.

We repeated the reaction reported in entry 5 by adding hydrogen peroxide in 1.1 eq portions at intervals of 30 min (entry 6 and Fig. 2) and we verified that the evolution of the reaction catalyzed by

Table 1
Oxidation of glycerol with H_2O_2 catalyzed by iron compounds^a.

Entry	Catalyst	[BPA(tot)]/[Fe]	Solvent	[H_2O_2]/[glycerol]	t (min)	Conv. ^b (%)	DHA Sel. (%)	GA Sel. (%)	FA Sel. (%)
1	$\text{Fe}(\text{OTf})_2$ + BPA	3	$\text{MeCN:H}_2\text{O}^c$	2.8	90	25	72	3	25
2	$[\text{Fe}(\text{BPA})_2(\text{OTf})_2]$ + BPA	3	$\text{MeCN:H}_2\text{O}^c$	2.8	90	18	74	4	22
3	$\text{Fe}(\text{OTf})_2$ + BPA	3	$\text{MeCN:H}_2\text{O}^c$	11.2 ^d	280	99	Traces	6	93
4	$\text{Fe}(\text{OTf})_2$ + BPA	3	$\text{MeCN:H}_2\text{O}^c$	11.2 ^e	36	96	2	6	92
5	$\text{Fe}(\text{OTf})_2$	0	$\text{MeCN:H}_2\text{O}^c$	11.2 ^e	36	99	Traces	Traces	99
6	$\text{Fe}(\text{OTf})_2$	0	$\text{MeCN:H}_2\text{O}^c$	4.4 ^d	106	100	Traces	1	98
7	$\text{Fe}(\text{OTf})_2$	0	$\text{MeCN:H}_2\text{O}^c$	4.2 ^e	6	97	Traces	Traces	96
8	$\text{Fe}(\text{OTf})_2$	0	H_2O	4.2 ^e	6	99	Traces	4	94
9	$\text{Fe}(\text{OTf})_2$ + BPA	3	H_2O	4.2 ^e	36	82	24	12	63
10 ^f	$\text{Fe}(\text{OTf})_2$	0	H_2O	4.2 ^e	6	99	1	3	95
11 ^g	$\text{Fe}(\text{OTf})_2$	0	H_2O	4.2 ^e	6	99 ^h	Traces ^h	3 ^h	95 ^h
12 ⁱ	$\text{Fe}(\text{OTf})_2$	0	H_2O	4.2 ^e	6	98 ^h	0 ^h	5 ^h	95 ^h
13 ^j	$\text{Fe}(\text{OTf})_2$	0	H_2O	4.2 ^e	16	98	Traces	2	97
14 ^k	$\text{Fe}(\text{OTf})_2$	0	H_2O	4.2 ^l	60	0	0	0	0
15	$\text{Fe}(\text{OTf})_3$	0	H_2O	4.2 ^e	6	99	Traces	1	99
16	FeCl_2	0	H_2O	4.2 ^e	6	99	Traces	4	95
17	FeCl_3	0	H_2O	4.2 ^d	6	99	Traces	3	97

^a Experimental conditions: $[\text{Fe}] = 1.0 \times 10^{-2}$ M; $[\text{glycerol}]/[\text{Fe}] = 35$. Isolated yields could not be provided, since all efforts to separate FA from reaction mixture were unsuccessful; therefore all yields are based on GC analyses (see Experimental section in Supplementary data).

^b Calculated as % of glycerol reacted.

^c $\text{MeCN:H}_2\text{O}$ ratio = 2:1.

^d Addition of H_2O_2 in portions of 40 μl (1.1 equivalents) each.

^e Addition of H_2O_2 in a single portion.

^f Reaction carried out in closed and thermostatted vessel.

^g A second load of fresh glycerol was added after the end of the first reaction and a second addition of H_2O_2 was repeated.

^h Overall results.

ⁱ A third load of fresh glycerol was added after the end of the second reaction and a third addition of H_2O_2 was repeated.

^j $[\text{glycerol}]/[\text{Fe}] = 100$.

^k Reaction carried out with *t*-BuOOH as oxidant.

^l $[\text{t-BuOOH}]/[\text{glycerol}]$ (*t*-BuOOH 70% w/w).

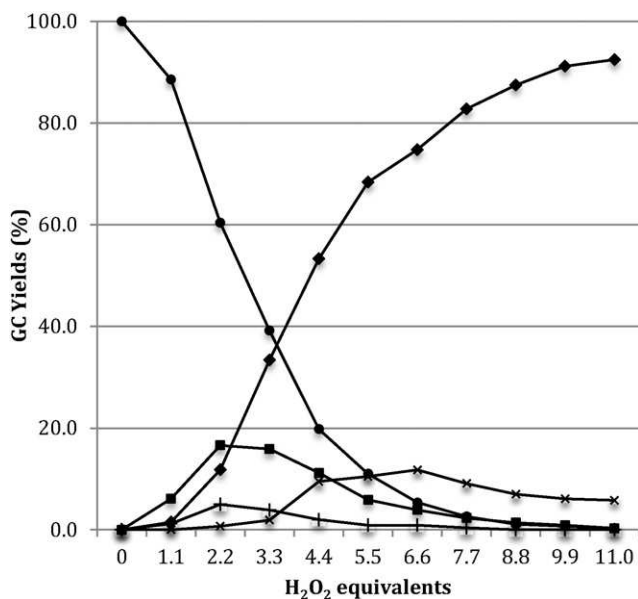


Fig. 1. Glycerol oxidation by H₂O₂ in MeCN:H₂O (2:1) catalyzed by Fe(OTf)₂ + BPA (1:3). Exp. conditions: [Fe] = 1.0×10^{-2} M; [glycerol]/[Fe] = 35; (●): glycerol; (◆): formic acid; (■): DHA; (⋈): glyceraldehyde; (×): glycolic acid.

Fe(OTf)₂ was the same either in the presence or in the absence of the co-catalyst BPA (compare entries 3 and 6); the main differences in the latter case were a lower production of DHA and glyceraldehyde and the use of fewer equivalents of hydrogen peroxide to obtain a complete glycerol oxidation.

In several reports of heterogeneously catalyzed glycerol oxidation, the reaction was proposed to occur *via* formation of intermediates such as DHA, glyceraldehyde, glycolic acid, glyceric acid and oxalic acid [30–32]. In the present case, the results shown in Figs. 1 and 2 indicate that the initial glycerol oxidation products were DHA and glyceraldehyde, which upon further addition of hydrogen peroxide were in turn oxidized to FA; in contrast, formation of glycolic acid was not followed

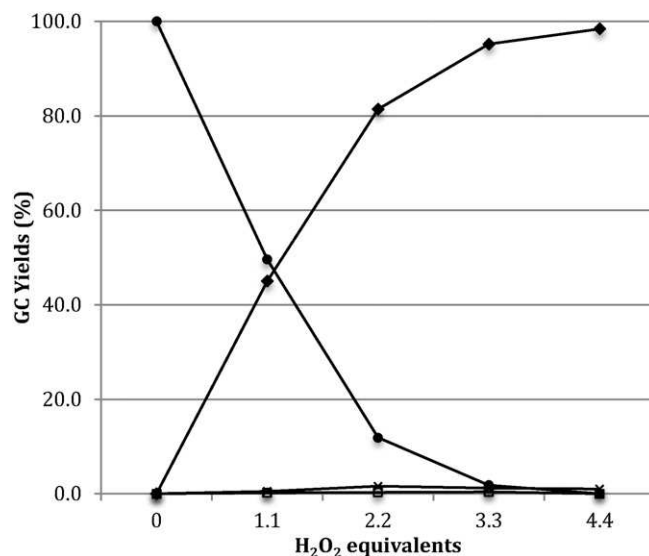


Fig. 2. Glycerol oxidation by H₂O₂ in MeCN:H₂O (2:1) catalyzed by Fe(OTf)₂. Exp. conditions: [Fe] = 1.0×10^{-2} M; [glycerol]/[Fe] = 35; (●): glycerol; (◆): formic acid; (■): DHA; (×): glycolic acid.

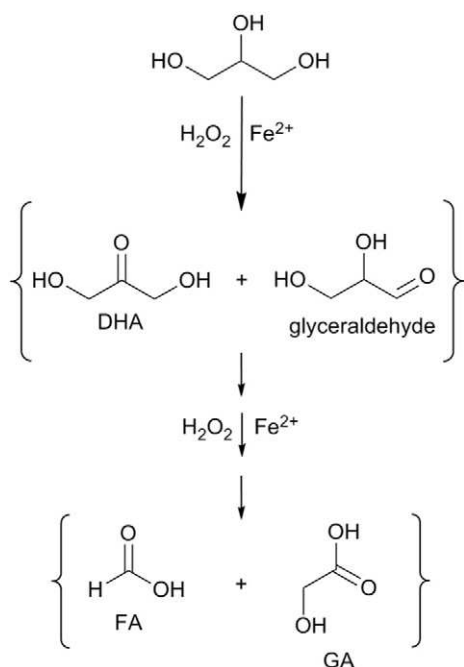
by further oxidation. Notably, other possible oxidation products were never detected in the reaction mixture.

The results of entry 6 and Fig. 2 evidence that after addition of 4.4 equivalents of H₂O₂ oxidation of glycerol to FA was complete under these experimental conditions, in agreement with the stoichiometry of the oxidation from glycerol to formic acid. Therefore, we carried out a reaction by slow addition of 4.2 equivalents of H₂O₂ to the reaction mixture (one drop every 15 s, 6 min altogether): glycerol oxidation was completed immediately after the last H₂O₂ addition, with no further reaction occurring afterwards (entry 7). A faster addition of hydrogen peroxide gave rise to somewhat lower conversion, probably due to partial hydrogen peroxide decomposition which is known to occur in the presence of iron salts [21,25].

We tested water as solvent to improve the sustainability of the reaction; the catalytic reactions performed in water gave similar results to those in acetonitrile/water, with only a slight decrease in FA selectivity (entry 8), thus confirming the full feasibility of the reaction in environmentally friendly setting. As already observed in acetonitrile/water mixture (see entries 4 and 5), also in water in the presence of BPA the reaction was less selective, giving mixtures of DHA, glycolic acid and formic acid (compare entries 8 and 9). These results can be interpreted in terms of formation of two catalytically active species, *i.e.* a free cationic iron species which promotes glycerol oxidation to formic acid on one hand, and on the other a Fe/BPA complex which is responsible for the glycerol oxidation to DHA.

In order to minimize the effects of temperature variations, we performed the reaction in a vessel closed by a serum cap and kept at constant temperature (21 °C) in a thermostatted oil bath: the results of such experiment (entry 10) were comparable to those obtained in an open vessel. At the end of the reaction a second catalytic cycle with a new glycerol load and subsequent oxidation by H₂O₂ addition was performed, followed by an analogous third cycle. The results, reported in entries 11 and 12 respectively, show for both catalytic cycles a remarkable selectivity in FA, with no apparent loss of catalytic activity. The only detectable side effect was the absence of DHA and a moderate increase of glycolic acid yield. The high catalytic activity was confirmed by increasing the catalytic ratio [glycerol]/[Fe] to 100 (entry 13), without significant variation on both glycerol conversion and FA selectivity.

The effect of choice of the oxidizing agent was tested by replacing hydrogen peroxide with another peroxide: use of *tert*-butyl hydroperoxide, a frequently employed oxidant in iron promoted alcohol



Scheme 2. Glycerol oxidation to formic acid (FA) and glycolic acid (GA).

oxidation [25,27] was totally ineffective in the catalytic reactions under investigation (entry 14).

We also tested the effect on the catalytic activity of both catalyst counterion and iron oxidation state (entries 15–17): the results were not affected by replacing either triflate with chloride, or Fe(II) with Fe(III) salts. Our findings on the catalytic activity of iron(III) salts, although not in agreement with those reported by Fenton [20], are however coherent with other literature reports of oxidation by hydrogen peroxide catalyzed by Fe(III) salts [21,22,24,26]. On the other hand, it is known that Fe^{2+} could be easily oxidized to Fe^{3+} in air or when exposed to oxidizing agents; that might account for the similar activity showed by FeCl_2 , FeCl_3 , $\text{Fe}(\text{OTf})_2$ and $\text{Fe}(\text{OTf})_3$.

In fact the underlying chemistry in Fenton- or Gif-type reactions, i.e. the oxidation systems based on Fe^{n+} with H_2O_2 , is very complex and still subject of studies and discussions [24,33,34], one of the most debated points being the iron oxidation states throughout the catalytic cycle. The two proposed mechanisms, i.e. a free radical mechanism as opposed to H_2O_2 nucleophilic addition to the metal centre, have been supported by mechanistic studies and it was shown that the actual reaction pathway depends on several experimental parameters, one of which is the pH of the reaction medium [24,34]. Although we do not have any positive proof indicating the actual mechanism of the reaction here described, the high selectivity and the absence of tar polymers might be both evidences (see Supplementary data) in favour of a non-radical pathway [24,35]; at the same time, the neat changes of colours during the first stages of the H_2O_2 addition – from orange to deep purple to clear yellow – might indicate fast variations through different iron oxidation states.

Further studies are presently in progress to shed light on the influence of the various experimental parameters – including the presence of added ligands – on the course of the catalytic reactions: all evidences obtained by us as well as by other groups may contribute to a better understanding of Fenton-type chemistry.

3. Conclusions

Glycerol was oxidized by hydrogen peroxide to formic acid with excellent selectivity in the presence of iron salts; the only by-product present in the final reaction mixture was glycolic acid. The reaction was carried out at r.t. either in acetonitrile/water or in water; at the end of the reaction the catalytic system was still active and oxidation of another glycerol load took place with comparable reaction rate and selectivity.

The catalytic reactions here described fully comply the requirements for a green process, from the point of view of metal, oxidant, reaction medium, experimental conditions as well as excellent selectivity and overall high atom efficiency.

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Appendix A. Supplementary data

Supplementary data to this article (Experimental Section and ESI-MS spectra) can be found online, at <http://dx.doi.org/10.1016/j.catcom.2016.05.014>.

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New Insights of the Fenton Reaction Using Glycerol as the Experimental Model. Effect of O₂, Inhibition by Mg²⁺, and Oxidation State of Fe

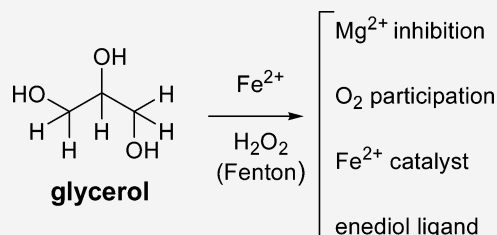
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ABSTRACT: The use of iron ions as catalyst of oxidation with hydrogen peroxide, known as the Fenton reaction, is important for industry and biological systems. It has been widely studied since its discovery in the 19th century, but important aspects of the reaction as which is the oxidant, the role of oxygen, and the oxidation state of Fe still remain unclear. In this work new mechanistic insights of the oxidation of carbohydrates by the Fenton reaction using glycerol as experimental model are described. The reaction was studied by means of oxidation reduction potential (ORP) measures. The stoichiometry was measured, showing the important role of oxygen for lowering H₂O₂ consumption under aerobic conditions. Evidence is provided to demonstrate that in this system Fe²⁺ generates a catalyst by reacting with a substrate to produce a complex, which gives rise to singlet oxygen after reacting with H₂O₂. This is the first time that the inhibitor effect of Mg²⁺ is reported in this reaction, and its participation in the mechanism is described. A rational mechanism for the oxidation of glycerol using the Fenton reaction under these specific conditions is proposed. The role of oxygen, the participation of Fe²⁺, and the inhibition by Mg²⁺ are fully demonstrated experimentally.



INTRODUCTION

The Fenton reaction has been known since 1894, when Fenton described the oxidation of tartaric acid with hydrogen peroxide in the presence of Fe²⁺ ions.¹ However, according to von Sonntag² the use of iron ions as catalysts of oxidation with hydrogen peroxide was described four decades before by Schönbein³ in 1857 for the oxidation of iodide with H₂O₂ in the presence of Fe²⁺. The Fenton reaction has important industrial applications⁴ and in biological systems.⁵ In the 20th century, Haber and Weiss⁶ studied exhaustively the decomposition of hydrogen peroxide with iron ions, and proposed the formation of the hydroxyl radical, HO•. This mechanism was criticized by several authors.⁷ Bray and Gorin⁸ and Barb et al.⁹ proposed the occurrence of (H₂O)₅Fe^{IV}=O (ferryl). In 1984 Sugimoto and Sawyer¹⁰ proposed that the products of the Fenton reaction with 1,2-diphenylethylene in acetonitrile were typical of the presence of singlet oxygen. The kinetics of this reaction was studied recently in detail by Rachmilovich-Calis et al.,¹¹ where they reported evidence for Fe (H₂O)₆²⁺. In 1999 Kremer¹² showed that FeO²⁺ acts as the key intermediate. Fenton chemistry at aqueous interfaces was also studied.¹³ There are several and exhaustive reviews about the Fenton reaction;^{2,14,15} however, the mechanism has not been yet fully elucidated, as stated by von Sonntag² and Barbusinski.¹⁴ In a 2015 paper¹⁶ there is still a discussion about the existence of HO• radical.

There are important aspects of the reaction that still remain unclear according to von Sonntag² and Barbusinski,¹⁴ as follows:

- Which is the oxidant, e.g., hydroxyl radical, ferryl ion, or singlet oxygen?
- Which is the stoichiometry of the Fenton reaction? How much hydrogen peroxide is consumed by the organic compound, and which is the effect of oxygen? Ingles¹⁷ in 1972 reported that the amount of hydrogen peroxide consumed in titration of carbohydrates in the presence of iron was higher if air was excluded from the system. The same effect was also described for aniline.¹⁸
- Why is the reaction slower when Fe³⁺ is used instead of Fe²⁺?

First of all, it is necessary to point out what is called “Fenton reaction”. There is some misunderstanding in the literature between two reactions that are not the same: (a) the original reaction described by Fenton in 1894 that was the **oxidation of organic compounds** by hydrogen peroxide in the presence of iron ions and (b) the Haber–Weiss cycle of decomposition of hydrogen peroxide in the presence of iron ions, **without**

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organic compounds. We should be clear about this definition to know what we are talking about.

In this paper we studied the Fenton reaction according to the definition “the oxidation of organic compounds by hydrogen peroxide in the presence of iron ions”.

The aim of this work is to elucidate several mechanistic aspects of the oxidation of carbohydrates by the Fenton reaction using glycerol as a model compound with the evidence obtained from experiments performed in our laboratories, and to propose a mechanism according to these results, comparing them with those of literature.

EXPERIMENTAL SECTION

Several procedures were used to study the Fenton reaction; for example, it is worthwhile to mention redox titration,¹⁷ cyclic voltammetry,¹⁹ COD (chemical oxygen demand),²⁰ ORP (oxidation–reduction potential measurement),²¹ stop flow spectrometry,¹¹ and UV spectroscopy.¹² In this work we applied several of the methods mentioned above like ORP, UV, COD, and H^+ concentration. We used some of them together in the same reaction batch that led us to accurate information about the mechanism. Moreover, we took into account the use of the iron ion only in catalytic concentration referred to H_2O_2 , thus being the ratio $Fe^{2+}/H_2O_2 < 10^{-1}$, as similarly reported by several authors for the treatment of wastes.²²

Instruments. ORP was measured with the HI 323 OB electrode (HANNA) calibrated with the standard solution HI 7022L (470 mV) and recorded with a computer interface (PROSKIT 1860 MT). COD was measured with a COD Thermo Reactor and DR2000 spectrophotometer (Hach Company). UV was measured with a spectrophotometer UV–Vis M-1700 (Metrolab). All chemicals were purchased from Aldrich. H_2O_2 was titrated with $KMnO_4$ before each set of reactions. All experiments were carried out by triplicate, under temperature control.

Reaction in the Presence of Oxygen. A 100 mL beaker provided with ORP and glass electrodes, thermometer, and a magnetic stirrer was placed in a thermostated bath, 50 mL of water with the desired concentration of substrate was added, and the pH was adjusted with diluted H_2SO_4 (1%). When the temperature was stabilized, solid $FeSO_4 \cdot 7H_2O$ was poured in, and the solution was stirred vigorously to favor air oxygen dissolution (Figure 1, point A). The required amount of H_2O_2 was added, this time was taken as t_0 of the reaction (Figure 1, point B), which was also indicated by the appearance of a yellow color (absorbance, λ 460 nm) that stayed along the reaction and faded at the end of it. The reaction was followed by ORP recording [E (mV) vs t (s)] until the ORP reached a minimum and then stayed constant (Figure 1, point E). When the ORP began to drop (Figure 1, point C), 100 μ L aliquots were taken to check the disappearance of H_2O_2 by the standard $K_2Cr_2O_7$ test (a green-blue color appeared in the presence of minute amounts of H_2O_2). When the test was negative, the end of the reaction was reached, and the whole H_2O_2 was consumed. This matched the ORP maximum slope (Figure 1, point D) that was checked by the first derivative.

Reaction in the Absence of Oxygen. A 100 mL four-neck flask provided with an ORP and glass electrodes, a thermometer, a rubber septum, and a magnetic stirrer was placed in a thermostated bath, 50 mL of water with the desired concentration of substrate was added, the pH was adjusted, and the temperature was stabilized. Nitrogen was bubbled through the rubber septum with a needle connected to a nitrogen tank

ORP GRAPHICS FOR MEASURING REACTION KINETICS

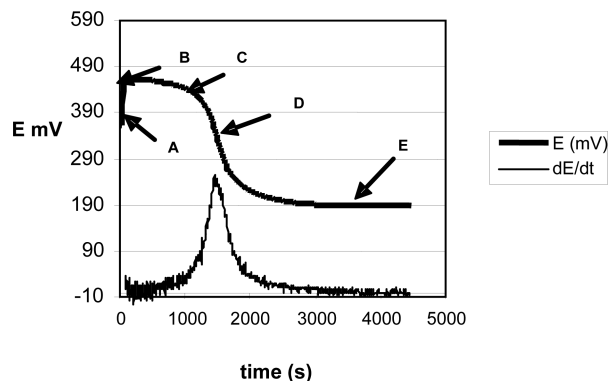


Figure 1. Change of ORP and first derivative during the Fenton reaction: (A) before adding H_2O_2 ; (B) just after adding H_2O_2 ; (C) just after consuming all H_2O_2 ; (D) ORP maximum slope (negative reaction to $K_2Cr_2O_7/H^+$); (E) ORP reaching a minimum, and staying constant.

and another needle for the exhaust. After bubbling for 30 min, solid $FeSO_4 \cdot 7H_2O$ was quickly poured in under positive nitrogen pressure to avoid air. When the salt was dissolved, the required amount of H_2O_2 was added by syringe and needle through the rubber septum. All the reaction was performed under nitrogen, and samples were taken with a syringe.

Reaction for Stoichiometric Purposes. In both cases, with and without oxygen, H_2O_2 was added dropwise until ORP remained constant in the upper value (Figure 1, point B), the positive dichromate test showed an excess of H_2O_2 , and iron hydroxide precipitates began to appear.

H^+ Concentration. It was recorded with the glass electrode as mV vs t (s) instead of plotting pH vs t for comparison with ORP in the same units. Moreover, mV vs t plots are directly proportional to H^+ concentration.

COD. It was measured at the beginning and at the end of the reaction, when required.

Measure of Fe^{2+} and Fe^{3+} Concentration. Concentrations of different species of iron were measured by the standard *o*-phenanthroline method.²³ A calibration curve was built by plotting the absorbance at 510 nm of the Fe^{2+} complex with *o*-phenanthroline (0.3% solution in water at pH 2.5). Fe^{3+} was measured after reduction to Fe^{2+} with NH_2OH (10% in water at pH 2.5).

Rate Measures and Calculations. Except for stoichiometric measurements, H_2O_2 was always used in shortage respect of the substrate to avoid side reactions with free iron when the substrate was exhausted. In this work we measured the time in which a known amount of H_2O_2 was consumed according to Figure 1, ($t_D - t_B$), reaction 1. For calculating k and other kinetics parameters we used the half-life method, $t_{1/2}$ (time required for a known amount of H_2O_2 decreasing by 50%), reactions 2 and 3. To check whether H_2O_2 was consumed in a linear way, a sample reaction was stopped at $t_{1/2}$ by rising pH to 10 with 0.1 M NaOH to precipitate iron hydroxides. After filtering, the pH was adjusted again to 2.0, and the residual H_2O_2 was titrated by permanganimetry. The concentration of H_2O_2 was just half of the initial.

$$\Delta t \text{ (s)} = (t_D - t_B) \quad (1)$$

$$\ln(0.5[H_2O_2]_0/[H_2O_2]_{10}) = \ln(0.5) = -kt_{1/2} \quad (2)$$

$$k = 0.693/t_{1/2} \quad (3)$$

RESULTS AND DISCUSSION

Stoichiometry. It is very important to know the stoichiometry of the Fenton reaction to get a deep insight into the mechanism. Data about this topic are scarce; there are few papers about this subject.^{17,4,24,25} Ingles¹⁷ used the Fenton reaction with an almost stoichiometric amount of iron to titrate carbohydrates, performing accurate measures of the uptake of hydrogen peroxide. He found that there was less required hydrogen peroxide if the reaction was carried out with oxygen than without it, pointing out the importance of oxygen in the Fenton reaction, but not investigating thoroughly the mechanistics of this result.

We tried several substrates, and we chose carbohydrates to study the mechanism of the Fenton reaction because they have antioxidant activity^{26,27} and their reactions have a neat end point that helps to analyze the experimental results.¹⁷

One set of reactions was performed in the presence of air with fast stirring to allow a quick exchange with the oxygen of air for keeping constant the O₂ concentration in the solution (3.1 × 10⁻⁴ M at 20 °C).

Another set of reactions was performed in the absence of oxygen by degassing the solution with nitrogen.

In all reactions, the total oxidation of the organic substrate by hydrogen peroxide was verified by an independent method. COD measures were performed at the beginning and at the end of the reactions to check the absence of organic oxidizable matter.

The reactions were carried out at temperatures between 20 and 50 °C that can be found in an industrial plant that uses this method for destroying organic wastes. Below 20 °C the reaction is too slow, and over 50 °C H₂O₂ begins to decompose.

We selected substrates in which the reaction was slow enough to measure the rate parameters minimizing error, taking into account that this is a heterogeneous reaction under fast stirring when it is performed in the presence of air.

The following substrates were studied: glycerol, sucrose, mannitol, lactose, glucose, fructose, and tartaric acid.

In all cases, it can be observed that less H₂O₂ is consumed in the presence of oxygen than in its absence (Table 1). The reaction was performed in triplicate, and on different days. The relationship between consumed hydrogen peroxide and substrate was always the same for each compound.

Measure of Reaction Rates. For the measure of the reaction rates, H₂O₂ was always used in shortage with respect to the substrate to avoid side reactions with free iron when the substrate was exhausted. In this work we measured the time in which a known amount of H₂O₂ was consumed (Table 2).

Different rates of reaction were observed, the reaction of tartaric acid being the fastest, and that of glycerol being the slowest. Consequently, glycerol was chosen as a model compound to carry out a detailed study of the kinetics, and other parameters of the reaction. As the reaction was slower, the error in the measures was smaller.

Effect of the Initial pH and H⁺ Variation during the Reaction. The reaction was performed with glycerol at pH 2.0 and 2.5. A larger pH difference could not be used because at pHs higher than 2.6 iron hydroxides began to precipitate, and at

Table 1. Stoichiometric Intake of H₂O₂ with and without O₂ and Comparison with CDO^a

S ^b	A	B	CDO initial	CDO final
Gly	4.1 ± 0.1	7.3 ± 0.1	2391 ± 10	<10
Suc	11.7 ± 0.1	24.8 ± 0.1	2231 ± 10	<10
Man	6.7 ± 0.1	13.2 ± 0.1	2245 ± 10	<10
Lac	11.5 ± 0.1	24.3 ± 0.1	2229 ± 10	<10
Glu	6.0 ± 0.1	12.5 ± 0.1	2120 ± 10	<10
Fru	6.0 ± 0.1	12.3 ± 0.1	2125 ± 10	<10
Tar	3.1 ± 0.1	5.2 ± 0.1	1050 ± 10	<10

^aConcentration of the substrate (S) = 2.0 ± 0.1 mg/mL; Fe²⁺ = (1.5 ± 0.1) × 10⁻³ M; T = 20 °C; pH 2.5 ± 0.1. CDO = mg O₂/L. A = mmol H₂O₂ /mmol S (with O₂); B = mmol H₂O₂/mmol S (without O₂).

^bGly (glycerol); Suc (sucrose); Man (mannitol); Lac (lactose); Glu (glucose); Fru (fructose); Tar (tartaric acid). Reaction blanks for each compound were carried out without H₂O₂ in the presence of air for 300 min; precipitates of iron oxides were the only products. Initial and final CODs were the same.

Table 2. Reaction Rate for Different Substrates Using the t_{1/2} Method^a

substrate (S)	k ₁ (1/s) × 10 ⁻³
glycerol	2.6 ± 0.1
sucrose	4.9 ± 0.1
mannitol	6.6 ± 0.1
lactose	6.6 ± 0.1
glucose	13.7 ± 0.1
fructose	26.2 ± 0.1
tartaric acid	38.7 ± 0.1

^aS (2.0 ± 0.1 mg/mL); H₂O₂ [(3.0 ± 0.1) × 10⁻² M]; Fe²⁺ [(1.5 ± 0.1) × 10⁻³ M]; T = 20.0 ± 0.1 °C with O₂.

pHs lower than 1.8 the reaction did not start. These measures provided important data to elucidate the reaction mechanism (Table 3). Other results about this subject have been previously reported,²⁸ but they were referred to the decomposition of H₂O₂ in the presence of iron ions **without** organic substrate.

Table 3. Rate of Reaction at Different pH^a

pH	k ₁ (1/s) × 10 ⁻³
2.0	0.8 ± 0.1
2.5	2.7 ± 0.1

^aVol = 50 mL; T = 21 °C; Fe²⁺ = (2.0 ± 0.1) × 10⁻³ M; H₂O₂ = (2.0 ± 0.1) × 10⁻² M; glycerol = (2.0 ± 0.1) × 10⁻² M.

A variation of the H⁺ concentration was observed along the reaction, increasing continuously, and remaining constant at the end of the reaction. This was due to the production of carbonic acid as a final product of the reaction with glycerol. This effect cannot be detected if the reaction is carried out in a buffer. Both measures with an ORP electrode and a glass electrode to check H⁺ concentration (mV) are shown in Figure 2 in the same plot for comparative purposes.

Relationship between the Initial Concentration of H₂O₂ and the Reaction Rate. The time of reaction according to different initial concentrations of H₂O₂ was studied (Figure 3), showing that Δt/ΔH₂O₂ (slope of the plot) was linear with r² = 0.991. No difference in the reaction rate was found because it is independent of the initial concentration of H₂O₂. This result is in agreement with a catalytic reaction, in which H₂O₂ concentration does not affect the rate-determining step.

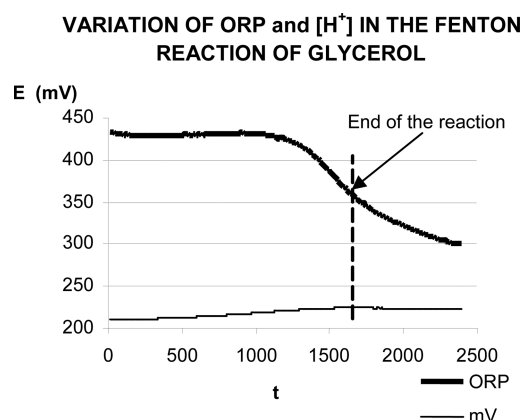


Figure 2. Variation of ORP and H⁺ in the Fenton reaction of glycerol. Vol = 50 mL; $T = 21.0 \pm 0.1$ °C; $\text{Fe}^{2+} = (2.0 \pm 0.1) \times 10^{-3}$ M; $\text{H}_2\text{O}_2 = (2.0 \pm 0.1) \times 10^{-2}$ M; pH = 2.5 ± 0.1 ; glycerol = $(2.0 \pm 0.1) \times 10^{-2}$ M.

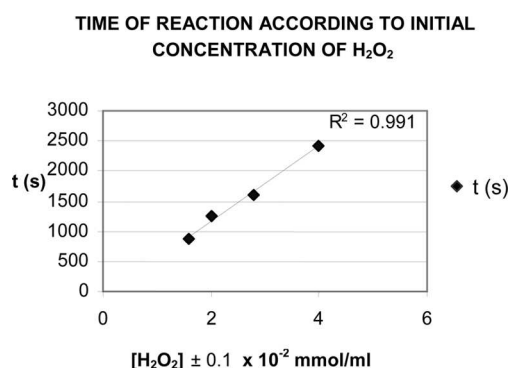


Figure 3. Time of reaction according to the initial concentration of H₂O₂.

Effect of Concentration of Fe²⁺ and Fe³⁺. The reaction rate for different initial concentrations of Fe²⁺ and Fe³⁺ was measured (Table 4), showing a linear dependence on iron ions.

Table 4. Reaction Rate for Different Concentrations of Fe²⁺ and Fe³⁺ ^a

Fe ²⁺ (M)	k_1 (1/s)	Fe ³⁺ (M)	k_1 (1/s)
$(1.5 \pm 0.1) \times 10^{-3}$	1.10×10^{-3}	1.5×10^{-3}	7.6×10^{-5}
$(2.0 \pm 0.1) \times 10^{-3}$	2.16×10^{-3}	2.0×10^{-3}	13.1×10^{-5}
$(2.5 \pm 0.1) \times 10^{-3}$	4.70×10^{-3}	2.5×10^{-3}	23.7×10^{-5}

^aVol = 50 mL; $T = 21.0 \pm 0.1$ °C; $\text{H}_2\text{O}_2 = (2.0 \pm 0.1) \times 10^{-2}$ M; pH = 2.5 ± 0.1 ; glycerol = $(2.0 \pm 0.1) \times 10^{-2}$ M.

When the reaction was started with Fe³⁺, it was at least 10 times slower than that started with Fe²⁺ at the same concentration (Table 4). This result provided important information about the mechanism as will be explained below.

Oxidation State of Fe in the Fenton Reaction. One important topic of the Fenton mechanism is the oxidation state of iron along the reaction. Different authors¹⁴ proposed that iron can participate as Fe²⁺, Fe³⁺, or Fe⁴⁺. To clear up this topic, we measured the concentration of iron species at the beginning and at the end of the reaction with the *o*-phenanthroline method (Table 5). In experiment A, the reaction was started with Fe²⁺, and in experiment B, it was started with Fe³⁺.

When the reaction was started with Fe²⁺, at the end of the reaction the same amount of Fe²⁺ was found as at the

Table 5. Concentration of Iron Species by the *o*-Phenanthroline Method^a

	reaction			
	A	B	C	D
1 (Fe ²⁺)	1.5 ± 0.1	0	1.5 ± 0.1	0
2 (Fe ³⁺)	0	1.5 ± 0.1	1.5 ± 0.1	0
3 (Fe ²⁺)	2.0 ± 0.1	0	2.0 ± 0.1	0
4 (Fe ³⁺)	0	2.0 ± 0.1	2.0 ± 0.1	0
5 (Fe ²⁺)	2.5 ± 0.1	0	2.5 ± 0.1	0
6 (Fe ³⁺)	0	2.5 ± 0.1	2.5 ± 0.1	0

^aA [Fe²⁺] initial (10^{-3} M); B [Fe³⁺] initial (10^{-3} M); C [Fe²⁺] final (10^{-3} M); D [Fe³⁺] final (10^{-3} M).

beginning. Instead, when the reaction was started with Fe³⁺, at the end of the reaction we only found Fe²⁺ in an amount equivalent to that of Fe³⁺ added at the beginning of the reaction. This result means that iron participates as a catalyst only as Fe²⁺, and when the reaction was started with Fe³⁺, this iron species first had to be reduced to Fe²⁺ before the reaction begins. This reduction step slows down the reaction with Fe³⁺.

Arrhenius Parameters for Fe²⁺ and Fe³⁺. To look for differences in the rate-determining step of the reaction with Fe²⁺ and Fe³⁺, we carried out the Arrhenius study for both reactions. Results are shown in Figure 4 and Table 6.

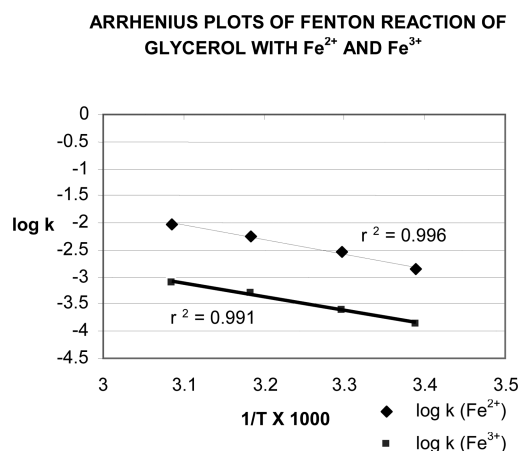


Figure 4. Arrhenius plot of Fenton reaction of glycerol with Fe²⁺ and Fe³⁺. Vol = 50 mL; $\text{Fe}^{2+} = \text{Fe}^{3+} = (1.5 \pm 0.1) \times 10^{-3}$ M; $\text{H}_2\text{O}_2 = (2.0 \pm 0.1) \times 10^{-2}$ mmol/m; pH = 2.5 ± 0.1 ; glycerol = $(2.0 \pm 0.1) \times 10^{-2}$ M.

Activation energies for Fe²⁺ and Fe³⁺ showed a difference of 2.5 kJ mol⁻¹ that was within the experimental error. Therefore, the rate-determining step of the reaction was the same for both iron species. The difference in the rate of reaction was due to the initial Fe³⁺ to Fe²⁺ reduction step as mentioned above.

Effect of Mg²⁺ and Ca²⁺ in the Fenton Reaction of Glycerol. We studied the incidence of Mg²⁺ and Ca²⁺ (Table 7). These are cations of biological importance²⁹ and can be also found in wastes that are prone to Fenton treatment. Data in literature about this subject are scarce.³⁰

A higher effect was observed with Mg²⁺ (the reaction was slower than with Ca²⁺). Therefore, a detailed study was carried out with this cation.

A set of reactions with a different Mg²⁺/Fe²⁺ ratio was performed as shown in Table 8. In all cases the reaction was slower than that without Mg²⁺, and the reaction rate drop has

Table 6. Arrhenius Data of Figure 4^a

T (°C)	T (K)	t (s)	1/T × 10 ³	log k(Fe ²⁺)	log k(Fe ³⁺)
22.0 ± 0.1	295 ± 0.1	960	3.39 ± 0.01	−2.8 ± 0.1	−3.8 ± 0.1
31.0 ± 0.1	304 ± 0.1	480	3.28 ± 0.01	−2.5 ± 0.1	−3.6 ± 0.1
41.0 ± 0.1	314 ± 0.1	240	3.18 ± 0.01	−2.2 ± 0.1	−3.3 ± 0.1
51.0 ± 0.1	324 ± 0.1	150	3.08 ± 0.01	−2.0 ± 0.1	−3.1 ± 0.1

^aE_a(Fe²⁺) = 50.8 kJ mol^{−1} E_a(Fe³⁺) = 48.3 kJ mol^{−1}.Table 7. Comparative Rate of the Fenton Reaction of Glycerol in the Presence of Mg²⁺ and Ca²⁺ Cations^a

cation	k ₁ (1/s)
without cation	2.6 × 10 ^{−3}
Mg ²⁺	0.6 × 10 ^{−3}
Ca ²⁺	2.0 × 10 ^{−3}

^aVol = 50 mL; T = 21 °C; Fe²⁺ = (2.0 ± 0.1) × 10^{−3} M; H₂O₂ = (2.0 ± 0.1) × 10^{−2} M; pH = 2.5; glycerol = (2.0 ± 0.1) × 10^{−2} M, Mg²⁺ = (4.0 ± 0.1) × 10^{−3} M; Ca²⁺ = (4 ± 0.1) × 10^{−3} M.Table 8. Reaction Rate of the Fenton Reaction of Glycerol with a Different Mg²⁺/Fe²⁺ Ratio^a

Mg ²⁺ × 10 ^{−3} M	Mg ²⁺ /Fe ²⁺	k ₁ (1/s)
0	0	(2.3 ± 0.1) × 10 ^{−3}
2	1	(1.9 ± 0.1) × 10 ^{−3}
4	2	(1.3 ± 0.1) × 10 ^{−3}
6	3	(0.9 ± 0.1) × 10 ^{−3}
8	4	(0.6 ± 0.1) × 10 ^{−3}

^aVol = 50 mL; T = 21.0 ± 0.1 °C; Fe²⁺ = (2.0 ± 0.1) × 10^{−3} M; H₂O₂ = (2.0 ± 0.1) × 10^{−2} M; pH = 2.5 ± 0.1; glycerol = (2.0 ± 0.1) × 10^{−2} M.

an inverse linear relationship with the increase of Mg²⁺ (Figure 5). According to these results the concentration of Mg²⁺ showed a significant inhibition effect on the reaction rate of glycerol.

Summary of the Results of the Study of the Fenton Reaction with Glycerol and Considerations about the Mechanism. (1) According to experimental results, the amount of H₂O₂ consumed in the absence of air is higher than in the presence of air (Table 1). Therefore, 1 mole of

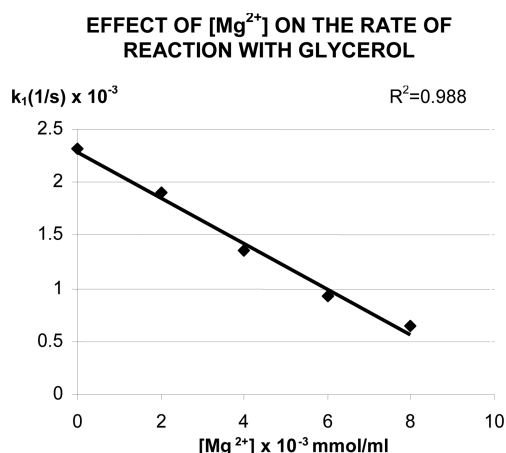
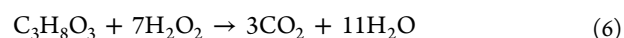
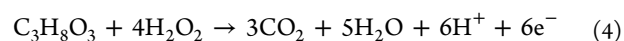


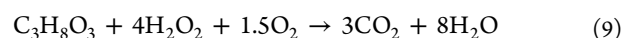
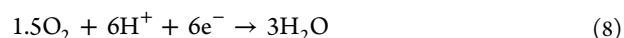
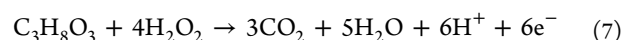
Figure 5. Effect of Mg²⁺ in the rate of the Fenton reaction with glycerol. Vol = 50 mL; T = 21.0 ± 0.1 °C; Fe²⁺ = (2.0 ± 0.1) × 10^{−3} M; H₂O₂ = (2.0 ± 0.1) × 10^{−2} M; pH = 2.5 ± 0.1; glycerol = (2.0 ± 0.1) × 10^{−2} M.

glycerol (C₃H₈O₃) needs 4.1 moles of H₂O₂ with air, and 7.3 moles of H₂O₂ without air for full oxidation to CO₂ and H₂O. This result was checked by an independent COD test at the beginning and at the end of the reaction. It means that **oxygen participates in the reaction**, being very important for the elucidation of the mechanism.

According to the stoichiometry of the reaction for the full oxidation of glycerol, if H₂O₂ is the only source of oxygen, theoretically 7 moles of H₂O₂ are necessary to complete the oxidation of 1 mol of glycerol to H₂O and CO₂. This fact can be explained by reactions 4–6.



In the presence of oxygen, 4 moles of H₂O₂ (experimentally, 4.1 moles; Table 1) are necessary for complete oxidation of 1 mol of glycerol. It means that oxygen dissolved in water participates as an oxidant in the Fenton reaction, which is possible because the redox potential of oxygen in water at pH 2.0 is high enough (1.002 V at 20 °C and 0.21 bar) for being a good electron scavenger, reactions 7–9.



(2) If the order of addition is changed, and to an iron solution in water at an adjusted pH (1.8 < pH < 2.6) is added a solution of glycerol and hydrogen peroxide, iron hydroxides precipitate, and the reaction does not take place. This happens only with glycerol.

However, if the acid solution of glycerol is prepared at 20 °C 20 min before adding iron, the reaction can be performed normally. This result means that it is necessary to reach the equilibrium between glycerol 1 and its dehydrated derivative (enol) 2, in which the *n* electron pair of oxygen increases the electron density of the alkene. This is the necessary ligand 2 to keep Fe²⁺ in solution for catalytic purposes. It is important to achieve enough concentration of 2 to produce the iron complex 5 that is needed as a catalyst of the reaction (Figure 6). This effect cannot be observed with other compounds of Table 2 because this step is too fast. The possible formation of hydroxyalkene derivatives has been previously reported by Ingles.¹⁷

(3) The reaction does not take place at pH < 1.8. Moreover, it is necessary to start the reaction at pH < 2.6 to avoid the precipitation of iron hydroxides. This result shows the relevance of pH control. At a lower pH the reaction is slower than at a higher pH. We attributed this effect to the protonation of the enol to produce compound 4 (Figure 7), in which the

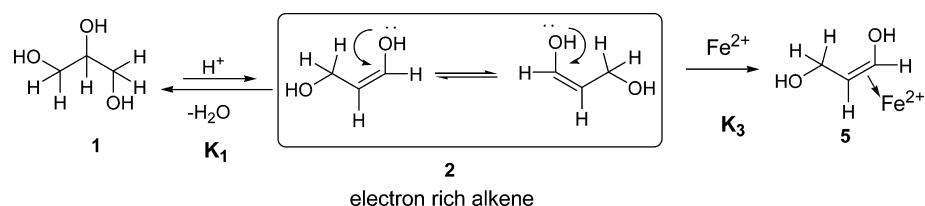


Figure 6. Partial dehydration of glycerol in acid media to produce ligand 2 and complex 5.

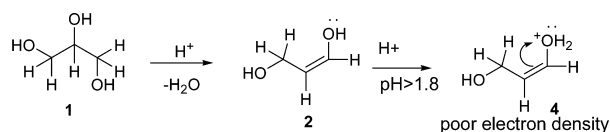


Figure 7. Protonation of 2 to produce the electron-poor compound 4.

electron density of the alkene is diminished, so that the concentration of 2 decreases with the consequent lowering of the concentration of 5 that is in the rate-determining step of the reaction.

The concentration of H_2O_2 has no effect on the rate of the reaction (Figure 3), thus meaning that the H_2O_2 addition step is too fast compared with other steps of the mechanism.

(4) The inhibition effect of Mg^{2+} on the reaction rate was studied, showing that the decreasing reaction rate keeps a linear relationship with the increasing of Mg^{2+} concentration (Table 8). Therefore, Mg^{2+} participates in the reaction mechanism similarly as H^+ as described above. Mg^{2+} reacts with the electron pair of the oxygen in the enol moiety, producing compound 3 (Figure 8) with the consequent decreasing of ligand 2 and catalyst 5. The role of carbohydrates in complex formation with magnesium was previously reported in the literature.^{31,32}

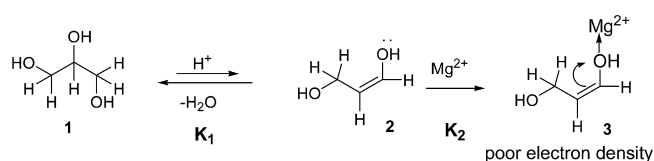
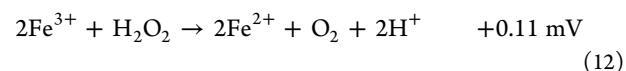
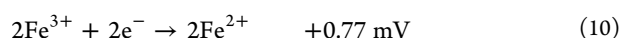
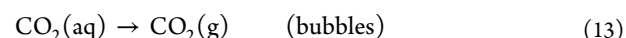


Figure 8. Proposed structure 3 for the reaction of Mg^{2+} with 2.

(5) The reaction started with Fe^{3+} as the only source of iron is too much slower than that started with the same concentration of Fe^{2+} (Table 6), but according to the Arrhenius plot, E_a is the same for both Fe^{2+} and Fe^{3+} reactions (Table 6; Figure 4). At the end of the reaction only Fe^{2+} was found at the same concentration as the initial concentration of Fe^{3+} (Table 5), which means that Fe^{3+} had to be reduced to Fe^{2+} by H_2O_2 before the reaction begins, as shown in reactions 10–12. This is the reason for a too much slower reaction with Fe^{3+} than with Fe^{2+} . As the activation energy for both Fe^{2+} and Fe^{3+} reactions is the same, then the difference in the reaction rate depends on the initial Fe^{2+} concentration. In fact, **Fe^{2+} is the only iron ion species that takes place in the Fenton reaction.** Therefore, starting with Fe^{3+} , the reaction is slow because at the beginning there is small amount of Fe^{2+} produced by the reduction of Fe^{3+} . The reaction rate increases as the concentration of Fe^{2+} increases. Moreover, the reaction is slower with Fe^{3+} than with Fe^{2+} not with another mechanism, but for the low concentration of Fe^{2+} .



(6) The H^+ concentration increases (pH decreases) along the reaction, and when it is over, it becomes stable (Figure 2), and bubbles begin to appear, reaction 13. The H^+ concentration increasing is resumed when more H_2O_2 is added. This pH variation is usually not observed when carrying out the reaction in buffers. We point out that this pH decrease is due to products like H_2CO_3 and CO_2 that produce bubbles when the pH is too low to reach the solubility equilibrium of gaseous CO_2 in water, reactions 13–15.



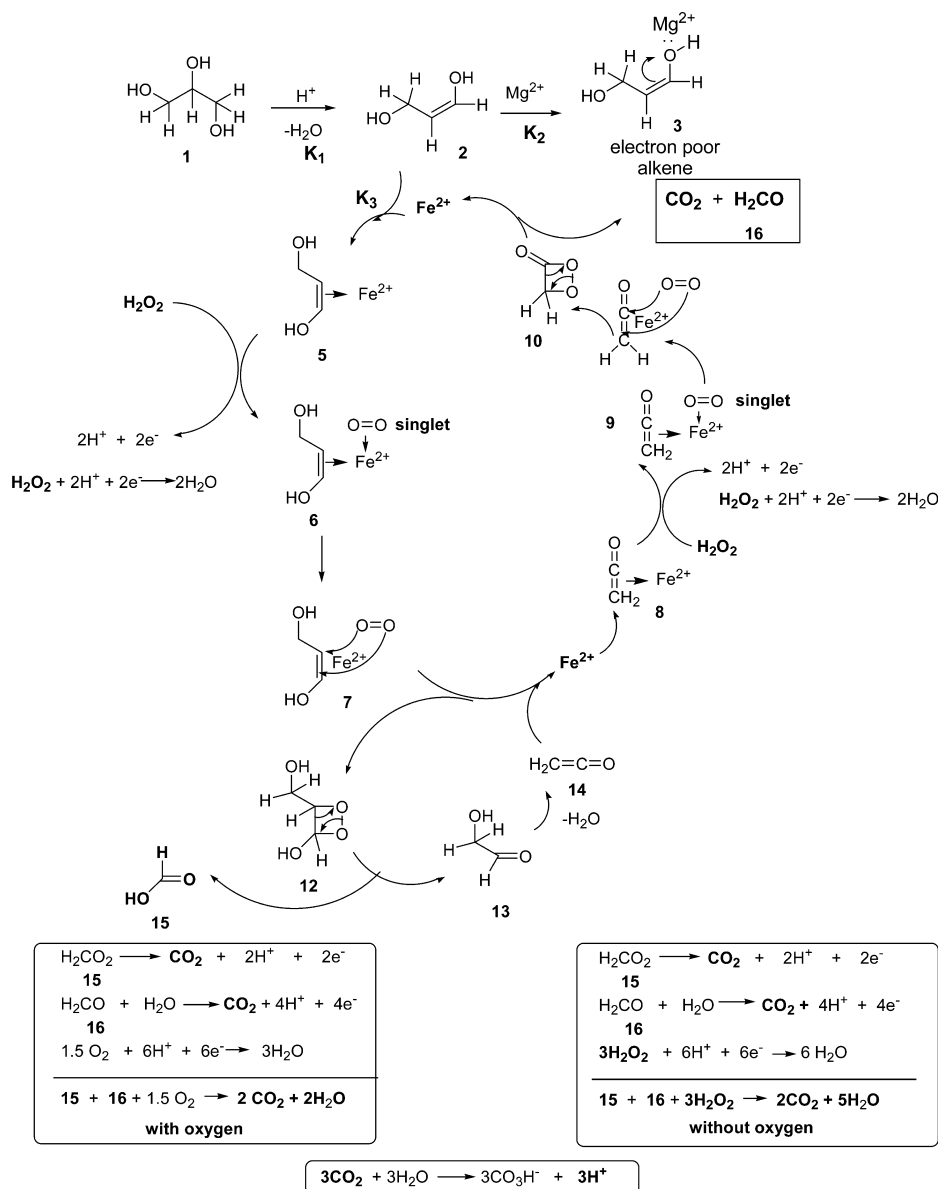
(7) The reaction takes place with glycerol and other polyols like mannitol, and carbohydrates like glucose, sucrose, and others. The presence of the 1,2,3-triol moiety is necessary for carrying out the reaction, as also reported by Ingles.¹⁷ We propose that it is due to the formation of an electron-rich enediol structure 2 that reacts with Fe^{2+} to produce a catalyst complex 5 that is the only intermediate responsible for the reaction rate. We have also found that methanol and ethanol do not react under these reaction conditions due to the impossibility of enol formation.

Discussion about the Mechanism. According to these results we propose the mechanism of Scheme 1.

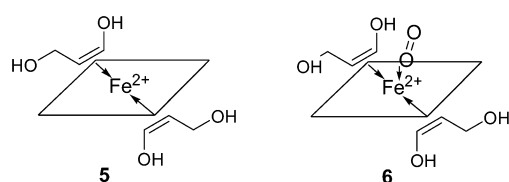
Step 1: Dehydration of Glycerol 1 To Form the Enediol 2. The kinetics of dehydration of glycerol at room temperature has been scarcely studied. There are reports on glycerol dehydration under extreme conditions, at high temperatures, and with very acidic media. This fact has been mentioned by Nimlos et al.,³³ who explained the mechanisms of glycerol dehydration in acid media and calculated the activation energy of reaction, which is 20–25 kcal/mol.

This is a high activation energy for this endothermic reaction of protonated glycerol, implying that the concentration of the dehydration product is too low at room temperature.

The H^+ concentration is critical in this step. A low pH is important to yield the enediol 2.²⁵ However, the pH cannot be too low (below 1.8) because the reaction does not start (Figure 7) and it cannot be too high (over 2.8) because iron hydroxides begin to precipitate. In the case of glycerol the formation of the enediol is slow; therefore, it is necessary some time to produce the required amount of enediol to form the complex 5 with Fe^{2+} . With other polyols like mannitol or glucose, this step is too much faster under these reaction conditions, as several enediol structures can be produced.

Scheme 1. Mechanism of the Fenton Reaction with Glycerol, Effect of Oxygen and Mg^{2+} 

Step 2: Formation of the Catalytic Complex 5. Taking into account the huge bibliography about the use of the Fenton reaction for degradation of organic compounds, many of them have common structural properties. They are alkenes or aromatics with electron-rich or electron donor properties that can produce a square planar complex with Fe^{2+} like **5** (Figure 9). This structure is necessary for two purposes: (a) to keep Fe^{2+} in solution to avoid iron hydroxides precipitation and (b) to produce a catalytic core to activate the organic compound for Fenton oxidation. (This is a structural requirement like in

Figure 9. Proposed structures of complexes **5** and **6**.

the hemo moiety in biological systems that has a square planar complex with a Fe^{2+} core to carry oxygen.)

According to the results shown in Table 4, the reaction rate increases linearly with the increasing iron concentration, so the Fe^{2+} concentration is in the rate-determining step of the reaction, and as it is in the core of the catalytic complex **5**, the reaction rate is directly related with the concentration of it, as shown in equations 18 and 19, which only depends on the concentration of Fe^{2+} , and hydroxy alkene **2**, equations 16 and 17.

$$K_3 = [\text{5}]/([\text{Fe}^{2+}][\text{2}]) \quad (16)$$

$$[\text{5}] = K_3[\text{Fe}^{2+}][\text{2}] \quad (17)$$

$$\Delta[\text{H}_2\text{O}_2]/\Delta t = k_1[\text{5}] \quad (18)$$

$$d[\text{H}_2\text{O}_2]/dt = k_1 K_3[\text{Fe}^{2+}][\text{2}] \quad (19)$$

Inhibition by Mg^{2+} . When Mg^{2+} is added, the reaction rate decreases linearly with the concentration of Mg^{2+} (Table 8).

The whole effect is that the amount of complex **5** decreases proportionally with Mg^{2+} increasing, equations 20–23.

$$K_2 = [3]/[\text{Mg}^{2+}][2] \quad (20)$$

$$[3] = K_2[\text{Mg}^{2+}][2] \quad (21)$$

$$[5] = [2]K_3[\text{Fe}^{2+}] - [2]K_2[\text{Mg}^{2+}] \quad (22)$$

$$[5] = [2](K_3[\text{Fe}^{2+}] - K_2[\text{Mg}^{2+}]) \quad (23)$$

This effect can be observed with glycerol because the concentration of **2** seems to be very low. However, it is more difficult to observe with other polyols or other compounds, in which the concentration of intermediates like **2** are higher, thus being necessary to increase the Mg^{2+} concentration too much to get a measurable effect on the reaction rate.

Step 3: Reaction of **5 with H_2O_2 .** In this step H_2O_2 reacts with **5** to produce complex **6** (Figure 9), in which oxygen is in the singlet state. Sugimoto and Sawyer¹⁰ previously reported the reaction products of several organic substrates like diphenylisobenzofuran and 9,10-diphenylanthracene, in which they found oxygenated products that are consistent with a singlet oxygen intermediate in acetonitrile. This important fact was barely mentioned by authors of papers and reviews about the mechanism of the Fenton reaction. The driving force is given by the formation of a singlet oxygen complex that can react very fast with the organic ligand, being present in the coordination sphere of the complex. Here, we report our results under these reaction conditions with these substrates occurring through a singlet oxygen mechanism, in a pH-dependent reaction. Therefore, we propose that singlet oxygen is formed in the first step of the reaction of H_2O_2 with the Fe^{2+} complex, which is in agreement with the structural and electronic requirements for the formation of a singlet dioxygen complex, as it is mentioned in literature.³⁴

The spectroscopic evidence that we have is the absorbance at 460 nm that appears after adding H_2O_2 to the reaction mixture and disappears when H_2O_2 is exhausted. This absorbance value is close to those of hemoprotein models of ferrous porphyrins with oxygen according to literature.^{35,36}

Also there is an important bibliography about the structural requirements of Fe^{2+} complexes for carrying oxygen in a reversible way.^{34,37,38}

Step 4: Intramolecular Reaction of Singlet Oxygen with Ligands. Singlet O_2 is a very reactive compound,³⁶ so that it can react in an intramolecular way with the activated substrate in the same coordination sphere, producing intermediate complex **7**, which can give rise to carbonyl compounds by $^1\text{O}_2$ 2 + 2 cycloaddition reactions,^{39–42} and CO_2 when it is fully oxidized.

Step 5: Recycling of Fe^{2+} . These partially oxidized compounds can leave the coordination sphere of the complex to produce intermediate oxidized compounds like **12–15** and leave Fe^{2+} to react either with another ligand **2** to regenerate complex **5** or with partially oxidized products like **14** to produce other complexes like **9** until yielding CO_2 .

This ketene **14** is a long-lived intermediate because of forming an iron(II)–ketene complex such as that previously reported.⁴³

The iron(II)–ketene complex reacts with singlet oxygen giving an unstable dioxetane intermediate, which finally yields CO_2 and water.

Step 6: Decreasing of pH Due to the Production of Carbonic Acid. In this last step, pH decreases due to the dissociation of carbonic acid that is the final product of the Fenton reaction.

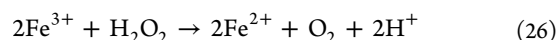
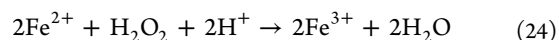
Participation of O_2 in the Mechanism. The participation of O_2 as electron scavenger is shown in this paper. When during the course of the reaction H_2O_2 is in shortage compared with the substrate like in stoichiometric studies (Experimental Section), in which H_2O_2 is added dropwise, the final oxidation of intermediates such as formaldehyde **15** ($E_{\text{red}} = -0.48$ V) and formic acid **16** ($E_{\text{red}} = -0.61$ V) to CO_2 is carried out by O_2 dissolved in water ($E_{\text{red}} = +1.10$ V at pH 3 and $p_{\text{O}_2} = 0.21$ bar) that is at constant concentration throughout the reaction. Therefore, when there is not enough H_2O_2 to perform the full oxidation to CO_2 , the first step is the addition to alkene to give dioxetane intermediates **10** and **12** before oxidizing **15** and **16** to CO_2 , and these intermediates are further oxidized by the O_2 dissolved in water.

If the same reaction is carried out without H_2O_2 in the presence of air, the only products will be those of oxidized iron. In this case, initial and final CODs are the same (Table 1, footnote). If some O_2 radicals were produced from the air in the absence of H_2O_2 , these radicals would be used for the irreversible oxidation of Fe^{2+} , but not to perform the oxidation of the organic compounds.

Moreover, if a free radical participated in the absence of H_2O_2 , some COD decrease related to the formation of oxidation products would be detected.

ORP of the Reaction Media. The redox potential of the solution that is measured by the ORP electrode can be explained by the ratio **6** (formally oxidized intermediate)/**5** (formally reduced intermediate) (Scheme 1).

- (1) Before adding H_2O_2 , **5** is the single reduced complex in the reaction media (point A in Figure 1).
- (2) When H_2O_2 is added, oxidized complex **6** appears, and the ratio **6**/**5** increases until reaching a maximum, so the ORP increases (points A to B in Figure 1).
- (3) When enough **6** is produced, the reaction begins and the ratio **6**/**5** is kept constant while H_2O_2 is present, so the ORP remains constant (points B to C in Figure 1).
- (4) When all H_2O_2 is consumed, **6** begins to decrease, so the ratio **6**/**5** decreases, and the ORP decreases until reaching a minimum (points C to E in Figure 1).
- (5) At this point more H_2O_2 can be added, if there is still enough substrate, ORP increases, and the reaction begins again. When there is not enough substrate, iron is oxidized and iron hydroxides precipitate appears, reactions 24–26.



Reaction of Other Substrates. A similar set of reactions can be proposed to explain the stoichiometry of the other compounds of Table 1, reactions 27–34, which now can be compared with theoretical calculations (Table 9).

Table 9. Complete Intake of H₂O₂ with and without O₂ and Comparison with Theoretical Calculations^a

S ^b	A		B	
	exper	calc	exper	calc
Gly	4.1 ± 0.1	4	7.3 ± 0.1	7
Suc	11.7 ± 0.1	12	24.8 ± 0.1	24
Man	6.7 ± 0.1	6.5	13.2 ± 0.1	13
Lac	11.5 ± 0.1	12	24.3 ± 0.1	24
Glu	6.0 ± 0.1	6	12.5 ± 0.1	12
Fru	6.0 ± 0.1	6	12.3 ± 0.1	12
Tar	3.1 ± 0.1	4	5.2 ± 0.1	5

^aConcentration of the substrate (S) = 2.0 ± 0.1 mg/mL; Fe²⁺ = (1.5 ± 0.1) × 10⁻³ M; T = 20 °C; pH 2.5 ± 0.1. CDO = mg O₂/L. A = mmol H₂O₂/mmol S (with O₂); B = mmol H₂O₂/mmol S (without O₂).

^bGly (glycerol); Suc (sucrose); Man (mannitol); Lac (lactose); Glu (glucose); Fru (fructose); Tar (tartaric acid). Exper = experimental; Calc = calculated.

Sucrose Lactose	Without O ₂	C ₁₂ H ₂₂ O ₁₁ + 24 H ₂ O ₂ →	12 CO ₂ + 35 H ₂ O	(27)
	With O ₂	C ₁₂ H ₂₂ O ₁₁ + 12 H ₂ O ₂ + 6 O ₂ →	12 CO ₂ + 23 H ₂ O	(28)
Glucose Fructose	Without O ₂	C ₆ H ₁₂ O ₆ + 12 H ₂ O ₂ →	6 CO ₂ + 18 H ₂ O	(29)
	With O ₂	C ₆ H ₁₂ O ₆ + 6 H ₂ O ₂ + 3 O ₂ →	6 CO ₂ + 12 H ₂ O	(30)
Mannitol	Without O ₂	C ₆ H ₁₄ O ₆ + 13 H ₂ O ₂ →	6 CO ₂ + 20 H ₂ O	(31)
	With O ₂	C ₆ H ₁₄ O ₆ + 6.5 H ₂ O ₂ + 3.25 O ₂ →	6 CO ₂ + 13.5 H ₂ O	(32)
Tartaric Acid	Without O ₂	C ₄ H ₆ O ₆ + 5 H ₂ O ₂ →	4 CO ₂ + 8 H ₂ O	(33)
	With O ₂	C ₄ H ₆ O ₆ + 3 H ₂ O ₂ + O ₂ →	4 CO ₂ + 6 H ₂ O	(34)

All results are in agreement with theoretical calculations of H₂O₂ consumption. This can be a useful tool to calculate the expense of H₂O₂ if the composition of a waste is roughly known, like in factories, in which sugar is the main raw material.

Comparative Rates of Reaction. The reaction rate depends on the concentrations of iron and hydroxyalkene type-2 (Figure 6; reactions 16–19), as demonstrated for glycerol. So, at a constant iron concentration and with a greater concentration of hydroxyalkene type-2, the rate of reaction is higher (Figure 6).

The concentration of the hydroxyalkene type-2 further depends on the capacity of formation of this alkene described as K₁, equations 35 and 36.

$$K_1 = [2]/[1] \quad (35)$$

$$[2] = [K_1][1] \quad (36)$$

Upon comparison of polyhydroxy compounds such as mannitol and glycerol, mannitol contains four secondary HO, whereas glycerol has one; therefore, mannitol will render a higher hydroxyalkene type-2 concentration than glycerol (K_{1man} > K_{1gly}). Then, the rate of reaction of mannitol will be greater than that of glycerol.

Fructose and glucose are both hexoses, but fructose is a ketose that contains a carbonyl group at C-2, which migrates in acid media, so readily stabilized hydroxyalkenes can be formed by dehydration (ΔG = -25.6 kcal/mol).⁴⁴ Therefore, the hydroxyalkene concentration is higher for fructose than mannitol (K_{1fru} > K_{1man}), and also the rate of reaction.

Glucose has one aldehydic carbonyl group but first isomerizes to fructose in acid media with an endothermic barrier of ΔG = +1.53 kcal/mol,⁴⁴ which makes K_{1glu} < K_{1fru}. Therefore, the rate of reaction is slower for glucose than for fructose.

In the case of the disaccharide sucrose, since both anomeric carbons are involved in the interglycosidic bond, a previous hydrolysis reaction step is required to obtain two monosaccharides. Therefore, the rate of reaction is less than that of

monosaccharides and polyhydroxy compounds. The other disaccharide lactose comprises only one anomeric carbon in the interglycosidic bond. Therefore, the rate of reaction is somewhat greater than that of sucrose and polyhydroxy compounds such as mannitol, but less than those of monosaccharides such as glucose.

In the case of tartaric acid the hydroxyalkene is conjugated with two carboxylic groups that make the hydroxyalkene production thermodynamically more favorable than those of carbohydrates (which have carbonyl groups), and any other compound of Table 2, so the reaction is the fastest of all them. According to these statements a scale of relative reactivities related to K₁ can be proposed.

$$K_{1tar} > K_{1fru} > K_{1glu} > K_{1lac} = K_{1man} > K_{1suc} > K_{1gly}$$

This explanation is very important for getting a clue on the mechanism, because it is clear that the rate of reaction strongly depends on the structure of the substrate taking into account its ability to produce hydroxyalkene intermediates. The reaction is faster when more hydroxyalkene is produced.

If the reaction were via free radicals or ferryl mechanisms, the structure of the substrate would have little or no influence in the reaction rate.

CONCLUSIONS

In this paper new mechanistic insights of the oxidation of carbohydrates by the Fenton reaction using glycerol as experimental model are described according to experimental results, showing the importance of ORP measures as an experimental tool to study this kind of reactions. We remark the use of glycerol as a model compound because the reaction is slow enough to reduce experimental error, the use of independent methods as the dichromate reaction to check the exhaust of H₂O₂, and COD to measure the full consumption of the organic substrates.

- (1) **Stoichiometry:** It was carefully measured, and these results were of great importance for understanding the role of oxygen in the mechanism.
- (2) **Importance of oxygen:** We disclosed and measured how oxygen participates in the Fenton reaction. In aerobic conditions it plays a key role in the mechanism; this is important in industrial operations as degradation of liquid waste by the Fenton reaction, because with an energetic aeration, significative amounts of H₂O₂ can be saved decreasing the cost of the process. Also it may have importance to explain some biological mechanisms.
- (3) **Oxidation state of Fe:** Many authors indicate that the Fenton reaction can be carried out indistinctly with Fe²⁺ or Fe³⁺ with only some differences in the reaction rate, depending on the initial oxidation state of Fe. However, all mechanisms described up to now indicate that Fe³⁺ participates in the Fenton reaction. **As far as we know, this is the first time that experimental evidence is provided, showing that Fe²⁺ is the only iron ion species that is involved in the Fenton reaction because it can produce the planar complex that is necessary for this kind of catalysis.** When Fe³⁺ is used, a previous reduction step to Fe²⁺ is required before the reaction starts. The difference in the reaction rate is due to accumulating enough Fe²⁺ upon reduction of Fe³⁺.
- (4) **Inhibitor effect of Mg²⁺:** As far as we know, this is the first time that the inhibitor effect of Mg²⁺ is reported in

the Fenton reaction with a rational involvement in the mechanism. It is necessary to point out the great importance that this effect may have in biological and industrial processes because Mg^{2+} is a widely distributed cation that can be found elsewhere.

- (5) **Structure of the catalytic intermediate:** We described the structural requirements that must fulfill the substrate to behave as a reactive ligand in the Fenton reaction in catalytic concentrations and room temperatures. They must be able to produce an electron-rich planar complex with Fe^{2+} to give rise to singlet oxygen after reacting with H_2O_2 . This is the explanation of why not all organic compounds can be oxidized by the Fenton reaction under mild reaction conditions.

Finally, in this paper a rational mechanism for the oxidation of glycerol using the Fenton reaction is proposed. Further extension of this mechanism is currently under study.

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

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