Journal of Molecular Catalysis A: Chemical 352 (2012) 1–20

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Catalysis A: Chemical

journal homepage: www.elsevier.com/locate/molcata

Review

Mechanisms of radical generation in the removal of phenol derivatives and pigments using different Fe-based catalytic systems

I. Magario[a,b], F.S. García Einschlag[c], E.H. Rueda[d], J. Zygadlo[b], M.L. Ferreira[e,*]

[a] Investigación y Desarrollo en Tecnología Química (IDTQ-Grupo Vinculado a PLAPIQUI), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Velez Sarsfield 1611, Ciudad Universitaria, X5016GCA Córdoba, Argentina
[b] Instituto Multidisciplinario de Biología Vegetal (IMBIV-UNC-CONICET), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611, Ciudad Universitaria, X5016GCA Córdoba, Argentina
[c] Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA-UNLP-CCT La Plata-CONICET), Diag. 113 y 64, Sucursal 4, C.C. 16, (B1900ZAA), La Plata, Argentina
[d] Departamento de Química (INQUISUR), Universidad Nacional del Sur, Av. Alem 1253, B8000CPB Bahía Blanca, Argentina
[e] Planta Piloto de Ingeniería Química (PLAPIQUI-UNS-CONICET), Camino a la Carrindanga km 7, B8000CPB Bahía Blanca, Argentina

ARTICLE INFO

Article history:
Received 8 July 2011
Received in revised form
13 September 2011
Accepted 1 October 2011
Available online 8 October 2011

Keywords:
Horseradish
Hematin
Biomimetics
Dyes
Lignin

ABSTRACT

Phenolic compounds removal is a very active research field due to occurrence and the toxicity of phenolic pollutants in industrial wastewaters. In order to make an a priori selection of the most efficient removal process for a target structure this contribution reviews and compares some of the mechanistic aspects of the oxidation in the presence of hydrogen peroxide and catalyzed by complexed iron which is the in-common element in Fenton systems, plant peroxidases and biomimetics. Different substrates were considered from the most basic phenol molecule to complex structures such as phenolic dyes and lignins. The reactivity of iron is related to its microenvironment generated by ligands and their electron withdrawing capacity thus conditioning the type of cleavage induced on hydrogen peroxide and the oxidation state change on iron upon reaction. The relative concentrations of organic to inorganic free radicals generated control the main catalytic action; i.e. from degradation up to mineralization in Fenton systems or oligomerization up to polymerization in plant peroxidases systems. Moreover, some reaction conditions as the peroxide concentration, the initial molar ratio of organic compounds to peroxide and the type of reaction solvent are identified as key factors to promote a desired action mechanism by peroxidases (and their biomimetics).

Contents

* Corresponding author at: Camino La Carrindanga Km 7, CC 717, 8000 Bahía Blanca, Prov. Buenos Aires, R. Argentina. Tel.: +54 291 486 1700x261; fax: +54 291 486 1600.
E-mail address: mlferreira@plapiqui.edu.ar (M.L. Ferreira).

1381-1169/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcata.2011.10.006

1. Introduction

Currently available methods for the removal of phenol/phenolic compounds from wastewaters (chemical oxidation, reverse osmosis, adsorption and others) are expensive, have regeneration problems and may produce themselves wastewaters with a high environmental impact [1,2]. Particularly contaminating wastewaters are those generated by textile and paper mill industries. These wastewaters include medium to low concentrations of dyes or pigments. The degradation of dyes is one of the most important research fields in wastewater treatments. Researchers have recently focused on enzymatic treatments. Many peroxidases such as lignin peroxidase, manganese peroxidase, soybean peroxidase, horseradish peroxidase (HRP), laccase, polyphenol oxidases, microperoxidases and azoperoxidases have been used for the removal of dyes in industrial effluents [3–6]. However, the enzymatic treatment has several drawbacks related to: (a) the need of enzyme immobilization for reuse; (b) the requirement of relatively low temperatures and narrow pH ranges of operation to guarantee high enzyme activity; (c) the deactivation of the enzyme through its immobilization and (d) the use of H_2O_2 as oxidant and the resulting enzyme inactivation. These issues have generated an active research field that comprises biomimetic catalysts and Fenton or Fenton-like catalysis in homogeneous systems (to study basic features), but mainly in supported or immobilized systems (if the goal is the application) [1].

Fenton and related reactions include reactions of peroxides (mainly H_2O_2) with iron ions to form active oxygen species. These active species can oxidize both organic and inorganic compounds. There are several reviews on Fenton mechanisms and applications of Fenton systems, including photo-assisted Fenton reaction, use of chelated iron, electro-Fenton reactions, and Fenton reactions using heterogeneous catalysts [7–11]. One of the main application fields of Fenton and related reactions is wastewater treatment. In particular, the removal of dyes and pigments from wastewater is an important sub-field that includes effluents of textile, pulp and paper mill industries. The extremely complicated chemistry of Fenton systems is nowadays understood in considerable detail [11]. Comparisons of Fenton or photo-assisted Fenton systems with other advanced oxidation processes (AOPs) are favorable to Fenton systems [12,13]. Fenton chemistry is being used to degrade contaminants in soil and groundwater. Research on heterogeneous reactions and the preparation of supported iron catalysts continues in an effort to understand and facilitate the reactions in soils, and to circumvent the problem of iron oxide sludge generation and disposal inherent to the homogeneous Fenton treatment of wastewaters [1].

Peroxidases are enzymes that contain a heme group, which is a porphyrinic ring with iron in the oxidation state +3. In this sense, peroxidases are a particular form of a chelated iron, where one of the ligands is a protein and the other ligands are nitrogen atoms of the porphyrinic ring. The similarity with compounds such as hemo or hematin has inspired the biomimetic approach. Biomimetics are compounds that maintain some structural characteristics of peroxidase cofactor without the complexity of the enzyme related to the protein. These biomimetics, even though they are much cheaper than enzymes, are in general less active.

One of the most studied substrates is phenol, which is frequently used as a simple model compound of more complex pollutants such as dyes, pigments and others. Among the most toxic phenolic compounds are the chloro- or nitro-substituted phenols. These compounds are used as pesticides and anti-bacterials [14].

Phenol is present in wastewaters discharged by resin manufacturing, petrochemical, oil-refining, paper mill, coking, and iron-smelting industries [15]. Phenol derivatives include anthraquinone dyes, an important group of dyes. Phenolic groups, besides being part of many dyes and pigments, are also the main moiety of lignin. Nowadays, high amounts of ligno-cellulosic wastes from paper and wood industries are generated, of which only 1–2% are reused. Therefore, their accumulation represents a serious environmental problem. Moreover, high-valuable products potentially obtainable from lignin degradation are misspent [16]. The enzymatic complex (Li-peroxidase, Mn-peroxidase and laccase) produced by white-rot fungi is able to degrade lignin up to mineralization. Hence, the application of well-known, commercially available and robust enzymes such as HRP is an attractive approach for lignin degradation. Recent studies on totally chlorine-free processes for pulping and bleaching involve the use of oxygen, ozone or hydrogen peroxide as oxidants, and enzymes or biomimetics as catalysts [17].

There are three main research fields in the heterogeneous catalytic degradation of phenols: the catalytic wet-peroxide oxidation [18], the catalytic ozonation [19] and the catalytic wet oxidation [20]. The catalysts used in wet-peroxide oxidation include metal-exchanged zeolites, hydrotalcite-like compounds, metal-exchanged clays and resins. The catalysts used in catalytic wet oxidation are transition metal oxides and supported noble metals [21].

The present work compares the similarities and differences among three types of catalytic systems: Fenton, HRP and biomimetics both in homogeneous and heterogeneous systems. Phenolic substrates and polyphenolic derivatives, such as lignin, were considered as model substrates. Several aspects and controversies related to reaction mechanisms will be analyzed in the following sections. Fig. 1 summarizes the kind of systems evaluated for phenol (and phenol derivatives) removal in this work.

2. Reaction mechanisms

2.1. Homogeneous Fenton systems

2.1.1. Definition

Fenton reaction involves the reduction of hydrogen peroxide by ferrous ions (+2 oxidation state), whereas Fenton-like processes involve the decomposition of hydrogen peroxide catalyzed by ferric ions (+3 oxidation state) or iron in reduced state (zero oxidation state).

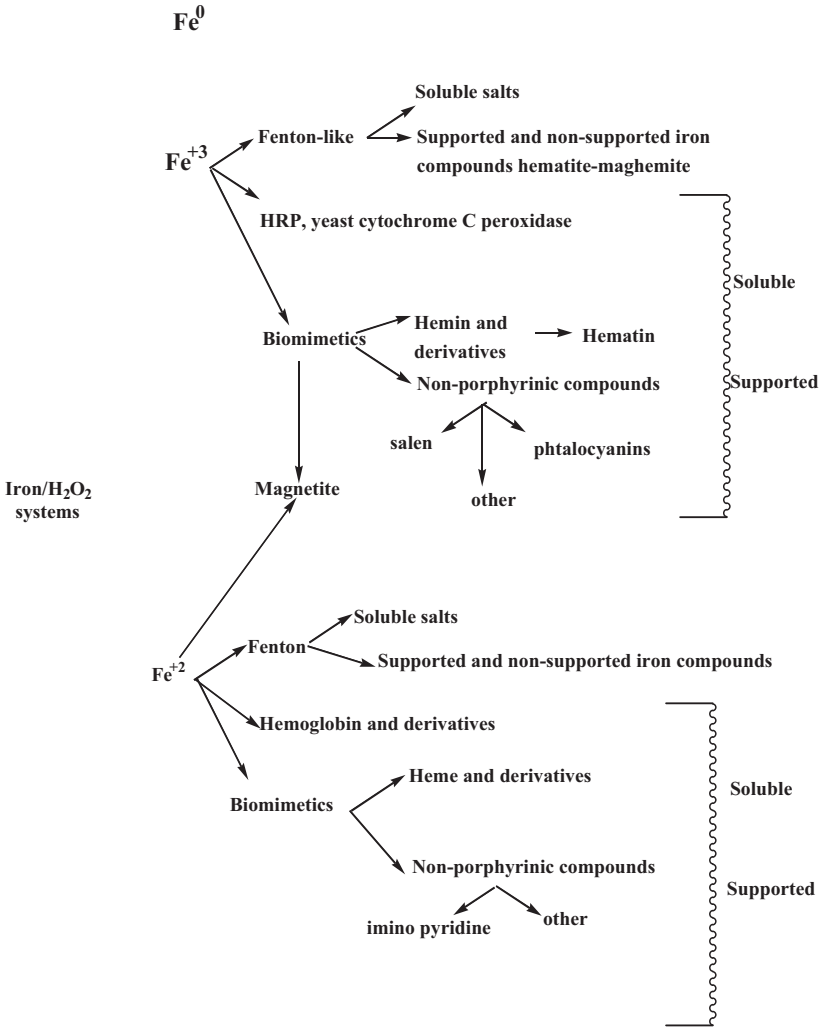


Fig. 1. Iron initial oxidation status, compounds and iron ligands presented in this review.

2.1.2. Iron chemistry

Iron ions exist in aqueous solution as hexa-coordinated complexes (see Fig. 2). In the case of Fe^{2+}, the main species to consider are $Fe(H_2O)_6^{2+}$, $Fe(H_2O)_5(OH)^+$ and $Fe(H_2O)_4(OH)_2$, abbreviated as Fe^{2+}, $FeOH^+$, and FeO, respectively. In the absence of strong ligands, the most important ferric species below pH 3.5 are $Fe(H_2O)_6^{3+}$, $Fe(HO)(H_2O)_5^{2+}$ and $Fe(HO)_2(H_2O)_4^+$, for simplicity they are summarized as Fe^{3+}. As pH increases, polymeric Fe^{3+} species, which finally lead to the formation of precipitates, are produced. The precipitates usually show the structure of the insoluble ferric oxyhydroxide [22]. Fe^{3+} hydrolyzed species are evidenced by turbidity and/or a slight yellow-orange color. By increasing pH, species such as $Fe_2(OH)_4^{2+}$ increase their concentration. On the other hand, below pH 3.5 and in the presence of hydrogen peroxide, Fe^{3+} and $FeOH^{2+}$ form complexes with H_2O_2 (see Fig. 2):

$$Fe^{3+} + H_2O_2 \leftrightarrow Fe(HO_2)^{2+} + H^+ \quad (1)$$

$$FeOH^{2+} + H_2O_2 \leftrightarrow Fe(OH)(HO_2)^+ + H^+ \quad (2)$$

$Fe(OH)_3$ precipitates at pH 4.5 and $Fe(OH)_2$ at pH 9.5. This aspect is very important as Fe^{2+} species/ions diffuse on the surface of Fe^{3+} species and could be oxidized by adsorbed oxidants [23].

The role of zero valent iron as remediation agent is being increasingly studied [24] and Fenton reactions are progressively discussed in Fe^0/H_2O_2 systems [25].

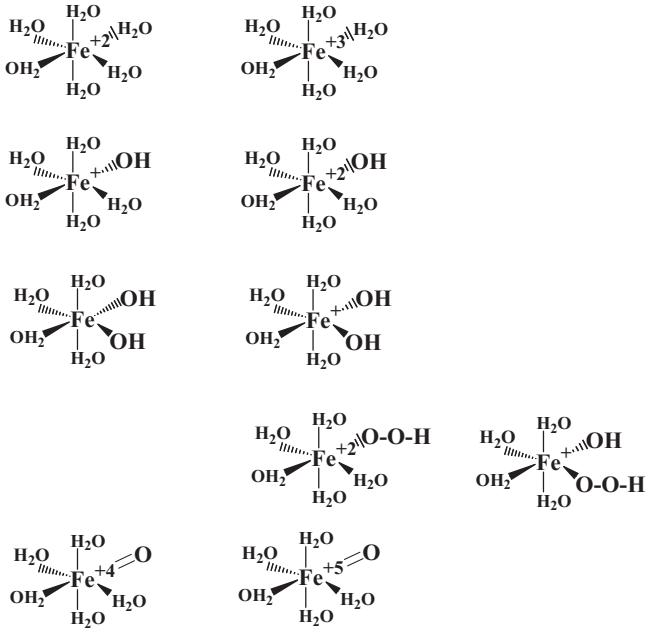


Fig. 2. Different structures of iron aqua-complexes. No iron charges are depicted.

2.1.3. Inorganic Fenton reactions

One of the best reviews on Fenton and Fenton-like systems was recently published by Pignatello et al. in 2006 [1]. The main facts on the Fenton systems will be briefly summarized based on this review and other published works [2,25].

Barb et al. [26–28] proposed a radical mechanism for the dark decomposition of H_2O_2 in acidic solutions and in the absence of organic compounds. It consists of the following set of reactions:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^\bullet \quad (3)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^\bullet + H^+ \quad (4)$$

$$H_2O_2 + HO^\bullet \rightarrow HO_2^\bullet + H_2O \quad (5)$$

$$Fe^{2+} + HO^\bullet \rightarrow Fe^{3+} + OH^- \quad (6)$$

$$Fe^{3+} + HO_2^\bullet \rightarrow Fe^{2+} + O_2 + H^+ \quad (7)$$

$$Fe^{2+} + HO_2^\bullet + H^+ \rightarrow Fe^{3+} + H_2O_2 \quad (8)$$

$$HO_2^\bullet + HO_2^\bullet \rightarrow H_2O_2 + O_2 \quad (9)$$

Reactions (3)–(9) are very well-known, and they are characterized in solutions without strongly coordinating ligands other than OH^- and H_2O or other redox species. Iron cycles between the +2 and +3 oxidation states. The oxidant that initiates the degradation of the target pollutant, the hydroxyl radical ($HO^\bullet$), is supposedly produced by reaction (3), which is pH-independent below pH 3. Reaction (4) is rate-limiting since its overall rate constant is about four orders of magnitude smaller than that associated with reaction (3). Hydroxyl radicals may react with H_2O_2 or Fe^{2+} (reactions (5) and (6)). Oxidation of Fe^{2+} by O_2 is negligible at pH values at which Fe^{3+} is soluble [1]:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -} \quad (10)$$

Therefore, reaction (10) does not contribute to Fenton reactions below pH 4. However, at pH values higher than 4, it may be important to increase the $O_2^{\bullet -}$ concentration.

Several results clearly presented in the review of Pignatello et al. [1] point to the participation of an additional oxidant besides $OH^\bullet$. These species could be a Fe^{3+}-hydrogen peroxide complex or a Ferryl species.

This species may have the iron in +2 or +3 oxidation state, depending on the kind of coordination of the OOH moiety and the nature of it (single bond? double bonds involved? are questions still not completely solved). Considering a different structure, even Fe=O may be included in the analysis.

$$Fe^{3+} + HOO^- \rightarrow Fe^{2+} + HOO^\bullet \quad (11)$$

$$[FeOOH] \rightarrow \left[Fe^{4+}O\right] + OH^\bullet \quad (12)$$

According to Kremer [29], ferryl species can also be formed by reaction (13),

$$Fe^{2+} + H_2O_2 \rightarrow FeO^{2+} + H_2O \quad (13)$$

and the decomposition of H_2O_2 by Fe^{3+} may occur through a Fe^{5+} oxo-species (reaction (14)) following the Dunford–Kremer proposal (see Table 1)

$$Fe^{3+} + HO_2^\bullet \rightarrow FeHO_2{}^{2+} \rightarrow FeO^{3+} + HO^\bullet \quad (14)$$

It is important to note that it has been widely accepted that, at typical pH values of Fenton systems, the reaction rates are mainly governed by $HO^\bullet$ radicals, while ferryl species only play a minor role. However, a review from some years ago from Dunford [30] questioned that and revised the publications on this topic. Dunford mentioned that Kremer pointed out an error in the analysis of Barb [26–28]. According to Kremer, the assumption that there is a steady state in Fe^{2+} is wrong given that $[Fe^{2+}]$ *should go* to zero in the Fenton system. As Dunford demonstrated, there is no obvious kinetic way to distinguish the mechanisms proposed using hydroxyl radical as intermediate or presenting two different intermediates (see Table 1). The manuscript of Dunford presents all the aspects of the controversy. Bossmann et al. provided evidence for ferryl ion being the active oxidant in Fenton Chemistry [31,32]. Bossmann et al. [31] presented an explanation based on the coordination chemistry of iron in aqueous solution. Because the formation of a hydrated Fe^{2+}–H_2O_2 complex is thermodynamically favored Bossman et al. proposed a ligand exchange reaction (H_2O_2 by H_2O) in the inner sphere of high spin Fe^{2+}. The reaction rate constant of this reaction ($2 \times 10^6\ M^{-1}\ s^{-1}$) is remarkable higher than the bimolecular Fenton reaction ($60–80\ M^{-1}\ s^{-1}$). This author proposed that an inner-sphere reaction takes place in $[Fe(OH)(H_2O_2)(H_2O)_4]^+$ and the intermediate iron(IV) complex ($[Fe(OH)_3(H_2O)_4]^+$, is formed. The intermediate iron(IV) complex may react further leading to the formation of a free hydroxyl radical and $[Fe(OH)(H_2O)_5]^{2+}$ (see Table 1, Bossman). The question the authors presented was whether hydroxyl radical production is not too slow to compete with direct electron transfer between the substrate and a hydrated higher-valent iron species. Bossman et al. [31] postulated that the reaction of a metal cation, as for instance Fe^{4+} with an aliphatic or an aromatic hydrocarbon proceeds exclusively by an electron-transfer mechanism because hydrogen exchange is not possible. However, the experiments described in reference cannot distinguish between Fe^{4+} and a hydroxyl radical complexed by Fe^{3+}. The latter species would possess exactly the same reactivity as Fe^{4+}.

The work reported by Wink et al. also supports Dunford ideas from references [33] and [34].

Despite the high rate constant value associated to the recombination of $HO^\bullet$ radicals, in most conditions this reaction plays only a minor role owing to the low concentration associated to this radical species in the bulk which limits its occurrence compared with other reactions involving the participation of non-radical species [35]. Furthermore, given the high reactivity of hydroxyl radicals, reactions (5 and 6) are major $HO^\bullet$ sinks that decrease the oxidizing power of the Fenton systems.

Recent publications show that some kind of consensus has been found among researchers who consider that both the "classical" (i.e., based on hydroxyl radicals) and "non-classical" (i.e., ferryl ion based) mechanisms coexist and predominate one or the other depending on the operation conditions [1,35–59].

Table 1 summarizes the mechanisms associated to Fenton systems reported by different authors. In Fenton systems at pH 3 there is no way to distinguish the hydroxyl radical from the ferryl one. When organic matter is degraded in aqueous solution and at pH 3 in almost every Fenton system a distribution of products very similar to those obtained with radiolysis, photocatalytic and also with TiO_2 (all of them in absence of iron) is found with Fenton and Fenton-like systems. From the analysis of the recent review of Pignatello et al. [1] the contribution of the ferryl group is considered not decisive, mainly at pH 3. However, it seems that the suspicion is widespread that at pH values near to neutral the ferryl group would be the intermediary and the mechanism of hydrogen peroxide decomposition would be mediated by it.

Beyond controversy, the hydroxyl radical may be generated by reaction of an organic radical with H_2O_2, without iron involved. Hydroxyl radicals may react with iron, H_2O_2 or recombine to generate H_2O_2 (with a $k = 6 \times 10^9\ M^{-1}\ s^{-1}$ [35]). In the case of phenol, there are reactions with superoxide that may produce phenoxyl radicals. One of the key aspects to consider in the most simple Fenton system is whether the hydroxyl radical is a free diffusive entity in aqueous solutions or it is coordinated to iron. The location of the hydroxyl radical or anion (free or coordinated) affects iron reactivity and reaction mechanisms.

Table 1
Comparison of mechanisms proposed for Fenton and Fenton-like.

Barb et al.	k in $M^{-1}\,s^{-1}$	Kremer	k in $M^{-1}\,s^{-1}$
$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^\bullet$ $\left(Fe^{IV}–OH?\right)$	53	$Fe^{2+} + H_2O_2 \leftrightarrow Fe^{2+} * H_2O_2 \rightarrow FeO^{2+} + H_2O$	55.4
$H_2O_2 + HO^\bullet \rightarrow HO_2^\bullet + H_2O$	2.7×10^7		
$Fe^{3+} + HO_2^\bullet \rightarrow Fe^{2+} + O_2 + H^+$	1.2×10^4 to 3.6×10^5	$FeO^{2+} + H_2O_2 \rightarrow Fe^{2+} + O_2 + H_2O$	
$Fe^{2+} + HO^\bullet \rightarrow Fe^{3+} + OH^-$ $\left(Fe^{III}–OH?\right)$	3.3×10^8	$FeO^{2+} + H^+ + Fe^{2+} \rightarrow 2Fe^{3+} + 2OH^-$	
$Fe^{2+} + HO_2^\bullet \rightarrow Fe^{3+} + HO_2^-$	1.8×10^3 to 2.5×10^4	$FeO^{2+} + Fe^{3+} \leftrightarrow FeOFe^{5+}$	
$Fe^{2+} + HO_2^\bullet \rightarrow Fe(HO_2)^{2+}$		$FeOFe^{5+} + H_2O_2 \rightarrow Fe^{2+} + Fe^{3+} + O_2 + H_2O$	
Bielski	k in $M^{-1}\,s^{-1}$	**Haber Willstätter cycle (HWC)**	
$HO_2^\bullet + HO_2^\bullet \rightarrow H_2O_2 + O_2$	8.3×10^5	$H_2O_2 + HO^\bullet \rightarrow O_2^{\bullet-} + H^+ + H_2O$	
$HO_2^\bullet + O_2^{\bullet-} \rightarrow H_2O_2 + O_2$	9.7×10^7	$O_2^{\bullet-} + H^+ + H_2O_2 \rightarrow OH^\bullet + O_2 + H_2O$	Haber Weiss reaction
Kozlov	k in $M^{-1}\,s^{-1}$	**Evidence against Haber Weiss reaction**	
$Fe^{3+} + HO_2^- \rightarrow Fe^{2+} + HO_2^\bullet$	9×10^5	$2K^+O_2^- + 2H_2O \rightarrow 2OH^- + O_2 + H_2O_2 + 2K^+$	Harcourt
Jiang et al.		No reaction between superoxide and H_2O_2	Dunford paper references
$Fe^{3+} + H_2O_2 \rightarrow Fe^{III}(HO_2)^{2+} + H^+$	3.1×10^{-3}	Bray and Gorin	
$FeOH^{2+} + H_2O_2 \rightarrow Fe^{III}(OH)(HO_2)^+ + H^+$	2×10^{-4}	$Fe^{3+} + H_2O \leftrightarrow Fe^{2+} + FeO^{2+} + 2H^+$	
$Fe^{III}(HO_2)^{2+} \rightarrow Fe^{2+} + HO_2^\bullet$	2.7×10^{-3}	$Fe^{2+} + H_2O_2 \leftrightarrow FeO^{2+} + H_2O$	
Reaction with an organic substrate- $OH^\bullet$ generation		$FeO^{2+} + H_2O_2 \rightarrow Fe^{2+} + O_2 + H_2O$	
$RH + O_2^{\bullet-} \rightarrow R^- + HO_2^\bullet$		$Fe^{2+} + H_2O_2 + 2H+ \leftrightarrow 2Fe^{3+} + 2H_2O$	
$RH + O_2^{\bullet-} \rightarrow R^\bullet + HO_2^-$			
$R^\bullet + H_2O_2 \rightarrow ROH + OH^\bullet$		Cahill and Taube	
$HR + OH^\bullet \rightarrow R^\bullet + H_2O$		$Fe^{2+} + FeO^{2+} + 2H^+ \rightarrow 2Fe^{3+} + H_2O$	
Global reaction $HR + H_2O_2 \rightarrow ROH + H_2O$			
$R^\bullet + O_2 \rightarrow ROO^\bullet$		Bossman	$k = 2 \times 10^6$
$R^\bullet + HO_2^\bullet \rightarrow RO^\bullet + OH^\bullet$		$Fe(OH)(H_2O)_5^{+} + H_2O_2 \leftrightarrow$	
		$Fe(OH)(H_2O)_4(H_2O_2)^+$	
$R^\bullet + Fe^{3+} \rightarrow R^+ + Fe^{2+}$		$Fe(OH)(H_2O)_4(H_2O_2)^+ \leftrightarrow Fe(OH)_3(H_2O)_4^{+}$	
$R^\bullet + Fe^{2+} \rightarrow R^- + Fe^{3+}$			
$Fe^{III}–OH \rightarrow Fe^{III}{=}O^+ + H^+$ $Fe^{IV}–OH \rightarrow Fe^{IV}{=}O^{2+} + H^+$		$Fe^{4+} + RH \rightarrow Fe^{3+} + RH^{\bullet+}$	Electron transfer

Mechanism of conversion of Fe^{2+} to Fe^{3+}

Barb et al.	Versus	Bray–Gorin–Dunford–Cahill–Tauble	
$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + OH^- + HO^\bullet$	Steady state in $OH^\bullet$ $v = 2k[Fe^{2+}]^*[H_2O_2]$ 10^7	$Fe^{2+} + H_2O_2 \leftrightarrow Fe^{2+} * H_2O_2 \rightarrow FeO^{2+} + H_2O$	Slow Fe(IV) in FeO^{2+}
$Fe^{2+} + HO^\bullet + H^+ \rightarrow Fe^{3+} + H_2O$		$Fe^{2+} + FeO^{2+} + 2H^+ \rightarrow 2Fe^{3+} + H_2O$ $v = 2k[Fe^{2+}]^*[H_2O_2]$; no $OH^\bullet$ involved	Fast

Reaction of Fe^{3+} with H_2O_2

Barb et al.	k	Dunford–Kremer	
$Fe^{3+} + H_2O_2 \leftrightarrow Fe^{2+} + HO_2^\bullet + H^+$	0.001–0.01	$Fe^{3+} + HO_2^- \leftrightarrow Fe^{3+}HO_2^-$ Compound I	
		$Fe^{3+}HO_2^- \rightarrow HO^- + FeO^{3+}$ Compound II	Fe(V) in FeO^{3+}
$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$	5×10^7	$FeO^{3+} + H_2O_2 \rightarrow Fe^{3+} + O_2 + H_2O$	

Regeneration of Fe^{2+}		Barton–Gif chemistry
$Fe^{3+} + H_2Q \leftrightarrow Fe^{2+} + HSQ^\bullet + H^+$	1–24	$Fe^{2+} + H_2O_2 \rightarrow Fe^{III}–O–O–H + H^+$; $Fe^{III}–O–O–H + H^+ \rightarrow Fe^{V}{=}O + H_2O$
$Fe^{3+} + HSQ^\bullet \leftrightarrow Fe^{2+} + Q + H^+$		Barton-sleeping beauty effect-activation of hydrocarbons to give alcohols or ketone
$OH\text{-}Ph\text{-}OH^\bullet + Q + H^+ \rightarrow HSQ^\bullet + OH\text{-}Ph\text{-}OH$		$R_2CH_2 + Fe^{V}{=}O + H^+ \rightarrow R_2CHFe^{III} + H_2O$
		$R_2CHFe^{III} + O_2 \rightarrow R_2CHOH + Fe^{V}{=}O$

It has been established that the hydroxycyclohexadienyl-like radicals formed may reduce Fe^{3+} indirectly through the catalytic mediation of quinone molecules [39,40]. The role of the quinones and semiquinones formed in the oxidation of phenol using Fenton Systems is not minor [39]. Quinones (Q) are π-electron acceptors in charge transfer complexes with π donor molecules. The formation of this kind of complexes in Fenton systems with phenol or phenolic substrates is certainly possible. When iron is initially present as Fe^{3+}, phenol degradation displays autocatalysis. The radical $OHPhOH^\bullet$ reacts with Q in acidic media to give the $HSQ^\bullet$ that may regenerate Fe^{2+}. The role of quinones in the regeneration of Fe^{2+} in Fenton systems is shown in Scheme 1. The hydroxylation of phenol in meta position has been neglected by Pontes et al. [11]. According to Alnaizy and Akgerman [60], the formation of resorcinol is about 1000 times lower than the formation of catechol (ortho-oxidation) and hydroquinone (para-oxidation).

2.1.4. Organic Fenton reactions

The reactions of hydroxyl radical with organic compounds are well-known. These reactions take place mainly by H abstraction (from C–H, N–H, or O–H bonds), by addition to C=C bonds or by addition to aromatic rings [37,38].

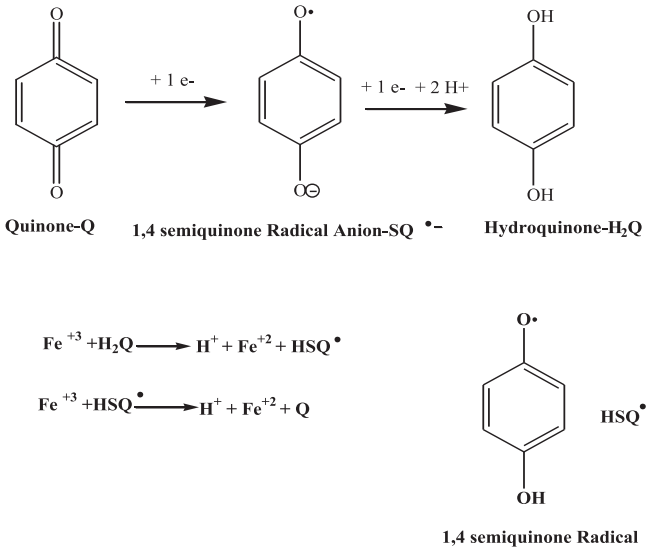
$$HO^\bullet + R–H \rightarrow H_2O + R^\bullet \quad (15)$$

$$HO^\bullet + C{=}C \rightarrow HO–C–C^\bullet \quad (16)$$

$$HO^\bullet + Ph–H \leftrightarrow Ph–H(OH)^\bullet \rightarrow \text{other secondary reactions} \quad (17)$$

where $Ph–H(OH)^\bullet$ represents hydroxycyclohexadienyl radicals.

As it was shown above in reaction (7), O_2 is produced by the reaction of iron with H_2O_2.



Scheme 1. Quinone and derivatives and their reactions with Fe^{3+}.

Reactions (15 and 16) are irreversible, whereas reaction (17) has been proposed as reversible [38].

$$HR^{\bullet} + O_2 \rightarrow R + HO_2^{\bullet} \quad (18)$$

$$R^{\bullet} + O_2 \rightarrow R\text{–}OO^{\bullet} \quad (19)$$

The bimolecular reaction of $R^{\bullet}$ with O_2 is very fast. The radicals may couple or disproportionate, and the overall process eventually leads to the mineralization of the organic matter to CO_2, H_2O, and inorganic acids. The produced organic radicals $ROO^{\bullet}$, $RO^{\bullet}$ and $R^{\bullet}$ may also react with iron species in the following sequence of reactions [41]. Reactions between $ROO^{\bullet}$ and molecular oxygen were also reported in the case of natural phenolic compounds, such as humic substances [42].

$$Fe^{2+} + ROO^{\bullet} \rightarrow Fe^{3+} + :OOR \quad (20)$$

$$Fe^{3+} + :OOR + H^{+} \rightarrow HOOR + Fe^{3+} \quad (21)$$

$$Fe^{3+} + :OOR + Fe^{2+} + 3H^{+} + 1e^{-} \rightarrow 2Fe^{3+} + ROH + H_2O \quad (22)$$

$$Fe^{3+}OOR + ROO^{\bullet} + H^{+} \rightarrow Fe^{2+} + O_2 + ROH + RO \quad (23)$$

Interestingly, the rate of reaction (20) has been reported as much higher than that of reaction (8). In addition, alkoxyl radicals consume Fe^{2+} rapidly according to Eq. (24):

$$Fe^{2+} + RO^{\bullet} + H^{+} \rightarrow ROH + Fe^{3+} \quad (24)$$

Depending on their structure, $R^{\bullet}$ radicals may reduce Fe^{3+} or oxidize Fe^{2+} through irreversible reactions:

$$Fe^{3+} + R^{\bullet} \rightarrow Fe^{2+} + R^{+} \quad (25)$$

$$Fe^{2+} + R^{\bullet} + H^{+} \rightarrow Fe^{3+} + RH \quad (26)$$

Iron species are known to react directly with some compounds of interest in Fenton applications, including organoperoxides, organic contaminants such as aniline and chlorophenol, hydrogenated quinones and dyes [43,44].

Table 1 summarizes the main reactions assigned to the Fenton system and it also includes the main reactions of organic radicals with H_2O_2 and O_2.

Pontes et al. [11] have used a stoichiometric kinetic model for phenol degradation by the Fenton process that includes 53 reactions and 26 compounds. The unknown model parameters were obtained by fitting to the experimental results. Sensitivity analysis was performed to determine the most influential kinetic parameters and assess the impact of the initial concentration and flow rate of reactants on the efficiency of the Fenton process to degrade phenol. Kang and Lee [14] have described the degradation of chlorophenols by the Fenton process using the same model for phenol degradation.

In the absence of UV radiation and Fe^{3+}-reducing organic compounds, reaction (4) (i.e., the reduction of Fe^{3+} by H_2O_2) becomes rate-limiting. On the other hand, when zero valent iron is used, an initial oxidation step is needed to begin the reaction [45].

The total mineralization of phenol requires 14 moles of H_2O_2.

$$C_6H_5OH + 14H_2O_2 \rightarrow 6CO_2 + 17H_2O$$

However, approximately 30 mol of hydrogen peroxide were required to mineralize 1 mol of phenol. Thus, only 35% of the hydrogen peroxide was efficiently used to mineralize the phenol. The degradation of phenol leads to formation of a mixture of byproducts, such as catechol, benzoquinone, resorcinol and hydroquinone [46]. There exist an important number of references addressing the formation of benzenediols resulting from Fenton-driven oxidation of phenol [47].

Organic compounds may act as iron-complexing agents. Thus, the redox potential of the couple ferrous/ferric iron is affected and the reaction rates of iron species with reactive oxygen species such as H_2O_2 and superoxide may also change. Chen and Pignatello demonstrated an improvement in oxidation performance with the addition of quinones and hydroquinones in their Fenton-phenol system [39].

Yoon et al. [48] studied the impact of the $[Fe^{2+}]_0/[H_2O_2]_0$ ratio on Fenton systems and classified them in three categories:

(a) High ratio of $[Fe^{2+}]_0/[H_2O_2]_0$ ($\geq$2)
Ferrous ion and hydrogen peroxide are mainly consumed within minutes. The presence of RH affects only the behavior of the concentration of the ferrous ion.
(b) Medium ratio of $[Fe^{2+}]_0/[H_2O_2]_0$ (=1)
(c) Low ratio of $[Fe^{2+}]_0/[H_2O_2]_0$ («1)

In the absence of RH, hydrogen peroxide decomposes slowly through ferric ion-induced radical chain reactions. The presence of RH changes the behavior of the hydrogen peroxide (i) no further hydrogen peroxide decomposition occurs just after the initial decrease of hydrogen peroxide (ii) the presence of excess RH can hinder the supposed reaction between $OH^{\bullet}$ and the ferrous ion.

The hydrogen peroxide decomposition, induced by the initial ferrous ion (ferrous system) in the presence of RH, is smaller than in the absence of RH. Considering the radical versus the non-radical view, an excellent review of the impact of the organic substrate has been published by Gozzo [49].

2.1.5. Biphasic Fenton kinetics

The removal of organic compounds in reaction mixtures starting with Fe^{2+} and having H_2O_2 in a large stoichiometric excess (100–1000 peroxide-to-iron molar ratio) generally exhibits a biphasic kinetic behavior. An initial fast degradation phase results from the high amount of $HO^{\bullet}$ produced by reaction (3) [50,51], followed usually by a much slower phase (with reaction (4) being rate-limiting). If H_2O_2 is in large excess, the extent of this phase will depend on the iron/organic compound molar ratio. The kinetic mechanism of atrazine degradation was explained by De Laat et al. [51] using biphasic Fenton mechanism whereas the mechanistic study was further dilucidated years later [52]. The $HO^{\bullet}$ radical initiated the decay of ATZ through alkylic-oxidation (alkylamino side chain oxidation), dealkylation (alkylic side chain cleavage), and/or dechlorination (hydroxylation at the chlorine site). Fig. 3 shows

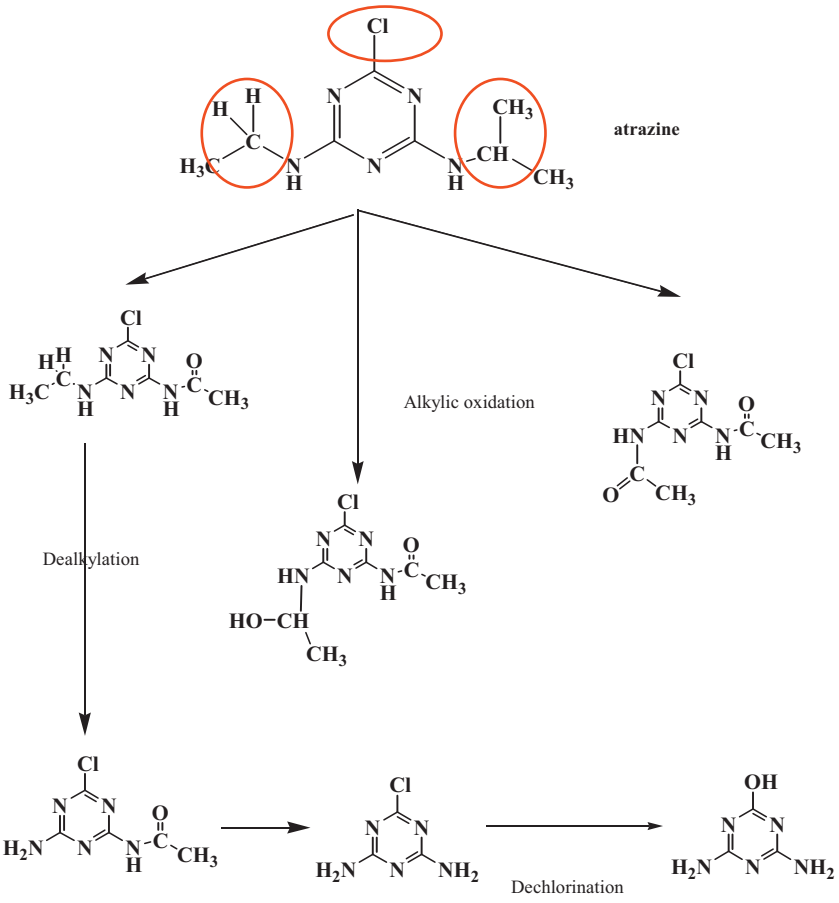


Fig. 3. Structure of atrazine and its oxidation pathway with Fenton system.

the structure of atrazine and its main degradation pathways in the presence of Fenton's reagent.

In reaction mixtures starting with a salt of Fe^{3+} and when aromatic compounds are the targets, there is usually an activation period that often results in a slow initial rate followed by a fast reaction phase [53,54]. The rate-limiting step in Fenton-like processes is usually a reductive dissociation of the Fe^{3+}–peroxide complex:

$$Fe^{3+}(HOO^-) \rightarrow Fe^{2+} + HOO^\bullet \quad (27)$$

This is similar to reaction (11) but this time the HOO anion is considered to be complexed with Fe^{3+}.

$$Fe^{3+}(HOO^-)(HO^-) \rightarrow Fe^{2+} + HOO^\bullet + HO^- \quad (28)$$

As stated above, during the degradation of phenolic compounds, the formation of hydroquinone-like intermediates and the generation of Fe^{2+} interact with each other. The reduction rate constant of Fe^{3+} by H_2O_2 is 0.001–0.01 M^{-1} s^{-1}. This reaction rate is too low to generate Fe^{2+} quickly enough. In consequence, the removal of the phenolic compounds in the initiation period is slow. However, hydroquinone-like intermediates produced in the initiation period reduce Fe^{3+} to Fe^{2+}. This important reaction results in an increase of Fe^{2+} concentration, which in turn accelerates the degradation of phenolic compounds as well as the formation of intermediates [55]. This electron-shuttle mechanism has been recently analyzed in detail [56]. Electron-donating substituents on the quinone ring inhibit the reaction, and naphthoquinones are better catalysts than benzoquinones [44]. In line with the above, the oxidation of substituted benzenes by Fe^{3+}/H_2O_2 system displays autocatalysis [44]. After a lag phase, a fast phase is initiated owing to the build-up of hydroquinone- and quinone-like products. This is accompanied by a sharp increase in Fe^{2+} concentration. Similarly, Fe^{3+}/H_2O_2 degradation of atrazine is accelerated when 1,2,4-trichlorobenzene is added. This is attributed to the enhanced regeneration of Fe^{2+} by hydroquinone/semiquinone intermediates from trichlorobenzene degradation [57].

It has been reported that Fe^{3+} rapidly oxidizes the *p*-hydroxyazo dye Acid Orange 20 (AO20) [58]. The reaction produces two equivalents of Fe^{2+}, a product that forms a reversible complex with Fe^{2+}, and 4% of 1,4-naphthoquinone. AO20 is in its hydrazone form, the predominant tautomer in polar solvents. Interestingly, *o*-hydroxyazo analogs, Acid Orange 7 and Acid Orange 10 are inert (see Fig. 4 for the structures of some azo dyes).

The mechanism of phenol removal using Fenton systems generates hydroxylated aromatic compounds. These compounds can be oxidized to quinones. Liotta et al. [21] presented a simplified scheme for phenol oxidation. Complete degradation was not observed, although the H_2O_2 to organic compound molar ratio was initially set to the stoichiometric value. This was explained in terms of the formation of strong iron(III)-complexing agents, such as oxalic acid. As the Fenton degradation proceeds, inorganic acids such as glyoxalic, maleic, oxalic, acetic, and formic acids increase their concentration if the reaction is carried out in the dark (see Fig. 5 for the structures of some strong Fe(III) complexing acids). Moreover, these acids are weakly reactive towards $HO^\bullet$. In contrast, under UV or visible irradiation these acids may be mineralized through Fe^{3+}-catalyzed photoreactions. Other authors presented more complicated schemes for the phenol oxidation [59,60].

Lee et al. showed that the reaction between oxygen and phenolate ion was much faster than that between phenol and oxygen (i.e. 10^7 times higher at 473 K). The chemistry of phenol above pH 10 is

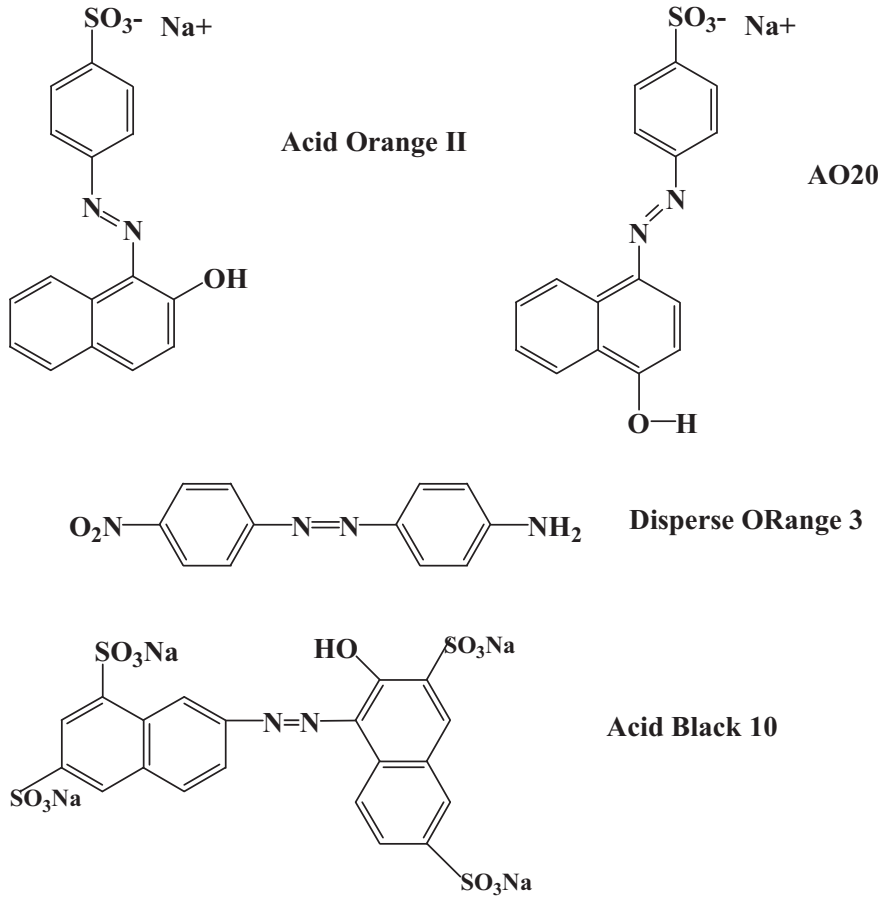


Fig. 4. Some common azo dyes.

that of the phenolate ion (pK = 9.1). The pH of the solution is then crucial to accelerate or not the radical formation [61].

Table 2 shows the main reactions of phenol and derivatives (phenolate and phenoxyl radical) with different species (H_2O_2 and derivatives).

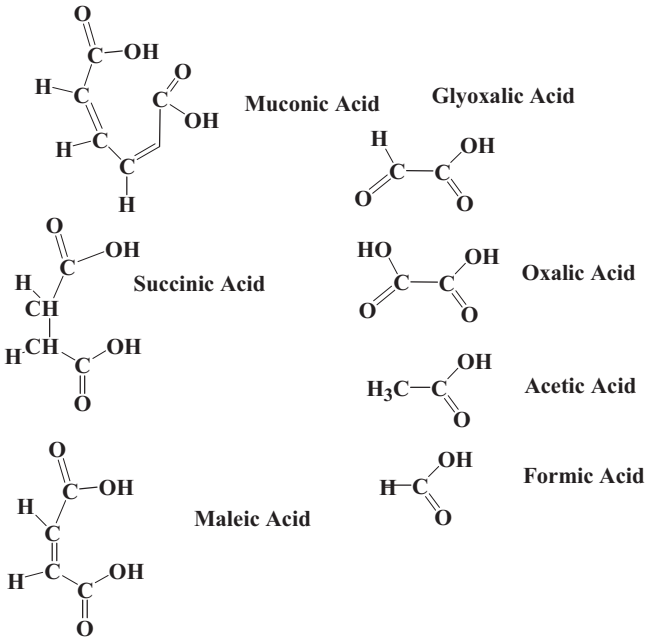


Fig. 5. Some strong complexing acids for iron.

2.2. Heterogeneous Fenton systems

Zazo et al. [59] pointed out that the major problem of Fenton homogeneous catalytic systems is the pH control and the production of toxic wastes that require further treatment. Heterogenization is required to reduce costs, pH sensitivity and the generation of secondary waste. Heterogeneous Fenton processes are very interesting because most of the iron remains in the solid phase and can be reused [62,63].

In terms of heterogenization, different approaches have been tested for the catalytic abatement of phenolic compounds:

- Use of *iron metal* as part of the catalytic system, e.g. iron metal tetrahedrally coordinated into a zeolitic framework [64–67].
- Immobilization of Fe^{2+} *and* Fe^{3+} in conventional supports with low environmental impact, e.g. silica [68], alumina, zeolites and resins [69,70].

Table 2
Main reactions of phenol and derivatives in presence of H_2O_2.

Reaction	Description
$2PhOH + H_2O_2 \rightarrow 2PhO^{\bullet} + 2H_2O$	Generation of radicals
$PhOH + OH^- \rightarrow PhO^- + H_2O$ at alkaline pH	
$PhO^- + O_2 \rightarrow PhO^{\bullet} + O_2^{\bullet -}$	
$2PhO^{\bullet} \rightarrow HOPh\text{-}PhOH$	Dimerization
$PhO^{\bullet} + O_2^{\bullet -} + H^+ \rightarrow O_2 + PhOH$	Repair and O_2 generation
$PhO^{\bullet} + O_2^{\bullet -} + H^+ \rightarrow HOO\text{-}HPh{=}O \longleftrightarrow HOO\text{-}Ph\text{-}OH$	Addition and peroxide generation
$HOO\text{-}HPh{=}O \rightarrow H_2O + O{=}Ph{=}O$	Condensation-quinone generation

- Use of *oxides* [71–74] immobilized in adequate supports such as those referenced for iron ions [75–77,15].

Iron leaching from the immobilized species, pH control, and the need of total mineralization of phenol (to avoid the increase of organic acids able to dissolve iron during phenol removal) are the main problems found in these systems. The observed catalytic activity often results not only from heterogeneous contribution, but also from the homogeneous contribution of the leached iron in the gradually acidified reaction mixture.

Among the most important mechanistic issues in heterogeneous systems are the *coordination* and the *oxidation state* of iron species found in oxides and zeolites. In relation to the oxidation states, surface iron species participate in processes similar to those presented in reactions (3–10). The differences are mainly related to: (a) the coordination of iron and the kind of ligand; (b) the environment of the solid surface versus the solution; (c) the interaction of the substrate with the surface through adsorption on iron active sites and (d) how the oxidation pathway may have a lower activation energy in immobilized (heterogeneous) systems compared to that associated with homogeneous systems.

Therefore the distribution, coordination and acidity of different surface species are critical factors to understand the reactivity regarding Fenton reactions. Iron oxides/hydroxides produced on the surface of heterogeneous catalysts may play the role of electron mediators. The relative contribution of each surface species is pH-dependent. Cornell and Schwertmann [78] identified four different surface oxide products on Fe metal in presence of Orange II dye: lepidocrocite (γ-FeOOH), goethite (α-FeOOH), ferroxyhite (δ-FeOOH), and akaganeite-like (β-FeOOH) species. These oxidized phases on metallic Fe showed differences in their reactivities and final structures after the interaction with the dye. In the case of magnetite (Fe_3O_4), it has been reported that at acidic pH the dominating surface species was $\equiv Fe(II,III)OH_2^+$. With increasing pH, the zeta potential decreased and the main species around pH of the point of zero charge was ≡Fe(II,III)OH. At alkaline pH, the dominating species was $\equiv Fe(II,III)O^-$ [79]. The magnetite catalyst exhibited low iron leaching, good structural stability and no loss of performance in the second reaction cycle of pentachlorophenol (PCP) degradation. Fenton-like oxidation of PCP was mainly controlled by a surface reaction mechanism [74]. Oxides containing only Fe^{3+}, i.e. hematite (α-Fe_2O_3) and maghemite (χ-Fe_2O_3), were nearly inactive for the H_2O_2 decomposition at 25 °C. On the other hand, freshly prepared magnetite (Fe_3O_4) showed a much higher H_2O_2 decomposition. This magnetite exposed to air for few weeks showed a much lower activity for the H_2O_2 decomposition [80]. The increase in activity caused by the addition of Fe metal is discussed in terms of the formation of Fe^{2+} surface species during the preparation of the composite and of an electron-transfer mechanism from Fe metal to Fe^{3+} during the Fenton reaction to regenerate the Fe^{2+} surface active species. A redox process Fe^{2+}/Fe^{3+} on superparamagnetic nanoparticles took place when an excess of H_2O_2 was added into the reaction solution to produce hydroxyl ($OH^\bullet$) and hydroperoxyl radicals ($HO_2^\bullet$). The hydroxyl radicals could destroy the benzene ring and finally produce H_2O and CO_2 [81].

The mechanism of phenol degradation with iron-based zeolites (Fe-ZSM-5, Fe-silicalite and Fe-TS-1) is considered to be the same as that associated with Fenton reactions in solution, however it should be pointed out that the results are often influenced by iron leaching [82]. The Fe^{n+} species in Zeolite ZSM5 form a distribution of isolated, dimeric or clustered sites, and Fe_2O_3 particles. In Fe-ZSM5, the additional species are those related to Al-containing regions via AlOFe bridges. Zecchina et al. found at least two types of Fe^{2+} sites characterized by different reactivities [82]. The structure of the oxidized center is probably $Fe^{4+}{=}O$ (or $Fe^{3+}{-}O^-$), although there are also oxo-bridged $Fe^{3+}O^{2-}Fe^{3+}$ structures. An isolated $(FeO)^{2+}$ structure is emerging as the preferred candidate for active adsorbed oxygen in these samples. This does not exclude the presence of a minor fraction of reduced Fe_xO_y clusters entrapped inside the framework cavities, where the formation of oxo-bridged species is more likely than on the surface.

Excellent recent reviews on the heterogeneous Fenton and Fenton-like reactions have been published, including several reactions mechanisms [83–85].

There are several mechanisms proposed for the oxidation of organic compounds on the surface of iron oxide catalysts through a Fenton-like reaction. These mechanisms include the radical mechanism proposed by Lin and Gurol in 1998 [86], the radical mechanism proposed by Kwan and Voelker in 2002 [87] and the non-radical mechanism proposed by Andreozzi et al. in 2002 [88] (see reference [83]). The review of Garrido Ramírez et al. includes an extensive bibliography where topics like transition metal-exchanged zeolites, pillared interlayered clays, iron oxide minerals and nano-catalysts are carefully revised and discussed [83].

According to the radical mechanism proposed by Lin et al. [86], the reaction is initiated by the formation of an inner-sphere complex between hydrogen peroxide and ≡Fe(III)–OH groups at the oxide surface. The excited state can be deactivated through dissociation of the peroxide radical, that reacts with other compounds. The peroxide radicals produced can react with Fe(II) and Fe(III), exposed on surface site. These free radicals can also react with hydrogen peroxide or with themselves.

On the other hand, Andreozzi et al. [88] have suggested a non-radical mechanism for the degradation of 3,4-dihydroxybenzoic acid. The adsorbed substrate (S) and hydrogen peroxide react on the catalyst surface, giving rise to reaction products and the regeneration of active sites. The main point, again, is whether the OH radical is free to diffuse or is coordinated to the iron on the surface. The difference between the two radical mechanisms proposed is the kind of surface species and the interaction with soluble free species (see Table 4 of reference [79]). Even when it may be accepted that the mechanism involves the formation of this radical, it is highly probable that the hydroxyl is not a free species, but it remains coordinated to the iron at surface. The coordination of different radical or ionic species would be different depending on steric hindrance on iron and the ability of these species to displace coordinated water or other ligands present on surface.

2.3. Homogeneous and heterogeneous HRP systems

2.3.1. Enzymatic cycle description

The general mechanism for homogeneous phenol (AH_2) transformation using HRP/H_2O_2 systems is:

$$\text{Native HRP} + H_2O_2 \rightarrow \text{Compound I} + H_2O \quad (29)$$

$$\text{Compound I} + AH_2 \rightarrow \text{Compound II} + AH^\bullet \quad (30)$$

$$\text{Compound II} + AH_2 \rightarrow \text{Native HRP} + AH^\bullet + H_2O \quad (31)$$

There are several excellent reviews on the formation of Compound I and Compound II when using HRP in phenol oxidation by H_2O_2 [89,90]. Fig. 6 shows a schematic representation of the catalytic cycle. In the native state of HRP, a pentacoordinated iron is in oxidation state +3. Everse [91b] has proposed the following notation for these intermediates: Compound I could be written as Protein($^\bullet$) $Fe^{IV}{=}O$, and Compound II as Protein–$Fe^{IV}{=}O$. Compounds I and II are considered to be ferryl complexes with $Fe^{IV}{=}O$ [91]. This implies that *the notation of oxidation state* for the transition metal is changed to the *valence notation* for intermediaries whose charge is difficult to assign. The joint analysis of Mössbauer spectroscopy data and the results obtained with model compounds indicated that Compound I contained Fe^{IV} and a porphyrin π-cation radical.

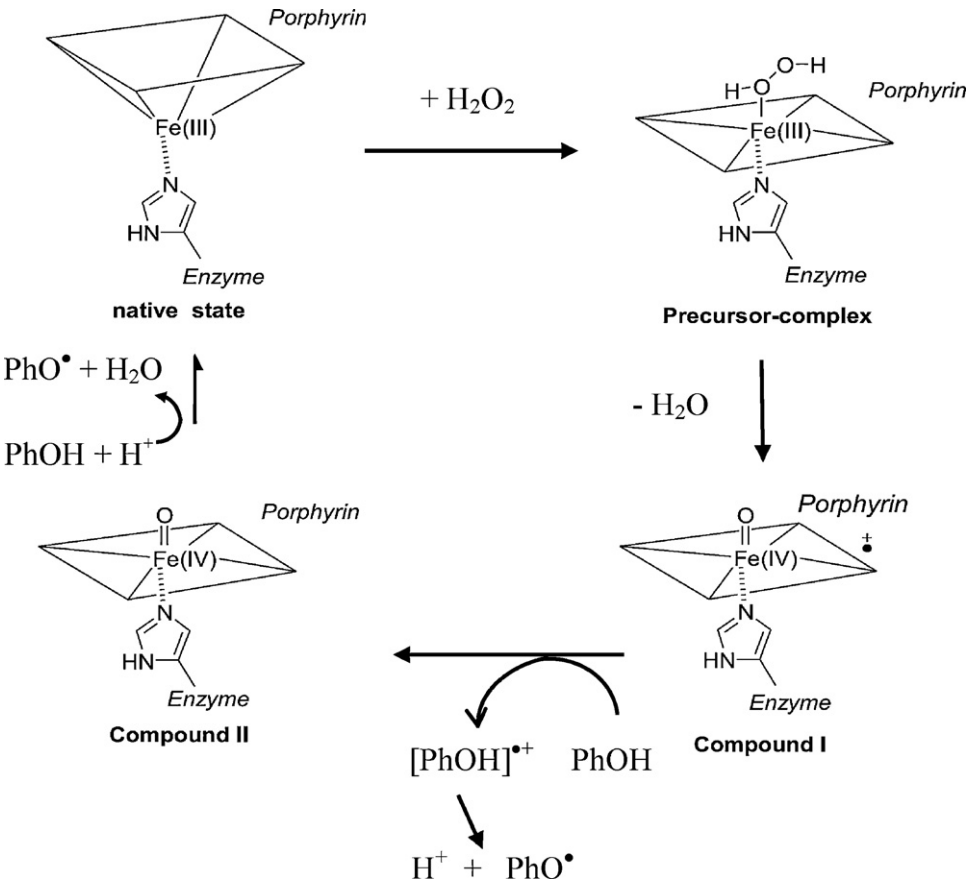


Fig. 6. Catalytic cycle of HRP.
With kind permission from Authors and Springer Science & Business Media: Advances in Polymer Sciences, Synthesis of Phenol Polymers Using Peroxidases, 194, 2006, page 8, Matthias Reihmann and Helmut Ritter, Scheme 2 and any original (first) copyright notice displayed with material.

At pH 7, the reduction of Compound I to Compound II was accompanied by the protonation of the enzyme. The one-electron oxidation of phenols was linked to the deprotonation of its radical cations, thereby yielding phenoxyl radicals [91].

The mechanism of phenol oxidation by HRP is called irreversible ping-pong mechanism [92]. Details of the formation of Compounds I and II by HRP are shown in Figs. 7 and 8, respectively. The enzyme first abstracts one proton from the hydrogen peroxide to the His–42. The peroxide coordinates side-on to the 5-fold Fe^{3+}. A heterolytic cleavage of the O–O bond is induced by the distal His–42 and the proximal His–170. The cleavage of the O–O bond liberates water as the leaving group, and later Compound I is formed. The formation of Compound I is sometimes parallel to the oxidation of an amino acid residue, instead of the porphyrin structure. Arg 38 residue helps with the formation and release of a water molecule needed for Compound I formation.

When Compound II is formed, the proton of the phenoxy radical is abstracted by a base. It is probable that the base is a distal His–42. This species oxidizes the second phenol molecule. The phenoxy radical is formed by one-electron transfer to the Fe^{IV}, and the native enzyme is regenerated. The catalytic cycle requires the formation of a second water molecule that remains coordinated to Fe^{III}.

In terms of pH profile, the pK_a of Compound I is 4.9 and can react in its alkaline or acidic form, whereas Compound II has a pK_a of 8.6 and for pH values higher than 9 Compound II looses reactivity. When the pH of the peroxidase solution is changed from 7 to 12, the Fe atom in the iron complex in the enzyme changes from a high spin Fe to a low spin Fe. It is now known that the sixth coordination position of iron is vacant in the native form and occupied by a hydroxide ion at high pH, the pK for the alkaline transition being in the 10.8–11.1 range [91].

Direct reduction of Compound I to Compound II by p-cresol shows that it is a one-electron reaction [91]. The phenol donates both a single electron and a single proton to Compound I to yield Compound II. Hydrogen atom donation by p-cresol results in the formation of a free radical that may dimerize to form a biphenol, or it may form Pummerer's ketone. In addition, it has been observed that one molar equivalent of phenol can convert Compound I to the native enzyme. The pH profile of Compound I reaction with p-cresol shows that plots of log k versus pH are linear up to pH 6. From pH 7 to 9 a different slope is observed, with a downward curvature beyond pH 9. Thus, Compound I reacts with the non-ionized form of p-cresol since it has a pK_a value of 10.01 [92].

Besides Compounds I and II, another species denoted as Compound III can also be formed under certain reaction conditions. Compound III has two different proposed structures: H^+-Fe^{II}-O_2 or H^+-Fe^{III}-$O_2{}^-$. The consensus is that the structure is mainly a ferric–superoxide complex. In contrast with dioxygen-carrying proteins, peroxidases are Fe^{3+} hemoproteins in their resting state, their reduction to the Fe^{II} state being rather difficult. However, they are very reactive towards hydrogen peroxide, much more than myoglobin is. An inactive species that is formed in excess of hydrogen peroxide is the compound called P670 or verdoheme. In the case of heme, verdoheme is considered to be formed from Compound I. At low H_2O_2 concentrations (below 1.0 mM in the absence of phenol), inactivation is regarded as reversible and attributed to the formation and accumulation of the catalytically inert Compound III. As H_2O_2 concentrations increase, an

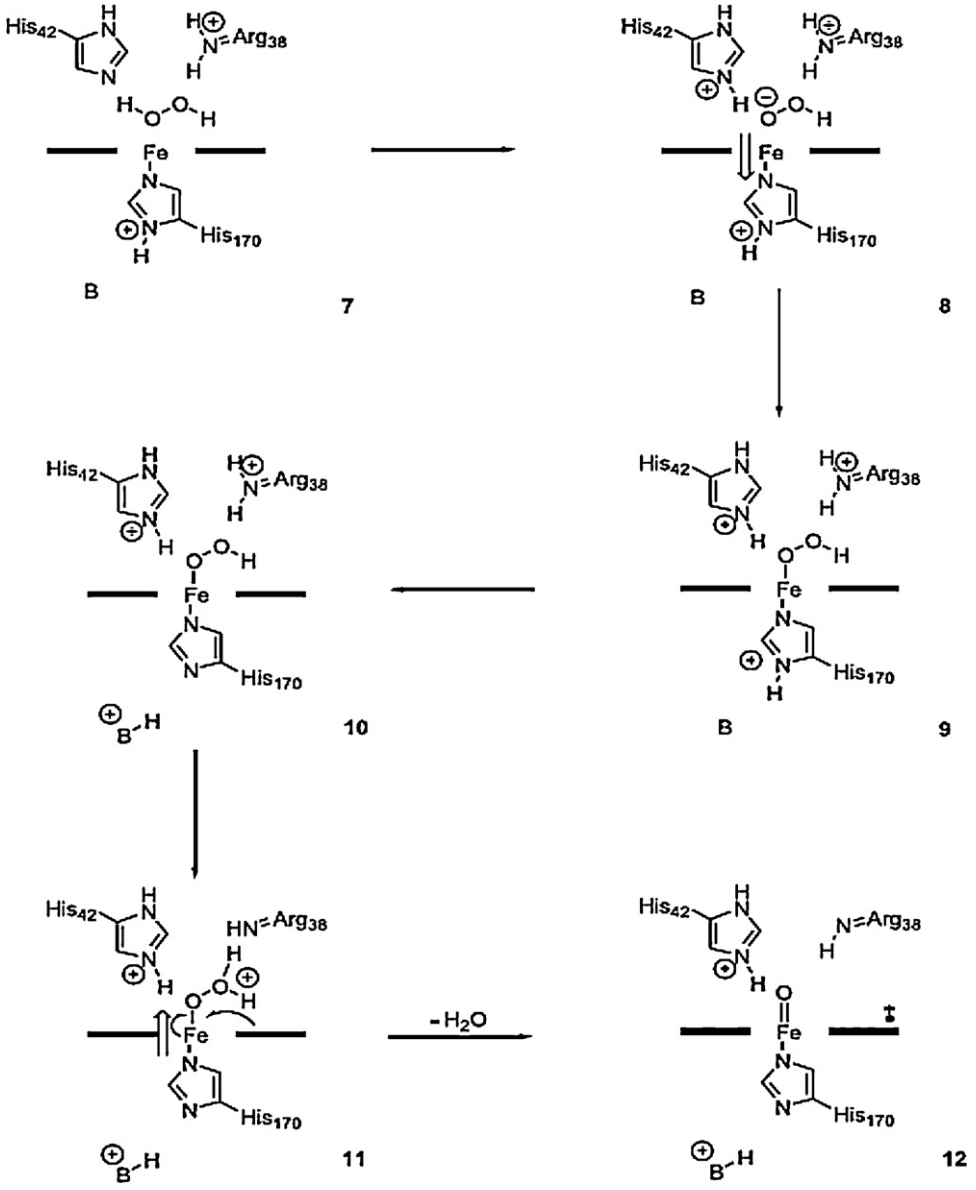


Fig. 7. Mechanism of Compound I formation.
With kind permission from Authors and Springer Science & Business Media: Advances in Polymer Sciences, Synthesis of Phenol Polymers Using Peroxidases, 194, 2006, page 9, Matthias Reihmann and Helmut Ritter, Scheme 3 and any original (first) copyright notice displayed with material. Numbers only show sequence of structures.

irreversible inactivation process becomes predominant [93]. The formation of P670 is the major inactivation process in which H_2O_2 acts as a suicide substrate in the HRP/H_2O_2 system. The mechanisms are still not completely understood. It is supposed that the P670 compound is formed through two different intermediates called 965 and 940 compounds. One mole of CO is evolved per mole of the verdohemoprotein formed from HRP [94].

There are other substrates (e.g. indoleacetate) that have provided insights on peroxidase reactions and mechanisms. Ferrous peroxidase is difficult to obtain, requiring a strong reducing agent and anaerobic conditions [91].

Table 3 shows a comparison between a ferryl-based mechanism and the mechanism generally accepted for HRP. It is accepted that the phenoxyl radical is generated by secondary reactions of the species resulting from the reaction of iron with hydrogen peroxide whatever the mechanism of iron/H_2O_2 is assumed. In this sense, the comparison to do is between Table 3 for HRP and Table 1 for Fenton system.

2.3.2. Phenol transformation by HRP/H_2O_2

2.3.2.1. Efficiency of enzymatic treatments. Polyphenol has been prepared using HRP/H_2O_2, and its structure was found to be a mixture of phenylene and oxyphenylene units [95]. It has been reported that the residual toxicity of HRP-treated phenolic wastewaters can be very high [96,97]. This toxicity depends on the kind of substrate, the reaction conditions (addition mode of HRP and hydrogen peroxide), and the use or not of additives (chitosan and polyethylenglycol-PEG). Phenolic solutions treated with HRP/H_2O_2, yielded near 95% phenol removal within 3 h through the formation of radicals (see Fig. 9). Intermediate dimers formed by radical coupling further reacted to produce polymers. Therefore, depending on the experimental conditions, the treatment of phenol contaminated wastewaters with HRP basically generates dimers, oligomers and polymers through radical coupling reactions. Toxic compounds were formed during the treatment of aqueous solutions of phenol, 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol and 2-methylphenol. The

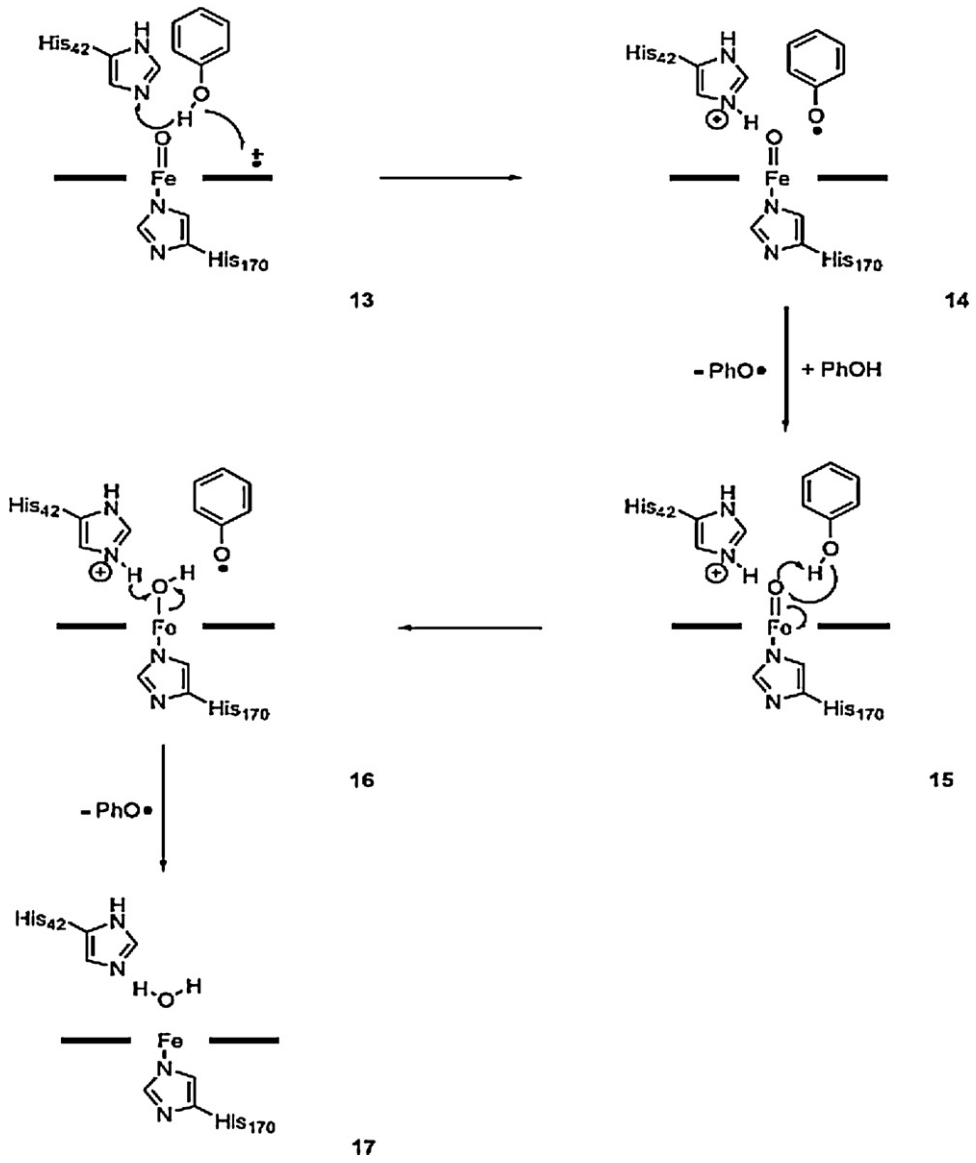


Fig. 8. Mechanism of Compound II formation and phenol oxidation.
With kind permission from Authors and Springer Science & Business Media: Advances in Polymer Sciences, Synthesis of Phenol Polymers Using Peroxidases, 194, 2006, page 11, Matthias Reihmann and Helmut Ritter, Scheme 4 and any original (first) copyright notice displayed with material. Numbers only show sequence of structures.

toxicities of HRP-treated solutions decreased within 21 h, though it remained high in the case of 2-methylphenol.

Ulson de Souza et al. [97] and Nicell et al. [98,99] have published several manuscripts on dye degradation and phenol polymerization using HRP and additives. Residual toxicity levels of HRP-treated phenol solutions correlated well with the UV/Visible spectra of these solutions, in particular with the absorbance at 400 nm. This wavelength matched the position of the quinone absorbance peaks. With the adequate selection of solvent composition and buffer pH, the structure and molecular weight of the produced polyphenol could be controlled. In wastewater treatment, the goal, if possible, is the production of the most insoluble material, with the highest molecular weight and the lowest solubility under the working conditions [100]. The use of HRP for the treatment of phenolic compounds is, compared to other conventional methods, efficient and not expensive [101]. Considerable efforts have been devoted at optimizing the HRP-catalyzed removal of phenols from aqueous solutions with no residual toxicity. An extension in the useful life of HRP has been achieved through the selection of an appropriate reactor configuration, enzyme immobilization and the use of additives. Additives such as sodium borate, gelatin, talc, NaY and PEG protect the enzyme from entrapment in the precipitating polymers, or from inhibition by oxidation products. A review of these and other results can be found in the work by Hamid and Khalil [102].

Immobilization offers some protective effect against inactivation of HRP [103]. Dalal and Gupta [104] used supported HRP enzyme immobilized by bio-affinity layering to convert the phenol into free radicals. The biocatalyst was used for 10 min, then it was removed and polymerization was allowed to continue in the absence of enzyme. By using this approach, immobilized HRP could bring about complete conversion of p-chlorophenol from synthetic wastewater. Bio-affinity-layered HRP preparation (1 IU ml/l) could be used five times successfully, with 100% conversion of pentachlorophenol.

Cheng et al. [105] observed that PEG improved the efficiency of phenol removal by forming a protective layer in the vicinity of the active center of HRP. PEG has a higher affinity to the polymer product than to the enzyme. Similar results were reported for the effect of PEG on HRP by Nazari et al. [106].

Table 3
Hematin mechanism versus HRP mechanism.

HRP mechanism – Dunford	Fenton mechanism ferryl-based for radical formation from AH_2
NativeHRP + H_2O_2 → HRP * H_2O_2 → Compound I + H_2O $k = 10^7$	$Fe^{2+} + H_2O_2 \leftrightarrow Fe^{2+} * H_2O_2 \rightarrow FeO^{2+} + H_2O$ slow heterolytic
Compound I + AH_2 → Compound II + AH•	$Fe = O^{2+} + AH_2 \rightarrow FeOH^{3+} + AH^{\bullet}$? Not probable
Compound II + AH_2 → NativeHRP + AH• + H_2O	$FeOH^{3+} + AH_2 \rightarrow Fe^{2+} + H_2O + AH^{\bullet}$? Not probable
2AH• → AH–AH	See Table 1

Gómez et al.	Hematin mechanism – H_2O_2 decomposition (Tappel, Bell)
Compound I + AH–AH → Compound II + AH–A•	Hematin(Fe^{III}) + HOOH → Hematin(Fe^{II}) + HOO• + H^+ heterolytic, favored in presence of alcohols
Compound II + AH – AH → NativeHRP + AH – A•	Hematin(Fe^{III}) + HOOH → Hematin(Fe^{III}) + HO• + HO•
	Hemolytic – less probable with organic substrates present
2AH–A• → AH–A–A–AH	Hematin(Fe^{III}) + HOOH → Hematin(Fe^{III}) * HO• + HO•
	Hematin(Fe^{III}) + HOOH → Hematin(Fe^{III}) + HOO• + H_2O
	Hematin(Fe^{II}) + O_2 → Hematin(Fe^{III}) + O_2^-

Dunford et al. secondary reactions	Hematin mechanism (Akkara, Bell, Descombes)-phenoxyl radical formation
HRP * H_2O_2 → Compound I	
Compound I + H_2O_2 → P965	Hematin(Fe^{III}) + HOOH → Hematin(Fe^{IV})O• + H_2O
P965 + H_2O_2 → P940 → verdohemoprotein (P6790) + CO	
Compound II + H_2O_2 → Compound III + H_2O	Hematin(Fe^{IV})O• + HOOH → Hematin(Fe^{IV})OH + HOO•
Compound III → NativeHRP + $O_2^{\bullet -}$ + H^+	Hematin(Fe^{IV})OH + HOOH → Hematin(Fe^{III}) + H_2O + HOO•
Compound III + PhOH → Compound I + PhO• + H_2O	Instead of HOOH, the H donor may be PhOH
	Hematin(Fe^{III}) + HOO• → Hematin(Fe^{II}) + H_2 + O_2
	Hematin(Fe^{III}) + $O_2^{\bullet -}$ → Hematin(Fe^{II}) + O_2
	Hematin(Fe^{II}) + $O_2^{\bullet -}$ + $2H^+$ → Hematin(Fe^{III}) + H_2O_2
	2Hematin(Fe^{III}) → Hematin(Fe^{III})–O–Hematin(Fe^{III}) + H_2O At alkaline pH
	Hematin(Fe^{III}) + H_2O_2 → Hematin(Fe^{III})OH–OH
	Hematin(Fe^{III})OH–OH + H_2O_2 → Hematin•(Fe^{IV})O + H_2O + O_2
	Hematin•(Fe^{IV})O + PhOH → Hematin(Fe^{IV})OH + PhO•
	Hematin(Fe^{IV})OH + PhOH → Hematin(Fe^{III})OH_2 + PhO•
	Hematin(Fe^{III})OH_2 → Hematin(Fe^{III}) + H_2O

2.3.2.2. Some mechanistic aspects. Akkara et al. [107] performed structural studies of poly(4-phenylphenol) prepared by HRP catalysis. Using FT-IR and CP/MAS (cross polarization magic angle spinning) ^{13}C NMR data, it was concluded that the major linkage was the C–C coupling through the *o*-positions. From the possible radicals formed, it is clear that the linkage through the *orto-para* positions was favored.

The generation of species present in the polyphenol is related to the following sequence of reactions [108]:

$$ROO^{\bullet} + AH_2 \rightarrow ROOH + AH^{\bullet} \quad (32)$$

$$ROO^{\bullet} + AH^{\bullet} \rightarrow ROO^{-} + AH^{\bullet +} \quad (33)$$

$$AH^{\bullet +} \leftrightarrow A^{\bullet} + H^{+} \quad (34)$$

The organic radicals may spontaneously react to form oligomers and polymers.

$$2AH^{\bullet} \rightarrow AH{-}AH \quad (35)$$

$$2AH{-}AH \xrightarrow{H_2O_2/HRP} 2AH{-}A^{\bullet} + 2H_2O \quad (36)$$

$$2AH{-}A^{\bullet} \rightarrow AH{-}A{-}A{-}AH \quad (37)$$

$$AH^{\bullet} + AH{-}A^{\bullet} \rightarrow AH{-}A{-}AH \quad (38)$$

The generation of the phenoxyl radical (AH•) and the phenoxyl radical cation ($AH^{\bullet +}$) explains the final polyphenol structure. The phenoxyl radical cation is strongly acidic and reactive, and it readily undergoes deprotonation, being its pK_a −5 [109].

Peroxidase is inactivated by free radicals and also by oligomeric and polymeric products formed in the reaction mixture, which attach to the enzyme and inactivate it [110]. In addition, it has been reported that the radicals formed using immobilized HRP remain in the surface or near the enzyme, increasing the rate of inactivation reactions due to protein denaturation by radical attacks [111]. Metal ions can coordinate to the oxidative site residues, leading to enzyme activation. Nazari et al. [106] reported a mechanism to prevent and control the suicide-peroxide inactivation of HRP by means of the use of Ni^{2+} ion. The addition of the latter ion was found to be useful in phenol removal and peroxidatic conversion of reducing substrates. The use of gels based on, for example, alginates, seems promising in terms of activity and reusability for phenol conversion at short times (approximately 1 h), but substantial improvements are still needed [112].

2.4. Homogeneous and heterogeneous biomimetic systems

Biomimetics of HRP based on iron have been studied for several years. Peroxidases are hemoenzymes that contain a ferriheme moiety. The Ferriheme is the Fe^{3+} complex of protoporphyrin IX. There are several problems related to the use of ferriheme as a biomimetic, and for this reason other metalloporphyrins have been explored. Stability, costs, separation and recovery, as well as the need of mediators to be active are the main issues to consider with metalloporphines. Therefore, many studies focus on the immobilization of metalloporphyrins. The coordination between properly featured solid supports and the central metal ions complexes within porphyrin macrocycles seems to be the most promising approach. Different biomimetic immobilized systems have been used as versatile peroxidase-like catalysts (e.g. Fe-5,10,15,20-tetrakis(pentafluorophenyl)porphine supported on pyridyl-functionalized, crosslinked polyvinyl alcohol [113] or imine complexes of iron such as 2,6 bis[1-2,6 diisopropylphenylimino-ethyl]pyridine Fechloride (FeB)) [114]).

Iron porphyrins, phtalocyanins and salen complexes (salen=N,N′-bis (salicylidene) ethane-1,2-diaminato) have also been explored as catalysts for the removal of phenol and its derivatives using hydrogen peroxide as oxidant [108,115,116]. Studies carried out with chlorinated phenols showed that, when the number of chlorine atoms on the chlorophenols decreased, the yield of the corresponding quinone also decreased and the number

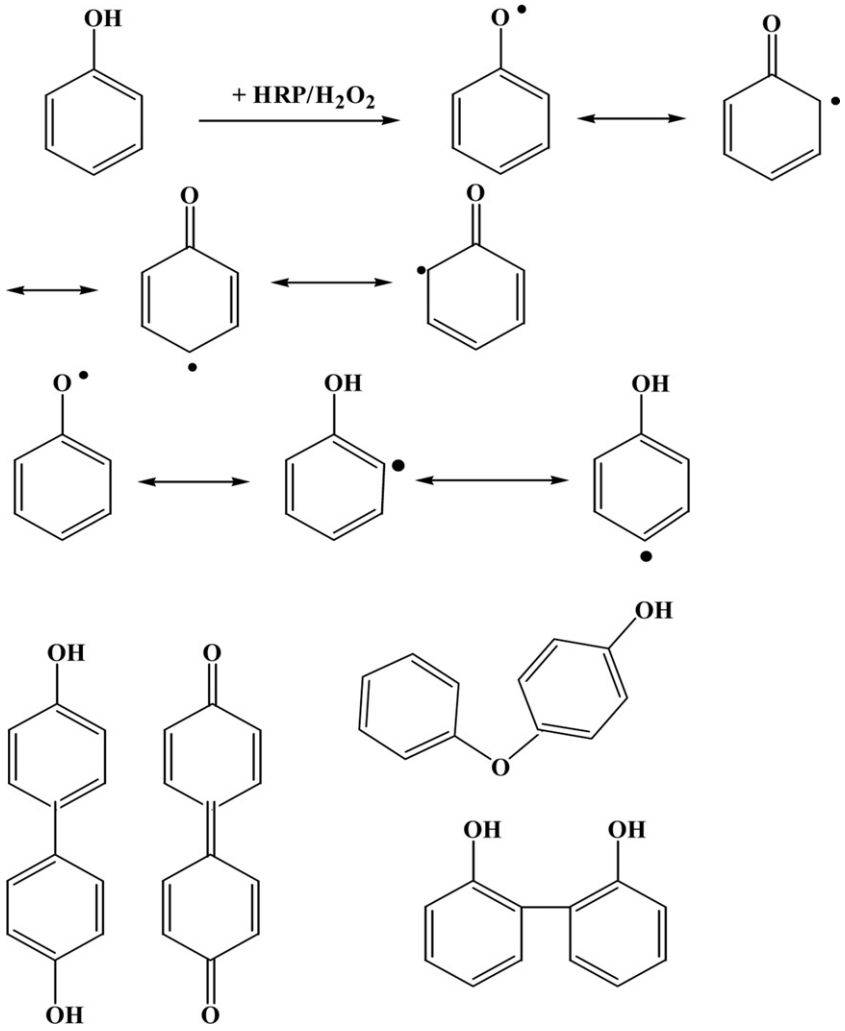


Fig. 9. Radicals (and their resonant forms) formed in the phenol oxidation using HRP/H_2O_2 and their main coupling products.

of detected products increased. This suggests that substrates containing additional C–Cl bonds could prevent polymerization reactions, which inhibit further degradation [115].

The water-soluble iron sulfoporphyrins and sulfophthalocyanines were found to be active for the oxidations of chlorophenols to chloroquinones in the presence of potassium monopersulfate. In general, potassium monopersulfate is obtained as a double salt called "Oxone". Several articles deal with potassium monopersulfate oxidation of chlorophenols using iron sulfoporphyrins and sulfophthalocyanines with different additives, such as humic acids [117–121].

Hemmert et al. proposed a model where the oxidation of the non-heme iron led to the formation of Fe^{IV}=O by one-electron oxidation of the iron ligand bis-di-2-pyridyl methyl amine (BDPMA). They demonstrated that neither the perferryl (through Fe^{V}=O) nor the hydroxyperoxy (Fe^{III}–OOH) routes were adequate to explain their results. They proposed two major pathways: one leading to benzoquinones, and the other one producing dimers through C–O or C–C coupling reactions [115]. Kamp and Lindsay Smith [122] reported a kinetic study of phenol oxidation in aqueous solution by a complex with **T**etra(2-N**M**ethyl**py**ridyl)**P**orphyrin OFe^{IV} (**T2MpyP**). A Hammett correlation analysis of the kinetic data showed that this analogue of Compound II of HRP oxidized phenols by hydrogen atom abstraction, in contrast with the enzyme where oxidation occured by electron-transfer [123].

It has been shown that hydrogen peroxide reversibly coordinates to the Fe porphyrin cation [124,125]. Bruice and coworkers proposed that the oxygen–oxygen bond of the coordinated hydrogen peroxide undergoes homolytic cleavage to produce a hydroxyl radical and one-electron oxidized Fe^{IV} porphyrin species [126,127]. Traylor and Xu proposed that acid-catalyzed heterolytic cleavage of the oxygen–oxygen bond produces an equivalent of water and a two-electron oxidized Fe^{IV} pi-radical cation species [124,125]. More recently, Nam and Han reported evidence indicating that both heterolytic and homolytic cleavages can occur simultaneously, and that the partitioning between the two pathways depends upon the composition of the porphyrin catalyst, the axial ligand, and the oxidant [128] (see Fig. 10). The one-electron oxidized Fe^{IV} species was shown to contribute exclusively to peroxide decomposition [129]. Hydroxyl and hydroperoxyl radicals are involved in porphyrin degradation [130] and the production of dioxygen [2,131].

Hydrogen peroxide coordinates to Fe in HRP more readily when it is more Lewis acidic and when intermolecular interactions between the solvent and hydrogen peroxide are minimized. Increasing the electron-withdrawing ability of the porphyrin ligand or the presence of an axial ligand promote the heterolytic cleavage relative to homolytic cleavage of the oxygen–oxygen bond of the hydrogen peroxide. In addition, the rate of heterolytic cleavage increases with the concentration and acidity of the protic solvent [126].

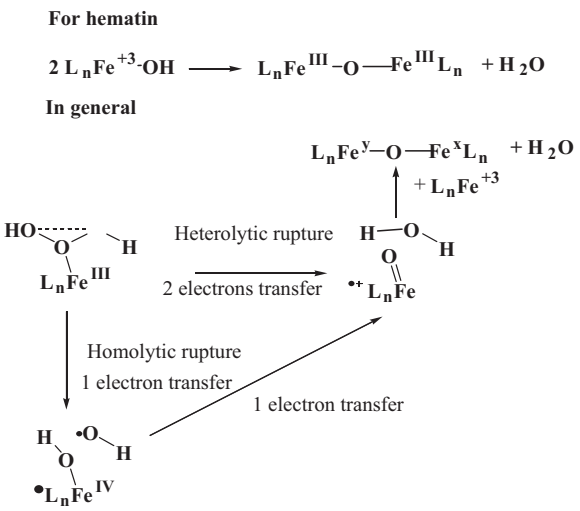


Fig. 10. Heterolysis and homolysis of peroxides in chelated iron-dimerization.

Porphyrin degradation is minimized by increasing the reaction rate and selectivity towards heterolytic cleavage. Supported and/or immobilized porphyrins are other important efforts for preventing the self-degradation. The degradation can also be reduced by halogenation of the phenyl groups attached to the porphyrin ring [132,133]. The formation of oxo-dimers is more favorable for electron-poor porphyrins, but *ortho*-fluoro and *ortho*-chloro substituents offer steric protection that precludes the formation of dimers under the reaction conditions tested [130]. Hemin catalysts are considered to suffer dimerization when, as in the case of hematin, a hydroxyl group is attached [134]. This dimerization is not allowed for the iron atom of HRP. The reaction rate of HRP is several orders of magnitude higher than that of hemin, pointing to a clear effect of the protein on the iron ability to activate H_2O_2.

The reaction of porphyrin with a base has been proposed by Bruice and Balasubramanian [126] (X is a ligand on Fe):

$$(\text{Porph})\text{Fe}^{\text{III}}(\text{X})(\text{H}_2\text{O}_2)+ : \text{B} \rightarrow (\text{Porph})\text{Fe}^{\text{IV}}(\text{X})(\text{O}) + \text{HO}^{\bullet} + \text{BH} \quad (39)$$

$$(\text{Porph})\text{Fe}^{\text{IV}}(\text{X})(\text{O}) + \text{HO}^{\bullet} + \text{BH} \rightarrow (''\text{Porph})\text{Fe}^{\text{IV}}(\text{X})(\text{O}) + \text{H}_2\text{O} + \text{B} : \quad (40)$$

It has been proposed that the biomimetic could be generated *in situ* through the use of metallic iron and EDTA in the presence of H_2O_2.

According to Bruice and Balasubramanian [126] Hematin catalyzes the decomposition of organic hydroperoxides via the same reactions reported for transition metal ions:

$$\text{Hematin}(\text{Fe}^{\text{III}}) + \text{ROOH} \rightarrow \text{Hematin}(\text{Fe}^{\text{II}}) + \text{ROO}^{\bullet} + \text{H}^{+} \quad (41)$$

On the other hand Tappel [135] proposed a scheme in which no change in the iron valence occurs, but that involves the homolytic scission of the oxygen–oxygen bond of the ROOH molecule that forms the alkoxy radical and hematin-bound hydroxyl radical:

$$\text{Hematin}(\text{Fe}^{\text{III}}) + \text{ROOH} \rightarrow \text{Hematin}(\text{Fe}^{\text{III}}) - \text{HO}^{\bullet} + \text{RO}^{\bullet} \quad (42)$$

$$\text{Hematin}(\text{Fe}^{\text{III}}) - \text{HO}^{\bullet} + \text{HOOH} \rightarrow \text{Hematin}(\text{Fe}^{\text{III}}) + \text{HOO}^{\bullet} + \text{H}_2\text{O} \quad (43)$$

The global reaction is:

$$\text{HOOH} + \text{ROOH} \rightarrow \text{RO}^{\bullet} + \text{HOO}^{\bullet} + \text{H}_2\text{O} \quad (44)$$

Among the major reactions reported for hematin, there are reactions involving the consumption of O_2, and reactions involving hydrogen peroxide as substrate and different kinds of organic peroxides. The following reaction is related to oxygen consumption:

$$\text{Hematin}(\text{Fe}^{\text{II}}) + \text{OO} \rightarrow \text{Hematin}(\text{Fe}^{\text{III}}) + \text{O}_2^{-} \quad (45)$$

Another possibility for hydrogen peroxide consumption would involve the following reaction sequence:

$$\text{HOOH} + \text{porph}(\text{Fe}^{\text{III}}) \rightarrow \text{HOO}^{\bullet} + \text{porph}(\text{Fe}^{\text{II}}) + \text{H}^{+} \quad (46)$$

$$\text{HOOH} + \text{porph}(\text{Fe}^{\text{II}}) \rightarrow \text{HO}^{\bullet} + \text{porph}(\text{Fe}^{\text{III}}) + \text{HO}^{-} \quad (47)$$

Apart from hydrogen peroxide, several organic peroxides have been explored as oxidants as well. Van der Zee and Cervantes [56] showed, using hematin and t-Bu-OOH, that the primary radical is not the peroxyl but the alkoxyl radical in the heme iron/hydroperoxide system. This mechanism was further supported by Bruice and Balasubramanian [126], who investigated the reaction between tert-butyl hydroperoxide and (meso-tetrakis(2,6-dimethyl-3-sulfonatophenyl)-porphinate)-Fe^{3+}, a water soluble and non-μ-oxo-porphyrin. From product analysis and UV/Vis spectroscopy studies, they concluded that the primary species in this reaction was most likely the alkoxyl radical.

There are few reports on hematin, but some of the selected ones propose a mechanism similar to that of HRP [114,136], whereas other report a mechanism like the one presented by Bruice and Balasubramanian [126] and worse, the work on mechanisms is scarce [137–140]. Besides hematin, other iron complexes such as FeB have been studied by our group. The main difference is the iron oxidation status: Fe^{3+} in the case of hematin, and Fe^{2+} in the case of FeB. Since a major drawback in the latter system is the lack of solubility of FeB in water, a proper immobilization is required in order to be applied to aqueous phenol treatment.

In the field of biomimetics, TAML (for *t*etra*a*mido *m*acrocyclic *l*igand) activators, developed by the Green Oxidation Chemistry (SA), are becoming extensively patented worldwide. The search for hydrolysis-resistant biomimetic systems is continuous, and research on the topic is especially active [140].

2.5. Comparative discussion

Table 3 shows the comparison of the mechanism accepted for HRP with that proposed for hematin. From the comparison, it seems that in the case of hematin, depending on the conditions, the chelated iron may produce radicals from the reaction with H_2O_2 and also generate phenoxyl radical through an HRP-like mechanism.

The overall picture in PhOH/H_2O_2 solutions with these systems would be after the analysis of this information:

(a) Fenton and Fenton-like systems have mainly non-chelated inorganic iron and the generation of radicals takes place through the reaction with $H_2O_2^{-}$ not necessarily through hydroxyl radicals that attack PhOH to generate phenoxyl radicals. Hydroxyl radicals may be generated later by the secondary reactions of phenoxyl radicals. Radicals and anions may be coordinated to the iron species.
(b) HRP attacks phenol through a three steps mechanism – involving H_2O_2 and PhOH – to generate phenoxyl radicals and water.
(c) Hematin, a hemin compound whose mechanism of action seems similar to that of HRP, but at lower rate of phenoxyl radical generation than in the HRP case. The homolytic rupture of H_2O_2 is also feasible due to the presence of the OH in hematin and then the generation of the hydroxyl radical may occur, especially in absence of phenol. When phenol is present, the heterolytic rupture of H_2O_2 would be favored.

Following the published literature on the topic of porphyrins reactions, it can be stated that in the presence of hemin the main H_2O_2 rupture would be heterolytic. Differences would arise in secondary reactions of porphyrin group with the generated radicals and the subsequent porphyrin degradation and the formation of oxo-dimers in the hematin versus HRP. The heme loss at low phenol/H_2O_2 concentration is predominantly caused by a radical attack in the case of HRP [141].

The catalase activity of HRP is likely to be influenced by bound water molecules in the active site. It has been suggested by Jones [142] that the absence or the presence of a water in the active site determines whether compound I reacts in a one-electron reduction process (which is the normal reaction for peroxidases) or in a two-electron reaction (as catalases do). In the case of hematin there is no "protection" for the coordination of further H_2O_2 and the catalase-like reaction would be an important competence for the peroxidatic reaction [94,143].

Hematin exists in aqueous solutions as a mixture of dimmers and monomers [114,136,144]. HRP is present as enzyme aggregates of several HRP molecules, such as it happens with proteins in aqueous solution. Other ions and species present in these solutions would affect coordination of iron, reactivity and steric hindrance to displacement of different ligands to coordinate H_2O_2 [91(a)].

3. Removal mechanisms of selected phenolic derivatives

3.1. General

A wide variety of phenolic compounds have been used as substrates for peroxidases. Among the compounds tested as model substrates are hydroxy-anthraquinones. The results of Arrieta-Báez et al. [137] suggest that horseradish and the *Senna angustifolia* peroxidases efficiently oxidize alizarin and purpurin (1,2-dihydroxy anthraquinones) to produce the respective bianthraquinones. Peroxidase synthesizes bi-anthraquinones from anthracenones or anthraquinones. Considering phenol derivatives, 1-napthol was one of the best characterized in terms of its mechanism of polymerizatoin with HRP/H_2O_2. Regarding naphtol degradation pathways, it was possible to detect several naphtol polymerization products (NPP). According to this model, 1-naphthol is initially transformed to free radicals or naphthoquinones by HRP. These reactive intermediates may undergo self-coupling with each other, or participate in cross-coupling reactions with previously generated polymerization products through C–C and C–O bond formation. Their results suggest that naphtol polymerization occurs preferentially through C–C bonding, which results in the production of oligomers with intact –OH groups. It is probable that several of these OH-containing oligomers retain a high degree of polarity. Unlike phenol polymerization products, which are predominantly less polar than the parent solute, naphtol polymerization resulted in the production of some NPP species that were more polar than 1-naphthol [138].

In addition, there are several studies related to the application of HRP in the removal of azo-dyes that include phenolic groups in their structure [139]. Since the present review is mainly focused in the mechanistic aspects rather than in experimental results, these studies will be only briefly discussed here.

3.2. Removal of phenolic dyes and pigments

Research on the mechanisms of dye removal using HRP published in the open literature is scarce. Direct Blue 54 and Direct Red 31 are sulfonic salts of complex azo dyes with multiple substituents (OH and NH_2). It was reported that the concentrations of H_2O_2 and Fe, pH, temperature and reaction time are the five leading factors affecting the extent of degradation [138,139]. The dyes are mineralized by Fenton's reagent [145]. Other published studies used Amido black 10B dye as substrate [146]. Our group recently studied the degradation of Alizarin and Eriochrome Blue Black R (EBBR) using free HRP or hematin in the presence of H_2O_2 [147,148]. The removal of synthetic dyes from wastewaters has been reviewed by Forgacs et al. [149].

3.2.1. Antraquinone dyes

Pirillo et al. [147] studied the degradation of alizarin in HRP/H_2O_2 and hematin/H_2O_2 systems using high [H_2O_2]. The analysis of UV/Vis spectra showed an efficient alizarin removal for both systems. However, in the presence of HRP the processes were much faster and the efficiencies somewhat higher than those observed in the presence of hematin. The profiles of dissolved oxygen concentration showed that alizarin removal in hematin/H_2O_2 systems involved O_2 consumption. In contrast, alizarin removal in HRP/H_2O_2 systems involved evolution of O_2. This was mainly associated with a catalase-like activity of HRP.

3.2.2. Azo dyes

Pirillo et al. [148] compared the behavior of Eriochrome Blue black R (EBBR) in HRP/H_2O_2 and Hematin/H_2O_2 systems. The comparison of UV/Vis spectra showed noticeable differences in the decolorization mechanism since, depending on the catalyst used, batochromic or hypsochromic shifts were observed for the spectral evolution of the reaction mixtures. An important increase of absorption around 760 nm was observed in the presence of hematin. This was attributed to the formation of compounds with a higher degree of conjugation. On the other hand, the main dye band of EBBR practically disappeared in the presence of peroxidase, and a new band appeared at 390 nm.

In addition, the profiles of dissolved oxygen concentration showed substantial differences during the first reaction stages, since O_2 was consumed in the presence of hematin but released in the presence of HRP. The consumption of oxygen during EBBR degradation in the presence of hematin was explained by taking into account the formation of organic peroxyl radicals during the initial steps of oxidative degradation, whereas the release of molecular oxygen in the presence of HRP was ascribed to the high catalase-like activity of peroxidase.

The differences observed between peroxidase and hematin catalyzed systems suggest different reaction pathways during the initial degradation stages of each catalyst: condensation reactions in the presence of hematin, and degradation in the presence of peroxidase.

3.3. Lignin removal

Lignin is a polymeric structure of the monomers p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. The reactivity and mechanism of catalytic lignin degradation are dependent both on the type of lignin bonds within monomers and on the topology of the reaction media. In native lignin, 50–70% of linkages are 8–O–4 bonds, whereas in synthetic lignin (*in vitro* polymerization of coniferyl alcohol units by HRP/H_2O_2) 8–8 and 8–5 linkages prevail [150]. Moreover, kraft lignin is highly modified with respect to native lignin, exhibiting a significant amount of 5–5 linkages and diphenylmethane substructures [16]. Therefore, the differences in the kinetic behavior observed between *in situ* and *in vitro* lignin degradation result from the fact that the degradation rate depends on several factors that include the physical state of the reaction media, the accessibility of the oxidant to lignin structures, and the availability of reactive sites [16,151].

The published literature on lignin degradation using HRP, iron-based biomimetics or Fenton systems is rather scarce. One of the reasons is the structural complexity of lignin. Fenton reagent's was effective in the degradation of lignin [152], residual black

liquor [153,154], and steam lignin [155]. High concentrations of H_2O_2 were required to achieve lignin degradation, whereas polymerization occurred at lower oxidant concentrations [153]. Fenton-produced $^{\bullet}OH$ radicals are involved in the lignin degradation by white-rot fungi (WRF). With these WRF a quinone redox cycle generates superoxide radicals, which reduce Fe^{3+} to Fe^{2+}, and this ion reacts with extracellular-produced H_2O_2 in a Fenton fashion [156]. In addition, Fenton-based treatment readily opened the structure of the ligno-cellulosic matrix in spruce wood, releasing cellulose fibrils from the matrix. Limited demethoxylation and side chain oxidation also occurred [157]. As reported by [158], the oxidation of a dimeric lignin model compound by Fenton's reagent was unspecific for the erythro or threo forms, in contrast with preferential degradation of the threo form by lignolytic enzymes. The efficiency of Fenton degradation of lignin and lignin model compounds was dramatically enhanced by the use of dihydroxybenzenes and cathecolate chelators (chelator-mediated Fenton reactions, CMFR) [159,160]. Dihydroxybenzenes chelate and reduce Fe^{3+} to Fe^{2+}, increasing its reactivity. Furthermore, these complexes probably participate in $^{\bullet}OH$ radical production [161]. This non-enzymatic process occurs in wood degradation by brown-rot fungi. Heterogeneous Fenton systems (FeZSM-5 zeolite) showed a much lower lignin oxidation rate, probably due to steric hindrance on the catalytic surface [152].

It was reported that peroxidase prefers low molecular weight substrates [82], however in the absence of phenols the enzyme catalyzes lignin oxidation [162]. HRP seems to display a ping-pong mechanism with lignin and lignin model compounds [163]. Although internal rearrangements of the lignin substrate may occur, the HRP/H_2O_2 system does not depolymerize lignin [163,164]. Indeed, preferential degradation of 8–5 and 8–1 lignin dimeric structures occurs instead of the degradation of 5–5 and 4–O–5 lignin substructures [164]. A number of reports deal with polymerization and copolymerization of lignin fragments by HRP/H_2O_2 [165–167]. The formation of phenoxy radicals able to participate in coupling reactions was confirmed in all these works. Veratryl alcohol is a typical non-phenolic lignin model compound and a secondary metabolite that acts as a mediator in the ligninase-catalyzed oxidation of lignin. Veratraldehyde and 2-hydroxymethyl-5-methoxy-2,5-cyclohexadiene-1,4-dione were the two major oxidation products when veratryl alcohol was treated by HRP/H_2O_2 in ionic liquids [168]. HRP exhibited a higher activity in ionic liquids than in aqueous medium, and this was attributed to the stabilization of the high-valent oxoiron (IV) π-cation radical (intermediate in the HRP mechanism) generated in the reaction.

Joo et al. [169] achieved de-polymerization of a polyphenol by HRP, which was previously produced by the same enzyme. High H_2O_2 concentration levels in a dioxane/aqueous 50:50 buffer system were required for depolymerization. The involvement of HRP in this degradation process was confirmed. It was proposed that HRP-Compound III is the active species in the degradation process due to its appearance under the depolymerization conditions at high H_2O_2 concentrations. Compound III of peroxidase is known as a ferric ion-superoxide complex that exists as a resonance structure: $H^+\text{-}Fe^{III}\text{-}O_2^{\bullet -} \leftrightarrow H^+\text{-}Fe^{II}\text{-}O_2$. There is a fairly general agreement that the ferric ion superoxide complex is the predominant species. The authors concluded that superoxide anions (O_2^{-}) are the main cause of depolymerization, since the reaction to form the native peroxidase from Compound III produces superoxide anions [169]. In addition, Durán et al. [170] also observed that HRP-Compound III was the most reactive intermediate acting on lignin. In fact, the second order rate constant of Compound III formation was determined to be 10-fold higher in 50% dioxane/buffer than in aqueous buffer [169]. This is in agreement with the findings of Dordick et al. [171], who also observed, at high H_2O_2 initial content, depolymerization of synthetic and natural lignin by HRP in dioxane, but not in aqueous solution. The scheme proposed by Joo et al. [169] states that at relatively high H_2O_2 concentrations Compound III can be easily formed via the Compound II* state. Therefore, phenol polymerization is slowed down and de-polymerization occurs under those conditions.

Zakzeski et al. [172] recently reviewed non-enzymatic lignin oxidations, including biomimetic systems with metal ions and oxidants different from iron and hydrogen peroxide, respectively. The oxidation behavior of iron porphyrins on isolated lignin and residual kraft lignin has not been much explored. There are few examples of iron porphyrins applied to the degradation of spruce lignin [151], kraft lignin [16,173] or sodium lignosulfonate [174]. In general, iron porphyrins are able to de-polymerize lignin and mimic the action of ligninolytic enzymes. The reactions involve 8–O–4 bond cleavages, side chain oxidations, ring opening and de-methoxylation reactions. 2-methoxyphenol, 4-hydroxybenzaldehyde, vanillin and vanillic acid were found as end-products [164,173]. On the other hand, re-polymerization may also occur [9,174]. However, coupling reactions were not observed when spruce wood, instead of isolated lignin, was treated. The latter result is probably associated with the immobilization of generated radicals in the cell wall [164]. Mn-porphyrins seemed also to suppress condensation reactions, and were therefore evaluated as being more suitable for lignin degradation [15]. Hemoglobin demonstrated superior selectivity and efficiency for ligno-sulfonate degradation than $FeSO_4$ or Fe-EDTA systems in a pulp model in the presence of cellulose at high H_2O_2 levels [174]. This indicates the involvement of a ligninase-like mechanism rather than an unselective Fenton-like mechanism. Type and position of the substituents in the aromatic ring seemed to play a significant role in the reaction mechanism and the formation of different products. In contrast, iron porphyrins were unable to cleave neither 5–5 byphenyl nor diphenylmethane substructures, which are present in significant amounts in Kraft lignins. Oxidized dimeric structures and p-quinones were formed instead [9]. Immobilized iron porphyrins were found to be active for the oxidation of lignin model compounds, and acted in a similar fashion as HRP [168] or lignin-peroxidases [113]. The activity of tetrakis (pentafluorophenyl) porphine-iron(III) chloride was dramatically enhanced upon addition of Mn^{2+} [113]. However, this approach is not environmentally friendly. Haikarainen et al. [116] obtained oligomeric structures of coniferyl alcohol in the presence of Fe-salen complexes. Racemic mixtures of 8–O–4, 8–5 and 8–8 oligomers were observed in a 1:1:1 proportion. The same regiochemistry was also observed with HRP-catalyzed reaction.

The lignin degrading action of porphyrins can also operate by a different mechanism, the active species being a hydroxyl radical instead of a superoxide radical anion [171,175]. High H_2O_2 levels deactivate metallic porphyrins due to homolytic O–O cleavage and hydroxyl radical generation. Hydroxyl radicals attack the phenolic rings and generate oxidized structures up to mineralization in a Fenton fashion. On the other hand, the aminoacidic conformation around the active site of HRP ensures the heterolytic O–O cleavage of H_2O_2. Axial coordination of the central metal on porphyrins with imidazole or pyridine has demonstrated to favor H_2O_2 heterolytic cleavage, thus protecting HRP activity [113]. Surprisingly, reported iron porphyrins having a degradative action on lignin were not axially coordinated. Data on porphyrins stability have not been reported in the open literature. Effective hydroxyl radical generation was observed during lignin degradation by hemoglobin [174].

4. Conclusions

The homogeneous Fenton reaction generates high amounts of radicals ($HO^{\bullet}$, $HO_2^{\bullet}$, $O_2^{\bullet -}$) that react with phenolic compounds

resulting in substrate degradation as the main reaction pathway. The heterogeneous Fenton systems can be highly effective to degrade phenolic compounds. Mimicking as much as possible the electronics of the homogeneous Fenton reaction improves the activity and stability of immobilized Fenton systems.

The HRP/H_2O_2 system generates a high initial concentration of species such as $AH^{\bullet}$ or $A^{\bullet -}$ depending on the pH of the reaction media and on the substituents in the phenolic moiety. The main non-organic radical involved in many reaction steps of HRP/H_2O_2 systems is $O_2^{\bullet -}$.

With the relatively high concentrations of inorganic radicals usually found in Fenton systems, the main pathway of phenols involves from degradation and phenyl ring opening to mineralization. On the other hand, at the rather high concentrations of organic radicals obtained during the first stages of HRP/H_2O_2 treatment, the preferred pathway is from dimerization to polymerization. At high H_2O_2 concentration, HRP may degrade phenolic compounds of high molecular weight. Biomimetics based on chelated iron share some mechanistic details with HRP, but the relative importance and stability of the structures proposed for Compounds I, II and III are different. Fenton and biomimetic systems seem to work through degradation reactions followed by either mineralization or the formation of strong Fe^{3+} complexes, such as those with certain carboxylic acids. Lignin fragments seem to be polymerized by HRP at typical reaction conditions, whereas lignin polymers are degraded using Fenton reaction or biomimetic catalysts.

The main *in vitro* application of HRP is the generation of phenoxy radicals through HRP-Compound I and HRP-Compound II intermediates. If the superoxide radical is the active species in the HRP-Compound III degrading action, the use of aprotic solvents could favor degradation over polymerization mechanism. HRP suffers from radical attacks and protein denaturation with the formation of compound P670 at high H_2O_2 concentration, whereas Hematin undergoes dimerization and probably porphyrin ring degradation in an oxidizing environment.

Based on the concluding remarks, different approaches are postulated in order to favor the oxidative degradation of a given substrate (monomeric or polymeric) when using HRP or biomimetics as catalysts and H_2O_2 as additive: (a) the control of the proportion of organic to inorganic radicals generated using different initial molar ratios among oxidant, organic compound and catalyst; (b) the modification of the different active species concentrations derived from HRP (or biomimetics) through the oxidant concentration and its feeding strategy in the reaction medium; (c) the selection of a suitable solvent to inhibit the disproportioning reaction of active inorganic radicals or a selection of ligands to the iron mimicking this effect; (d) the immobilization in suitable supports to avoid secondary reactions affecting HRP or its biomimetics.

Acknowledgements

The authors acknowledge the financial support of CONICET (Argentina) and the UNS (PGI 24/Q022) of Bahía Blanca, Argentina; ANPCyT (no. PICT 2005 33919) and UNLP (no. X559).

References

[1] J.J. Pignatello, E. Oliveros, A. MacKay, Crit. Rev. Environ. Sci. Technol. 36 (1) (2006) 1–84.
[2] F. Derek, L. Laine, I.F. Cheng, Microchem. J. 85 (2007) 183–193.
[3] A. Bhunia, S. Durani, P.P. Wangikar, Biotechnol. Bioeng. 72 (5) (2001) 562–567.
[4] L. Levin, L. Papinutti, F. Forchiassin, Biores. Technol. 94 (2) (2004) 169–176.
[5] K. Knutson, S. Kirzan, A. Ragauskas, Biotechnol. Lett. 27 (11) (2005) 753–758.
[6] Q. Husain, Crit. Rev. Biotechnol. 26 (4) (2006) 201–221.
[7] G. Busca, S. Berardinelli, C. Resini, L. Arrighi, J. Hazard. Mater. 160 (2–3) (2008) 265–288.
[8] B. Ai Ni Soon, H. Hameed, Desalination 269 (1–3) (2011) 1–16.
[9] E.G. Garrido-Ramírez, B.K.G. Theng, M.L. Mora, Appl. Clay Sci. 47 (3–4) (2010) 182–192.
[10] I. Oller, S. Malato, J.A. Sánchez-Pérez, Sci. Total Environ. 409 (20) (2011) 4141–4166.
[11] R.F.F. Pontes, J.E.F. Moraes, A. Machulek Jr., J.M. Pinto Jnal, Hazard. Mater. 176 (1) (2010) 402–413.
[12] S. Esplugas, J. Giménez, S. Contreras, E. Pascual, M. Rodríguez, Water Res. 36 (4) (2002) 1034–1042.
[13] R. Munter, Proc. Estonian Acad. Sci. Chem. 50 (2) (2001) 59–80.
[14] N. Kang, D.S. Lee, J. Yoon, Chemosphere 47 (9) (2002) 915–924.
[15] C.P. Huang, Y.H. Huang, Appl. Catal. A: Gen. 346 (1–2) (2008) 140–148.
[16] C. Sánchez, Biotechnol. Adv. 27 (2) (2009) 185–194.
[17] C. Crestini, R. Saladino, P. Tagliatesta, T. Boschi, Bioorg. Med. Chem. 7 (9) (1999) 1897–1905.
[18] A. Santos, P. Yustos, S. Rodriguez, E. Simon, F. Garcia-Ochoa Jnal, Hazard. Mater. 146 (3) (2007) 595–601.
[19] K. He, Y.M. Dong, Z. Li, L. Yin, A.M. Zhang, Y.C. Zheng Jnal, Hazard. Mater. 159 (2–3) (2008) 587–592.
[20] Kyoung-Hun Kim, Son-Ki Ihm, J. Hazard. Mater. 186 (1) (2011) 16–34.
[21] L.F. Liotta, M. Gruttadauria, G. Di Carlo, G. Perrini, V. Librando, J. Hazard. Mater. 162 (2–3) (2009) 588–606.
[22] R.N. Sylva, Rev. Pure Appl. Chem. 22 (1972) 115–130.
[23] M.J. Lottering, L. Lorenzen, N.S. Phala, J.T. Smit, G.A.C. Schalkwyk, Miner. Eng. 21 (1) (2008) 16–22.
[24] C. Noubactep, Water SA 36 (5) (2010) 663–670.
[25] D.F. Laine, I.F. Cheng, Microchem. J. 85 (2) (2007) 183–193.
[26] W.G. Barb, J.H. Baxendale, P. George, K.R. Hargrave, Nature 163 (4148) (1949) 692–694.
[27] W.G. Barb, J.H. Baxendale, P. George, K.R. Hargrave, Trans. Faraday Soc. 47 (1951) 462–500.
[28] W.G. Barb, J.H. Baxendale, P. George, K.R. Hargrave, Trans. Faraday Soc. 47 (1951) 591–616.
[29] M.L. Kremer, Phys. Chem. Chem. Phys. 1 (15) (1999) 3595–3605.
[30] H.B Dunford, Coord. Chem. Rev. 233–234 (2002) 311–318.
[31] S.H. Bossmann, E. Oliveros, S. Göb, S. Siegwart, E.P. Dahlen, L. Payawan Jr., M. Straub, M. Worner, A.M. Braun, J. Phys. Chem. A 102 (1998) 5542–5550.
[32] S.H. Bossmann, E. Oliveros, S. Göb, M. Kantor, A. Göppert, A.M. Braun, L. Lei, P.L. Yue, Prog. React. Kinet. 26 (2001) 113–137.
[33] D.A. Wink, C.B. Wink, R.W. Nims, P.C. Ford, Environ. Health Perspect. 102 (Suppl. 3) (1994) 11–15.
[34] D.A. Wink, R.W. Nims, J.E. Saavedra, W.E. Utermahlen Jr., P.C. Ford, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 6604–6608.
[35] E. Brillas, I. Sirés, A. Mehmet Oturan, Chem. Rev. 6570 (109) (2009) 6570–6631.
[36] E. Neyens, J. Baeyens, J. Hazard. Mater. B98 (2003) 33–50.
[37] G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, J. Phys. Chem. Ref. Data 17 (2) (1988) 513–886.
[38] C. Von Sonntag, H.P. Schuchmann, Z.B. Alfassi, Peroxyl Radicals, John Wiley and Sons, New York, 1997, pp. 173–234.
[39] R. Chen, J.J. Pignatello, Environ. Sci. Technol. 31 (8) (1997) 2399–2406.
[40] R. Chen, J.J. Pignatello, J. Adv. Oxidation Technol. 4 (1999) 447–453.
[41] C. Mansano-Weiss, C.H. Cohen, D. Meyerstein, J. Inorg. Biochem. 91 (1) (2002) 199–204.
[42] M. Fukushima, K. Tatsumi, S. Nagao, Environ. Sci. Technol. 35 (2001) 3683–3690.
[43] M. Fukushima, K. Tatsumi, K. Morimoto, Environ. Sci. Technol. 34 (2000) 2006–2013.
[44] M. Fukushima, K. Tatsumi, Environ. Sci. Technol. 35 (2001) 1771–1778.
[45] M. Kallel, C. Belaida, T. Mechichib, M. Ksibia, B. Elleuch, Chem. Eng. J. 150 (2009) 391–395.
[46] H. Kusic, N. Koprivanac, A.L. Bozic, Chem. Eng. J. 123 (2006) 127–137.
[47] J. Poerschmann, U. Trommler, J. Chromatogr. A 1216 (2009) 5570–5579.
[48] J. Yoon, Y. Lee, S. Kim, Water Sci. Technol. 44 (5) (2001) 15–21.
[49] F. Gozzo, J. Mol. Catal. A: Chem. 171 (2001) 1–22.
[50] J. De Laat, H. Gallard, Environ. Sci. Technol. 33 (16) (1999) 2726–2732.
[51] J. De Laat, H. Gallard, S. Ancelin, B. Legube, Chemosphere 39 (15) (1999) 2693–2706.
[52] K.H. Chan, W. Chu, J. Hazard. Mater. 118 (1–3) (2005) 227–237.
[53] D. Nichela, L. Carlos, Appl. Catal. B: Environ. 82 (1–2) (2008) 11–18.
[54] D. Nichela, M. Haddou, F. Benoit-Marquié, M.-T. Maurette, E. Oliveros, F.S. García Einschlag, Appl. Catal. B: Environ. 98 (2010) 171–179.
[55] Y. Du, M. Zhou, L. Lei, J. Hazard. Mater. 136 (3) (2006) 859–865.
[56] F.P. Van der Zee, F.J. Cervantes, Biotechnol. Adv. 27 (3) (2009) 256–277.
[57] H. Gallard, J. De Laat, Chemosphere 42 (4) (2001) 405–413.
[58] A.A. MacKay, J.J. Pignatello Helvetica, Chim. Acta 84 (9) (2001) 2589–2600.
[59] J.A. Zazo, J.A. Casas, A.F. Mohedano, M.A. Gilarranz, J.J. Rodríguez, Environ. Sci. Technol. 39 (23) (2005) 9295–9302.
[60] R. Alnaizy, A. Akgerman, Adv. Environ. Res. 4 (2000) 233–244.
[61] C. Lee, J. Yoon, Chemosphere 56 (2004) 923–934.
[62] S. Papia, D. Vujevia, N. Koprivanac, D. Ainko, J. Hazard. Mater. 164 (2–3) (2009) 1137–1145.
[63] S. Parsons, Advanced oxidation processes for water and wastewater treatment, IWA Publishing, London, 2004.
[64] K. Fajerwerg, H. Debellefontaine, Appl. Catal. B: Environ. 10 (4) (1996) L229–L235.

[65] G. Ovejero, J.L. Sotelo, F. Martínez, J.A. Melero, L. Gordo, IE&CR 40 (18) (2001) 3921–3928.
[66] E.V. Kuznetsova, E.N. Savinov, L.A. Vostrikova, V.N. Parmon, Appl. Catal. B: Environ. 51 (3) (2004) 165–170.
[67] J.S. Choi, S.S. Yoon, S.H. Jang, W.S. Ahn, Catal. Today 111 (3–4) (2006) 280–287.
[68] N. Crowther, F. Larachi, Appl. Catal. B: Environ. 46 (2) (2003) 293–305.
[69] R.M. Liou, S.H. Chen, Chemosphere 55 (9) (2004) 1271–1280.
[70] R. Liou, M.S.H. Chen, M.Y. Hung, C.S. Hsu, J.Y. Lai, Chemosphere 59 (1) (2005) 117–125.
[71] M.C. Lu, Chemosphere 40 (2) (2000) 125–130.
[72] R.L. Valentine, H.C.A. Wang, J. Environ. Eng.-ASCE 124 (1) (1998) 31–38.
[73] P. Baldrian, V. Merhautova, J. Gabriel, F. Nerud, P. Stopka, M. Hrubý, M.J. Benes, Appl. Catal. B: Environ. 66 (3–4) (2006) 258–264.
[74] X. Xue, K. Hanna, M. Abdelmoula, N. Deng, Appl. Catal. B: Environ. 89 (3–4) (2009) 432–440.
[75] D. Wang, Z. Liu, F. Liu, X. Ai, X. Zhang, Y. Cao, J. Yu, T. Wu, Y. Bai, T. Li, X. Tang, Appl. Catal. A: Gen. 174 (1–2) (1998) 25–32.
[76] F. Martínez, J.A. Melero, J.Á. Botas, M.I. Pariente, R. Molina, Ind. Eng. Chem. Res. 46 (13) (2007) 4396–4405.
[77] J.A. Melero, G. Calleja, F. Martínez, R. Molina, M.I. Pariente, Chem. Eng. J. 131 (1–3) (2007) 245–256.
[78] R.M. Cornell, U. Schwertmann, The Iron Oxides: Structure, Properties, Reactions, Occurrence and Uses, Wiley-VCH Verlag GmbH & Co. KgaA, Weinheim, 1996.
[79] Z.X. Sun, F.W. Su, W. Forsling, P.O. Samskog, J. Colloid Interface Sci. 197 (1) (1998) 151–159.
[80] F.C.C. Moura, M.H. Araujo, R.C.C. Costa, J.D. Fabris, J.D. Ardisson, W.A.A. Macedo, R.M. Lago, Chemosphere 60 (8) (2005) 1118–1123.
[81] S. Zhang, X. Zhao, H. Niu, Y. Shi, Y. Cai, G. Jiang, J. Hazard. Mater. 167 (1–3) (2009) 560–566.
[82] A. Zecchina, M. Rivallan, G. Berlier, C. Lamberti, G. Ricchiardi, Phys. Chem. Chem. Phys. 9 (27) (2007) 3483–3499.
[83] E.G. Garrido-Ramírez, B.K.G. Theng, M.L. Mora, Appl. Clay Sci. 47 (2010) 182–192.
[84] S. Navalon, M. Alvaro, H. Garcia, Appl. Catal. B: Environ. 99 (2010) 1–26.
[85] G. Zelmanov, R. Semiat, Water Res. 42 (2008) 492–498.
[86] S.S. Lin, M.D. Gurol, Environ. Sci. Technol. 32 (1998) 1417–1423.
[87] W.P. Kwan, B.M. Voelker, Environ. Sci. Technol. 36 (2002) 1467–2147.
[88] R. Andreozzi, A. D'Apuzzo, R. Marotta, Water Res. 36 (2002) 4691–4698.
[89] M. Reihmann, H. Ritter, Adv. Polym. Sci. 194 (2006) 1–49.
[90] C. Zazza, M. Aschi, A. Palma, Chem. Phys. Lett. 428 (2006) 152–156.
[91] (a) B. Dunford, Heme Peroxidases, Wiley-VCH, 1999, p. 55;
(b) J. Everse, Biol. Med. 24 (7–8) (1998) 1338–1346.
[92] J.L. Gómez, A. Bódalo, E. Gómez, A.M. Hidalgo, M. Gómez, Am. J. Biochem. Biotechnol. 1 (2) (2005) 115–120.
[93] K.J. Baynton, J.K. Bewtra, N. Biswas, K.E. Taylor, Biochim. Biophys. Acta (BBA)–Protein Struct. Mol. Enzym. 1206 (2) (1994) 272–278.
[94] R. Nakajima, I. Yamazaki, J. Biol. Chem. 255 (1980) 2067–2071.
[95] (a) H. Uyama, H. Kurioka, Colloid Surf. A 153 (1–3) (1999) 189–194;
(b) H. Uyama, H. Kurioka, J. Polym. Sci. Part A: Polym. Chem. 35 (8) (1997) 1453–1459.
[96] M. Wagner, J.A. Nicell, Water Res. 36 (16) (2002) 4041–4052.
[97] S.M.A.G. Ulson de Souza, E. Forgiarini, A.A. Ulson de Souza, J. Hazard. Mater. 147 (3) (2007) 1073–1078.
[98] J.A. Nicell, K.W. Saadi, Biores. Technol. 54 (1) (1995) 5–16.
[99] J.A. Nicell, K.W. Saadi, I.D. Buchanan, Biores. Technol. 54 (1995) 5–16.
[100] T. Oguchi, S.I. Tawaki, Macromol. Rapid Commun. 20 (7) (1999) 401–403.
[101] C. Regalado, B.E. García-Almendárez, M.A. Duarte-Vázquez, Phytochem. Rev. 3 (1–2) (2004) 243–256.
[102] M. Hamid, R. Khalil, Food Chem. 115 (4) (2009) 1177–1186.
[103] J.L. Gómez, A. Bódalo, E. Gómez, J. Bastida, A.M. Hidalgo, M. Gómez, Enzyme Microb. Technol. 39 (5) (2006) 1016–1022.
[104] S. Dalal, M.N. Gupta, Chemosphere 67 (4) (2007) 741–747.
[105] J. Cheng, S. Ming Yu, P. Zuo, Water Res. 40 (2) (2006) 283–290.
[106] K. Nazari, N. Esmaeili, A. Mahmoudi, H. Rahimi, A.A. Moosavi-Movahedi, Enzyme Microb. Technol. 41 (3) (2007) 226–233.
[107] J.A. Akkara, K.J. Senecal, D.L. Kaplan, J. Polym. Sci. Part A: Polym. Chem. 29 (11) (1991) 1561–1574.
[108] S. Kobayashi, H. Higashimura, Prog. Polym. Sci. 28 (6) (2003) 1015–1048.
[109] E.J. Land, G. Porter, E. Strachan, Trans. Faraday Soc. 57 (1961) 1885–1893.
[110] J. Hernandez-Ruiz, M.B. Arnao, A.N.P. Hiner, F. García-Canovas, M. Acosta, J. Biochem. 354 (2001) 107–1114.
[111] C. Xialing, M. Lin, J. Biochem. Tech. 1 (4) (2009) 92–95.
[112] I. Alemzadeh, S. Nejati, J. Hazard. Mater. 166 (2–3) (2009) 1082–1086.
[113] P. Zucca, F. Sollai, A. Garau, A. Rescigno, E. Sanjust, J. Mol. Catal. A: Chem. 306 (1–2) (2009) 89–96.
[114] M.L. Ferreira, Macromol. Biosci. 3 (3–4) (2003) 179–188.
[115] C. Hemmert, M. Renz, B. Meuinier, J. Mol. Catal. A: Chem. 137 (1–3) (1999) 205–212.
[116] A. Haikarainen, J. Sipila, P. Pietikainen, A. Pajunen, I. Mutikainen, Bioorg. Med. Chem. 9 (6) (2001) 1633–1638.
[117] M. Fukushima, H. Ichikawa, M. Kawasaki, A. Sawada, K. Morimoto, K. Tatsumi, Environ. Sci. Technol. 37 (2003) 386–394.
[118] M. Fukushima, A. Sawada, M. Kawasaki, H. Ichikawa, K. Morimoto, K. Tatsumi, Environ. Sci. Technol. 37 (2003) 1031–1036.
[119] M. Fukushima, K. Tatsumi, J. Mol. Catal. A: Chem. 245 (2006) 178–184.
[120] S. Rismayani, M. Fukushima, A. Sawada, H. Ichikawa, K. Tatsumi, J. Mol. Catal. A: Chem. 217 (1–2) (2004) 13–19.
[121] M. Fukushima, K. Tatsumi, Bioresour. Technol. 97 (2006) 1605–1611.
[122] N.W.J. Kamp, J.R. Lindsay Smith, J. Mol. Catal. A: Chem. 113 (1–2) (1996) 131–145.
[123] N. Colclough, J.R. Lindsay Smith, J. Chem. Soc. Perk. Trans. 2 (6) (1994) 1139–1149.
[124] T.G. Traylor, F. Xu, J. Am. Chem. Soc. 109 (20) (1987) 6201–6202.
[125] T.G. Traylor, F. Xu, J. Am. Chem. Soc. 112 (1) (1990) 178–186.
[126] T.C. Bruice, P.N. Balasubramanian, J. Am. Chem. Soc. 110 (23) (1988) 7890–7892.
[127] A. Almarsson, T.C. Bruice, J. Am. Chem. Soc. 117 (16) (1995) 4533–4544.
[128] W. Nam, H.J. Han, J. Am. Chem. Soc. 122 (36) (2000) 8677–8684.
[129] I.D. Cunningham, T.N. Danks, J.N. Hay, I. Hamerton, S. Gunathilagan, Tetrahedron 57 (31) (2001) 6847–6853.
[130] N.A. Stephenson, A.T. Bell, J. Am. Chem. Soc. 127 (24) (2005) 8635–8643.
[131] J.A. Howard, Peroxyl and Related Radicals, Springer, Berlin, 1997.
[132] (a) M. Fukushima, K. Tatsumi, Environ. Sci. Technol. 39 (2005) 9337–9342;
(b) M. Fukushima, Biomacromolecules 8 (2007) 386–391.
[133] (a) M. Fukushima, J. Mol. Catal. A: Chem. 286 (2008) 47–54;
(b) M. Fukushima, S. Shigematsu, J. Mol. Catal. A: Chem. 293 (2008) 103–109.
[134] C. Walling, M. Kurz, H.J. Schugar, Inorg. Chem. 9 (4) (1970) 931–937.
[135] A.L. Tappel, Autoxidation and Antioxidants, 1, Wiley & Sons, W. O. Lundberg, New York, 1961, pp. 325–366.
[136] J.A. Akkara, J. Wang, Macromolecules 33 (7) (2000) 2377–2382.
[137] D. Arrieta-Báez, R. Roman, R. Vazquez-Duhalt, M. Jiménez-Estrada, Phytochemistry 60 (6) (2002) 567–572.
[138] F. Xu, D.E. Koch, C.K. In, R.P. Hunter, A. Bhandari, Water Res. 39 (11) (2005) 2358–2368.
[139] C. López, A.G. Valade, B. Combourieu, I. Mielgo, B. Bouchon, J.M. Lema, Anal. Biochem. 335 (1) (2004) 135–149.
[140] T.J. Collins, Acc. Chem. Res. 35 (9) (2002) 782–790.
[141] Q. Huang, Q. Huang, R.A. Pinto, K. Griebenow, R. Schweitzer-Stenner, J. Wa Weber Jr., J. Am. Chem. Soc. 127 (2005) 1431–1437.
[142] P. Jones, J. Biol. Chem. 276 (2001) 13791–13796.
[143] G.H. Carlsson, P. Nicholls, D. Svistunenko, G.I. Berglund, J. Hajdu, Biochemistry 44 (2005) 635–642.
[144] M.J. Dascombe, M.G.B. Drew, H. Morris, P. Wilairat, S. Auparakkitanon, W.A. Moule, S. Alizadeh-Shekalgourabi, P.G. Evans, M. Lloyd, A.M. Dyas, P. Carr, F.M.D. Ismail, J. Med. Chem. 48 (17) (2005) 5423–5436.
[145] P.K. Malik, S.K. Saha, Sep. Purif. Technol. 31 (3) (2003) 241–250.
[146] J.H. Sun, S.P. Sun, G.L. Wang, L.P. Qiao, Dyes Pigments 74 (3) (2007) 647–652.
[147] S. Pirillo, F.S. García Einschlag, E.H. Rueda, M.L. Ferreira, Ind. Eng. Chem. Res. 49 (15) (2010) 6745–6752.
[148] S. Pirillo, F.S. García Einschlag, M.L. Ferreira, E.H. Rueda, J. Mol. Catal. B: Enzyme 66 (1–2) (2010) 63–71.
[149] E. Forgacs, T. Cserhati, G. Oros, Environ. Int. 30 (2004) 953–971.
[150] R. Hatfield, W. Vermerris, Plant Physiol. 126 (4) (2001) 1351–1357.
[151] (a) B. Kurek, I. Artaud, J. Agric. Food Chem. 44 (7) (1996) 1953–1959;
(b) B. Kurek, M.J. Martínez-Iñigo, Polym. Degrad. Stab. 59 (1–3) (1998) 359–364.
[152] O.A. Makhotkina, S.V. Preis, E.V. Parkhomchuk, Appl. Catal. B: Environ. 84 (3–4) (2008) 821–826.
[153] E. Araujo, A.J. Rodríguez-Malaver, A.M. González, O.J. Rojas, N. Peñaloza, J. Bullón, M.A. Lara, N. Dmitrieva, Appl. Biochem. Biotechnol. – Part A Enzyme Eng. Biotechnol. 97 (2) (2002) 91–103.
[154] F. Torrades, S. Saiz, J.A. García-Hortal, J. García-Montaño, Environ. Sci. Technol. 25 (1) (2008) 92–98.
[155] G. Bentivenga, C. Bonini, M. D'Auria, A. De Bona, Biomass Bioeng. 24 (3) (2003) 233–238.
[156] V. Gómez-Toribio, A.B. García-Martín, M.J. Martínez, Á.T. Martínez, F. Guillén, Appl. Environ. Microbiol. 75 (12) (2009) 3944–3953.
[157] V. Arantes, Y. Qian, J. Biol. Inorg. Chem. 14 (8) (2009) 1253–1263.
[158] C. Bohlin, P.O. Andersson, K. Lundquist, L.J. Jonsson, J. Mol. Catal. B: Enzyme 45 (1–2) (2007) 21–26.
[159] D. Contreras, J. Freer, J. Rodríguez, Int. Biodeterior. Biodegrad. 57 (1) (2006) 63–68.
[160] V. Arantes, A.M.F. Milagres, J. Hazard. Mater. 141 (1) (2007) 273–279.
[161] D. Contreras, J. Rodríguez, J. Freer, B. Schwederski, W. Kaim, J. Biol. Inorg. Chem. 12 (7) (2007) 1055–1061.
[162] D.L. Kaplan, Phytochemistry 18 (12) (1979) 1917–1919.
[163] R. Hartenstein, E.F. Neuhauser, R.M. Mulligan, Phytochemistry 16 (11) (1977) 1855–1857.
[164] B. Kurek, B. Monties, Enzyme Microb. Technol. 16 (2) (1994) 125–130.
[165] J.L. Popp, T.K. Kirk, J.S. Dordick, Enzyme Microb. Technol. 13 (12) (1991) 964–968.
[166] A. Guerra, A. Ferraz, A.R. Cotrim, F.T. Da Silva, Enzyme Microb. Technol. 26 (5–6) (2000) 315–323.
[167] S. Groenqvist, L. Viikari, M.L. Niku-Paavola, M. Orlandi, C. Canevali, J. Buchert. Appl. Microb. Biotechnol. 67 (4) (2005) 489–494.
[168] A. Kumar, N. Jain, S.M.S. Chauhan, Synlett 3 (2007) 411–414.
[169] H. Joo, H.J. Chae, J.S. Yeo, Y.J. You, Proc. Biochem. 32 (4) (1997) 291–296.

[170] N. Durán, H. Mansilla, C.C.L. Leite, A. Faljoni-Alario, J. Inorg. Biochem. 34 (2) (1988) 105–115.
[171] J.S. Dordick, M.A. Marletta, A.M. Klivanov, Biochemistry 83 (1986) 6255–6257.
[172] J. Zakzeski, P.C.A. Bruijnincx, A.L. Jongerius, B.M. Weckhuysen, Chem. Rev. 110 (6) (2010) 3552–3599.
[173] O. Suparno, A.D. Covington, C.S. Evans, J. Chem. Technol. Biotechnol. 80 (1) (2005) 44–49.
[174] C.C. Walker, T.J. McDonough, R.J. Dinus, K.E.L. Eriksson, Holzforschung 55 (4) (2001) 391–396.
[175] J.F. Kadla, H.M. Chang, ACS Symp. Series 785 (2001) 108–129.