Chapter

A History of the Fenton Reactions (Fenton Chemistry for Beginners)

Rafael Ovalle

Abstract

A deceptively simple mixture, ferrous sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), tartaric acid ($C_4H_6O_6$), and water (H_2O), initiated a century-long argument and a convoluted hunt to understand the oxidation mechanism(s) initiated by the combination of these components. Fenton's discovery rallied a legion of scientists, including two Nobel Winners, to find an explanation for the chemistry discovered when a graduate student mixed a couple of random chemicals, producing a molecule that became purple in strong base. Those investigation uncovered three separate branches of iron/oxygen chemistry, the Hydroxyl Radical [HO•], the Ferryl-Oxo Ion $[Fe = O]^{+2}$, and the Perferryl-Oxo Ion $[Fe = O]^{+3}$. Today their uses include chemical modifications [either untargeted and random [HO•] or targeted and selective $[Fe = O]^{+2}$, $[Fe = O]^{+3}$ dehydrogenations and/or oxygen additions] to effective and green oxidation and mineralization of persistent organic wastes.

1. Introduction

The Initial Experiments. In 1876, Henry John Horstman Fenton first discovered the enhanced oxidizing power of ferrous ions (Fe^{+2}), hydrogen peroxide (H_2O_2) on tartaric acid ($C_4H_6O_6$). When Fenton added sodium hydroxide (NaOH) to the mixture, the solution became bright purple [1]. Fenton made a decision to find out what that purple molecule was. That goal became his career and immortalized his name in the annals of chemistry [2].

Eighteen years later, Fenton repeated the experiment, again adding to a tartaric acid solution, a catalytic amount of $FeSO_4$, followed by H_2O_2 with the molar ratio of each factor: $C_4H_6O_6$ / H_2O_2 / Fe^{+2} = 1.0: 1.0: 'catalytic'. Fenton then isolated the reaction product by sequentially precipitating the acid with heavy metal ions, weighing the salt to calculate the molar formula, re-purifying the acid, then repeating the process with a different cation, thus calculating the empirical formula of the new acid. The new acid bound one divalent cation ion or two monovalent cations ions per molecule, and thus was a di-acid. Fenton determined: 1) the molecule had the empirical formula $C_4H_4O_6$; 2) was a 4-carbon di-acid; 3) produced by abstraction of two hydrogen atoms from tartaric acid [3].

Fenton (1896) assumed that the 4-chain backbone was not severed, limiting his options to three possible structures: 1) loss of two hydrogen from a single middle carbon, forming a hydroxy-, keto-, di- carboxylic acid: (2-hydroxy-3-oxosuccinic acid); or 2) loss of two hydrogen from the internal adjacent carbon atoms forming a double bond with the HO- groups either in: 2a) *trans-* conformation: 2-, 3-, di-hydroxyfumaric acid, or 2b) *cis-* conformation: 2-, 3-, di-hydroxymaleic acid (Eq. (1)).
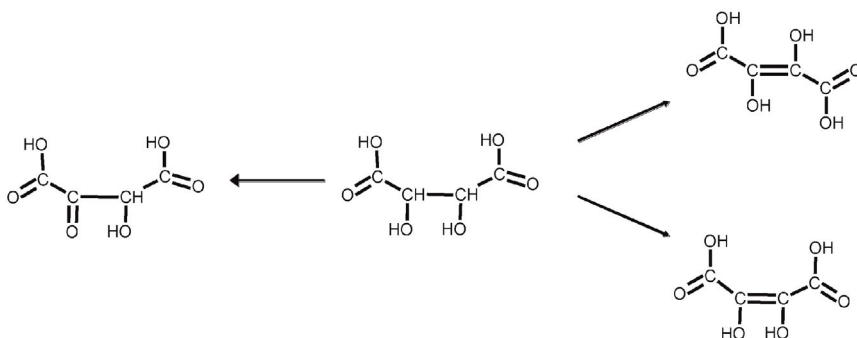
$$\text{Fenton's Initial Guess for } C_6H_4O_6. \hspace{2cm} (1)$$

The first structure was eliminated when the di-acid failed to form a hydrazone with phenylhydrazine (an aldehyde/ketone reactive agent). The assumption that the molecule had two internal hydroxyl groups was verified when the molecule formed a 4-carbon di-ester, di-anhydride with either acetyl chloride or acetic anhydride.

The structure of the molecule was finalized by reaction with aniline. Fenton knew (from literature) that the 1:1 product of aniline and fumaric acid ($C_4H_2O_4$: $-C_2H = HC_3-$ in *trans-*) was soluble in water, whereas the 1:1 product of aniline and maleic acid ($C_4H_2O_4$, $-C_2H=HC_3-$ in *cis*) was insoluble in water. The aniline derivative of the unknown acid was also insoluble in water. Fenton concluded that Fe^{+2}/H_2O_2 oxidized tartaric acid to 2-, 3-, di-hydroxy-maleic acid: a loss of two H• atoms in *cis-* orientation, forming a double bond and creating a previously unknown molecule [4].

2. Early Fe^{+2}/H_2O_2 investigations

Following the initial discovery, Fenton tested the range of his new reagent. Fenton & Jackson (1899) oxidized aliphatic alcohols, polyalcohols, and benzoic, then $FeSO_4$ followed by H_2O_2, added stepwise, in molar ratios of (1: 0.1–0.25: 1), adding the peroxide gradually in small amounts.

The aliphatic alcohols: (CH_3OH, CH_2CH_5OH, n-C_3H_7OH, i-C_3H_7OH, and n-$C_5H_{11}OH$), did not produce any visible changes in temperature or precipitates with phenylhydrazine, therefore Fenton assumed that these molecules were non-reactive [5]. Merz & Waters (1947) commented that the 1:1 alcohol: H_2O_2 ratio used in the experiments by Fenton & coworkers was so high that the alcohols were oxidized directly to organic acids. Fenton & coworkers would have discovered this result if: a) they had assayed their samples for acids, and/or b) assayed the alcohols with different concentrations of H_2O_2] [6].

On the other hand, the polyalcohols ($C_2H_6O_2$, $C_3H_8O_3$, $C_4H_{10}O_4$, $C_5H_{12}O_5$, and $C_6H_{14}O_6$) showed temperature increases and release of gases, whereas the H_2O_2 only controls showed no reaction for either group. When the oxidized polyalcohols were

reacted with phenylhydrazine forming osazones, indicating that hydroxyl groups of the polyols were oxidized to carbonyls, forming aldoses and/or ketoses. The oxidation of hydroxyl groups in the polyalcohols to aldehydes or ketones required a loss of two hydrogen atoms (H•) from the molecular formula.

Benzoic acid ($C_7H_6O_2$) was oxidized to salicylic acid (o-$C_7H_6O_3$) as determined by a robust violet color when ferric ion was added to the solution indicating that an exchange of a -H atom with an -OH group adjacent to the -COOH on the benzene ring [5].

Fenton & Jones (1900) repeated testing the oxidizing abilities of Fe^{+2}/H_2O_2 on a larger set of aliphatic and polyhydroxy acids. Their method was to prepare a 1 M solution of reagent in H_2O at 0°C, add $FeSO_4$ to 0.125 M, then add H_2O_2 to 0.25 M (-$FeSO_4$ was the control). The authors again reported that aliphatic acids appeared non-reactive while polyhydroxy acids showed vigorous and energetic reactions. The oxidized acids reacted with phenylhydrazine, and the precipitates were purified by crystallization, confirming that hydroxyl groups were oxidized to ketones or aldehydes and the molecules identified by melting point determinations. The oxidation of benzoic acid to salicylic acid was also confirmed [7].

Collectively, Fenton (1896) and Fenton et al. (1899, 1900) presented evidence for three different reactions: 1) carbon–carbon double bond formation with loss of 2 H-; 2) carbon–oxygen double bond formation with loss of 2 H- (both aldehydes and ketones; and 3) oxygen addition to phenol.

2.1 Isolation of glucosones

Following Fenton's lead, Cross, Bevan, & Smith (1898) oxidized glucose with $FeSO_4$ / H_2O_2 and isolated "glucosone" (2-keto-, glucose). The experiment consisted of: 1) in 100 mL H_2O, 4% or 10% glucose; 2) $FeSO_4$ added to a concentration of $1/10^4$ ratio with glucose, and 3) H_2O_2 was gradually added to a final 1:1 molar glucose/ H_2O_2 with stirring on ice [8]. Theorizing that 2-keto-, glucose would be indigestible, the authors used yeast to scavenge unoxidized glucose. After filtering out the yeast, the authors found that the solution still contained carbonyl molecules, as indicated by reduction of CuO. The solution retained 20% of the reducing power of the original glucose solution. The oxidized glucose solution also increased in acidity. After drying (105°F / 40.6°C), the dried residue comprised 88% of the weight of glucose including 3.8% furfural.

The solids were resuspended in chilled water and reacted with phenylhydrazine (PHZ), a reversible carbonyl-reactive label). The rationale was that while glucosone and glucose are likely to be equally soluble in most solvents, the double substituted glucosazone was expected to have a different solubility profile from the gluco-hydrazone. The glucosone reacted with 2 moles of PHZ, whereas glucose reacts with only one mole of PHZ, therefore their solubility in differences in organic solvents would be greater than the unlabeled molecules. After purification, PHZ-labeling was reversed with H_2SO_4, allowing analysis of purified glucosone. The authors repeated their method with fructose and sucrose recovering only glucosone from all three sugars implying: 1) fructose oxidized to glucosone; and 2) sucrose hydrolyzed to glucose and fructose.

Morell *et al.* (1899–1905) conducted a larger and more detailed survey of the oxidation of monosaccharides to corresponding -osones with Fenton's reagent. Following Cross & Bevan's method [9–14]. Morell et al. (1899a) expanded the study of the osones to include galactose, rhamnose, arabinose, and mannose [9].

Morell et al. (1899b) increased glucose to glucosone yields by: 1) slow addition of H_2O_2, 2) controlling temperature with refrigeration, 3) controlling pH and precipitating

organic acid with (Pb(OAc)$_2$). This method increased glucosone yield to 10%. Morell et al. purified PHZ-glucosazone and PHZ-mannosazone from their corresponding PHZ-hydrazones, but were not able to purify PHZ-arabinosazone, PHZ-rhamnosazone, and PHZ-galactosazone from their PHZ-hydrazone contaminants [10].

Morrell *et al*. (1900) oxidized glucose, fructose, arabinose, rhamnose, galactose, maltose, lactose and sucrose, then labeled both aldoses and osones with PHZ. The authors increased purity by precipitating the saccharic acids with Pb(OAc)$_2$, while controlling pH with Ba(OH)$_2$. PHZ-rhamnosazone was also separated from its PHZ-hydrazone, but not galactose or arabinose [11].

Morrell *et al*. (1902) found that reacting a glucosone with bromine severed the C2-C3 bond and oxidized C3 to a carboxylic acid, the final product being erythronic acid (trihydroxy-butyric acid), which was then dehydrated to butyric acid with nitric acid. The identity of erythronic acid was confirmed by calculating formula weights of the lead and barium salts [12].

Morell et al. (1903) oxidized aldoses with Fenton's reagent, precipitate organic acids with Pb(OAc)$_2$ and Ba(OH)$_2$, then label the oxidation mixture for carbonyl groups with methyl-, phenyl-hydrazine (MPHZ). However, galactose and arabinose MPHZ-osazones were not separable from their MPHZ-hydrazones. The authors tested bromo-, phenylhydrazine (BPHZ) and found that BPHZ-arabinosazone was easily separable from BPHZ-arabinose hydrazone using benzene as the solvent [13].

Morrell & Bellars (1905) retested purification of all the aldose osazones with BPHZ. BPHZ-labeling after Fenton oxidation allowed sharp separations of BPHZ -osazones from the corresponding hydrazones in benzene, thus achieving the goal of acquiring pure osazones after Fe^{+2}/H$_2$O$_2$ oxidation [14]. [Considering that both Cross & Bevan (1898) and Morell et al. (1899) stated that the purified osones were tasted, the implication is that both groups were investigating the osones as non-caloric sweeteners].

2.2 Isolation of aldonic acids and other byproducts

Cross & Bevan (1898) surveyed the by-products yielded by Fenton oxidation of aldoses to glucosones. The secondary products included: tartronic acid, ($\sim$ 8%), acetic acid ($\sim$5%), formic acid ($\sim$15%), and furfural(s) ($\sim$ 4%). Missing carbohydrate mass was assumed to be lost as carbon dioxide (CO$_2$). The authors noted that Fenton oxidation of glucose produced furfural, but fructose did not; on the other hand, glucose produced lower amounts of dicarboxylic acids and pentoses [8]. Cross & Bevan (1899) oxidized a 2% solution of furfural with H$_2$O$_2$ and a catalytic amount of FeSO$_4$. Formic, acetic acid, and a red precipitate identified as pyromucic acid were isolated. The authors also reported that Fe^{+2}/H$_2$O$_2$ oxidized benzene to phenol, followed by additional hydroxylations [15].

Morrell et al. (1903) used Pb(OAc)$_2$ and Ba(OH)$_2$ to precipitate the sugar acid impurities in the quest to purify the osones. From the oxidation of glucose and fructose, the experimenters recovered the several polyhydroxy acids after precipitation with Pb^{+2} or Ba^{+2} ions. After removal of Pb^{+2} and Ba^{+2} ions with H$_2$SO$_4$, the solubilized acids were identified as glycolic and oxalic. The Pb^{+2} / Ba^{+2} soluble acids were separately precipitated as calcium salts and identified as glyoxylic and trihydroxy-butyric acids [13].

In sum, Cross & Bevan (1898, 1899) and Morrell et al. (1899–1903) confirmed that Fenton's reagent was responsible for the following reactions: dehydrations producing C=C bonds, C-C bond cleavages, and oxygen atom additions forming hydroxyl, aldehyde, ketone, and carboxylic acid groups, creating new classes of organic molecules.

3. Joint history of the hydroxyl radical and ferryl-oxo ion

Prof. Henry J. H. Fenton died in 1929 without knowing the mechanism of the reagent that he discovered. Within three years of his death, two competing mechanisms naming two different intermediate molecules were published.

3.1 Hydroxyl radical

In 1932, Fritz Haber & Joseph Weiss (1932) published in *Naturwissenschaften* (Science of Nature), and again in Proceedings of the Royal Society of London: A (1934), that the hydroxyl radical (HO•) is the oxidative intermediate responsible for Fenton's observation.

The authors proposed that the Fe^{+2} ion donates an electron to the peroxide molecule, cleaving the O-O bridge producing a hydroxyl radical (HO•) and a hydroxide ion (HO^-). The hydroxyl radical then attacks another peroxide molecule, forming superoxide, eventually generating oxygen [16, 17].

3.2 Ferryl-oxo ion

On the other side of the Atlantic Ocean, William Bray & H. M. Gorin published in Journal of the American Chemical Society (1932) that oxygen production after addition of Fe^{+2} ions to H_2O_2 in H_2O is due to creation of the ferryl-oxo ion $(Fe = O)^{+2}$ (**Figure 2**). After creation, the ferryl-oxo ion then reacts with another ferryl-oxo molecule to produce oxygen gas, recycling the ferrous ion. The authors proposed that an oxygen atom (•O•) is abstracted by a Fe^{+2} ion from the peroxide molecule, forming the ferryl-oxo ion $(Fe = O^{+2})$ (no net change in charge) and H_2O [18].

[Bray & Gorin named the molecule 'ferryl ion', but the term is currently used for the Fe^{+4} ion (without oxygen) [19]. To avoid confusion, the Bray & Gorin molecule is named here 'ferryl-oxo' ion. For similar reason, Barton's 'perferryl ion' will be named 'perferryl-oxo ion'].
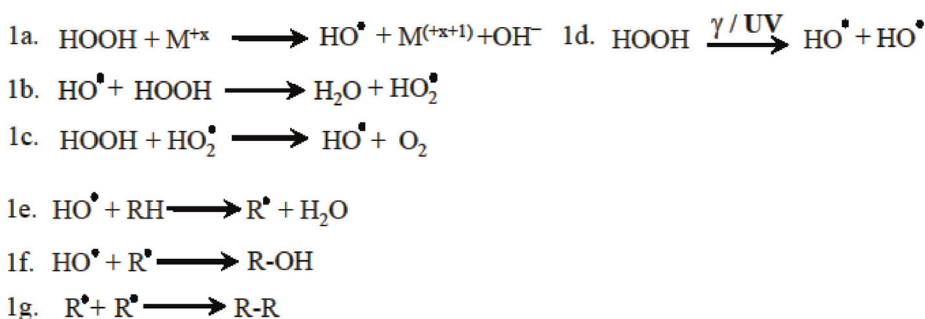
These two papers divided the scientific community into partisan camps with sports-like fanaticism that continues today. Champions of the hydroxyl radical theory include: JD Rush, WH Koppenol [20–23], C. Walling [24–26], M. Kremer [27–29], JH Merz, WA Waters, [6, 30, 31], as well as many others. The scientists who argued for the existence of the ferryl-oxo ion included JT Groves [32–35], DA Wink [36–38], and DT Sawyer [39], among many others.

3.3 Oxidative behavior of the hydroxyl radical

Only exceeded by a fluorine (F^0) atom, the powerful hydroxyl (HO•) radical is the second strongest electrophile, and will even oxidize chlorine ion(s) (Cl^-) to elemental chlorine (Cl^0) or gas (Cl_2) [40]. A hydroxyl radical will strip an electron from an element (except F^-) or H• from a hydrocarbon (**Figure 1**).

Hydroxyl radicals (HO•: **Figure 1**) are created by:

1. Donation of an electron to H_2O_2 from a transition metal ion (**Figure 1a**) (with *exception* of iron and copper ions [41]) produces HO• radicals via secondary electron transfer from the ion to peroxide. The O-O bond of peroxide scissions, producing a hydroxyl ion (HO^-) and hydroxyl radical (HO•);

1a. $HOOH + M^{+x} \longrightarrow HO^\bullet + M^{(+x+1)} + OH^-$ 1d. $HOOH \xrightarrow{\gamma/UV} HO^\bullet + HO^\bullet$

1b. $HO^\bullet + HOOH \longrightarrow H_2O + HO_2^\bullet$

1c. $HOOH + HO_2^\bullet \longrightarrow HO^\bullet + O_2$

1e. $HO^\bullet + RH \longrightarrow R^\bullet + H_2O$

1f. $HO^\bullet + R^\bullet \longrightarrow R\text{-}OH$

1g. $R^\bullet + R^\bullet \longrightarrow R\text{-}R$

References:
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1d: Dorfman, L. M., Adams, G. E. Nat. Stand. Refer, Data Sys. 1973, 46. NSRDS: NBS-46 National Bureau of Standards, U.S. Dept. Comm.
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1e-g. Balavoine, G.; Barton, D.; Boivin J.; Gref A. Tetrahedron Lett. 1990, 31 659-62. DOI: 10.1016/S0040-4039(00)94595-7 :
Barton, D.; Beviere, S.; Chavasiri, W.; Csuhai, E.; Doller, D.; Liu, W. J. Am. Chem. Soc. 1992, 114 2147-56. DOI: 10.1021/ja00032a032
Schuchardt, U.; Jannini, M. J.; Richens, D. T.; Guerreiro, M. C.; Spinacé, E.V. 2001, Tetrahedron, 57(14) 2685-8. DOI: 10.1021/ja00032a032

Figure 1.
Hydroxyl radical reactions.

2. Splitting H_2O_2 with UV (λ = 253.7 nm) or γ- radiation (from ^{60}Co) produces two hydroxyl radicals:

$$H\text{-}O\text{-}O\text{-}H + (UV) \rightarrow HO\bullet$$
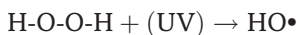
Hydrogen Peroxide is Cleaved by UV Rays to Hydroxyl Radicals   (2)
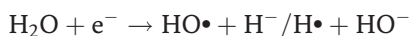
(See (Eq. (2)) (**Figure 1d**) [42];

3. UV flash irradiation of oxygen donating molecules, such as N2O to create oxygen atoms ($\bullet O\bullet$) that react with water molecules:

$$H_2O + \bullet O\bullet \rightarrow 2\,HO\bullet$$

Water Molecule and Oxygen Atom Form Hydroxyl Radicals   (3)

(Eq. (3)) [43, 44]; or.

4. Ionizing water with electrically produced β-rays to produce radicals

$$H_2O + e^- \rightarrow HO\bullet + H^-/H\bullet + HO^-$$

Water Molecule is Cleaved by High Energy Electrons to Hydroxyl Radicals, Hydroxyl Ions, Hydride Radicals, and Hydrogen Atoms   (4)
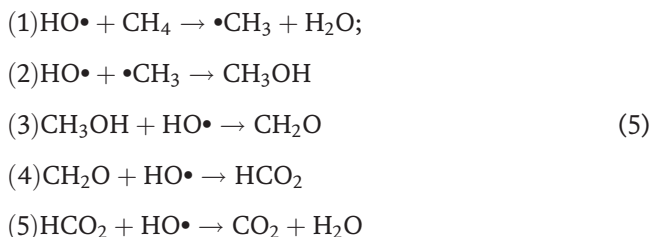
(Eq. (4)) [42];
(H$\bullet$ radicals likely combine with each other, as also H$^-$ & H$^+$ ions; thus escaping as H_2 gas) [42, 45].

Once created, hydroxyl radicals (HO$\bullet$) will oxidize an element, ion, or compound, by extracting an electron to form HO$^-$ and a cation, increase the valence of another ion by +1, or abstract H- from an X-H bond of organic molecule, forming H_2O and an organic radical (**Figure 1e**).

3.3.1 Oxidation of hydrocarbons (alkanes) by hydroxyl radicals

A hydroxyl radical abstracts H• from an alkane to create a C• radical and water (**Figure 1e**) [46, 47]. A second HO• is required to collide with C• to form an alcohol (**Figure 1f**), thus a high HO•/ substrate ratio is required to produce alcohols. Subsequent HO• hydroxyl attacks to the same carbon atom progressively oxidizes and adds oxygen atoms, producing aldehydes/ketones, then organic acids, and finally, carbon dioxide [31, 42, 48]. An example of a hydroxyl radical reaction sequence for oxidation of methane:

$$(1) HO\bullet + CH_4 \rightarrow \bullet CH_3 + H_2O;$$

$$(2) HO\bullet + \bullet CH_3 \rightarrow CH_3OH$$

$$(3) CH_3OH + HO\bullet \rightarrow CH_2O \qquad (5)$$

$$(4) CH_2O + HO\bullet \rightarrow HCO_2$$

$$(5) HCO_2 + HO\bullet \rightarrow CO_2 + H_2O$$

Sequential Oxidation of Methane to Carbon Dioxide by Hydroxyl Radicals

(Eq. (5)) (**Figure 1f**) [25].

There is no guarantee that a second HO• will collide with a carbon radical to make an alcohol.

Under low HO• concentrations, long-lived hydrocarbon radicals fuse to each other to make large complex hydrocarbons via $R_1C\bullet\bullet CR_2$ fusions (**Figure 1g**). The hydroxyl radical oxidation of methane can also follow:

$$(1) HO\bullet + CH_4 \rightarrow \bullet CH_3 + H_2O; \qquad (6)$$
$$(2) CH_3\bullet + \bullet CH_3 \rightarrow CH_3 - CH_3$$
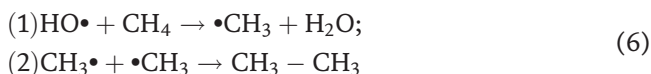
Partial Oxidation of Methane by Hydroxyl Radicals Allow C-C Fusions
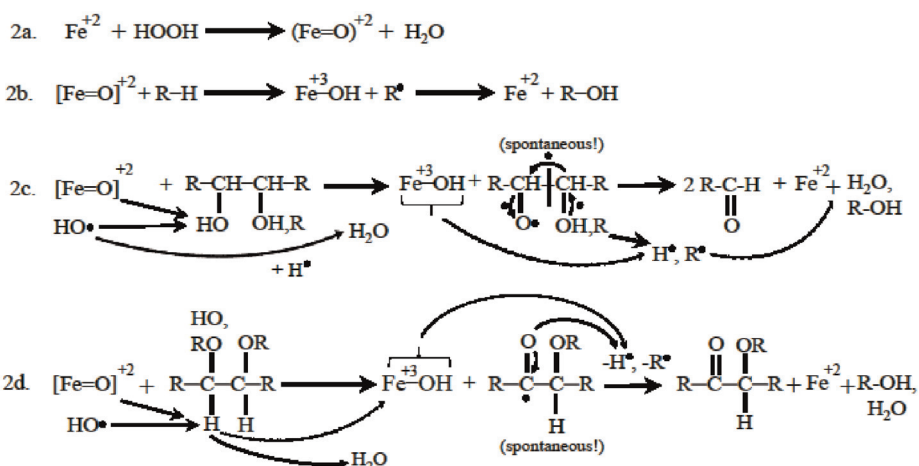
(Eq. (6)) (**Figure 1g**) [25].

Thus carbon–carbon fusions are a hallmark of hydroxyl radical reactions [42, 49].

The HO• radical is: 1) uncharged, and 2) will abstract an electron any atom (except F^-) or H• from a molecule it collides with, thus the (HO•) radical is an indiscriminant oxidant. Its oxidation profile determined by accessibility and rate of diffusion. In the oxidation of simple alkanes, the oxidation preference is: 1° H > 2° H > 3 H°.

Hydroxyl radicals (HO•) [17] can be created by H_2O_2 receiving an electron from a transition metal ion [41], or by splitting an oxygen donating molecule, either H_2O_2, with UV light or radiation [42, 50] or N_2O (aq.) with UV light [43, 44]. Hydroxyl radicals can be quenched/scavenged by reducing agents [51] including aliphatic alcohols [50], DMSO [52], acetate ions [22], polyols [53], H_2S [54], and NO [55]. These reagents are included as radical traps where HO• radical oxidations are undesirable.

3.3.2 Hydroxyl radical oxidation of alcohols

Waters (1946) reported that HO• radical oxidation of ethylene glycol ($CH_2(OH)$-$CH_2(OH)$) produced both glycoaldehyde ($CH(O)$-$CH_2(OH)$) and formaldehyde (2x: CH_2O). To determine which H• abstraction caused C-C bond cleavage, Waters

Figure 2.
Ferryl-Oxo ion reactions.

oxidized pinacol, which has no available O-**C-H** bonds, to acetone ($(CH_3)_2C=O$) as the only product. Thus, Waters (1946) proved that H• abstraction from the hydroxyl oxygen (H-C-***O-H***) bond of a 1-, 2-, diol causes C-C bond cleavage, and H• abstraction from an ***H-C***-O-H bond of a 1-, 2-, diol causes localized C=O bond formation [56] (see ferryl-oxo ion: **Figure 2c** and **d**).

Droege & Tully (1986a,b) oxidized gaseous ethane (^{1}H, ^{2}H, and mixed) (46)] and propane (^{1}H, ^{2}H, and mixed) [47] with UV-activated N_2O & H_2O to compare oxidation rates of the terminal vs. center carbons, and test the isotope effect on HO• oxidation for different positions of the ethane and propane molecules. The authors found that there was no difference in positional abstraction for hydrogen vs. deuterium at 1° (ethane & propane) or 2° positions (propane only); however C-C chain fusions increased with temperature.

Baxley & Wells (1998) oxidizing tertiary alcohols with HO• radicals in air. HO• radicals were generated by UV activation of CH_3ONO, NO, and O_2 gases. H-abstraction from the sole -OH group caused C-C cleavage producing a ketone, a hydrocarbon and water. Abstractions from C-H bonds produced either addition of a second hydroxyl group or fusions producing long chain diols, however the authors noted that the hydroxyl group of 2-butanol was targeted more frequently than of 2-pentanone [48].

3.3.3 Hydroxyl radical oxidation of diols, polyols, and carbohydrates

Gilbert and King (1981, 1984) oxidized glucose with HO• generated by Ti^{+3}/H_2O_2. Using electron spin resonance (ESR) the authors concluded that HO• radical produced

carbon (C•) radicals at all positions in equal ratios, indicating distributed attack by HO• toward all carbon positions in glucose, the established signature of HO• oxidations [57, 58].

Dizdaroglu & Von Sonntag reacted glucose [43] and cellobiose (44) with HO• generated from UV irradiation of N_2O saturated H_2O. By mass spectroscopy, the authors identified several 6-carbon derivatives of glucose including gluconic and glucuronic acids, several hexosuloses, hexodialdose, and. Several dehydro-hexoaldoses, proposing that addition of H• or HO• radicals occurred after abstraction of –H or –OH groups from glucose. The authors determined for both carbohydrates, all carbons were oxidized equally.

In addition to 6-carbon molecules, Von Sonntag and coworkers reported fragmentation of glucose into various aldoses, formaldehyde, formic acid, carbon dioxide, and carbon monoxide, representing different C-C bond cleavages. The authors did not explain the origins of the C-C bond cleavage products.

In summary, the hydroxyl radical (HO•) is a powerful but non-selective oxidant. It can abstract electrons from any molecule or element with exception of fluorine. Hydroxyl radicals will abstract hydrogen atoms (H•) from organic molecules from any accessible C-H, O-H, or N-H (59) bond at rates proportional to accessibility by simple diffusion [43, 46, 57].

3.4 Oxidative behavior of the ferryl-oxo ion

The ferryl-oxo $[(Fe = O)^{+2}]$ ion, a less powerful oxidant than the HO• radical, is created by oxygen abstraction from H_2O_2 (**Figure 2a**). The oxidizing power of the $(Fe = O)^{+2}$ ion is moderately stronger than the strength of C-H bond of an alkane and roughly equivalent to the C-H bond strength of benzene; the $(Fe = O)^{+2}$ ion is reported not to abstract H• from anhydrous acetonitrile ($CH_3-C \equiv N$) [59]. Though weaker than the HO• radical, the $(Fe = O)^{+2}$ ion is a discriminatory oxidant, abstracting H from the weakest X-H bond in a molecule and oxidizing molecules with the weakest X-H bonds in a mix of molecules (**Figure 2b**) [32–35]. In the oxidation of simple alkanes, the oxidation preference is: 3° H > 2° H > 1° H (**Figure 2**).

### 3.4.1 Ferryl-oxo ion oxidation of hydrocarbons (alkanes)

Groves & coworkers demonstrated that oxidation of alkanes by $(Fe = O)^{+2}$ in non-aqueous environments produced alcohols without carbon–carbon fusions. Addition of -OH groups to alkanes was both *regio-* and *stereo-*selective. Using isotopic $H_2^{18}O_2$ / $H_2^{16}O$, Groves and coworkers found that the peroxyl oxygen was incorporated as the hydroxyl oxygen 90% of the time. Groves et al. termed the effect 'oxygen rebound' [32–35].

Groves et al. proposed a two-step mechanism to explain their results (**Figure 2b**):

1. The ferrous ion abstracts oxygen from peroxide forming the ferryl-oxo ion with a coordinate double bond;

2. The ferryl-oxo ion $[(Fe = O)^{+2}]$ abstracts H• from a C-H bond creating a C• radical and ferric hydroxide $[(Fe^{+3}OH)$ or $(Fe-OH)^{+3}]$ (**Figure 2b**);

3. The oxygen of ferric hydroxide ($Fe^{+3}OH$) reacts with the C• radical, producing an alcohol (RC-OH), and regenerating the Fe^{+2} ion.

Unlike HO•, the $[Fe = O]^{+2}$ is both stereo- and regio- selective. For hydrocarbons, the H-C oxidation preference order is: 3° C-H > 2° C-H > 1° C-H. The basis of the rebound effect is likely due to attraction of the electrophile C• radical and the nucleophile oxygen (•O•) of the $(Fe-OH)^{+3}$ ion. The oxygen is added to the same bond position on the oxidized carbon.

[Fenton's original reaction: (Eq. (7)) violates Groves' model because tartaric acid oxidation follows a different pathway:

$$C_4H_6O_4 + FeSO_4 + H_2O_2 + H_2O \rightarrow C_4H_4O_4 + 2H_2O$$
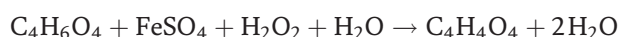
Fenton's First Reaction: Oxidation of Tartaric Acid by $FeSO_4/H_2O_2$ (7)

Erik Hückel's double bond resonance theory states that molecules with 4N + 2 unpaired electrons in conjugated (staggered) double bonds are extraordinarily stable. In the oxidation of tartaric acid, the abstraction of the first H• from C2 is followed by ejection of the second H• from C3 to form a C=C bond, because the central C=C bond is conjugated to the flanking C=O bonds of the terminal carboxylic acids. Thus, Fenton's molecule was resistant to further oxidations, allowing him to discover it].

3.4.2 Ferryl-oxo ion oxidization of alcohols

Ferryl-oxo ion $[(Fe = O)^{+2}]$ oxidation of oxygen-containing organic molecules behaves differently from hydrocarbon oxidations (**Figure 2c** and **d**). Carbon and hydrogen have similar affinity for electrons, therefore the electron pair is shared equally and in a hydrocarbon, hydrogen-carbon all bonds are about equal strengths. Oxygen (O) heteroatoms have a higher affinity for electrons than carbon or hydrogen atoms, making the C-O bond stronger than a C-C bond, while weakening other bonds extending from the hydroxyl carbon significantly [19, 60–62].

As an example, when (HO•) oxidizes ethanol, H• abstraction occurs indifferently from any of the six C-H positions, producing roughly 50% ethylene glycol and 50% acetaldehyde yield. On the other hand, when ethanol is oxidized by $(Fe = O)^{+2}$ ion, the bond strengths of the methyl C-H bonds are ∼96 kCal/mole, whereas the hydroxyl C-H bond strengths are ∼81.6 kCal/mol and the O-H bond strength is ∼104 kCal/mole (60). Because the $(Fe = O)^{+2}$ ion has the higher probability of abstracting H• from a hydroxyl carbon (due to bond strength) or hydroxyl oxygen (due to charge attraction) rather methyl carbons, acetaldehyde will be formed in preference to ethylene glycol [60, 63–66].

3.4.3 Ferryl-oxo ion oxidation of diols, polyols, and carbohydrates

Following the Coon & White (1977) discovery of the Fe^{+3}-heme core in cytochrome P450 and its ability to sever the O-O bond, and oxidize $NADPH_2$ [67, 68], Okamoto et al. (1985) mimicked the ability of enzyme $P450_{scc}$ to split a C-C bond of a diol in 1-, 2-, bis-(4-methoxyphenyl)ethane-l,2-diol using Fe^{+3} ion, O_2, and a reductant (N-benzyl-3-carbamoyl-1,4-dihydropyridine) [69].

Okamoto et al. (1988) found that Fe^{+2} + H_2O_2 could substitute for Fe^{+3} and O_2 to cleave diols to paired aldehydes. Using various inhibitors and/or substituting ferric for ferrous ion, the authors concluded that $(Fe = O)^{+2}$ was created and was the oxidant that cleaved the 1-, 2-, diols (**Figure 2c**). The authors also discovered that when one

hydroxyl group was substituted, paired aldehydes formed, but when both hydroxyl groups were blocked, no aldehydes were produced (**Figure 2d**) [70].

Sugimoto and Sawyer (1985a) reported that Fe^{+2} and two moles of H_2O_2 oxidized alkenes (hydrocarbons with double bonds) to paired aldehydes formed by $(Fe = O)^{+2}$. The authors proposed that Fe^{+2} ion and H_2O_2 caused dioxygen addition to a double bond, forming a dioxetane, that then scissioned to a diol; a second oxidation $(Fe = O)^{+2}$ scissioned the diol to paired aldehydes. The authors saw similar oxidative behavior when CH3-O-O-H and *p*-Cl-Ph-O-O-H were substituted for H_2O_2 [71].

Thus 1-, 2-, diols produce the same products when oxidized by either HO• (56) or $(Fe = O)^{+2}$ [68] oxidants indicating that the formation of paired aldehydes is faster than oxygen addition reactions of alkanes. [Though contemporary, the Sawyer's and Oka's research teams did not appear to be aware of each other, or of Waters (1946)].

The rationale for asymmetric cleavage of diols (**Figure 2c** and **d**) is due to the additive weakening of the C-C bond between the two hydroxyl groups [19, 60, 61, 65]. When H• is abstracted from a hydroxy oxygen of a diol pair, the weakest bond of the oxygen-centered (H-C-O•) carbonyl radical is the C-C bond between the diol pair (•O-R_1CH ~ R_2CH-OH); electron abstraction from the C ~ C bond produces paired aldehydes (**Figure 2c**). However, when H• is abstracted from a C-H bond of a hydroxyl carbon, the weakest bond of the diol group is the O-H bond opposite the C• radical (H ~ O-R_1C•); the hydrogen atom is ejected from carbon-centered (H ~ O-C•) carbonyl radical to form the carbonyl bond. Abstraction of H• from a tertiary -OH group can cause ejection of a C• radical to form the C=O bond (**Figure 2d**) [60–66].

[Fenton's oxidation of tartaric acid should have produced two products: 2-, 3-, dihydroxy-, maleic acid [HOOC-C(OH) = C(OH)-COOH] *and* glyoxalic acid [HO-C(O)-C(O)H. Waters (1946), Okamoto et al. (1988), and Sugimoto et al. (1984, 1985a) indicates that Fenton could have discovered both oxidation products].

3.5 Comparison of ferryl-oxo ion and hydroxyl radical oxidizations

Though HO• radical and $(Fe = O)^{+2}$ ion both create a C• radical, the differences between the two oxidants is: 1) HO• is a 1 e$^-$ oxidant, whereas $(Fe = O)^{+2}$ ion is a 2 e$^-$ oxidant, thus two independent HO• oxidations are required to make a hydroxyl group; and 2) reducing agents that trap HO• radicals and thus halt HO•-based oxidations, do not disrupt ferryl-oxo ion oxidations. The most likely explanation radical quenching by the ferryl-oxo ion is the proximate distance of Fe^{+3}-O-H and C• radical is coupled with likely nucleophile / electrophile attraction, allowing rapid re-reaction to occur [36–38].

The noted crypto-HO• positional effect seen in Fe^{+2}/H_2O_2 catalyzed oxidations is likely due to localized binding of Fe^{+2} ions to a substrate that has O heteroatoms when it added to the substrate prior to H_2O_2 [22, 23, 72] addition.

4. Perferryl-oxo ion

4.1 Early history of the perferryl-oxo ion

Fenton conducted Fe^{+3}/H_2O_2 experiments but did not note any reactions and assumed that no reaction(s) had occurred [5, 7]. However, on the other side of the English Channel, Fenton's contemporaries found contrary evidence.

Spring (1895) mixed H_2O_2 solutions with different pure chemical substances noting which substances caused oxygen gas release. Spring noted that both ferrous and ferric chlorides decomposed H_2O_2 and released oxygen gas [40].

Ruff (1898) used basic ferric acetate and H_2O_2 to oxidize gluconic acid to arabinose and carbon dioxide, a C_1-C_2 bond cleavage with oxidations of both C_1 and C_2, the reaction now known as 'Ruff's degradation' [73].

Bohnson (1921) noted that a solution of a ferric salt in water, dilute enough to show only very slight color, turns brightly lavender with '1 or 2 drops' of 30% H_2O_2 followed by O_2 gas release from the solution. After bubbling ends, no residual H_2O_2 remained in the solution, indicating complete decomposition. The author observed that when H_2O_2 is added to Fe^{+3} salts, a lavender color appears briefly. Bohnson speculated that the color represented a transient higher Fe oxidation state. Bohnson trapped the lavender pigment with cold KOH coloring the solution red, then $Ba(OH)_2$, forming a red gelatinous precipitate. Washing the precipitate with HCl released chlorine gas. Bohnson determined the empirical formula of the precipitate: barium ferrate ($BaFeO_4$), thus isolating the Fe^{+6} oxidation state as FeO_4^{-2} (ferrate) ion. Bohnson also prepared potassium ferrate (K_2FeO_4) by bubbling chlorine gas through a solution of $Fe(OH)_3$/KOH solution, producing a deep lavender color; addition of $Ba(Cl)_2$ to the lavender solution again formed the red precipitate: $BaFeO_4$ [74].

Bohnson (1921) also demonstrated direct conversion of ethanol to acetic acid. Bohnson noted that addition of Fe^{+3} ions to an H_2O_2 solution produced oxygen gas, but addition of ethanol to the H_2O_2 solution prior addition of Fe^{+3} ions disrupted oxygen evolution, leading the author speculated that ethyl alcohol was oxidized to acetaldehyde or acetic acid. Bohnson also compared of oxidation by ethanol by Fe^{+2}/H_2O_2 vs. Fe^{+3}/H_2O_2 and found that Fe^{+2}/H_2O_2 produces acetaldehyde, then acetic acid, whereas Fe^{+3}/H_2O_2 oxidized ethanol to acetic acid primarily, with only trace amounts of acetaldehyde detected. Bohnson proposed that Fe^{+3}/H_2O_2 oxidized ethanol directly to acetic acid, bypassing acetaldehyde formation [74].

Walton & Christensen (1926) compared the oxidation of ethanol with $FeSO_4$/H_2O_2 or $Fe_2(SO_4)_3$/H_2O_2 under anhydrous conditions. Separately assaying for acetaldehyde and acetic acid, the authors noted that when ethanol is oxidized with $FeSO_4$/H_2O_2 acetaldehyde appeared before acetic acid, whereas when ethanol is oxidized by $Fe_2(SO_4)_3$/H_2O_2 acetic acid appears long before acetaldehyde, proving that Fe^{+3}/H_2O_2 oxidation exhibits non-Fenton-like behavior, thus confirming Bohnson (1921) [75].
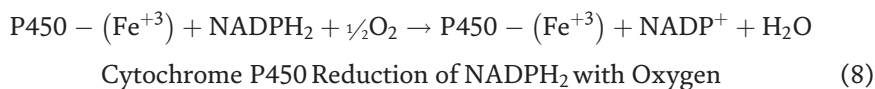
Wieland & Franke (1928) reported that under strong acidic conditions Fe^{+3}/H_2O_2 oxidized formic acid (HCOOH) to CO_2 and H_2O, and dihydroxymaleic acid (HOOC-(OH)C=C(OH)-COOH) to 2,3 dioxo-propanoic acid (HOOC-C(O)-C(O)-COOH) and CO_2 [76].

Goldschmidt & Pauncz (1933) investigated the $Fe_2(SO_4)_3$/H_2O_2/ethanol reaction and confirmed that ethanol was oxidized directly to acetic acid. The authors also explained that Fenton & Jackson (1899) and Fenton & Jones (1900) did not detect aldehydes from aliphatic alcohols because the 1:1 molar ratio of H_2O_2 and alcohol was sufficient to oxidize all the alcohol to organic acids [77].

Even as late as 1989, Fe^{+3}/H_2O_2 oxidation articles appeared noting unusual oxidations. Sanderson et al. (1989) submitted a patent for co-synthesis of *t*-butanol and *t*-butyl peroxide from *t*-butane by Fe^{+3}/H_2O_2, showing addition of either a hydroxyl or a peroxyl group to the 3° carbon without explanation of mechanism [78].

4.2 Oxidative behavior of the perferryl-oxo ion

White & Coon (1977) summarized the discovery of the mechanism of respiration by mitochondrial enzyme cytochrome P450. Cytochrome P450 uses a Fe^{+3} ion chelated in a heme ring to conduct the reduction: (Eq. (8)) [67, 68].
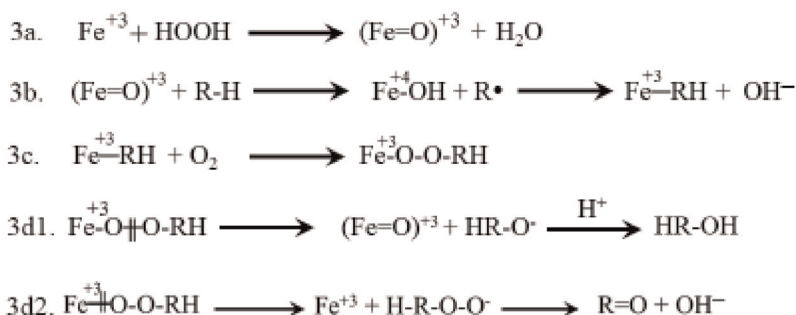
$$P450 - \left(Fe^{+3}\right) + NADPH_2 + \tfrac{1}{2}O_2 \rightarrow P450 - \left(Fe^{+3}\right) + NADP^+ + H_2O$$

Cytochrome P450 Reduction of $NADPH_2$ with Oxygen (8)

Responding to the discovery that the critical enzyme of respiration forms a $Fe^{+3} = O$ intermediate to split the dioxygen molecule, Barton et al. (1983) sought to mimic the biological reaction using chelated Fe^{+3} ions and peroxide ion (O_2^{-2}) instead of oxygen as a new process to oxidize hydrocarbons to alcohols. Working with alkanes (R1-CH_2-R2), Barton expected that pyridine-chelated Fe^{+3} ions and potassium peroxide (K_2O_2) would produce alcohols (**Figure 3**) [54].

What Barton did not expect was that the reaction produced a mix of alcohols [R1-HC(OH)-R2] *and ketones* (R1-(C=O)-R2). Direct oxidation of hydrocarbons to ketones, a single step $4e^-$ oxidation and oxygen addition, was new to organic chemistry. For the oxidation of simple alkanes, the oxidation preference of the Fe^{+3}/H_2O^2 oxidant is: 3° H > 2° H > 1 H°. Barton et al. (1983) also found that they could manipulate the alcohol/ketone ratio by choosing iron chelators with different N/O ratios.

To understand the reaction mechanism, Barton and co-workers studied the oxidation of adamantine ($C_{10}H_{16}$, 4 tertiary, 6 secondary, 0 primary C-H groups). Despite the preponderance of secondary carbons, Barton's reactant favored oxidation of tertiary vs. secondary carbons by a 5:1 margin indicating that the oxidant behaved similar to the ferryl-oxo ion, but single step ketone addition was never reported for $(Fe = O)^{+2}$ oxidations [54].

Sugimoto & Sawyer (1985b) confirmed and extended Barton's findings by using Fe^{+3} and H_2O_2 to oxidize hydrocarbons molecules with double and triple bonds, isolating epoxides (R1-C-^{O}-C-R2) and oxetanes (R1-C-$^{O-O}$-C-R2) [79].

Seven years elapsed until Barton and coworkers resolved the perferryl-oxo structure and oxidation mechanism (**Figure 3**).

3a. $Fe^{+3} + HOOH \longrightarrow (Fe=O)^{+3} + H_2O$

3b. $(Fe=O)^{+3} + R\text{-}H \longrightarrow Fe^{+4}\text{-}OH + R\bullet \longrightarrow Fe^{+3}\text{--}RH + OH^-$

3c. $Fe^{+3}\text{--}RH + O_2 \longrightarrow Fe^{+3}\text{-}O\text{-}O\text{-}RH$

3d1. $Fe^{+3}\text{-}O\text{||}O\text{-}RH \longrightarrow (Fe=O)^{+3} + HR\text{-}O^\cdot \xrightarrow{H^+} HR\text{-}OH$

3d2. $Fe^{+3}\text{||}O\text{-}O\text{-}RH \longrightarrow Fe^{+3} + H\text{-}R\text{-}O\text{-}O^- \longrightarrow R=O + OH^-$

References:
3a. Barton, D.; Gastiger, M.; Motherwell, W. J. Chem. Soc. Chem. Comm. 1983, 41-3. DOI: 10.1039/C39830000041 ; Barton, D.; Csuhai, E.; Doller, D.; Ozbalik, N.; Balavoine, G. PNAS 1990, 87 3401-04. DOI: 10.1073/pnas.87.9.3401.

3b, 3c. Barton, D.; Doller, D. Acc. Chem. Res. 1992, 25, 504-12. DOI: 10.1021/ar00023a004 : Schuchardt, U.; Jannini, M. J.; Richens, D. T.; Guerreiro, M. C.; Spinacé, E.V. 2001, Tetrahedron, 57(14) 2685-8. DOI: 10.1021/ja00032a032 : Barton, D.; Beviere, S.; Chavasiri, W.; Csuhai, E.; Doller, D.; Liu, W. J. Am. Chem. Soc. 1992, 114 2147-56. DOI: 10.1021/ja00032a032 :

3d.1,2. Barton, D.; Doller, D. Acc. Chem. Res. 1992, 25, 504-12. DOI: 10.1021/ar00023a004 : Schuchardt, U.; Jannini, M. J.; Richens, D. T.; Guerreiro, M. C.; Spinacé, E.V. 2001, Tetrahedron, 57(14) 2685-8. DOI: 10.1021/ja00032a032 : Sugimoto, H.; Spencer, L.; Sawyer, D. PNAS 1987, 84 1731-33. DOI: 10.1073/pnas.84.7.1731

Figure 3.
*Perferryl-Oxo ion reactions.*

Couching their model on the accepted behavior of Fe^{+3} nucleus of cytochrome P450 [80–82], Barton et al. (1990) proposed $(Fe = O)^{+3}$ as the reaction product of Fe^{+3} and H_2O_2 or Fe^{+2} and $O_2^{\bullet -}$ (superoxide anion) (**Figure 3a**). [Barton et al. (1990–8) wrote the structure of the perferryl-oxo ion as $[Fe^V=O]$. The $(Fe^V=O^{(-2)})$ and $(Fe = O)^{+3}$ formulas are equivalent for atoms, bonds, and net charge].

Barton et al. (1990): 1) proposed a bifurcated pathway leading either to alcohol or ketone formation, showing that the alcohol/ketone ratio could be varied with addition of dianisyl telluride, and 2) determined that both alcohol and ketone formation occurred in two steps, choosing different non-reversible paths at the second reaction [83, 84].

Barton and Doller (1992) mapped out steps of the pathway of perferryl-oxo ion oxidation of hydrocarbons (**Figure 3b–d**):

Step 1 (**Figure 3b**): Formation of Fe^{+4}-C-R intermediate. Using diphenyl-diselenide (Ph-Se-Se-Ph), or phenyl selenol (Ph-Se-H), Barton trapped the Fe^{+4}-C-R intermediate as a stable (Fe^{+3}-Ph-Se-C-R) intermediate as detected by mass spectroscopy (structure not specified).

Step 2 (**Figure 3c**): Oxygen Insertion to form Fe^{+3}-O-O-C-R intermediate. Comparing $^{16}O_2$ and $^{18}H_2O_2$, the authors detected primarily ^{16}O-labeled alcohols and ketones indicating that O_2 (not O_2^{-2}) formed the dioxygen bridge. The authors proposed that in an anoxic environment, peroxide is oxidized to dioxygen by ferric ions in sufficient quantities to complete the reaction as follows: [Eq. (9)]

$$2\,Fe^{+3} + O_2^{-2} \rightarrow 2\,Fe^{+2} + O_2$$

Reduction of H_2O_2 to O_2 by Ferric Ions   (9)
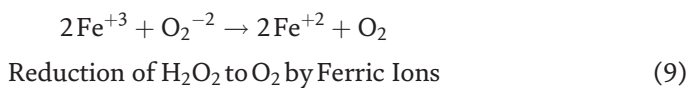
Step 3 (**Figure 3d** (1 & 2)): Bifurcated Pathways Arise from Differential Cleavage of the O-O Bridge. The Fe^{+3}-O-O-C-R intermediate is the branch point between the 2e- and 4e- oxidative pathways: a) scission of the Fe^{+3}-O-|-O-R bond produces an alkoxide (R-O$^-$) and the $(Fe = O)^{+3}$ ion (**Figure 3d**.1); b) scission of the Fe^{+3}-|-O-O-R bond produces Fe^{+3} ion and a peroxyl ($^-$O-O-R) ion which then degrades to a ketone (R = O), and an oxide ion (O^{-2}) (**Figure 3d**.2).

Barton and Doller (1992) trapped the ferric-peroxy-carbon (Fe^{+3}-O-O-C-R) cleavage intermediates with tri-methoxy phosphine (P(OMe)$_3$). P(OMe)$_3$ reacted with either oxygen (R-C-O*-O-Fe^{+3} or R-C-O-O*-Fe^{+3}) trapping potential oxygen bridge cleavage products as R-C-O-P(OMe)$_3$ and R-C-O-O-P(OMe)$_3$ respectively. Thus Barton and Doller (1992) explained the mechanism of bifurcated production of alcohols or ketones from alkanes by perferryl-oxo $(Fe = O)^{+3}$ ion (85). Barton's oxidation scheme was confirmed by Schuchardt et al. (2001) [55].

Barton's perferryl-oxo ion oxidation theory explains Ruff's oxidation gluconic acid to arabinose (1898) [71] the one-step conversion of ethanol to acetic acid observed by Bohnson (1921) [72], Walton & Christensen (1926) [75], Goldschmidt & Pauncz (1933) [75], and the co-synthesis of *t*-butanol and *t*-butyl peroxide from *t*-butane by Sanderson (1989) [76].

4.3 Comparison of $(Fe = O)^{+2}$ and $(Fe = O)^{+3}$ ion chemistry

Both $(Fe = O)^{+2}$ (**Figure 2**) and $(Fe = O)^{+3}$ ions (**Figure 3**) abstract H• from the weakest C-H bond present in a molecule to form ferric ($Fe^{+3}OH$) or ferryl hydroxide ($Fe^{+4}OH$) and a C• radical respectively [85].

The electrophilic ferric and ferryl hydroxides react 'instantaneously' with the nucleophilic C• radical, but the resulting intermediates are different. Ferric hydroxide donates HO• to the C• radical, regenerating the ferrous ion, ending the cycle [33], however the ferryl atom attacks the C• radical (ejecting the hydroxyl group) to form the ferryl-carbon (Fe^{+4}-C) intermediate [83]. Oxygen (O_2) insertion into the (Fe^{+4}-C) bond creates the bifurcated oxidative pathways not available to either ferryl-oxo ion or hydroxyl radical [86].

Sugimoto et al. (1987), using ^2H and ^{18}O labeled ethanediols, determined that H• abstraction by $(Fe = O)^{+3}$ from the hydroxyl oxygen of a diol group [R_1-HC(O*H*)-HC(OH)-R_2] causes C-C bond cleavage, producing paired aldehydes [R_1-HC=O + R_2-HC=O], whereas H• abstraction from the carbon backbone produces hydroxy-ketones [R_1-C(O)-HC(OH)-R_2] [87].

5. Mixed Fenton oxidation systems

5.1 Untangling oxidation behaviors arising when Fe^{+2} ions, H_2O_2, and H_2O are present

The Fenton reaction (Fe^{+2} + H_2O_2 + H_2O) has been shown to generate three powerful oxidants: 1) (HO•) radical [16, 17]; 2) $(Fe = O)^{+2}$ ion [18]; and 3) $[Fe = O]^{+3}$ ion [86, 88].

Sugimoto & Sawyer (1985a & 1985b) proposed that both ferrous and ferric ions can abstract an •O• atom from H_2O_2, thus explaining how ferrous and ferric spontaneously reorganize to form the secondary oxidants ferryl $(Fe = O)^{+2}$ and perferryl $(Fe = O)^{+3}$ ions, respectively.

Sugimoto and Sawyer (1984) and (1985b) compared $(Fe = O)^{+2}$ and $(Fe = O)^{+3}$ oxidations, respectively, in anhydrous CH_3CN or 90% CH_3CN/10% H_2O with several organic and inorganic molecules. In anhydrous CH_3CN, ferryl-oxo ions oxidation produced only 2-electron oxidations, primarily dehydrations or hydroxyl additions, while perferryl-oxo ions produced both 2-, and 4- electron oxidations. Neither oxidant produced 1- electron oxidations.

In aqueous acetonitrile (CH_3CN/H_2O), single electron oxidations, characteristic of HO• were observed including: 1) carbon–carbon fusions; 2) oxidation of Fe^{+2} ions; and 3) reduction of Fe^{+3} ions to Fe^{+2} ions. The authors proposed that HO• radicals are created by ferryl-oxo and perferryl-oxo ions only when water is present, implying that H• abstraction from water produces HO• radicals via the formula: $(Fe = O)^{+2,+3}$ + H_2O• HO• + $Fe^{(+3,+4)}$OH [59, 61].

Sawyer et al. (1993) tested the oxidizing capability of Fe^{+2} ions and organic peroxides (R-O-O-H) 1) under anhydrous conditions in the presence vs. absence of O_2, and 2) under anoxic conditions with anhydrous (Fe^{+2}) or partially hydrated ($Fe^{+2}(H_2O)_2$) conditions. The authors found evidence of $1e^-$ oxidations either when O_2 or H_2O_2 were present, indicating 1) that $(Fe = O)^{+2}$ reacted with H_2O to form HO• radicals, or 2) with O_2, creating O_2• [16], which then reacted with (R-O-O-H) to generate HO• radicals [39]. On the other hand, Hage et al. (1995) found that in the conversion of benzene to phenol, if a small amount of H_2O was added, the efficiency of conversion was increased, but other $1e^-$ signature products were not detected [89].

Sawyer et al. (1996) surveyed the oxidizing abilities of $Fe^{+2,+3}$, Cu^{+2}, Co^{+2}, and Mn^{+2} ions in anhydrous solvents with ROOH, with/out O_2. Under an argon atmosphere, only the hydroxyl radical sources produced chain fusion events, none of the

ions did. When air (20% O_2) was substituted, all of the ions showed oxidation patterns consistent with HO• radicals. The authors concluded that the metal ions, activated by peroxide, reacted with solubilized O_2, producing superoxide ($O_2^{\bullet-}$ or $HO_2^{\bullet}$), which in turn reacted with H_2O_2 to generate reactive singlet oxygen (•O•) which then reacts with R-C-H to produce HO• radicals [41].

Barton et al. (1995, 1996) seconded the research of Sawyer's group, confirming that absent H_2O, ferryl-oxo and perferryl-oxo ions perform distinct and distinctive 2- (and 4-) electron oxidations without mixing the unique chemistries of either ion [86, 90].

Mwebi (2005) also confirmed that when Fe^{+2} ions, H_2O_2, and H_2O are reacted in aqueous conditions, all three secondary oxidants [$(Fe = O)^{+2}$, $(Fe = O)^{+3}$, and HO•] arise in that either $(Fe = O)^{+2}$ and $(Fe = O)^{+3}$ ions can abstract H• from H_2O to create the HO• radical, the HO• radical can oxidize Fe^{+2} ions to Fe^{+3} ions, and H_2O_2 can reduce Fe^{+3} ions to Fe^{+2} ions [51].

5.2 Biological occurrence and utilization of the Fenton reagent

Oceans covered Earth 4.4 billion years ago [91], evidence of bacteria dates back 3.5 billion years ago (92), and evidence of oxygenic photosynthesis 2.3 billion years ago [91, 92]. From at least that time living organisms have evolved to defend against, and/ or, utilize Fenton chemistry.

The use of the Fenton reagents to kill organisms or degrade biopolymers is widely distributed in the biosphere. Saprophytic fungi use Fenton reagents to degrade polysaccharides of woody plant tissues [93], including cellulose [93–96], callose [97], and hemicelluloses [98].

On the other side of the eukaryote kingdom, mammalian leukocytes and neutrophils pump Fe^{+2} ions [99, 100] and H_2O_2 into phagosomes to produce oxygen radicals [101] to effect pathogen killing [102–107]. For both nutrient mobilization and pathogen killing, these oxidants target external glycan including cell walls to cause cell lysis and/or internal glycans such DNA and RNA to facilitate death of bacteria and eukaryote parasites.

Moore and coworkers incubated *Saccharomyces cerevisiae* cells with an Fe^{+2}-chelating anti-cancer medication. Treated and control cells were stained, fixed, and thin-sectioned for electron microscopy. While studying chromosome damage the authors observed damage to the yeast cell walls by the anti-cancer drug [108–110].

Following Moore's lead, Lipke and coworkers treated [35]S -labeled *S. cerevisiae* cells with the an Fe^{+2}-binding anti-cancer medication, then compared protein levels release into growth media from treated and control cells [111], and cell lysis rates of treated and control cells after adding *Arthrobacter luteus* (Zymolyase) protease [112].

In Lim et al. (1995), the authors noted that pretreatment with yeast cells with an Fe^{+2}-binding anti-cancer agent increased cell lysis rates by Zymolyase protease with: 1) Fe^{+2} + O_2 or Fe^{+3} + O_2, but not Ca^{+2}, Co^{+2}, Cu^{+2}, Mn^{+2}, Mg^{+2}, and Zn^{+2} ions; 2) H_2O_2 could substitute for O_2; and 3) Fe^{+2}/H_2O_2 and Fe^{+3}/H_2O_2 alone also accelerated yeast cell lysis; 4) H_2O_2 only controls did not accelerate Zymolyase lysis rates [112].

To understand the basis of cell wall weakening by Fe^{+2}/H_2O_2, Ovalle et al. (2001) elected to separately test pure analogs of carbohydrates and proteins found in yeast wall [113]. Ovalle et al. (2001) assumed that partial oxidation of fungal wall monosaccharides would oxidize hydroxyl groups to aldehydes and/or carboxylic acids and developed a method for separating carbohydrates from 0 to 20 glucan units on polyacrylamide gels. Surveying the available literature of the time, the authors followed the method of Ahrgren et al. (1975) where dextran was preincubated with $FeSO_4$ prior to addition of H_2O_2 [114].

Ovalle et al. (2001) [113] labeled the aldehyde groups of glucose, maltose, maltotriose and enzymatically digested laminaran with 8- amino, 1-, 3-, 6-, naphthalene trisulfonate (ANTS) and NaCNBH3, by the method of Klock & Starr (1998) [115], to have glucan ladders to estimate degree of polymerization of carbohydrate chains separated by polyacrylamide gel electrophoresis. Ovalle et al. (2001) modified the method Klock & Starr (1998) to visualize carboxylic acids and by quenching aldehydes with NaBH4, then crosslinking ANTS to carboxylic acids with N-hydroxysuccinamide (NHS) and N-ethyl-N-(3-aminopropyl) carbodiimide (EDC) [116]. Ovalle et al. (2001) separately visualized de novo aldehydes and de novo carboxylic acids (after quenching aldehydes with $NaBH_4$) on 10% stacking/ 20% running acrylamide gels.

Ovalle et al. (2020) [117] used the method of Ovalle et al. (2001) to determine if Fe^{+2}/H_2O_2 would oxidize algal laminaran (d.p. ≈ 50–60 glucose units; 97–99% β1–3 glu / 1–3% β1–6 glu). To optimize metal ion-carbohydrate interactions, $FeSO_4$ was incubated with carbohydrate for 1 min prior to addition of H_2O_2. The final ratio (glucose monomer: Fe^{+2}: H_2O_2 = 10:1:1) was chosen to oxidize 10% of glucose monomers and reduce the likelihood of a secondary oxidation of glucose fragments to 1% maximum. Unoxidized laminaran did not enter that stacking gel. $NaIO4^-$ oxidized laminaran entered the stacking gel but stopped at the stacking gel/running gel interface. Fenton-oxidized laminaran produced smears, when labeled for either aldehyde or carboxylate groups. Enzyme- (Zymolyase) digested laminaran were used as glucan ladders when labeled for aldehydes or organic acids.

To label glucan fragments so as to be suitable for positive ion mass spectroscopy [118, 119], Ovalle et al. (2020) substituted tert-butyl ester of tyrosine (TBT) for ANTS with no other changes required. Ovalle et al. (2020) observed the elution of TBT-labeled glucan fragments with masses consistent with six classes of TBT-labeled molecules: aldoses, dialdoses, uronic acids, hexosuloses, aldonic acids (unlabeled), and hexulosonic acids (unlabeled) (**Figure 4**).
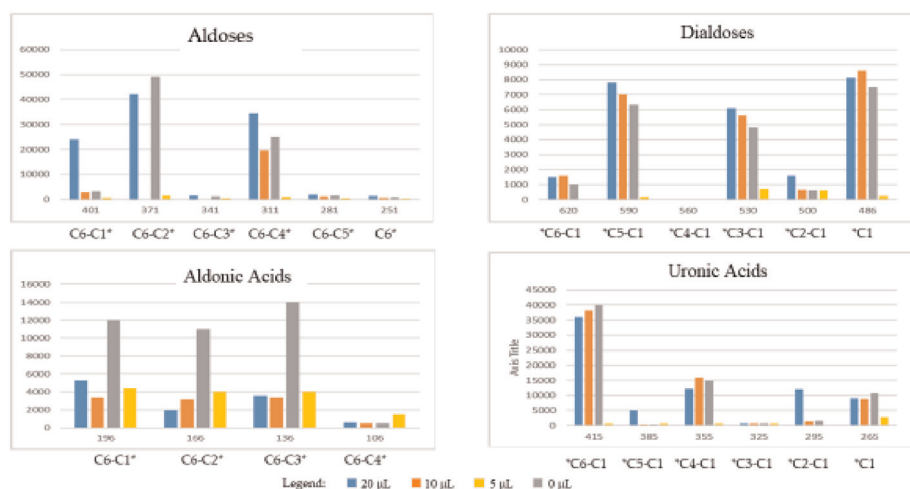
Method: In 100 mL of 1 mM NaH₂PO₄, 3.24 mg Laminaran, 0-20 mL of 100 mM Fe(NH₄)₂(SO₄)₂, & 0-20 mL of 100 mM H₂O₂. Order of addition as listed. Incubation with Fe⁺² was 1 min before addition of H₂O₂. *Position of C in glucose where fragment oxidation occurred. Samples assayed by positive ion HPLC/MS as per Ovalle et al. (2001) and (2020). Aldehydes labeled with TBT, carboxylic acids unlabeled. Net mass shown without carrier ion (H⁺, NH₄⁺, or Na⁺).

Figure 4.
Comparison of particles of four molecule classes from Laminaran after Fenton oxidation.

6. The authors concluded

1. Aldose / dialdose pairs arose from glucose by H• abstraction from an unsubstituted hydroxyl groups at O2, O4, or O6, and were mediated by $[Fe = O]^{+2}$ ion after Fe^{+2} ion was bound to a site where it was activated by H_2O_2. Diol-splitting reactions are consistent with oxidation by both HO• and $(Fe = O)^{+2}$ oxidants, however the ratios of the aldose / dialdose pairs were uneven, implying bias oxidations, hallmark of the $(Fe = O)^{+2}$ ion.

2. Uronic and aldonic acids were produced by ketone addition to a hydroxyl carbon (except at C1). The reaction is consistent with oxidation by Barton's perferryl-oxo ion.

3. As ferric ions were not added to the assay, Fe^{+2} ions must have been oxidized by HO• radicals.

4. Though present, the biased distribution of fragments excludes HO• radicals as the primary oxidant, HO• radicals are partially credited for non-zero values of infrequent carbohydrate fragments. Thus, Ovalle et al. (2020) observed all three Fenton oxidants directly or indirectly in the aqueous Fe^{+2}/H_2O_2 oxidation of laminaran.

6.1 Current applications of the Fenton oxidants

The Fenton Oxidants (HO•, $Fe = O^{+2}$, and $Fe = O^{+3}$) are being investigated as molecular scissors for insertion of reactive functional groups into otherwise inert substrates, such as carbohydrates. Oxidation of hydroxyl groups to carbonyl or carboxylic acid groups will allow them to act as carriers for various molecules with ramification in many sectors.

6.1.1 Hydroxyl radical oxidation of carbohydrates

Neyra et al. (2014, 2015) used a catalytic amount Fe^{+2} ions to produce HO• radicals from H_2O_2 to oxidize hydroxyl groups of acetylneuraminic acid monomers (2014) and tetramers (2015) to carbonyl and/or carboxylic groups. The goal of the experiment was to modify the sugars to create anchors for proteins so as to create vaccine adjuvant platforms [120, 121].

6.1.2 Perferryl-oxo oxidations of carbohydrates

Sorokin et al. (2004), using 'heme'-chelated Fe^{+3} ions, oxidized glucose monomers in starch fibers at C2 and C3 to produce acid / aldehyde pairs without hydrolyzing the flanking glycosidic bridges. The dual oxidations allow for two independent modifications of the glucose monomers in the starch chain [122–124].

6.1.3 Ozone-Fenton systems

Ozone (O_3) is being considered as an alternative to H_2O_2. Ozone gas can be activated by UV ($O_3{\rightarrow}O_2$ + •O•) to produce oxygen radicals, or by reaction with iron

ions (Fe^{+2}, $Fe^{+3} + O^3 \rightarrow Fe = O^{+2}$, $Fe = O^{3+} + O_2$, thus producing each Fenton oxidant without water as a byproduct.

Pestovsky (2004, 2005, 2006) reacted Fe^{+2} ion with O_3 in aqueous buffer as an alternative method of creating $(Fe = O)^{+2}$ ion. The signature of HO• radicals: 1 e$^-$ oxidations, were not detected for the oxidation of several classes of organic molecules [125–127].

Bataineh (2015a), and Bataineh et al. (2012, 2015b) compared the oxidation of DMSO with Fe^{+2} and O_3 in aqueous phosphate vs. acetonitrile solvents. In acetonitrile the primary product was $DMSO_2$, an oxygen addition reaction. In buffered H_2O, ethane and methylsulfinate were the primary products, indicating fragmentation of DMSO occurred by HO• oxidation [128–130].

Enami et al. (2014) fired microjets of aqueous $FeCl_2$ into sprays of either O_2 or O_3/ O_2 mixed gases. Particles detected by negative ion MS proved that Fe^{+2} and O_3 produces new particles not seen in $FeCl_2$ or $FeCl_2/O_2$ sprays [131].

6.1.4 Fenton systems for bioremediation

Fenton oxidants are gaining popularity as agents of bioremediation because of their ability to mineralize toxic organic molecules without contamination by ecologically damaging elements (halogens, heavy metal ions, etc.).

6.1.4.1 Ozone (O_3) for bioremediation with (HO•) radical or $(Fe = O)^{+3}$ ion

Turan-Ertas & Gurol (2002) compared ozone (O_3) against Fe^{+3}/H_2O_2 in the degradation of diethylene glycol [$(HO-CH_2-CH_2)O$], a toxic byproduct of the synthesis of ethylene glycol. The authors compared the diethylene glycol oxidation profile by O_3 and Fe^{+3}/H_2O_2. Both procedures were effective in degrading diethylene glycol, however the Fe^{+3}/H_2O_2 oxidation produced fewer and simpler products [132].

7. Fenton chemistry for beginners

1. In 1894, John HJH Fenton published his discovery that $FeSO_4$ and H_2O_2 produced oxidations not mimicked by other methods known at the time.

2. In 1932 & 1934, Fritz Haber & Joseph Weiss proposed the existence of HO• (hydroxyl radical) and $HO_2•^-$ (superoxide anion) as the principal oxidants of Fenton's reaction. Merz and Waters (1947) were among the first to propose that HO• radical oxidizes organic molecules by H• abstraction.

3. In 1932, William Bray & H. M. Gorin proposed $(Fe = O)^{+2}$ (ferryl-oxo ion) as the principal oxidant of the Fenton's reaction. Groves and coworkers proposed (in anhydrous solvents) oxygen rebound phenomena, to explain abstraction of H-, followed by addition of HO- to the same carbon to create of alcohols from alkanes in a single step. The debate raged for decades until umpired by D. T. Sawyer and coworkers.

4. Though 4e$^-$ oxidations by Fe^{+3}/H_2O_2 were observed by Ruff in 1898, and thereafter for nearly 100 years, Derek H. R. Barton & coworkers proposed the

structure of the oxidant as $(Fe = O)^{+3}$ (perferryl-oxo ion) in 1990, and the bifurcated oxygen addition mechanism in 1992.

5. Donald T. Sawyer & coworkers investigated the behaviors of several transition metal ions in aqueous and anhydrous systems. Sawyer and coworkers proved: 1) H_2O is not a spectator molecule; in the absence of water, Fe^{+2} and Fe^{+3} ions do not produce HO• radicals, thus explaining why Groves saw only $2e^-$ oxidations in anhydrous media, while Rush and others observed $1e^-$ oxidations in aqueous systems; 2) in aqueous system HO• radicals can oxidize ferrous ions to ferric ions; and 3) $HO_2•^-$ radicals can reduce ferric ions to ferrous ions, thus in water all three oxidants are present.

6. Though each oxidant has a singular profile seen in the oxidation of hydrocarbons, different oxidative behaviors are seen with organic molecules containing oxygen. Oxygen causes $(Fe = O)^{+2}$ and $(Fe = O)^{+3}$ ions to target hydrogens that are bonded to hydroxyl carbons and hydroxyl oxygens. Abstraction of H• from an O-H bond in molecules with adjacent hydroxyl groups causes C-C cleavage of diols for all three oxidants.

7. Because of competition between the oxidants for targets, the order of addition of reagents alters the outcome of the assay. Fenton's method was to begin with substrate, add H_2O_2, and then $FeSO_4$. In this sequence, when Fe^{+2} (or Fe^{+3}) ions are activated by peroxide, ferryl-oxo (or perferryl-oxo) ions will react with adjacent H_2O molecules, producing HO• radicals, that then diffuse to the substrate, oxidizing H-X bonds by accessibility.

   Addition of Fe^{+2} (or Fe^{+3}) ions first allows the metal ions to associate with and/or chelate onto the substrate. Addition of H_2O_2 now creates the ferryl-oxo (or perferryl-oxo) ions adjacent to the substrate, increasing the likelihood of in situ oxidation at the ion's binding site, creating uneven product profiles, as observed in Ovalle et al. (2020). Allowing binding of metal ions to substrates before addition of H_2O_2 can explain observations of non-canonical "crypto-hydroxyl-" substrate oxidations previously observed by many authors.

8. This summary is not an exhaustive history, nor is it the full collection of all the articles I read. However, it took me many years to both acquire and understand the chemistry of each oxidant. I did not address other metal/peroxide systems (such as copper-Fenton chemistry) here as it was not relevant to either Ovalle et al. (2001) nor (2020).

   This article is written as a guide for newcomers so that they have a head start in finding the papers they need for their research. Welcome to the club!

8. Conclusions

John H. J. H. Fenton did not know that his discovery would enthrall a legion of researchers, be championed by two Noble laureates, and create three separate fields of peroxide oxidation chemistry: hydroxyl radicals (HO•), ferryl-oxo ions $(Fe = O)^{+2}$ and perferryl-oxo ions $(Fe = O)^{+3}$.

Fenton's successors required a full century to explain the ramification of these reactants. These three simple molecules continue to generate novel research investigations in chemistry, physics, and biology. I am proud to be among Fenton's successors.

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Conflict of interest

I declare I have no financial or other interests, aside from the telling of the history of, and unusual chemistry of the Fenton reaction and its investigators.

Author details

Rafael Ovalle
Graduate School of CUNY, New York City, N.Y., USA

*Address all correspondence to: ovalle.rafael1@gmail.com

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