Generation of hydroxyl radicals from reactions between a dimethoxyhydroquinone and iron oxide nanoparticles

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The hydroxyl radical (·OH) is a powerful oxidant that is produced in a wide range of environments via the Fenton reaction (Fe2+ + H2O2 → Fe3+ + ·OH + OH-). The reactants are formed from the reduction of Fe3+ and O2, which may be promoted by organic reductants, such as hydroquinones. The aim of this study was to investigate the extent of ·OH formation in reactions between 2,6-dimethoxyhydroquinone (2,6-DMHQ) and iron oxide nanoparticles. We further compared the reactivities of ferrihydrite and goethite and investigated the effects of the O2 concentration and pH on the generation of ·OH. The main finding was that the reactions between 2,6-DMHQ and iron oxide nanoparticles generated substantial amounts of ·OH under certain conditions via parallel reductive dissolution and catalytic oxidation reactions. The presence of O2 was essential for the catalytic oxidation of 2,6-DMHQ and the generation of H2O2. Moreover, the higher reduction potential of ferrihydrite relative to that of goethite made the former species more susceptible to reductive dissolution, which favored the production of ·OH. The results highlighted the effects of surface charge and ligand competition on the 2,6-DMHQ oxidation processes and showed that the co-adsorption of anions can promote the generation of ·OH.

The hydroxyl radical (·OH) is a powerful oxidant that is produced in a wide range of environments1. This radical degrades organic compounds1–3, which makes it harmful if produced in excess and in close proximity to cells. Such oxidative stress reactions can have a range of adverse effects, and ·OH production may be accelerated by reactive nanoparticles4. The oxidative power of ·OH can also be favourably exploited, for instance in pollutant and wastewater treatments. Soil remediation studies have demonstrated that belowground injection of hydrogen peroxide (H2O2) effectively degrades organic contaminants, particularly in the presence of iron minerals5–8. Similar processes occur in terrestrial systems without human interference and have been suggested to play an important role in the degradation of natural organic matter, thereby contributing to the terrestrial carbon cycle9–14.

The Fenton reaction

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \text{OH}^- \quad (1) \]

is an important source of ·OH, and to trigger this reaction, Fe2+ and H2O2 must co-exist. In homogeneous solutions, the generation of ·OH is strongly pH dependent. At very acidic pH values, H2O2 is stabilized as H3O2+, which is deprotonated and subsequently disproportionates into O2 and H2O with an increase in the pH3. A pH increase also promotes the oxidation of Fe2+15, and therefore, the Fenton reaction has an optimum pH between 3 and 4.2. The regeneration of Fe2+ is accomplished either by oxygen species (e.g., the Haber-Weiss mechanism) or by organic reductants. H2O2 is produced via the enzymatic reduction of O2 or by redox-active metabolites10,16. In this respect, hydroquinones are an interesting class of organic metabolites because they can reduce both O2 and Fe3+, thereby generating both Fenton reagents17–19. In fact, certain fungi have evolved wood-degrading machinery that partially depends on the ·OH generated from reactions between 2,5-dimethoxy-1,4-hydroquinone (2,5-DMHQ (1,4-(CH3O)2-C6H2(OH)2)) and Fe3+20–24. In oxic environments, the reductive dissolution of Fe(III) oxides may provide the only source of Fe2+, which affects the pH dependence and mechanism of ·OH generation20–24. Moreover, reactive Fe(III) oxide nanoparticles

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can provide catalytically active surfaces for \( \text{H}_2\text{O}_2 \) generation and degradation\(^{2,25}\). This feature makes the reactions between such nanoparticles and redox-active metabolites particularly interesting because of the possibility of forming Fenton reagents at the particle surfaces, which increases the likelihood that molecules accumulated at these interfaces will be oxidized. Recently, reactions between 2,6-dimethoxy-1,4-hydroquinone (2,6-DMHQ \((1,4-(\text{CH}_3\text{O})_2-2,6-\text{C}_6\text{H}_2(\text{OH}))\)) and iron oxides were shown to cause reductive dissolution as well as catalytic hydroquinone oxidation in the presence of \( \text{O}_2 \)^{26}, but whether these reactions also generate significant amounts of \( \cdot\text{OH} \) remains unknown.

The detection and quantification of the short-lived \( \cdot\text{OH} \) species (lifetimes of ca. \( 10^{-3} \) to \( 10^{-6} \) s in aqueous solutions)\(^{27,28}\) are challenging, and often, \( \cdot\text{OH} \) has to be captured and measured with a probe molecule. Recent results showed that terephthalic acid (TPA \((\text{C}_6\text{H}_4(1,4-(\text{CO}_2\text{H})_2))\)) is a suitable probe for \( \cdot\text{OH} \) detection;\(^{29}\) thus, this probe was used to address the main question of this study: to what extent is \( \cdot\text{OH} \) formed by reactions between a hydroxylated probe molecule and iron oxide nanoparticles? The reactivity of ferrihydrite and goethite nanoparticles was further compared, and the influence of the \( \text{O}_2 \) level and \( \text{pH} \) was investigated. The hydroxylated probe \((\text{hTPA(C}_6\text{H}_4(1,4-(\text{CO}_2\text{H})_2)-2-\text{OH}))\) has been suggested\(^{30-32}\) but not shown to adsorb to surfaces below a neutral \( \text{pH} \). Thus, the effect of probe adsorption is also addressed herein.

**Results and Discussion**

The addition of any probe, be it a fluorescence or spin-trap probe, or buffer has the potential to modify the redox reactions that occur at the iron oxide surface and thus affect the generation of \( \cdot\text{OH} \). We begin, therefore, by discussing the adsorption of the probe molecules and compare the 2,6-DMHQ-iron oxide redox reactions under aerobic and anaerobic conditions in the absence and presence of TPA; these conditions are characterized by initial dissolved oxygen concentrations of 270–310 and 0–20 \( \mu \text{M} \), respectively (see Methods section).

**Interactions between the TPA/hTPA probe molecules and the iron oxide surface.** The dicarboxylic structure of the TPA probe molecule indicates that it should have a strong affinity for iron oxide surfaces\(^{29-37}\). Indeed, a previous study examined the adsorption of TPA on goethite, and the maximum surface coverage \((\Gamma_{\text{max}})\) was determined to be 0.5–0.6 \( \mu \text{mol/m}^2 \) at \( \text{pH} 5.0 \)^{38}. This result is in good agreement with the adsorption onto ferrihydrite at \( \text{pH} 4.5 \) that was determined in the current study \((\Gamma_{\text{max}} \sim 0.5 \mu \text{mol/m}^2, \text{Fig. 1a})\) and indicates that the surface saturation is rather low relative to that of strongly adsorbing ions, such as phosphate and arsenate, whose surface saturation on iron oxides typically reaches approximately 2.0–2.5 \( \mu \text{mol/m}^2 \)^{39,40}. Moreover, previous IR data suggest that TPA adsorption on goethite occurs via outer-sphere interactions\(^{38}\), i.e., TPA adsorbs via either surface hydration-shared ion pairs or solvent-surface hydration-separated ion pairs\(^{41}\). In the former class, the ligands are hydrogen-bonded to water molecules or hydroxyl groups in the surface layer, while in the latter class, the ligands retain the first solvation shell, and thus, at least one water molecule separates the ligands from the surface layer. The similarity between the IR spectra of the deprotonated TPA in solution and the spectra of TPA adsorbed onto ferrihydrite and goethite indicates that deprotonated outer-sphere complexes predominate on both iron oxides (Fig. 1b), although we cannot distinguish different outer-sphere species based on these data. Nevertheless, we can conclude that at the total TPA concentration \((1.5 \mu \text{mol/m}^2)\) used in the 2,6-DMHQ-iron oxide experiments, the surfaces are saturated with probe molecules, which exist predominantly as outer-sphere surface species. Hence, TPA should be in close proximity to any radical-generating processes that occur at the water-iron oxide interface.

The oxidized probe, hTPA, also adsorbed onto the iron oxides (Fig. 2). In this case, the IR spectra of the adsorbed hTPA were distinctly different from that of the aqueous counterpart (Fig. 2b), indicating direct coordination of the probe to the Fe\(^{+}\) at the surface, i.e., inner-sphere surface coordination. This type of coordination was expected since a hydroxyl group was introduced at the position ortho to one of the carboxyl groups, yielding a structural fragment similar to salicylate, which has been shown to adsorb to metal oxide surfaces through inner-sphere interactions\(^{42,43}\). The inner-sphere coordination mode was facilitated by the formation of
six-membered chelate structures. To determine the total amount of oxidized probe, this fraction of adsorbed hTPA must be desorbed (see SI and Fig. S3).

Effects of the TPA probe on the 2,6-DMHQ-iron oxide redox reactions. As mentioned above, hydroquinones are oxidized by iron oxide surfaces via two main reaction pathways, namely, reductive dissolution (2) and surface catalysis (3):

\[
\text{FeOOH} + \text{H}_2\text{Q} \rightarrow \text{Fe}^{2+} + \text{Q} + 4\text{OH}^{-} \quad \text{(2)}
\]

\[
\text{H}_2\text{Q} + \text{O}_2 \xrightarrow{\text{catalyst}} \text{Q} + \text{H}_2\text{O}_2 \quad \text{(3)}
\]

Reactions (2) and (3) can be distinguished by comparing the oxidation reactions under aerobic and anaerobic conditions because surface catalysis is strongly \(\text{O}_2\)-dependent while reductive dissolution is not. Our results showed that \(\text{O}_2\) had a large effect on 2,6-DMHQ oxidation, particularly in the presence of goethite (Fig. 3). At pH 4.5 under aerobic conditions, 100% of the hydroquinone was oxidized by goethite after 4 h, while only ca. 30% was oxidized anaerobically. The corresponding values in the ferrihydrite system under aerobic and anaerobic conditions were 100% and 90%, respectively. These results corroborate previous findings showing that goethite mainly mediated catalytic 2,6-DMHQ oxidation, whereas the reaction with ferrihydrite exhibited a large contribution from reductive dissolution, which was evidenced by the extensive 2,6-DMHQ oxidation observed under anaerobic conditions.

The extent of aerobic 2,6-DMHQ oxidation increased further at pH 7.0; otherwise, similar differences between aerobic and anaerobic conditions as those at pH 4.5 were observed (Fig. 3c,d). Interestingly, substantial oxidation was also observed at pH 7.0 under anaerobic conditions. This oxidation is likely caused by reductive dissolution, which will be favoured if the \(\text{Fe}^{2+}\) concentration is maintained at low concentrations through re-oxidation by the low \(\text{O}_2\) concentrations that exist also under conditions where we tried to exclude oxygen. In addition, these oxygen trace concentrations can also contribute to the catalytic oxidation of 2,6-DMHQ.

The \(\text{O}_2\) dependence and differences in reactivity between ferrihydrite and goethite were not dramatically affected by the presence of the TPA probe, although some changes in the extent of oxidation were observed (Fig. 3). TPA had an inhibiting effect on aerobic 2,6-DMHQ oxidation in the presence of goethite, which is in line with the occurrence of competitive reactions between the TPA anions and quinone species at the water-iron oxide interface. Since the catalytic pathway dominated aerobic oxidation, the results indicated that TPA interfered with the 2,6-DMHQ and/or \(\text{O}_2\) surface species involved in the catalytic process. Note that under anaerobic conditions, the 2,6-DMHQ oxidation by goethite in absence and presence of TPA was similar, which is consistent with the fact that both reductive dissolution and catalysis contribute to 2,6-DMHQ oxidation, as discussed in the paragraph above (Fig. 3b,d).

In contrast to the results for goethite, TPA increased the extent of 2,6-DMHQ oxidation by ferrihydrite under both aerobic and anaerobic conditions at pH 4.5 and 7.0 (Fig. 3a,c). The contribution of reductive dissolution was substantial in the presence of ferrihydrite, and thus, the TPA-promoted oxidation of 2,6-DMHQ is likely related to the reductive mechanism. The reason for this effect is not clear, but the outer-sphere adsorption of TPA on ferrihydrite will reduce the positive surface charge, which in turn will promote the adsorption of protons. Previously proposed mechanisms of reductive ferrihydrite dissolution suggest that protonation may affect the kinetics either via a preceding reaction step involving non-reductive dissolution or via surface reconstruction after \(\text{Fe}^{2+}\) dissolution. Both scenarios could explain the increased extent of 2,6-DMHQ oxidation in the presence of TPA.
Apparently, in the case of ferrihydrite, the relative contributions from reductive dissolution and catalytic oxidation were such that the rate-increasing effects induced by TPA out-weighed the competitive ones under aerobic conditions (Fig. 3a). Overall, the effects of TPA on 2,6-DMHQ oxidation further corroborated the differences between ferrihydrite and goethite.

Aqueous Fe$^{2+}$ is generated via the reductive dissolution of iron oxides caused by 2,6-DMHQ oxidation. It follows that the large difference in the contribution of this reaction resulted in a corresponding difference in the generation of Fe$^{2+}$ from ferrihydrite and goethite (Fig. 4); note that no results were reported for the pH 7.0 experiments because the Fe$^{2+}$ concentrations were below the limit of detection (LoD) of 2.0 μM. Under anaerobic conditions at pH 4.5, the Fe$^{2+}$ concentration reached levels of approximately 120 μM in the presence of ferrihydrite, while merely ca. 30 μM was dissolved from goethite. Re-adsorption of Fe$^{2+}$ under these conditions is very low, as shown by previous studies, and therefore, these results were a direct reflection of the magnitude of reductive dissolution. In the presence of O$_2$, the Fe$^{2+}$ concentrations decreased for both iron oxides (Fig. 4, black lines), and relative to the anaerobic conditions, the effect was largest in the case of goethite. In a previous study, we showed that the re-oxidation of Fe$^{2+}$ in the presence of ferrihydrite was low at pH 4.5, and therefore, the reduced Fe$^{2+}$ concentration indicated an increase in the contribution from the catalytic oxidation of 2,6-DMHQ at the expense of reductive dissolution. Some re-oxidation of Fe$^{2+}$ likely occurred in the presence of goethite, as indicated by the decreases in the concentration after ca. 100 min (Fig. 4b).

The addition of TPA had only small effects on the Fe$^{2+}$ concentrations except under aerobic conditions in the presence of ferrihydrite, where the concentration decreased dramatically in the presence of TPA (Fig. 4a). A similar effect was also observed in the presence of acetate buffer (Fig. S4). The results from the experiment performed in the absence of TPA showed that this decrease in the Fe$^{2+}$ concentration cannot be caused by oxidation via O$_2$. Instead, similar effects were observed when 30 μM H$_2$O$_2$ was added to the aerobic 2,6-DMHQ-ferrihydrite experiment (Fig. 4a). The reason for these low Fe$^{2+}$ concentrations will be further discussed below. Importantly, under anaerobic conditions, the release of Fe$^{2+}$ from ferrihydrite in the absence and presence of TPA was very similar despite the fact that 2,6-DMHQ oxidation was faster when TPA was present (c.f. Figs 3a and 4a). Furthermore, the faster generation of 2,6-dimethoxy-1,4-quinone (2,6-DMBQ (1,4-(CH$_3$O)$_2$-2,6-C$_6$H$_2$O$_2$)) in the presence of TPA (Fig. S5) implied that for every 2,6-DMHQ molecule oxidized to 2,6-DMBQ via reductive dissolution, the release

Figure 3. Oxidation of 90 μM 2,6-DMHQ (0.44 μmol/m$^2$) at pH 4.5 (black: aerobic and green: anaerobic) and pH 7.0 (red: aerobic and blue: anaerobic) in the presence of ferrihydrite (a,c) and goethite (b,d). Solid lines represent the experiments conducted in the presence of 300 μM TPA, and the dashed lines represent those conducted in the absence of TPA.
of Fe^{2+} from ferrihydrite was slowed by the presence of TPA (Fig. S6). This finding is consistent with the effects of electrostatic interactions on the desorption kinetics and the fact that the adsorption of TPA should lower the surface charge, which in turn will slow the desorption of cations, including Fe^{2+} 44.

**Generation of hydroxyl radicals.** In line with the parallel reductive dissolution and catalytic oxidation processes, which likely produce both Fenton reagents, the reactions between 2,6-DMHQ and the iron oxides generated ·OH, as indicated by the oxidation of the TPA probe (Fig. 5). These results were in general agreement with those of previous investigations of similar reactions in homogeneous solutions. Kerem et al. reported the spontaneous production of H_2O_2 by the reaction between 2,5-DMHQ and FeCl_3 in aqueous solution and further observed the cleavage of polyethylene glycol (PEG), which was presumably caused by ·OH16. These findings were supported by Jensen et al. who detected rapid O_2 consumption in the same reaction12.

Hence, the reaction between hydroquinones and Fe^{3+} in both the liquid and solid states generates ·OH, but as our results showed, the amount of ·OH formed via the heterogeneous reaction is strongly dependent on the experimental conditions and the form of solid Fe^{3+} (Fig. 5). These results can be rationalized by considering the one-electron 2,6-DMHQ oxidation reactions of the reductive dissolution and catalytic oxidation processes (Fig. 6). H_2O_2 is formed by the disproportionation or reduction (not included in Fig. 6) of superoxide (-HO_2), which is generated via the reduction of O_2 by the hydroquinone and semiquinone (reactions R_2, R_4 and R_5 in Fig. 6). Based on these reactions, reducing the O_2 supply will also reduce the generation of H_2O_2, and thus, the very low amounts of ·OH produced under anaerobic conditions in the presence of both ferrihydrite and goethite (Fig. 5) were a direct consequence of the shortage of H_2O_2.

**Figure 4.** Generation of Fe^{2+} from reactions between 90 μM 2,6-DMHQ (0.44 μmol/m^2) and (a) ferrihydrite and (b) goethite at pH 4.5. The black and green lines represent aerobic and anaerobic conditions, respectively. The experiments were carried out in the absence of TPA (dashed lines), in the presence of 300 μM TPA (solid lines), and in the presence of both 30 μM of H_2O_2 and 300 μM TPA (dotted lines). Note that the y-axis scales in (a) and (b) are different. The error bars are standard deviations of triplicate measurements. Some of these error bars are smaller than the size of the symbols and are therefore not distinguishable.

**Figure 5.** Generation of ·OH from reactions between 90 μM 2,6-DMHQ (0.44 μmol/m^2) and (a) ferrihydrite and (b) goethite in the presence of 300 μM TPA. The black and red lines represent pH 4.5 and 7.0, respectively. The experiments were carried out under aerobic conditions (solid lines) and anaerobic conditions (dashed lines). The error bars are standard deviations of triplicate measurements. Some of these error bars are smaller than the size of the symbols and are therefore not distinguishable.
On the other hand, the stark contrast in -OH production by ferrihydrite and goethite of almost an order of magnitude at pH 4.5 under aerobic conditions (Fig. 5) is largely caused by the difference in the contribution of reductive dissolution. The oxidation of 2,6-DMHQ and the generation of Fe$^{3+}$ both showed that the rate and extent of reductive dissolution was far greater in the presence of ferrihydrite than in the presence of goethite (Figs 3 and 4). This difference is in agreement with the higher reduction potential of ferrihydrite compared with that of goethite and the fact that reductive dissolution by 2,6-DMHQ is effective at much higher dissolved Fe$^{2+}$ concentrations in the presence of ferrihydrite. The initial 2,6-DMHQ oxidation reaction followed approximately first-order kinetics (Fig. S7), and the difference in the first-order rate constants between the aerobic and anaerobic conditions with ferrihydrite was a factor of 1.7, while the corresponding difference for goethite was a factor of 9.0. This difference indicates that the rates of catalytic oxidation and reductive dissolution were much closer for ferrihydrite than for goethite. Thus, not only is more Fe$^{2+}$ generated from ferrihydrite, but also, the timing of the production of Fe$^{2+}$ and H$_2$O$_2$ seems to be better synchronized, which enhances the efficiency of the Fenton reaction and thereby contributes to the difference between ferrihydrite and goethite in -OH production.

According to this reasoning, the increase in the rate of reductive ferrihydrite dissolution induced by TPA that was discussed above will favour the Fenton reaction. Although the reductive rate increased, the release rate of Fe$^{2+}$ into solution did not increase (cf. Figs 3a and 4a), implying that during the dissolution process, more Fe$^{2+}$ temporarily accumulated at the ferrihydrite surface in the presence of TPA. This accumulation increases the likelihood of the simultaneous presence of Fe$^{2+}$ and H$_2$O$_2$ at the surface and further reinforces the efficiency of the Fenton reaction. This enhanced efficiency was also indicated by the decrease in the Fe$^{2+}$ concentration in the aerobic ferrihydrite experiment at pH 4.5, where the generated H$_2$O$_2$ more effectively oxidized Fe$^{2+}$ in the presence of TPA (Fig. 4a). Moreover, previous studies have shown that the adsorption of oxyanions and low-molecular-weight organic acids inhibits H$_2$O$_2$ decomposition. Hence, by preventing the decomposition of H$_2$O$_2$ (Fig. 6, reaction R$_5$) the adsorption of TPA may further promote the Fenton reaction. Our results show that both the TPA probe and acetate buffer (Fig. S4) influence the redox reactions and -OH generation. In fact, TPA can be considered a reasonable model of the low-molecular-weight aromatic carboxylic acids present in natural organic matter, and therefore, the results provide some indication of processes that may occur in more complex soil environments.

Finally, the reduction in -OH production at pH 7.0 was likely caused by several factors. The results of the anaerobic experiments at this pH indicated that reductive dissolution dominated (although some 2,6-DMHQ oxidation might be caused by low concentrations of O$_2$), but at the same time, the rate of catalytic oxidation increased substantially, as indicated by the very rapid oxidation of 2,6-DMHQ under aerobic conditions at pH 7.0 (Fig. 3). This increase in rate is a consequence of the increase in the deprotonation of 2,6-DMHQ (Fig. 4), which is further amplified by the rapid re-oxidation of Fe$^{3+}$ at pH 7.0 (Fig. 6, reaction R$_0$). In addition, H$_2$O$_2$ is less stable at pH 7.0 and decomposes into H$_2$O and O$_2$, a reaction that is catalysed by iron oxide surfaces (Fig. 6, reaction R$_{10}$). Hence, both Fenton reagents are destabilized at pH 7.0.

In a broader sense, the collective results of this study add to the evidence suggesting that the potential toxicity of iron oxide nanoparticles is partly related to their role as nanoreactors that generate -OH. In this respect, the presented results provide new evidence that both Fenton reagents can be generated from reaction with a biological organic reductant, and no separate source of H$_2$O$_2$ is needed. The present study, which was conducted...
at a soil-relevant pH, also supports the idea that extracellular hydroquinones or similar reducing metabolites produced by soil-living fungi can disrupt the associations between soil organic matter and iron oxide via the generation of ·OH and thereby liberate accessible nutrients into the soil environment.

**Materials and Methods**

2,6-DMHQ, ferrozine [3-[(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine], and 2-hydroxyterephthalic acid were acquired from Sigma-Aldrich, Sweden. Disodium terephthalate was purchased from Alfa Aesar, Sweden. All chemicals were of pro analysis quality or better, and all solutions were made with ultra-pure water (<2 µS) that was boiled for at least 1 h. After cooling, the water was thoroughly purged with N₂ for 1 h and finally stored in sealed glass bottles at 4 °C.

Six-line ferrihydrite (Fe₂O₃·9H₂O) was synthesized according to the method of Schwertmann and Cornell. First, 20 g of Fe(NO₃)₃·9H₂O (Merck) was dissolved in 2 L of preheated distilled water under rapid stirring. The solution was kept at 75 °C for 10–12 minutes and then rapidly cooled to room temperature. The obtained suspension was transferred to a dialysis bag and dialyzed until the conductivity was <10 µS/cm. The solid concentration of ferrihydrite in the final suspension was 1.7 g/L, and the specific surface area (SSA) was estimated to be ca. 300 m²/g. The ferrihydrite used in this study was produced by this method and was used in the following experiments for the study of ·OH generation and ·OH scavenging.

Goethite (α-FeOOH) was prepared according to the method of Hiemstra and van Rijsemijik. A solution of 2.5 M NaOH (EKA Chemicals) was slowly added into a solution of 0.5 M Fe(NO₃)₃·9H₂O while stirring and sparging with N₂ until the pH reached approximately 12. The product was aged at 60 °C for one week and then transferred to a dialysis bag, as above. The solid concentration of goethite in the final suspension was 10.6 g/L. A subsample was freeze dried, and the SSA was determined to be 66.5 ± 0.5 m²/g by applying the Brunauer–Emmett–Teller (BET) equation to the N₂ adsorption data obtained by means of a Micromeritic Gemini VII 2390a instrument. Prior to storage at 4 °C in the dark, the mineral suspensions were thoroughly purged with N₂ to remove any carbonate species in solution and at the mineral surface. The goethite structure was confirmed by XRD, and the typical needle-shaped morphology was identified by TEM (Figs S1 and S2).

Isotherms of TPA and hTPA adsorption on ferrihydrite were determined from batch experiments. Various volumes of TPA and hTPA stock solutions were added to 115 ml/L ferrihydrite in a 0.1 M NaCl medium. Aliquots were filtered through a 0.2 µm syringe filter after 5, 15, 30, 60 and 120 min. TPA was measured spectrophotometrically using a NanoDrop 2000 instrument (Thermo Scientific) at 242 nm, and each data point was an average of five measurements per aliquot. The TPA concentration was calculated from a linear standard (0–500 µM), resulting in a limit of detection (LoD) of 0.5 µM. The hTPA concentration was measured by fluorescence at an excitation wavelength of 315 nm and an emission wavelength of 425 nm using an LS 50 B luminescence spectrometer (Perkin Elmer) with 0.1 M NaCl as the blank. The hTPA concentration was calculated from a linear standard curve (0–500 nM) with a LoD of 0.5 nM. The large difference in the total concentrations of TPA and hTPA explored in this study was motivated by the conditions of the 2,6-DMHQ–iron oxide experiments, which contained ca. 300 µM TPA and 100–6000 nM hTPA.

The adsorption of TPA and hTPA onto ferrihydrite and goethite was also characterized by infrared (IR) spectroscopy using the simultaneous infrared and potentiometric (SIPT) method described by Loring et al. and Krusina et al. Ferrihydrite and goethite over-layers were prepared on an attenuated total reflectance (ATR) ZnSe crystal by evaporating 0.2 mL of a ferrihydrite (1.7 g/L) or goethite (2.0 g/L) suspension while stirring and sparging with N₂ until the pH reached approximately 12. The product was aged at 60 °C for one week and then transferred to a dialysis bag, as above. The solid concentration of goethite in the final suspension was 10.6 g/L. A subsample was freeze dried, and the SSA was determined to be 66.5 ± 0.5 m²/g by applying the Brunauer–Emmett–Teller (BET) equation to the N₂ adsorption data obtained by means of a Micromeritic Gemini VII 2390a instrument. Prior to storage at 4 °C in the dark, the mineral suspensions were thoroughly purged with N₂ to remove any carbonate species in solution and at the mineral surface. The goethite structure was confirmed by XRD, and the typical needle-shaped morphology was identified by TEM (Figs S1 and S2).

Iron(III) oxide nanoparticles was studied in batch experiments at pH values of 4.5 and 7.0 with 50 mL of a 0.1 M NaCl suspension containing 200 m²/L iron oxide and 90 µM 2,6-DMHQ. For the experiments in the presence of TPA, TPA was added 60 s before the generation of ·OH and hydroquinone oxidation products from the reactions between 2,6-DMHQ and/or H₂O₂, and the suspension was thoroughly mixed on an end-over-end rotator. Sample aliquots were removed from the suspension at different time points between 5–240 min. All solutions were measured at the required pH with 40 mM HCl or NaOH in 0.1 NaCl medium prior to the experiment (except in experiments in which the interference from acetate buffer was tested) and the experiments were performed at different oxygen levels, denoted as anaerobic and aerobic in the following text. Prior to the anaerobic experiments, all solutions were sparged with N₂ for 20–30 minutes, and the experiments were carried out in a glove bag (Sigma-Aldrich) purged with N₂. The aerobic experiments were conducted...
under atmospheric conditions with \([O_3] = 270–310 \mu M\) in the initial suspensions. The \(O_3\) concentrations were measured with an Orion Star A213 dissolved oxygen meter (Thermo Scientific).

The 2,6-DHQ and 2,6-dimethoxybenzoquinone (2,6-DBQ) concentrations were determined by HPLC. Freshly prepared solutions of 2,6-DHQ and 2,6-DBQ were analyzed as standards to determine the HPLC response factors. Chromatographic separation was accomplished by a Dionex Ultimate 3000 system (Thermo Scientific) equipped with an RS variable wavelength detector and a 4.6 × 150 mm, 3.5 µm XDB-C18 Eclipse column (Agilent, USA), and this equipment was controlled by Chromelon® software. The column flow rate was 1.0 mL/min, and the solvent system consisted of H2O:acetonitrile/formic acid in a 90:10:0.1 ratio. The injection volume was 25 µL, and chromatograms were recorded at wavelengths of 282 and 290 nm for 2,6-DHQ and 2,6-DBQ, respectively.

The Fe\(^{2+}\) concentration was determined by ferrozine assay\(^54\). A filtered sample with a volume between 0.5 and 2.0 mL was mixed with 100 µL of 1% (wt/vol) aqueous ferrozine and diluted to a final volume of 2.3 mL with 0.1 M NaCl or acetate buffer. The absorbance of the solution was measured after 5 min with an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) at 562 nm. Either 0.1 M NaCl or 0.1 M acetate buffer was used as the blank. The Fe\(^{2+}\) concentration was determined from a linear standard curve (0–100 µM) made from FeSO\(_4\). This experimental method resulted in a LoD of 2.0 µM.

The -OH species was detected via its reaction with TPA and the formation of the hydroxylated product, hTPA\(^26\). The hTPA concentration was determined by fluorescence spectroscopy as described above, and further experimental details are provided in SI.

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