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**Author(s):** Xiao, Yihua; Carena, Luca; Näsi, Marja-Terttu; Vähätalo, Anssi V.

**Title:** Superoxide-driven autocatalytic dark production of hydroxyl radicals in the presence of complexes of natural dissolved organic matter and iron

**Year:** 2020

**Version:** Accepted version (Final draft)

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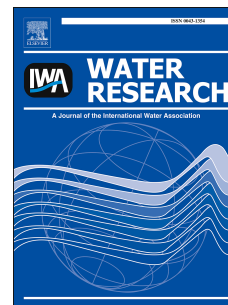
Xiao, Y., Carena, L., Näsi, M.-T., & Vähätalo, A. V. (2020). Superoxide-driven autocatalytic dark production of hydroxyl radicals in the presence of complexes of natural dissolved organic matter and iron. *Water Research*, 177, Article 115782.

<https://doi.org/10.1016/j.watres.2020.115782>

# Journal Pre-proof

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Yihua Xiao, Luca Carena, Marja-Terttu Näsi, Anssi V. Vähätalo



PII: S0043-1354(20)30319-5

DOI: <https://doi.org/10.1016/j.watres.2020.115782>

Reference: WR 115782

To appear in: *Water Research*

Received Date: 22 November 2019

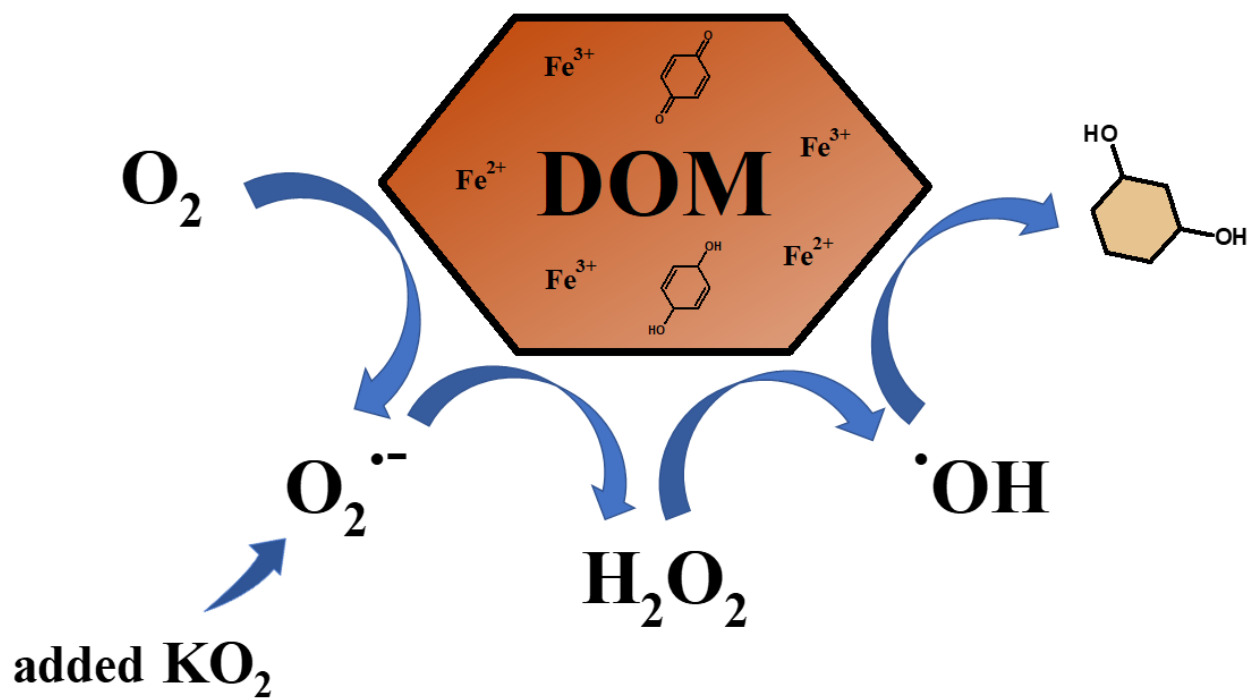
Revised Date: 31 March 2020

Accepted Date: 1 April 2020

Please cite this article as: Xiao, Y., Carena, L., Näsi, M.-T., Vähätalo, A.V., Superoxide-driven autocatalytic dark production of hydroxyl radicals in the presence of complexes of natural dissolved organic matter and iron, *Water Research* (2020), doi: <https://doi.org/10.1016/j.watres.2020.115782>.

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1 Superoxide-driven autocatalytic dark production of  
2 hydroxyl radicals in the presence of complexes of  
3 natural dissolved organic matter and iron

4 *Yihua Xiao*<sup>1,2\*</sup>, *Luca Carena*<sup>3</sup>, *Marja-Terttu Näsi*<sup>1</sup>, *Anssi V. Vähätalo*<sup>1</sup>

5 <sup>1</sup>Department of Biological and Environmental Science, University of Jyväskylä, 40014  
6 Jyväskylä, Finland

7 <sup>2</sup>School of Environmental & Municipal Engineering, Qingdao University of Technology,  
8 266033 Qingdao, China

9 <sup>3</sup>Dipartimento di Chimica, Università di Torino, Via Pietro Giuria 5, 10125 Torino, Italy

10

11 \*Corresponding author: [yihua.y.xiao@jyu.fi](mailto:yihua.y.xiao@jyu.fi)

12 **Abstract**

13 We introduced superoxide as potassium superoxide ( $\text{KO}_2$ ) to artificial lake water containing  
14 dissolved organic matter (DOM) without or with introduced ferric iron complexes (DOM-Fe),  
15 and monitored the production rate of hydroxyl radicals as well as changes in the absorption  
16 and fluorescence properties of DOM. The introduction of  $\text{KO}_2$  decreased the absorption by  
17 DOM but increased the spectral slope coefficient of DOM more with complexed ferric Fe  
18 than without it. The introduction of  $\text{KO}_2$  increased the fluorescence of humic-like  
19 components in DOM without introduced ferric Fe but resulted in the loss of fluorescence in  
20 DOM with introduced ferric Fe. A single introduction of  $13 \mu\text{mol L}^{-1} \text{KO}_2$  produced  $10 \mu\text{mol}$   
21  $\text{L}^{-1}$  and  $104 \mu\text{mol L}^{-1}$  hydroxyl radicals during a week-long experiment without and with the  
22 introduced DOM-Fe complexes, respectively. The production rate of hydroxyl radicals  
23 decreased exponentially with time but levelled off and continued several days in DOM with  
24 introduced ferric Fe. These findings suggest that in the presence of DOM-Fe complexes,  
25 superoxide can trigger an autocatalytic Fenton reaction that produces hydroxyl radicals and  
26 breaks down DOM.

27 **Keywords:** dissolved organic matter, iron, superoxide, hydroxyl radicals, production rate,  
28 absorption

29

## 30 **1 Introduction**

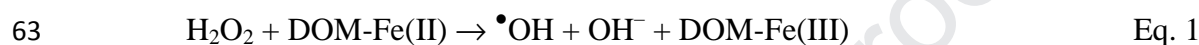
31 Dissolved organic matter (DOM) is a heterogeneous mixture of organic compounds  
32 and plays important roles in natural and engineered systems. In soils and freshwaters, the  
33 majority of DOM consists of humic substances that primarily originate from terrestrial plant  
34 litter after biotic and abiotic transformations (Piccolo, 1996; Tranvik, 1988). Humic DOM  
35 binds ferric iron, Fe(III), into complexes, DOM-Fe(III), and keeps poorly soluble Fe(III) in  
36 dissolved form (Fujii et al., 2014). Humic DOM contains aromatic and quinone-like moieties,  
37 which occur in three redox-states (quinones, semiquinones and hydroquinones) and can  
38 mediate reactions between electron donors and acceptors (Aeschbacher et al., 2010; Chen &  
39 Pignatello, 1997; Garg et al., 2018; Yuan et al., 2016).

40 The enzymatic hydrolysis of humic DOM and its intracellular metabolism is  
41 inefficient, because the large size of molecular aggregates, chemical heterogeneity, and non-  
42 hydrolysable bonds limit the microbial transformation of humic DOM (Arnosti, 2004).  
43 Abiotic photochemical reactions mineralize humic DOM and account for one tenth of CO<sub>2</sub>  
44 emissions in freshwaters (Aarnos et al., 2018; Koehler et al., 2014). The remaining 90% of  
45 DOM is mineralized through mechanisms that are poorly known.

46 Extracellular reactions between DOM and reactive oxygen species (ROS) can explain  
47 a part of DOM transformations (Mostovaya et al., 2017; Page et al., 2012; Trusiak et al., 2018;  
48 Waggoner et al., 2017). The first step in the formation of ROS is a one-electron reduction of  
49 O<sub>2</sub> to superoxide (O<sub>2</sub><sup>•-</sup>). Numerous processes produce O<sub>2</sub><sup>•-</sup>: (i) photochemical reactions  
50 (Micinski et al., 1993; Fujii & Otani, 2017; Zhang & Blough, 2016; Text SIV in supporting  
51 information (SI)), (ii) abiotic dark oxidation of reduced metals or organic matter (Garg et al.,  
52 2018; Gil-Lozano et al., 2017; Page et al., 2012; Yuan et al., 2016) and (iii) biological  
53 processes both in light and dark (Diaz et al., 2013; Diaz & Plummer, 2018; Imlay, 2004;  
54 Zhang et al., 2016). O<sub>2</sub><sup>•-</sup> reacts with the redox-active metals (e.g., Fe and copper) and

55 quinone-like moieties of DOM, but it has otherwise limited reactivity with aqueous DOM  
56 (Garg et al., 2011, 2018; Hayyan et al., 2016; Yuan et al., 2016).

57  $O_2^{\bullet-}$  can be reduced further to hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals ( $\bullet OH$ ).  
58 Bimolecular disproportionation and the disproportionation catalyzed by reduced metals or  
59 DOM transform  $O_2^{\bullet-}$  to  $H_2O_2$  (Goldstone & Voelker, 2000; Ma et al., 2010).  $O_2^{\bullet-}$  can reduce  
60 DOM-Fe(III) to DOM-Fe(II) (Rose & Waite, 2005). DOM-Fe(II) as well as inorganic Fe(II)  
61 can react with  $H_2O_2$  through the Fenton reaction and produce highly reactive  $\bullet OH$  that breaks  
62 down DOM (Southworth & Voelker, 2003; Voelker et al., 1997).



64 According to the stoichiometry of the Fenton reaction (Eq. 1), the Fe(III)-catalyzed  
65 production of  $\bullet OH$  requires three  $O_2^{\bullet-}$  radicals, two for the formation of  $H_2O_2$  and one for the  
66 formation of DOM-Fe(II). However, the stoichiometry of the Fenton reaction ( $\bullet OH$ -to- $O_2^{\bullet-}$   
67 ratio = 0.33) ignores a well-known fact that  $\bullet OH$  generates radical species that can regenerate  
68 the Fenton reactants and autocatalyze the Fenton reaction (e.g., Chen & Pignatello, 1997; Gil-  
69 Lozano et al., 2017). The degree of autocatalysis is poorly known, although it has high  
70 importance when the efficiency of the Fenton reaction is evaluated in natural or engineered  
71 systems.

72 The present study estimates the dark production rates of  $\bullet OH$  in artificial lake water  
73 from  $O_2^{\bullet-}$  (introduced as potassium superoxide,  $KO_2$ ) in the presence of DOM with or  
74 without introduced Fe(III). The production rates of  $\bullet OH$  were quantified from the reaction  
75 between  $\bullet OH$  and coumarin (Louit et al., 2005) and after accounting for the major scavengers  
76 of  $\bullet OH$  in the artificial lake water. In this study we demonstrate that the cumulative  
77 production of  $\bullet OH$  from  $O_2^{\bullet-}$  in a-week-long experiment exceeds the  $\bullet OH$  yield of the Fenton  
78 reaction by several folds and extensively modifies the spectroscopic properties of DOM.

79

## 80 2 Materials and methods

### 81 2.1 Materials and reagents

82 DOM was extracted from a water sample collected during the fall turnover of Lake  
83 Valkea-Kotinen in southern Finland. This small headwater lake is acidic (pH 5.4) with high  
84 concentration of DOC ( $10\text{--}12\text{ mg DOC L}^{-1} = \sim 20\text{ mg DOM L}^{-1}$ ) and total Fe ( $\sim 5\text{ }\mu\text{M}$ ; Einola  
85 et al., 2011; Vähätalo et al., 2003). In Lake Valkea-Kotinen, the mean molecular mass of  
86 DOM is  $1130\text{--}4000\text{ g mol}^{-1}$ , the content of humic substances and aromatic groups is 75% and  
87 45–67%, respectively (Vogt et al., 2004).

88 The extraction of DOM followed the method by Dittmar et al. (2008) but included an  
89 addition of 0.01 M sodium fluoride (NaF, Sigma-Aldrich) in filtered ( $<0.2\text{ }\mu\text{m}$ ) and acidified  
90 (pH 2) lake water. At pH 2, Fe(III) binds poorly on DOM and preferentially forms ferric  
91 fluoride complex (Gao & Zepp, 1998). Ferric fluoride and fluoride ions were rinsed out of the  
92 column with 0.01 M HCl (Dittmar et al. 2008) to yield extracted DOM with a very low  
93 content of fluoride and Fe. The extraction removed 96.6% of Fe from lake water and the  
94 DOM extracts contained 8.5 nmol Fe/mg DOM (Table 1). The chemicals ( $>97\%$  pure) were  
95 bought from Sigma Aldrich. Iron(III) chloride hexahydrate ( $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ ) and  $\text{KO}_2$  were the  
96 sources of Fe(III) and  $\text{O}_2^{\bullet -}$ , respectively. Coumarin and 7OH-coumarin were the probes for  
97  $\bullet\text{OH}$  (Burgos Castillo et al., 2018). The aqueous solutions were prepared in ultrapure water  
98 (resistivity  $18\text{ M}\Omega\cdot\text{cm}$ ; SG ultrapure water system, SG WATER), but were later modified to  
99 artificial lake water by a salt solution mixture (Table S1). Glassware was soaked overnight in  
100 0.1 M HCl and carefully rinsed with ultrapure water six times prior to use.

### 101 2.2 Experimental setup

102 The experiment consisted of four treatments prepared in triplicates (Table 1):

- 103 1) “control” – extracted DOM (8.5 nmol Fe/mg DOM) dissolved in artificial lake water;
- 104 2) “ $\text{KO}_2$ ” – like (1) but with introduced  $\text{KO}_2$ ;



105 3) “Fe” – like (1) but Fe(III) was introduced as DOM-Fe(III) (1000 nmol Fe(III)/mg  
106 DOM);

107 4) “Fe + KO<sub>2</sub>” – a combination of (2) and (3).

108 For the preparation of DOM-Fe(III), the acidic (pH 2) DOM solution (50 mg L<sup>-1</sup> in  
109 ultrapure water) received 1 mM Fe(III) and was titrated to pH 5 with NaOH and HCl,  
110 approximating the ambient pH of Lake Valkea-Kotinen. During the titration, the binding sites  
111 of DOM suppressed the hydrolysis of ferric Fe and DOM-Fe(III) was formed (Karlsson &  
112 Persson, 2012). According to an equilibrium speciation model (Visual Minteq 3.1), the DOM  
113 extract was able to bind Fe(III) entirely and accordingly visual precipitates were absent at any  
114 phase of the experiment. The “control” and “KO<sub>2</sub>” treatments were titrated in the same way  
115 but without the introduced Fe. All treatments received the stock solution of coumarin to the  
116 final concentration of 10 μM (Table 1) and inorganic component of artificial lake water  
117 (Table S1).

118 The “KO<sub>2</sub>” and “Fe + KO<sub>2</sub>” treatments received an alkaline solution of KO<sub>2</sub> (2 g KO<sub>2</sub>  
119 in 100 mL 0.05 M NaOH) to a 13 μM final concentration (Table 1). Similar magnitudes of  
120 O<sub>2</sub><sup>•-</sup> form instantly during the oxidation of reduced organic matter or metals (Liao et al.,  
121 2019; Minella et al., 2015; Page et al., 2013; Trusiak et al., 2018; Zhang & Yuan, 2017), with  
122 a few days of microbial metabolism (Zhang et al., 2016) or with 0.17–few days of solar  
123 irradiation depending on water quality (Cooper & Zika, 1983; Micinski et al., 1993; Text SIV  
124 in SI). The introduction of KO<sub>2</sub> increased the pH of non-buffered artificial lake water to 12.2,  
125 where the reduction rate of DOM-Fe(III) to DOM-Fe(II) by O<sub>2</sub><sup>•-</sup> is faster than bimolecular  
126 disproportionation of O<sub>2</sub><sup>•-</sup> (see Text SI in SI). The reaction medium was titrated back to pH 5  
127 with HCl. Finally, all treatments were incubated at 20 °C in the dark with a headspace of air.

### 128 2.3 UV-Vis spectral analysis

129 After 26 h and a week (168 h) of incubation, the absorbance of chromophoric DOM

130 (CDOM) was measured with a UV-Vis spectrometer (Lambda 850, PerkinElmer) from 200  
131 nm to 700 nm at 1 nm intervals. The absorption coefficient was calculated as,

$$132 \quad a_{\lambda} = 2.303 \times A_{\lambda}/L \quad \text{Eq. 2,}$$

133 where  $a_{\lambda}$  ( $\text{m}^{-1}$ ) is the absorption coefficient at wavelength  $\lambda$ ,  $A_{\lambda}$  (unitless) is absorbance, and  
134  $L$  is the path length of the cuvette ( $L = 0.01$  m). The changes in  $a_{\lambda}$  were quantified at 410 nm  
135 as  $a_{410}$ , an indicator of water color (Hongve et al., 2004). The spectral slope coefficient ( $S_{275-}$   
136  $_{295}$ ), which indicates the molecular mass of DOM, was calculated from ln-transformed  
137 absorption coefficient between 275 nm and 295 nm (Helms et al., 2008).

#### 138 2.4 Fluorescence analysis and PARAFAC

139 Samples for fluorescence analysis were stored at 4 °C after collection and measured  
140 within 3 weeks. Fluorescence EEMs were measured with a LS 55 luminescence spectrometer  
141 (PerkinElmer). The samples were scanned with an excitation wavelength (Ex) from 240 nm  
142 to 450 nm at 5 nm intervals and emission wavelength (Em) from 300 nm to 600 nm with 0.5  
143 nm intervals. The slit width for both Ex and Em was set to 5 nm. Blank and Raman samples  
144 from ultrapure water were measured prior to actual samples (Murphy et al., 2003).

145 PARAllel FACtor analysis (PARAFAC) was run in Matlab R2015b (Mathworks,  
146 USA) using the drEEM toolbox (version 0.3.0). The raw EEM dataset ( $n = 48$ ) was corrected  
147 for spectral bias, inner filter effects and background signals (measured with ultrapure water).  
148 In the end, all EEMs were normalized to the area of Raman peak collected with ultrapure  
149 water at Ex = 275 nm to compensate for daily fluctuations in lamp intensity (Kothawala et al.,  
150 2016; Murphy et al., 2013). The fluorescent components were validated with multiple split-  
151 half tests. The validation was constrained by a Tucker congruence coefficient (TCC >0.95).  
152 Finally, the maximum fluorescence intensities ( $F_{\text{max}}$ , in Raman unit, R.U.) of components  
153 were reported.

154 2.5 Calculations of the cumulative production of  $\bullet\text{OH}$  radicals

155 The samples for the quantification of coumarin and 7OH-coumarin were collected at 0,  
156 3, 6, 20, 26, and 168 h. These samples were frozen immediately after collection and analyzed  
157 later. Coumarin and 7OH-coumarin were measured by high performance liquid  
158 chromatography (HPLC) equipped with UV-Vis absorbance and fluorescence detectors  
159 (Louit et al., 2005). The quantification of the two compounds was carried out by means of the  
160 UV-Vis absorbance detector for coumarin (absorption wavelength = 280 nm) and  
161 fluorescence detector for 7OH-coumarin (excitation wavelength = 320 nm; emission  
162 wavelength = 450 nm). The Text SII in SI reports the details of HPLC technique.

163 The formation rates of  $\bullet\text{OH}$  were quantified from the reaction between coumarin and  
164  $\bullet\text{OH}$ . This reaction has a second-order rate constant of  $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  and produces a few  
165 hydroxycoumarin isomers, including 7OH-coumarin with a yield of 0.047 (Burgos Castillo et  
166 al., 2018). We calculated the production rates of  $\bullet\text{OH}$  along the course of the experiment by  
167 quantifying periodically the concentrations of 7OH-coumarin and coumarin as well as  
168 accounting for the scavenging of  $\bullet\text{OH}$  by DOM,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , coumarin and 7OH-coumarin.  
169 The calculations assumed a steady-state between the scavenging and the formation rate of  
170  $\bullet\text{OH}$ . The production rates of  $\bullet\text{OH}$  radicals were integrated over the course of the experiment  
171 for the cumulative production of  $\bullet\text{OH}$ . The detailed procedure for calculations is described in  
172 the SI.

## 173 2.6 Statistical analyses

174 The statistical difference between the triplicated treatments and control (DOM alone  
175 treatment) was assessed using paired  $t$  tests with two-tailed distributions. The significance  
176 level was set at  $P < 0.05$ .

## 177 3 Results

### 178 3.1 Changes in absorption spectra

179 The introduction of  $\text{KO}_2$  did not change the absorption coefficient  $a_{410}$  in an early  
180 phase of the experiment (at 26 h) but decreased it by 25% by the end of the experiment (at  
181 168 h) compared to DOM in artificial lake water without  $\text{KO}_2$  (“control” vs “ $\text{KO}_2$ ”, Figure  
182 1a). In the “Fe” treatment, the introduced DOM-Fe(III) consistently kept  $a_{410}$  at a higher level  
183 than in the control (Figure 1a). When introduced with DOM-Fe(III),  $\text{KO}_2$  decreased  $a_{410}$  by  
184 18% already at 26 h and by 66% over the entire experiment compared to the control (“Fe +  
185  $\text{KO}_2$ ”, Figure 1a).

186 In comparison with the control treatment,  $\text{KO}_2$  increased the spectral slope coefficient  
187 ( $S_{275-295}$ ), while DOM-Fe(III) decreased it (Figure 1b). When introduced with Fe,  $\text{KO}_2$   
188 increased  $S_{275-295}$  by 20% at 26 h and by 54% at the end of the experiment (Figure 1b).

### 189 3.2 Changes in fluorescent intensities of PARAFAC components

190 The four components of fluorescent DOM identified by the EEM-PARAFAC  
191 associated with humic substances (Comp 1–2, Figure 2 and Table S2), 7OH-coumarin (Comp  
192 3; Figure S1) and protein-like DOM (Comp 4, Figure 2 and Table S2). After 168 h, the  
193 introduction of  $\text{KO}_2$  had increased the fluorescence of humic-like components 1 and 2 by 39%  
194 and 18%, respectively, in comparison to the control treatment (“ $\text{KO}_2$ ”, Figure 3). The added  
195 associated Fe(III) quenched the fluorescence of humic-like components 1–2 (“Fe”, Figure 3).  
196 In the presence of DOM-Fe(III),  $\text{KO}_2$  reduced the fluorescence of components 1–2 relative to  
197 the control treatment and decreased the fluorescence of component 4 to negligible level (“Fe  
198 +  $\text{KO}_2$ ”, Figure 3). Component 3 was detected in all treatments (Figure 3) indicating that  $\bullet\text{OH}$   
199 radicals transformed coumarin (Table 1) into 7OH-coumarin, as explained in the following  
200 section.

201 3.3 Production of  $\bullet\text{OH}$ 

202 The formation rate of  $\bullet\text{OH}$ ,  $R_f^{\bullet\text{OH}}(t)$ , was assessed from the measured concentrations  
203 of coumarin and 7OH-coumarin (Figure SIII-1&2) accounting for the other scavengers of  
204  $\bullet\text{OH}$  as described in the Text SIII in SI. In the beginning of the experiment,  $R_f^{\bullet\text{OH}}(t_0)$  was  
205 0.0031–0.0034  $\text{nM s}^{-1}$  in the “Fe” treatment and the control, which did not receive  $\text{KO}_2$   
206 (Figure 4; Table SIII-1). The introduction of  $\text{KO}_2$  resulted in  $R_f^{\bullet\text{OH}}(t_0)$  of 0.039  $\text{nM s}^{-1}$  and  
207 1.14  $\text{nM s}^{-1}$  in the “ $\text{KO}_2$ ” and “ $\text{KO}_2 + \text{Fe}$ ”-treatments, respectively (Figure 4; Table SIII-1).  
208 In the “ $\text{KO}_2 + \text{Fe}$ ” treatment, the measured  $R_f^{\bullet\text{OH}}(t_0)$  was nearly identical to the  
209 corresponding rate of 1.05  $\text{nM s}^{-1}$  calculated based on a simple kinetic model (Figure SI-2).  
210 Briefly, the calculated rates are based on the kinetics for the following sequence of reactions:  
211 (i) the reduction of DOM-Fe(III) to 13  $\mu\text{M}$  DOM-Fe(II) by  $\text{O}_2^{\bullet-}$ , (ii) the reduction of  $\text{O}_2$  to  
212  $\text{O}_2^{\bullet-}$  by DOM-Fe(II), (iii) the disproportionation of  $\text{O}_2^{\bullet-}$  to  $\text{H}_2\text{O}_2$  and (iv) the Fenton reaction  
213 (Eq. 1) between  $\text{H}_2\text{O}_2$  and DOM-Fe(II). The good match between the measured and the  
214 calculated  $R_f^{\bullet\text{OH}}(t_0)$  in the “ $\text{KO}_2 + \text{Fe}$ ” treatment suggest that, (i)  $\text{O}_2^{\bullet-}$  induced the formation  
215 of  $\bullet\text{OH}$  in the presence of DOM-Fe(III) and (ii) the reaction stoichiometry (e.g.,  $\bullet\text{OH}$ -to- $\text{O}_2^{\bullet-}$   
216 ratio = 0.33 of Eq. 1) described the measured initial rates well.

217 The formation rates of  $\bullet\text{OH}$  decreased exponentially with time in all treatments, and  
218 after 10 h levelled at 0.15  $\text{nM s}^{-1}$  in the “ $\text{KO}_2 + \text{Fe}$ ” treatment (Figure 4, Table SIII-1). In the  
219 “ $\text{KO}_2 + \text{Fe}$ ” treatment, the prolonged formation of  $\bullet\text{OH}$  is consistent with the changes in  
220 DOM that took place mostly after 26 h (Figure 1), but inconsistent with a simple kinetic  
221 model (Text SI-3). The simple kinetic model incorrectly suggests the depletion of  $R_f^{\bullet\text{OH}}(t)$   
222 within a few minutes (Text SI-3) in contrast to the measured  $R_f^{\bullet\text{OH}}(t)$ , which lasted tens of  
223 hours (Figure 4).

224 The cumulative production of  $\bullet\text{OH}$  was computed as the integral of  $R_f^{\bullet\text{OH}}(t)$  for the

225 first 10 hours or for the entire length of the experiment (168 h, Table 2, Eq. SIII-1). In all  
226 treatments, the majority of  $\bullet\text{OH}$  was produced after 10 h (Table 2). In the treatments with  
227 introduced  $\text{KO}_2$ , the cumulative production of  $\bullet\text{OH}$  was 9.9 and 104  $\mu\text{M}$  in the “ $\text{KO}_2$ ” and  
228 “ $\text{KO}_2 + \text{Fe}$ ” treatments, respectively, over the entire length of the experiment (Table 2). The  
229 yields of  $\bullet\text{OH}$  per introduced 13  $\mu\text{M}$   $\text{KO}_2$  were 0.76  $\bullet\text{OH}/\text{O}_2^{\bullet-}$  and 8  $\bullet\text{OH}/\text{O}_2^{\bullet-}$  in the “ $\text{KO}_2$ ”  
230 and “ $\text{KO}_2 + \text{Fe}$ ” treatments, respectively. The measured yields exceeded the stoichiometric  
231 yield (0.33  $\bullet\text{OH}/\text{O}_2^{\bullet-}$  in Eq. 1) by a factor of 2.3 and 24 in the “ $\text{KO}_2$ ” and “ $\text{KO}_2 + \text{Fe}$ ”  
232 treatments, respectively, and indicated an autocatalytic formation of  $\bullet\text{OH}$  from  $\text{O}_2^{\bullet-}$  in the  
233 presence of DOM-Fe.

234

## 235 **4 Discussion**

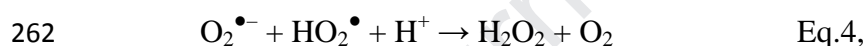
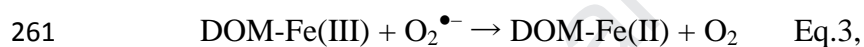
### 236 4.1 $\bullet\text{OH}$ production

237 As explained in the Method-section 2.2, the amount of introduced  $\text{O}_2^{\bullet-}$  in this study is  
238 environmentally relevant but here we compare the cumulative productions of  $\bullet\text{OH}$  (0.23–104  
239  $\mu\text{M}$ ) in our week-long experiment to those reported earlier. An oxidation of reduced DOM or  
240 metals produces  $\bullet\text{OH}$ . For example, the oxidation of Arctic surface and soil waters produce  
241 0.2–4.5  $\mu\text{M}$   $\bullet\text{OH}$  over 24 hour oxidation (Page et al., 2013); the oxidation of hypolimnetic  
242 water accumulatively produces 0.2–4.5  $\mu\text{M}$   $\bullet\text{OH}$  (Minella et al., 2015); the oxidation of pyrite  
243 can produce 7.5–135  $\mu\text{M}$   $\bullet\text{OH}$  within 7 hours (Zhang & Yuan, 2017); and the oxidation of  
244 river sediments can accumulatively produce 57–1479  $\mu\text{mol kg}^{-1}$   $\bullet\text{OH}$  within 48 hours (Liao  
245 et al., 2019). Thus, the cumulative productions of  $\bullet\text{OH}$  in this study are broadly similar to  
246 those reported earlier from various environmental processes.

### 247 4.2 Stoichiometric production of $\bullet\text{OH}$ from superoxide and DOM-Fe(III)

248 In this study, the production of  $\bullet\text{OH}$  is orders of magnitude larger in the presence than

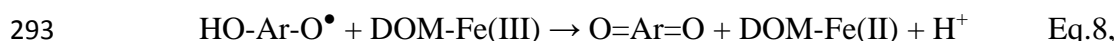
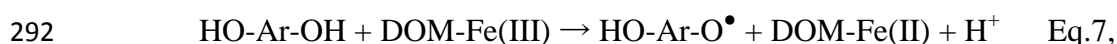
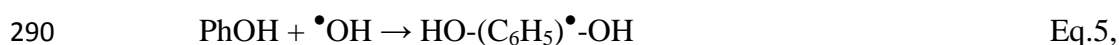
249 the absence of introduced  $\text{KO}_2$ , therefore,  $\text{O}_2^{\bullet-}$  is responsible for the extensive production of  
250  $\bullet\text{OH}$ . The reaction pathway from  $\text{O}_2^{\bullet-}$  to  $\bullet\text{OH}$  is beyond the scope of the present study,  
251 because we did not measure the intermediates such as DOM-Fe(II) or  $\text{H}_2\text{O}_2$ . Our simple  
252 kinetic model, however, successfully predicts the measured  $R_f^{\bullet\text{OH}}(t_0)$  in the “ $\text{KO}_2 + \text{Fe}$ ”  
253 treatment and may provide a mechanistic explanation for the initial  $\bullet\text{OH}$  production rates  
254 (Text SI). According to this simple model, the reaction pathway starts with the reduction of  
255 DOM-Fe(III) to DOM-Fe(II) by  $\text{O}_2^{\bullet-}$  (Eq. 3, Text SI). Later, bimolecular disproportionation  
256 generates  $\text{H}_2\text{O}_2$  (Eq. 4). At this stage, the reduction of  $\text{O}_2$  by DOM-Fe(II) is the source of  
257  $\text{O}_2^{\bullet-}$  (Text SI). Finally,  $\text{H}_2\text{O}_2$  reacts with DOM-Fe(II) (Eq. 1, Text SI). The reactive oxygen  
258 species can maintain the redox cycling of the iron catalyst and the production of  $\bullet\text{OH}$  from  
259 the oxidant ( $\text{H}_2\text{O}_2 = 2[\text{O}_2^{\bullet-} + \text{H}^+]$ ) according to the stoichiometry of the Fenton reaction  
260 (Pignatello et al. 2006; Text SI).



263 DOM facilitates the formation of  $\bullet\text{OH}$  through the Fenton reaction in many ways  
264 (Georgi et al., 2007). When DOM makes complexes with Fe(III) at  $\text{pH} > 3.5$ , it keeps Fe(III)  
265 in soluble reactive form (Zhang & Zhou, 2019). At low pH (for instance,  $\text{pH} = 5$  in this  
266 study), the deprotonated carboxylic groups of DOM are favorable ligand for Fe(III) and the  
267 concentration of a major competing ligand, hydroxyl ion ( $\text{OH}^-$ ), is low (Bhattacharyya et al.,  
268 2019; Lee et al., 2019; Neubauer et al., 2013; Zhang & Zhou, 2019). Mildly acidic conditions  
269 (like in the present study) are favorable for the Fenton reaction, which breaks down humic  
270 substances most efficiently at  $\text{pH} 4\text{--}5$  rather than in more acidic or basic solutions (Wu et al.,  
271 2010). Additionally,  $\text{H}_2\text{O}_2$  reacts faster with DOM-Fe(II) than with inorganic Fe(II) (Voelker  
272 & Sulzberger 1996; Zhang & Zhou, 2019).

## 273 4.3 Autocatalysis of the Fenton reaction

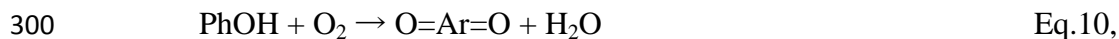
274 In our study, the cumulative production of  $\bullet\text{OH}$  is larger ( $0.76\text{--}8 \bullet\text{OH}/\text{O}_2^{\bullet-}$ ) than  
 275 expected from the introduced  $\text{O}_2^{\bullet-}$  according to the stoichiometry of the Fenton reaction  
 276 ( $0.33 \bullet\text{OH}/\text{O}_2^{\bullet-}$ ). The reactions between  $\bullet\text{OH}$  and the phenolic moieties of DOM can explain  
 277 the autocatalysis of the Fenton reaction in this study (Voelker & Sulzberger, 1996, Chen &  
 278 Pignatello, 1997). Those reactions generate hydroquinone-like DOM and  $\text{O}_2^{\bullet-}$  (Voelker &  
 279 Sulzberger, 1996, Chen & Pignatello, 1997, Duesterberg & Waite, 2007). The reactions  
 280 between  $\bullet\text{OH}$  and phenols have been examined earlier with model compounds (phenol and  
 281 hydroxybenzoic acid) that mimic the aromatic moieties of DOM (Chen & Pignatello, 1997;  
 282 Duesterberg & Waite, 2007). Firstly, an addition of  $\bullet\text{OH}$  to phenol (PhOH) generates a  
 283 dihydroxycyclohexadienyl radical ( $\text{HO}-(\text{C}_6\text{H}_5)\bullet\text{-OH}$ , Eq.5), which transforms into  
 284 hydroquinone ( $\text{HO-Ar-OH}$ ) in a reaction that consumes  $\text{O}_2$  and generates  $\text{O}_2^{\bullet-}$  (Eq.6, Chen &  
 285 Pignatello, 1997; Voelker & Sulzberger, 1996). Secondly, the transformation of  
 286 hydroquinone to semiquinone radical ( $\text{HO-Ar-O}\bullet$ ) reduces DOM-Fe(III) to DOM-Fe(II)  
 287 (Eq.7, Chen & Pignatello, 1997; Duesterberg & Waite, 2007). Finally, a semiquinone radical  
 288 reduces another DOM-Fe(III) when undergoing oxidation to quinone ( $\text{O=Ar=O}$ ) (Eq.8, Chen  
 289 & Pignatello, 1997; Duesterberg & Waite, 2007).



295 The four consecutive reactions (Eq. 5–8) described above produce three reducing  
 296 equivalents ( $\text{O}_2^{\bullet-}$  and/or DOM-Fe(II)) that re-generate the Fenton reactants and thus the



297 production of  $\bullet\text{OH}$  gets autocatalyzed through the Fenton reaction and DOM oxidation. Note  
298 that the net reaction of this process (from Eq.1 to Eq.9, with the exception of Eq.2) is the  
299 oxidation of phenol to quinone (Eq.10).



301 This autocatalysis can continue as long as water contains  $\text{O}_2$  and DOM contains  
302 aromatic moieties that  $\bullet\text{OH}$  can transform into hydroquinones. In this study, the headspace of  
303 air serves as a source of dissolved  $\text{O}_2$  to our solutions like the atmosphere is a source of  $\text{O}_2$  to  
304 surface waters. The high (45–67%) aromaticity of DOM used in this study (Vogt et al., 2004)  
305 provides a large reservoir of aromatic moieties that  $\bullet\text{OH}$  can transform into hydroquinones.  
306 Because highly aromatic humic substances and Fe are abundant in soils and freshwaters, the  
307 potential for the autocatalyzed Fenton reaction is high in these environments.

#### 308 4.4 Fenton reaction without introduced $\text{O}_2^{\bullet-}$

309 Our experiments show that even without introduction of  $\text{O}_2^{\bullet-}$ , DOM-Fe can produce  
310 hydroxyl radicals at low amounts that are similar to  $< 0.5 \mu\text{M}$   $\bullet\text{OH}$  produced during aeration  
311 of Arctic surface waters (Page et al., 2012; Trusiak et al., 2018). Since Lake Valkea-Kotinen  
312 has typically anoxic hypolimnion in late summer and it is surrounded by peaty soils, the  
313 DOM extract used in the present study may contain Fe or quinone-like moieties in a reduced  
314 state. The reduced hydroquinone-like moieties or reduced metals (e.g., Fe(II)) associated to  
315 DOM can reduce  $\text{O}_2$  to  $\text{O}_2^{\bullet-}$  and initiate the sequence of reactions leading to the Fenton  
316 reaction (Garg et al., 2018; Page et al., 2013, 2014). In this study, the external supply of  
317 DOM-Fe(III) doubled the  $\bullet\text{OH}$  production compared to DOM extract alone with low content  
318 of Fe and further emphasizes the Fenton reaction as a source of  $\bullet\text{OH}$ . Although an abiotic  
319 dark formation of  $\bullet\text{OH}$  is low in oxic surface waters without external source of  $\text{O}_2^{\bullet-}$  (Trusiak  
320 et al., 2018; this study), an episodic mixing of reduced DOM or redox sensitive metals to an

321 oxic environment can promote an extensive production of  $\bullet\text{OH}$  (Minella et al., 2015; Page et  
322 al., 2012, 2013; Trusiak et al., 2018).

#### 323 4.5 Effects of $\text{O}_2^{\bullet-}$ and Fe on the absorption spectra of CDOM

324 In this study, the introduction of external  $\text{O}_2^{\bullet-}$  eventually led to a CDOM breakdown  
325 and increased the value of  $S_{275-295}$  (Figure 1). These changes in CDOM are related to the  
326 produced amount of  $\bullet\text{OH}$  radicals and indicate that  $\bullet\text{OH}$  rather than  $\text{O}_2^{\bullet-}$  breaks down DOM  
327 (Goldstone et al., 2002; Pignatello et al., 2006; Rush & Bielski, 2005; Waggoner et al., 2017;  
328 Wu et al., 2010; this study). The changes in CDOM found in this study indicate a breakdown  
329 of DOM into smaller less aromatic molecules (Helms et al., 2008) as found earlier in the  
330 reactions between DOM and  $\bullet\text{OH}$  (Goldstone et al., 2002; Pignatello et al., 2006).

#### 331 4.6 Effects of $\text{O}_2^{\bullet-}$ and Fe on the fluorescence spectra of CDOM

332 In our study, the introduction of  $\text{KO}_2$  without external supply of Fe(III) increases the  
333 fluorescence of humic-like components (Figure 3), which agrees with the involvement of  
334 hydroquinones in the autocatalysis of the Fenton reaction (Chen & Pignatello, 1997;  
335 Duesterberg & Waite, 2007). The hydroxylation of aromatic moieties into hydroquinones by  
336  $\bullet\text{OH}$  can explain the increase in fluorescence and no change in absorption in the first 26 h  
337 (Figure 1a and 3), because hydroquinone-moieties have high fluorescence and absorption  
338 (Cory et al., 2005). Additionally, the breakdown of DOM by  $\bullet\text{OH}$  decreases the molecular  
339 size of DOM and increases the spectral slope coefficient (Figure 1b), which are both related  
340 to an increase in the quantum yield of fluorescence (Boyle et al., 2009; Ghigo et al., 2020;  
341 Senesi, 1990). The complexation of Fe quenches fluorescence of DOM (Cabaniss, 1992; Du  
342 et al., 2018; Poulin et al., 2014; Pullin et al., 2007; Figure 3), because Fe promotes internal  
343 conversion and intersystem crossing of the first excited singlet state as well as a ligand to  
344 metal charge transfer, i.e., processes that compete with fluorescence (Senesi, 1990). The  
345 reduction in fluorescence in the “Fe +  $\text{KO}_2$ ” treatment (Figure 3) is, instead, attributed to the

346 breakdown of DOM by the extensive amount of  $\bullet\text{OH}$ , suggesting that there is an optimum in  
347 fluorescence emission as a function of DOM molecular weight or aromaticity.

348

## 349 **5 Conclusions**

350 This study shows that  $\text{O}_2^{\bullet-}$  can induce the production of  $\bullet\text{OH}$  in the presence of complexes  
351 between Fe and humic DOM. The production of  $\bullet\text{OH}$  can exceed the stoichiometry of Fenton  
352 reaction by 2–24 folds. The autocatalysis of Fenton reaction observed in the present study  
353 emphasizes the role of  $\text{O}_2^{\bullet-}$  as an efficient transformer of organic matter. As numerous  
354 processes (photochemistry, abiotic dark oxidation, and biology) can produce  $\text{O}_2^{\bullet-}$ ,  
355 superoxide-driven Fenton reactions likely transform natural organic matter and contaminants  
356 in diverse terrestrial and freshwater environments.

357

## 358 **Author Contributions**

359 Y.X., L.C., and A.V.V. all contributed to the preparation, writing, and editing the manuscript.

360 Y.X. and M.-T.N. contributed to the sample collection and measurements. All authors  
361 reviewed the manuscript.

362

## 363 **Declaration of competing interest**

364 The authors declare no competing financial interest.

365

## 366 **Acknowledgements**

367 This study was financially supported by the Academy of Finland Grant (No. 295709) and  
368 Taishan Scholar Foundation of Shandong Province (No. tsqn201909126). The stay of L.C. in  
369 Jyväskylä was financially supported by the Erasmus+ Traineeship programme. L.C.

370 acknowledges Compagnia di San Paolo (Torino, Italy) for financially supporting his Ph.D.  
371 fellowship.

372

### 373 **Appendix A. Supplementary data**

374 Supplementary data to this article can be found online at \*\*\*.

375

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604

605 **Tables**

606 **Table 1.** Experimental design. The initial concentrations of DOM, complexed Fe, KO<sub>2</sub>, and  
 607 coumarin in the treatments made in artificial lake water (Table S1).

Treatments	DOM (mg L <sup>-1</sup> )	Fe (μM)	KO <sub>2</sub> (μM)	Coumarin (μM)
control	20	0.17*	--	10
KO <sub>2</sub> ***	20	0.17*	13	10
Fe	20	20**	--	10
Fe + KO <sub>2</sub> ***	20	20**	13	10

608 --, no addition of KO<sub>2</sub>. \* residual Fe in extracted DOM (8.5 nmol Fe/mg DOM), \*\* introduced  
 609 as DOM-Fe complex. \*\*\* In the treatments “KO<sub>2</sub>” and “Fe + KO<sub>2</sub>”, the introduction of 13 μM  
 610 KO<sub>2</sub> increased pH to 12.2, which was soon titrated with HCl back to the same pH 5 as in the  
 611 other treatments.  
 612

613 **Table 2.** Cumulative production of  $\bullet\text{OH}$  radicals ( $\mu\text{M}$ ) in the treatments.

614

Time Interval	control	Fe	KO <sub>2</sub>	Fe + KO <sub>2</sub>
0–10 h	0.09	0.11	1.32	16.3
0–168 h	0.23	0.57	9.88	103.5

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616 **Figure captions**

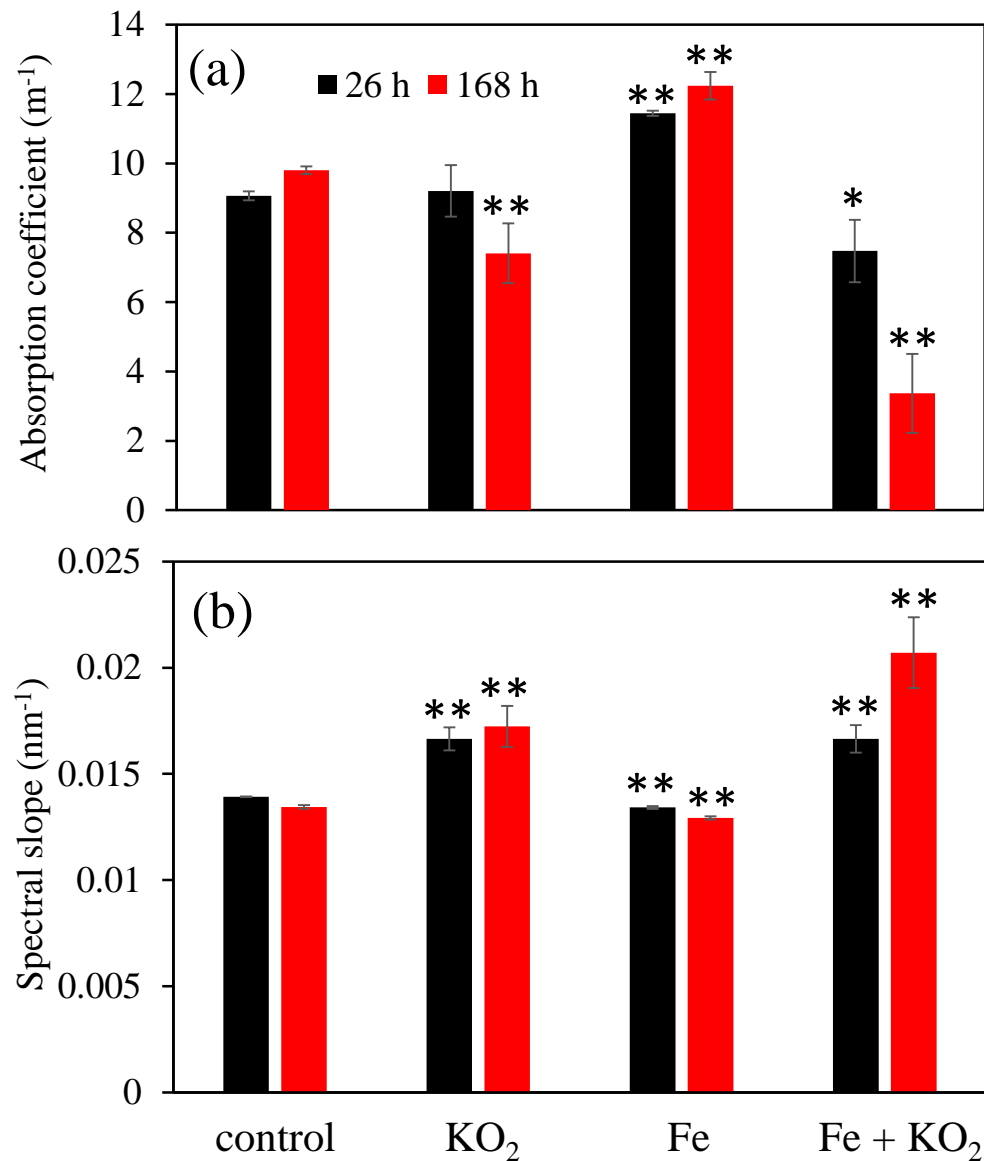
617 **Figure 1.** Absorption coefficient of CDOM at 410 nm ( $a_{410}$ ) and spectral slope coefficient  
618 ( $S_{275-295}$ ) after 26 h and 168 h incubations in the three treatments and the control. Table 1  
619 explains the treatments. Stars indicate a significant difference between the treatments and the  
620 control, \*  $P < 0.05$  and \*\*  $P < 0.01$ . Error bars show standard deviations of three replicated  
621 treatments.

622  
623 **Figure 2.** Overlaid spectra of four components (PARAFAC model). The figure shows six  
624 unique splits vs. the overall model. Dot lines indicate excitation spectra and solid lines  
625 indicate emission spectra. The excitation and emission maxima of each components are  
626 shown in Table S2. The loadings in the Y-axis indicate the normalized component intensity  
627 in the PARAFAC modeling.

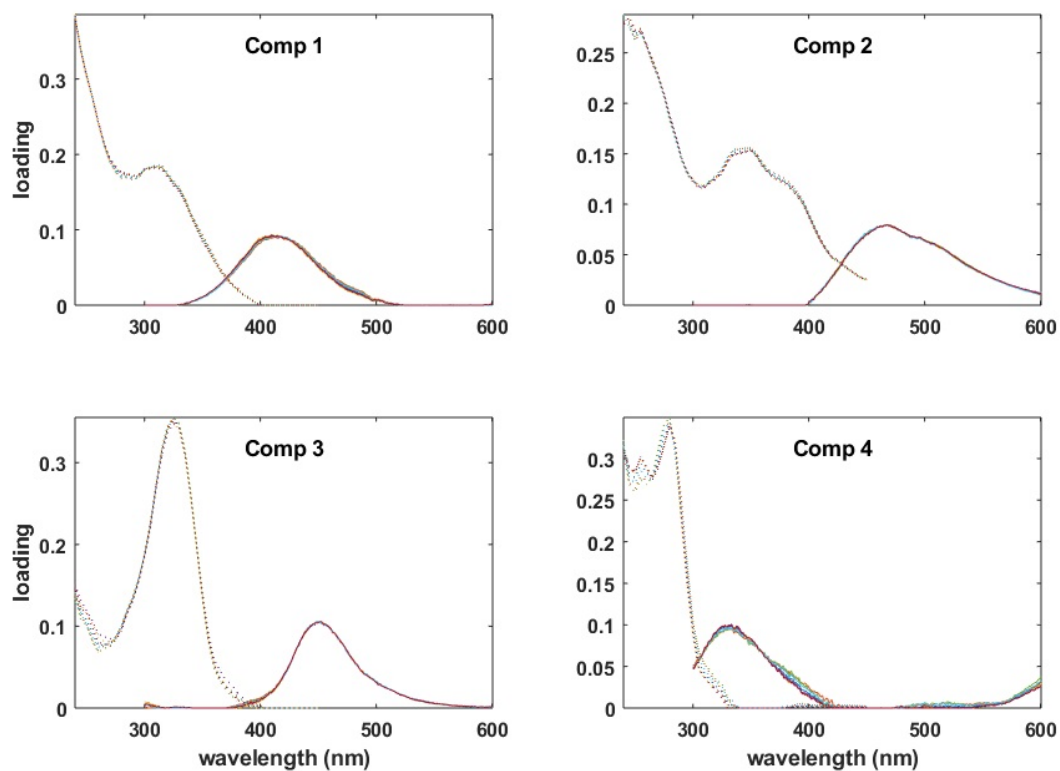
628  
629 **Figure 3.** Fluorescent intensities at 168 h of four components obtained from EEM-  
630 PARAFAC modeling (Figure 2). The fluorescence of component 4 was negligible in the  
631 “Fe+KO<sub>2</sub>” treatment. Stars indicate a significant difference between treatment and the control,  
632 \*  $P < 0.05$  and \*\*  $P < 0.01$ . Error bars show standard deviations of three replicated  
633 treatments.

634  
635 **Figure 4.** Computed formation rate of •OH in the treatments at selected times (■). The blue  
636 lines represent the fitting functions from which the cumulative production of •OH radicals  
637 were calculated. The R<sup>2</sup> parameter shows the goodness of the fit. See SI for the fitting  
638 functions. Note the orders of magnitude differences in the scales of Y-axes.

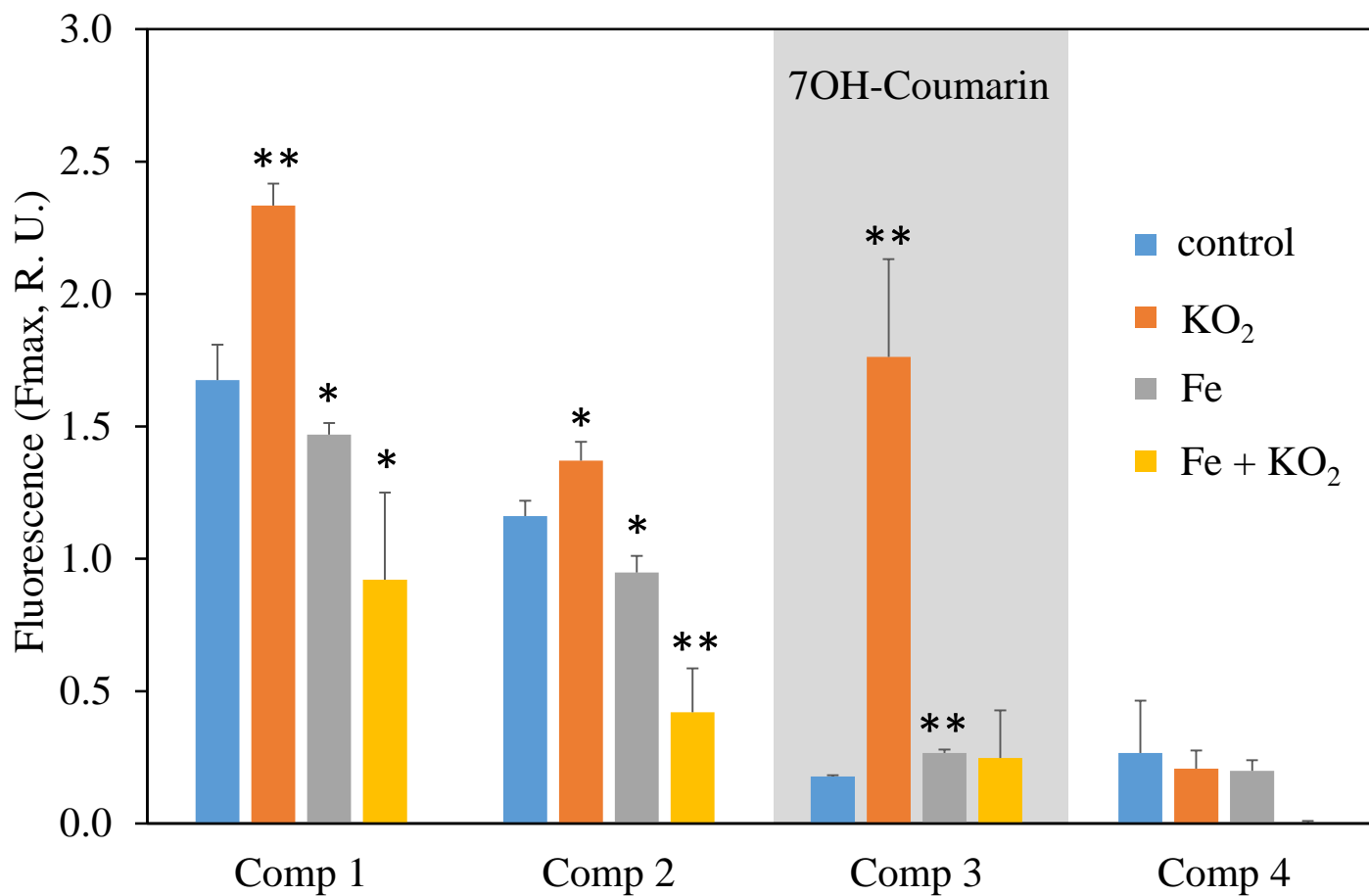




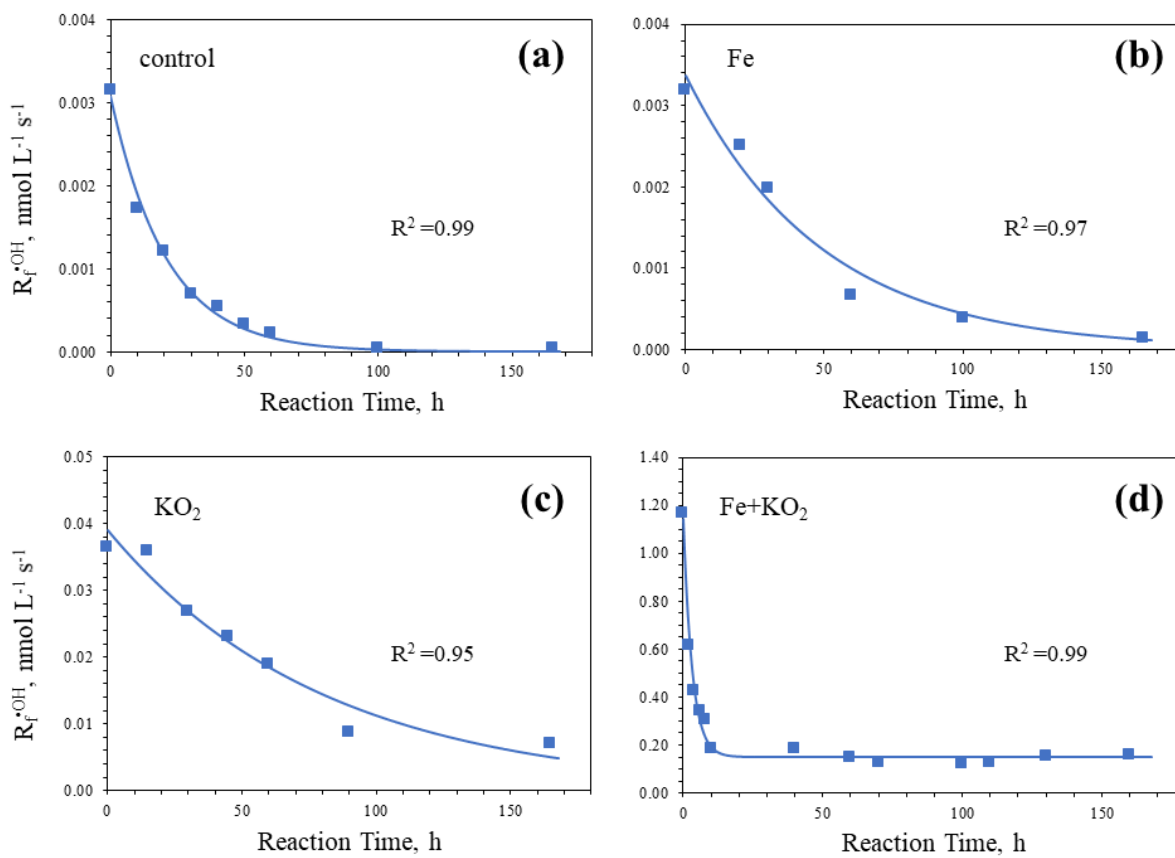
**Figure 1.** Absorption coefficient of CDOM at 410 nm ( $a_{410}$ ) and spectral slope coefficient ( $S_{275-295}$ ) after 26 h and 168 h incubations in the three treatments and the control. Table 1 explains the treatments. Stars indicate a significant difference between the treatments and the control, \* *P* < 0.05 and \*\* *P* < 0.01. Error bars show standard deviations of three replicated treatments.



**Figure 2.** Overlaid spectra of four components (PARAFAC model). The figure shows six unique splits vs. the overall model. Dot lines indicate excitation spectra and solid lines indicate emission spectra. The excitation and emission maxima of each components are shown in Table S2. The loadings in the Y-axis indicate the normalized component intensity in the PARAFAC modeling.



**Figure 3.** Fluorescent intensities at 168 h of four components obtained from EEM-PARAFAC modeling (Figure 2). The fluorescence of component 4 was negligible in the “Fe+KO<sub>2</sub>” treatment. Stars indicate a significant difference between treatment and the control, \*  $P < 0.05$  and \*\*  $P < 0.01$ . Error bars show standard deviations of three replicated treatments.



**Figure 4.** Computed formation rate of  $\bullet\text{OH}$  in the treatments at selected times (■). The blue lines represent the fitting functions from which the cumulative production of  $\bullet\text{OH}$  radicals were calculated. The  $R^2$  parameter shows the goodness of the fit. See SI for the fitting functions. Note the orders of magnitude differences in the scales of Y-axes.

**Highlights**

- $O_2^{\bullet-}$  can induce the production of  $\bullet OH$  in the presence of DOM and Fe complexes
- The production of  $\bullet OH$  exceeds the stoichiometry of Fenton reaction by 2–24 folds
- $\bullet OH$  produced from  $O_2^{\bullet-}$  extensively modified the spectroscopic properties of DOM
- $O_2^{\bullet-}$  can trigger an autocatalytic Fenton reaction and produce  $\bullet OH$  to break down  
DOM
- $O_2^{\bullet-}$  driven Fenton reaction may explain DOM transformation in multiple  
environment

## **Supplementary Information**

### **Superoxide-driven autocatalytic dark production of hydroxyl radicals in the presence of complexes of natural dissolved organic matter and iron**

Yihua Xiao<sup>1,2\*</sup>, Luca Carena<sup>3</sup>, Marja-Terttu Näsi<sup>1</sup>, Anssi V. Vähätalo<sup>1</sup>

<sup>1</sup>Department of Biological and Environmental Science, University of Jyväskylä, 40014 Jyväskylä, Finland

<sup>2</sup>School of Environmental & Municipal Engineering, Qingdao University of Technology, 266033 Qingdao, China

<sup>3</sup>Dipartimento di Chimica, Università di Torino, Via Pietro Giuria 5, 10125 Torino, Italy

This Supplementary Information contains:

**Table S1&S2**

**Figure S1**

**Text SI:** Calculations on the  $O_2^{\bullet-}$  disproportionation and DOM-Fe(III) reduction by  $O_2^{\bullet-}$  in treatment of “Fe +  $KO_2$ ” (Scheme SI-1, Figure SI-1 & SI-2)

**Text SII:** HPLC analysis for detecting coumarin and 7OH-coumarin.

**Text SIII:** Calculating the cumulative production of  $\bullet OH$  radicals by using the transformation of coumarin into 7-hydroxycoumarin as a  $\bullet OH$  probe reaction (Scheme SIII-1, Figure SIII-1 & SIII-2, and Table SIII-1).

**Text SIV:** Modeling  $O_2^{\bullet-}$  photoproduction in lake water (Figure SIV-1 & SIV-2).

**References**

**Table S1.** Composition of artificial lake water. The concentrations refer to the final concentrations in the beginning of the experiment.

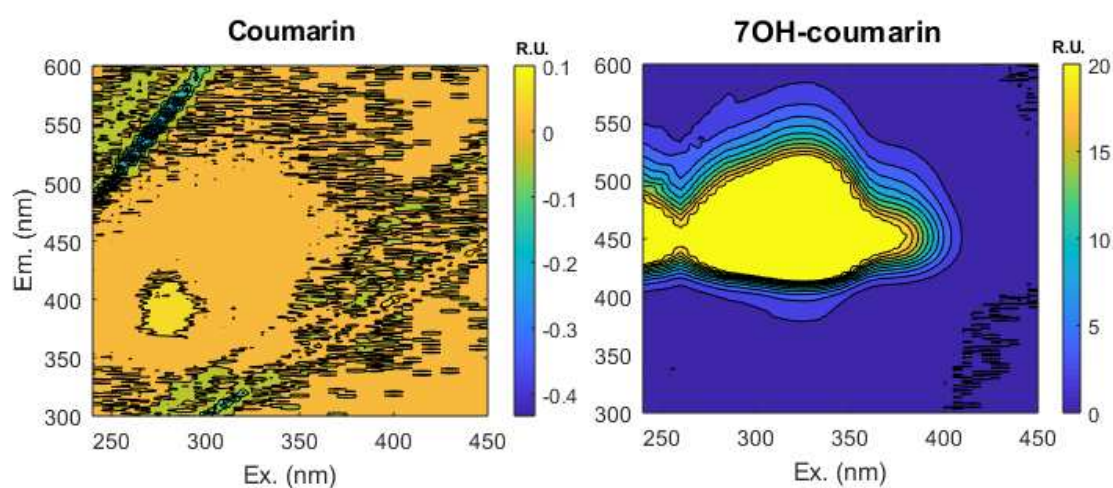
	Final concentration ( $\mu\text{mol L}^{-1}$ )
$\text{Na}_2\text{SO}_4$	54
KCl	7.9
$\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$	28.8
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	60
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.31
$\text{NaHCO}_3$	0.04
$\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$	82
$\text{NaNO}_3$	5.0
$\text{NH}_4\text{Cl}$	0.03
$\text{C}_3\text{H}_{17}\text{Na}_2\text{O}_6\text{P} \cdot 6\text{H}_2\text{O}^*$	21

\* $\beta$ -glycerophosphate disodium salt hydrate

**Table S2.** Description of four fluorescence components identified by PARAFAC

Component	Excitation/Emission maxima (nm)	Description
Comp 1	240(310)/415	Humic-like material with low molecular weight and aromaticity, common in marine environment but is also widely found in (boreal) freshwater, originated from biological activity (Coble et al., 1998; Gu et al., 2018; Kothawala et al., 2014; Zhang et al., 2009)
Comp 2	240(350)/459	Terrestrially-derived humic-like material with high molecular weight and aromaticity, is widely found but highest in forested environments and wetlands (Coble et al., 1990; Kothawala et al., 2014; Parlanti et al., 2000)
Comp 3	325/453	7OH-coumarin (Louit et al., 2005)
Comp 4	280/333	Tryptophan-like amino acid, free or bound on proteins indicating more degraded materials (Cory et al., 2005; Kothawala et al., 2014; Stedmon et al., 2003)

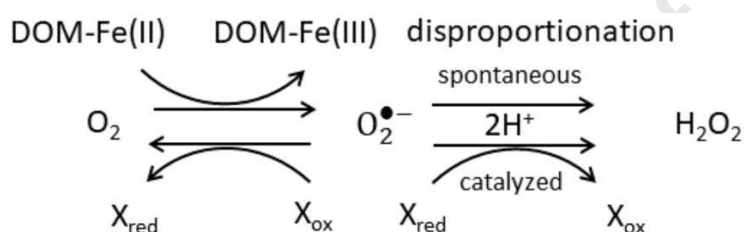




**Figure S1.** Excitation emission matrices for coumarin (left,  $10 \mu\text{mol L}^{-1}$ ) and 7OH-coumarin (right,  $10 \mu\text{mol L}^{-1}$ ). Note that the fluorescence of coumarin (given in units relative to the Raman of  $\text{H}_2\text{O}$ ; R.U.) is nearly negligible

### Text SI: Calculations on the $O_2^{\bullet-}$ disproportionation and DOM-Fe(III) reduction by $O_2^{\bullet-}$ in treatment of “Fe + $KO_2$ ”

This section will evaluate the reactions of superoxide ( $O_2^{\bullet-}$ ) in the beginning of treatment “Fe +  $KO_2$ ” containing  $20 \mu\text{mol L}^{-1}$  DOM-Fe(III) and artificial lake water (Table S1). The Scheme SI-1 shows that  $O_2^{\bullet-}$  can be either reduced to  $H_2O_2$  or oxidized to  $O_2$  (Fujii & Otani, 2017). Disproportionation can convert  $O_2^{\bullet-}$  to  $H_2O_2$  either spontaneously or through the catalysis by reduced metals or DOM, marked collectively as  $X_{\text{red}}$  in Scheme SI-1. Alternatively, oxidized DOM or metals ( $X_{\text{ox}}$ ) can oxidize  $O_2^{\bullet-}$  to  $O_2$  (Scheme SI-I).



**Scheme SI-1.** Possible fates of superoxide in the treatment “Fe +  $KO_2$ ” (modified from Fujii & Otani, 2017).  $X_{\text{red}}$  and  $X_{\text{ox}}$  refer to reduced and oxidized forms of metals or DOM.

In this study, the hydroxyl radical ( $\bullet\text{OH}$ ) production was an order of magnitude higher in the “ $KO_2$  + Fe” treatment with DOM-Fe(III) than in the “ $KO_2$ ” treatment with DOM alone. This finding suggests that the reaction of  $O_2^{\bullet-}$  with DOM-Fe(III) (from  $X_{\text{ox}}$  to  $X_{\text{red}}$  in Scheme SI-1) played a major role in the production of  $\bullet\text{OH}$ . Artificial lake water contained  $0.31 \mu\text{mol L}^{-1}$  Mn(II) (i.e.,  $X_{\text{red}}$  in Scheme SI-1), which can catalyze disproportionation of  $O_2^{\bullet-}$  to  $H_2O_2$  in addition to bimolecular disproportionation. One should notice that disproportionation requires also  $H^+$  (Scheme SI-1), and thus the process is sensitive to pH. In this study, the introduction of  $KO_2$  in  $0.05 \text{ mol L}^{-1}$  NaOH solution resulted in  $13 \mu\text{mol L}^{-1}$   $O_2^{\bullet-}$  concentration but at the same time increased the pH of artificial lake water to 12.2. The calculations below indicate that at pH 12.2 the rate of disproportionation was negligible sink compared to DOM-Fe(III).

#### (1) Disproportionation of superoxide and hydroperoxy radical

The conjugate acid of  $O_2^{\bullet-}$ , hydroperoxy radical ( $HO_2\bullet$ ) forms when a superoxide anion ( $O_2^{\bullet-}$ ) accepts a hydrogen ion:



For the equation of 1:

$$K_{HO_2} = [O_2^{\bullet-}] [H^+] / [HO_2\bullet],$$

the equilibrium constant,  $K_{\text{HO}_2}$ , is  $1.6 \times 10^{-5} \text{ L mol}^{-1}$  (Bielski et al., 1985).

Spontaneous disproportionation of  $\text{HO}_2^\bullet$  and  $\text{O}_2^{\bullet-}$  to  $\text{H}_2\text{O}_2$  and molecular  $\text{O}_2$  can proceed either through:



with a second order rate constant,  $k_2 = 8.3 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1}$  (Bielski et al. 1985) or through:



with a second order rate constant,  $k_3 = 9.7 \times 10^7 \text{ L mol}^{-1} \text{ s}^{-1}$  (Bielski et al., 1985).

Because  $\text{HO}_2^\bullet$  is in an equilibrium with  $\text{O}_2^{\bullet-}$  (Eq. SI-1), it is convenient to examine their concentrations together:  $\text{O}_2^* = \text{HO}_2^\bullet + \text{O}_2^{\bullet-}$ . In this case, the two separate disproportionation reactions can be presented together as:



where the second order rate constant,  $k_4$ , can be calculated with the combination of  $k_2$  and  $k_3$  accounting for the equilibrium between  $\text{O}_2^{\bullet-}$  and  $\text{HO}_2^\bullet$  (Eq. SI-1):

$$k_4 = (k_2 + k_3 (K_{\text{HO}_2}/[\text{H}^+])) (1 + K_{\text{HO}_2}/[\text{H}^+])^{-2} \quad (\text{Bielski et al., 1985}).$$

The formation rate of  $\text{H}_2\text{O}_2$  or molecular  $\text{O}_2$  ( $\text{mol L}^{-1} \text{ s}^{-1}$ ) through disproportionation is:

$$R_{\text{H}_2\text{O}_2, \text{O}_2} = k_4 [\text{O}_2^*][\text{O}_2^*] \quad (\text{Eq. SI-5})$$

The rate of  $\text{O}_2^*$  consumption ( $\text{mol L}^{-1} \text{ s}^{-1}$ ) is:

$$R_{\text{O}_2^*} = -2k_4 [\text{O}_2^*][\text{O}_2^*] \quad (\text{Eq. SI-6})$$

The temporal kinetics of  $\text{O}_2^*$  concentration ( $\text{mol L}^{-1}$ ) can be described as:

$$[\text{O}_2^*] = [\text{O}_2^*]_0 / (1 + 2k_4 [\text{O}_2^*]_0 t) \quad (\text{Eq. SI-7})$$

where  $[\text{O}_2^*]_0$  refers to the initial concentration of  $\text{O}_2^*$ .

and the half-life of  $\text{O}_2^*$  (s) is:

$$t_{1/2, \text{O}_2^*} = (2k_4 [\text{O}_2^*]_0)^{-1} \quad (\text{Eq. SI-8})$$

At pH 12.2,  $k_4 = 3.83 \text{ L mol}^{-1} \text{ s}^{-1}$  and the half-life of the introduced  $\text{O}_2^*$  through disproportionation was 10054 s (= 2.79 hours) when calculated according to Eq. SI-8.

Manganese can react with superoxide at fast rate:



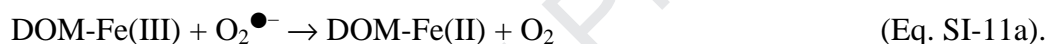
and in this study convert  $0.31 \mu\text{mol L}^{-1}$  (or 2.4% of introduced  $\text{KO}_2$ ) into manganous superoxide (Wuttig et al., 2013). In seawater,  $\text{MnO}_2^+$  behaves like  $\text{O}_2^{\bullet-}$  and its major fate is disproportionation (Wuttig et al., 2013). At pH 12.2 of present study, the rate of disproportionation of  $\text{MnO}_2^+$  is slow, if it behaves like  $\text{O}_2^{\bullet-}$  (see above). Instead,  $\text{MnO}_2^+$  is expected to form an equilibrium with Mn(II) and  $\text{O}_2^{\bullet-}$  (Wuttig et al., 2013):



and  $\text{O}_2^{\bullet-}$  will be eventually consumed by DOM-Fe(III) at pH 12.2 in the beginning of the “ $\text{KO}_2 + \text{Fe}$ ” treatment.

### (2) Reduction of DOM-Fe(III) by $\text{O}_2^{\bullet-}$

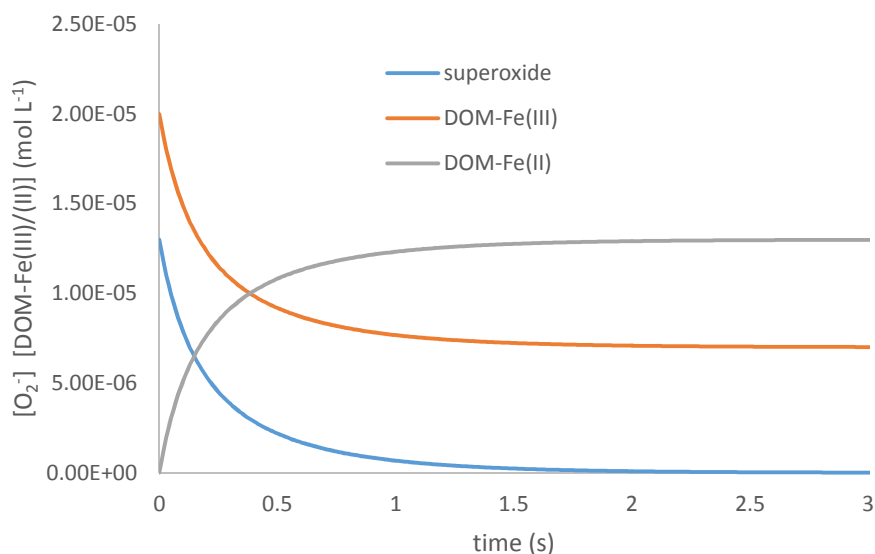
The reduction of DOM-Fe(III) by  $\text{O}_2^{\bullet-}$  can be expressed as,



The rate of superoxide consumption, DOM-Fe(III) loss and DOM-Fe(II) formation can be calculated as:

$$\text{rate} = k_{11} [\text{O}_2^{\bullet-}] [\text{DOM-Fe(III)}] \quad (\text{Eq. SI-11b}).$$

The second-order rate constant for the reaction SI-11 ( $k_{11} = 2.8 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1}$ ) has been determined at pH 8 using Suwannee River fulvic acid (SRFA) as a source of DOM (Garg et al., 2007). Using  $k_{11} = 2.8 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1}$ , the reaction Eq. SI-11 consumed  $\text{O}_2^{\bullet-}$  and produced  $13 \mu\text{mol DOM-Fe(II)}$  within about two seconds after the introduction of  $\text{KO}_2$ .



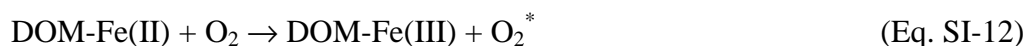
**Figure SI-1.** Calculated (Eq. SI-11) concentrations of superoxide ( $O_2^{\bullet-}$ ), DOM-Fe(III) and DOM-Fe(II) in the beginning of “Fe +  $KO_2$ ” treatment at pH 12.2.

3) *The Fenton process in the beginning of experiment after the adjustment of pH to 5*

After the introduction of  $KO_2$  and rapid reduction of DOM-Fe(III) by  $O_2^{\bullet-}$ , the pH of artificial lake water was adjusted to 5. Under those conditions, the concentration of superoxide was initially negligible, but the water contained  $13 \mu\text{mol L}^{-1}$  DOM-Fe(II) (Figure SI-1). The artificial lake water was in contact with atmosphere and contained  $264 \mu\text{mol L}^{-1}$  dissolved  $O_2$  according to the solubility of  $O_2$  to fresh water at  $+25^\circ\text{C}$ .

The oxidation of DOM-Fe(II) can lead to the production of  $\bullet\text{OH}$  through the following sequence of reactions.

Dissolved  $O_2$  can oxidize DOM-Fe(II):



and produce  $O_2^*$  with  $k_{12} = 100 \text{ L mol}^{-1} \text{ s}^{-1}$  determined for SRFA (Garg et al., 2007). The loss of DOM-Fe(II) through Eq. SI-12 is calculated in Figure SI-2a.

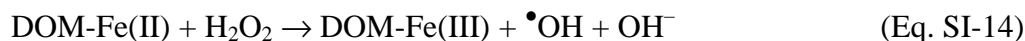
DOM-Fe(II) can also dissociate:



with  $k_{13} = 8 \times 10^{-4} \text{ s}^{-1}$  (Garg et al., 2007). Immediately after the pH adjustment to 5, the calculated rate of dissociation ( $1.04 \times 10^{-8} \text{ mol L}^{-1} \text{ s}^{-1}$ ; Eq. SI-13) is an order of magnitude

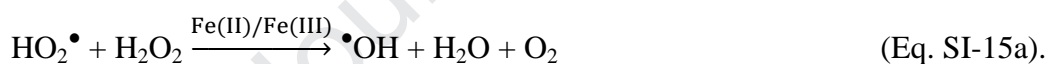
lower than the oxidation of DOM-Fe(II) by  $O_2$  ( $3.6 \times 10^{-7} \text{ mol L}^{-1} \text{ s}^{-1}$ ; Eq. SI-12). This indicates that Eq. SI-12 was primarily responsible for the production of  $O_2^*$  and for simplicity the dissociation of DOM-Fe(II) is omitted in kinetic modelling presented in Figure SI-2.

At pH 5, the spontaneous disproportionation of  $O_2^*$  (Eq. SI-4;  $k_4 = 2.3 \times 10^7 \text{ L mol}^{-1} \text{ s}^{-1}$ ) leads to a fast production of  $H_2O_2$  (Figure SI-2a). DOM-Fe(II) reacts faster with  $H_2O_2$  (Eq. SI-14;  $k_{14} = 1.75 \times 10^4 \text{ L mol}^{-1} \text{ s}^{-1}$ ) (Pignatello et al., 2006) than with  $O_2$  (Eq. SI-12):

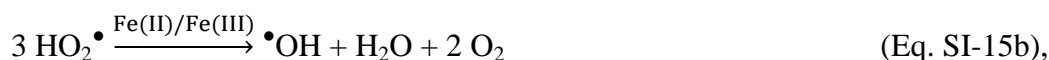


and produces hydroxyl radicals (Figure SI-2b). In the beginning of experiment, the calculated maximum rate of  $\bullet\text{OH}$  ( $1.05 \times 10^{-9} \text{ mol L}^{-1} \text{ s}^{-1}$ ) is similar to the measured rate of  $\bullet\text{OH}$  production ( $R_f^{\bullet\text{OH}}(t_0) = 1.14 \times 10^{-9} \text{ mol L}^{-1} \text{ s}^{-1}$ ; Figure 4d).

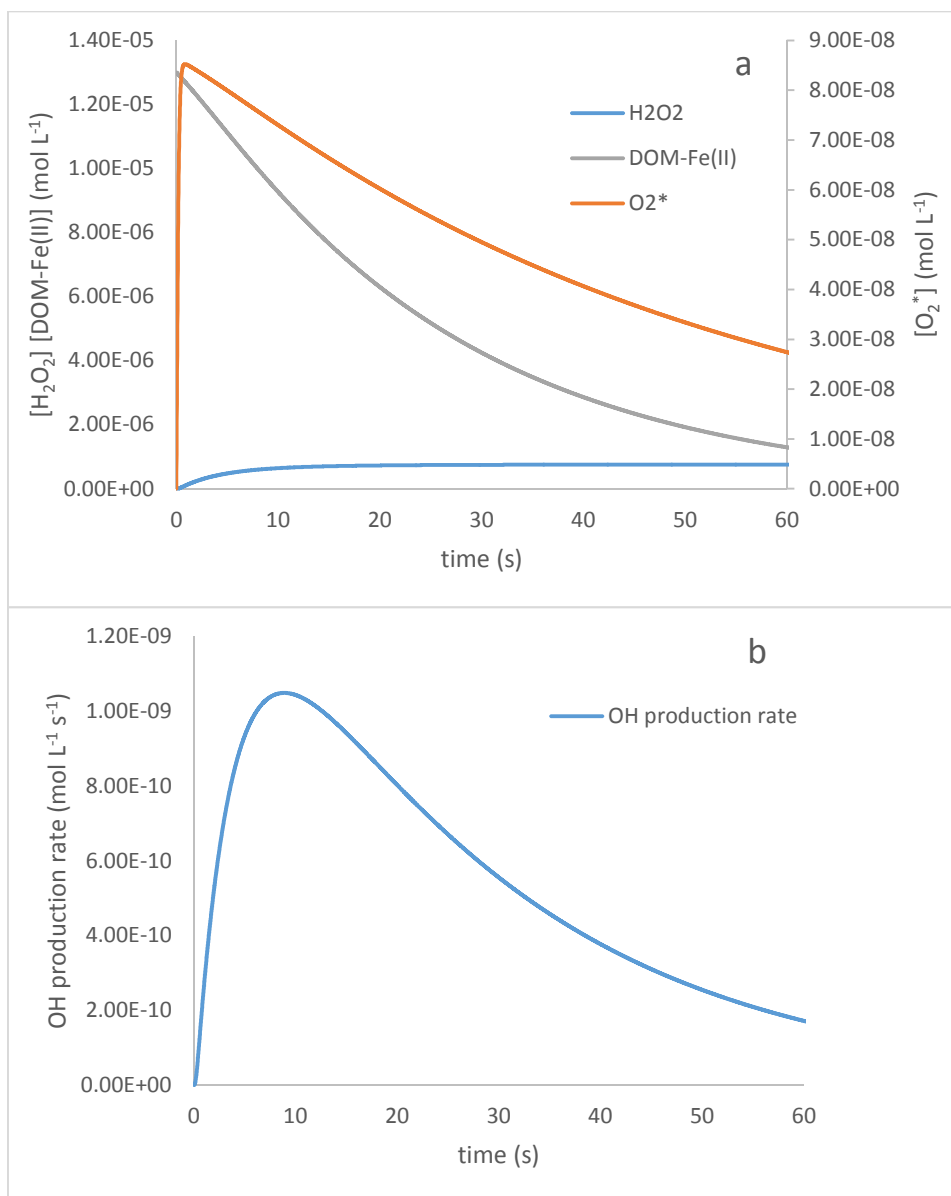
The simple kinetic model (Figure SI-2) can explain the initial rate of  $\bullet\text{OH}$  production in “Fe +  $KO_2$ ” treatment, but it fails to describe the later kinetics of  $\bullet\text{OH}$  production and the cumulative amounts of produced  $\bullet\text{OH}$  (Figure 4d;  $104 \mu\text{mol L}^{-1} \bullet\text{OH}$  in 168 h, Table 2). The simple kinetic model (Figure SI-2) predicts that the formation of  $\bullet\text{OH}$  stops in about three minutes, and produces cumulatively  $3.8 \mu\text{mol L}^{-1}$  of  $\bullet\text{OH}$  with a final residual concentration of  $0.8 \mu\text{mol L}^{-1} H_2O_2$ . The stoichiometry of  $\bullet\text{OH}$  production in the kinetic model (Figure SI-2) approximates iron-catalyzed Haber-Weiss process (Rush & Bielski, 1985):



When the formation of  $H_2O_2$  through disproportionation (Eq. SI-4) is included in Eq. SI-15a, it becomes:



The reaction Eq. SI-15 represents the theoretical maximum yield of  $\bullet\text{OH}$  ( $\bullet\text{OH}/3 \text{O}_2^*$ ) from superoxide through Fe catalysis, which is  $4.3 \mu\text{mol L}^{-1}$  of  $\bullet\text{OH}$  from  $13 \mu\text{mol L}^{-1} \text{O}_2^*$ . As the measured yield of  $\bullet\text{OH}$  ( $104 \mu\text{mol L}^{-1} \bullet\text{OH}$  in 168 h, Table 2) was 24-fold higher than the theoretical yield from  $\text{O}_2^*$  ( $4.3 \mu\text{mol L}^{-1} \bullet\text{OH}$ , Eq. SI-15b), the production of  $\bullet\text{OH}$  in the “Fe +  $KO_2$ ” treatment must have included an autocatalytic process.



**Figure SI-2.** The calculated concentrations of DOM-Fe(II),  $\text{H}_2\text{O}_2$  and  $\text{O}_2^*$  (a) and the production rate of hydroxyl radicals. The calculations on the oxidation of DOM-Fe(II) (Eq. SI-12), disproportionation of  $\text{O}_2^*$  (Eq. SI-4) and the Fenton reaction (Eq. SI-14).

**Text SII: HPLC analysis for detecting coumarin and 7OH-coumarin**

The HPLC system was a Shimadzu LC-30AD equipped with SIL-30AC autosampler, CTO-20AC column oven (set at 30 °C), DGU-20A5R degassing unit, SPD-M20A PDA detector and RF-20A XS fluorescence detector. Chromatographic runs were carried out with a reverse phase chromatography column Bridge Columns XBridge™ C18 (2.5 µm) in a gradient mode with a mixture of two eluents (A and B). Eluent A was 0.3% acetic acid with 99.7% ultrapure water, while B was 100% acetonitrile. The flow rate was 0.3 mL min<sup>-1</sup>. The relevant elution gradients were: 10% of B from 0 to 0.5 min, then linear gradient to 45% of B from 0.5 to 6 min, followed by a fast linear gradient to 75% of B for 6–6.5 min; 75% of B was then kept from 6.5 to 9 min and then followed by a linear gradient to the initial condition 10% of B at 9.5 min, the same gradient was kept until 12.5 min for stabilizing the system. The injection volume was 5 µL. The first sample was run two times to make sure the gradient solvent was fully stabilized in the system. The retention times were 5.23 min for coumarin and 3.64 min for 7OH-coumarin. Quantification of the two compounds was carried out by means of the PDA detector for coumarin (absorption wavelength = 280 nm) and of the fluorescence detector for 7OH-coumarin (excitation wavelength = 320 nm; emission wavelength = 450 nm,).



**Text SIII: Calculating the cumulative production of  $\bullet\text{OH}$  radicals by using the transformation of coumarin into 7-hydroxycoumarin as a  $\bullet\text{OH}$  probe reaction**

The cumulative production of  $\bullet\text{OH}$  radicals per unit of volume over a defined time ( $[\bullet\text{OH}]_{\Sigma}$ , mol L<sup>-1</sup>) was described as a definite integral over time:

$$[\bullet\text{OH}]_{\Sigma} = \int_{t_0}^{t_1} R_f^{\bullet\text{OH}}(t) dt \quad (\text{Eq. SIII} - 1),$$

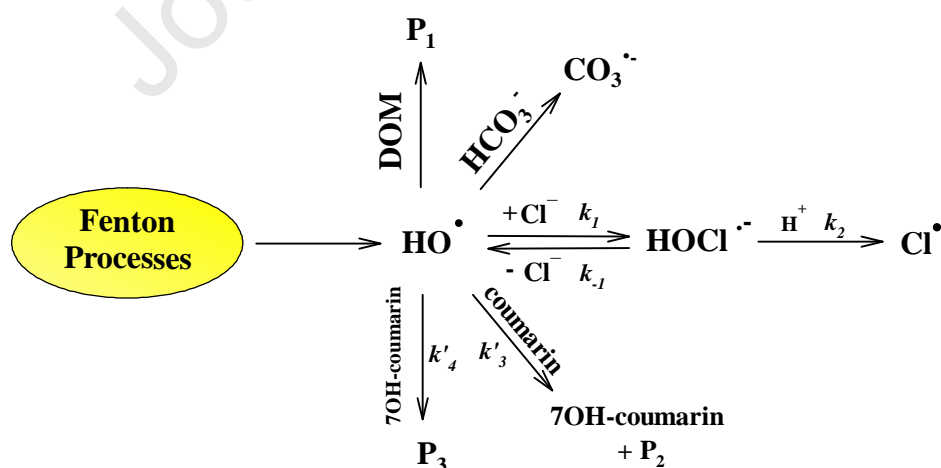
where  $t_0$  and  $t_1$  represent the time in the beginning of experiment and at the selected time  $t_1$ , respectively, and  $R_f^{\bullet\text{OH}}(t)$  is the formation rate of  $\bullet\text{OH}$  radicals at time  $t$  (mol L<sup>-1</sup> s<sup>-1</sup>).

$R_f^{\bullet\text{OH}}(t)$  was calculated from the scavenging rate of hydroxyl radicals. Because  $\bullet\text{OH}$  radicals react with their scavengers almost at diffusion-controlled rates, the total scavenging rate of  $\bullet\text{OH}$  at time  $t$  approximates  $R_f^{\bullet\text{OH}}(t)$ . When the formation and scavenging rates are nearly the same, the  $\bullet\text{OH}$  concentration at time  $t$  approximates a steady-state. In the experimental solutions, the scavengers (see Scheme SIII-1) set the steady-state concentration of  $\bullet\text{OH}$  radicals ( $[\bullet\text{OH}]_{s.s.}$ ) to:

$$[\bullet\text{OH}]_{s.s.} = \frac{R_f^{\bullet\text{OH}}(t)}{k'_{Scav} + k_{Cou}^{\bullet\text{OH}}[Cou]_t + k_{7OHCou}^{\bullet\text{OH}}[7OHCou]_t} \quad (\text{Eq. SIII} - 2),$$

where  $[Cou]_t$  and  $[7OHCou]_t$  are the concentrations of coumarin and 7-hydroxycoumarin (7OH-coumarin) at time  $t$ , respectively (Figures SIII-1&2), and  $k'_{Scav}$  represents scavenging by the artificial lake water (Table SIII-1).  $k'_{Scav}$  accounted for the major scavengers of  $\bullet\text{OH}$  in the artificial lake water: DOM (DOC = 11.35 mg C L<sup>-1</sup>), chloride (*vide infra* for its concentration) and bicarbonate anions ( $4 \times 10^{-6}$  mol L<sup>-1</sup>), and thus  $k'_{Scav} = k_{DOM}^{\bullet\text{OH}} \text{DOC} + k_{Cl^-}^{\bullet\text{OH}}[Cl^-] + k_{HCO_3^-}^{\bullet\text{OH}}[HCO_3^-]$ . The modeling used the initial concentrations of Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> (Table SIII-1). In terms of DOM, we assumed that the reactions between hydroxyl radicals and DOM resulted primarily in the transformation products that reacted with hydroxyl radical like reported for DOM in literature (Westerhoff et al. 2007). Therefore, we used the initial concentrations of DOM in the calculations (Table SIII-1). The modeling also ignored the reactions of  $\bullet\text{OH}$  radicals with the different coumarin degradation byproducts (P<sub>2</sub> and P<sub>3</sub> Scheme SIII-1), as well as those with the iron species, because we did not measure the

temporal variation of these compounds. However, this should not cause significant modeling errors, because these constituents had lower concentrations than the major scavenger DOM, making negligible their role in the total  $\bullet\text{OH}$  radicals scavenging. The reaction rate constants were from literature:  $k_{\text{Cou}}^{\bullet\text{OH}} = 5.6 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ ;  $k_{7\text{OH}\text{Cou}}^{\bullet\text{OH}} = 6.1 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ ;  $k_{\text{DOM}}^{\bullet\text{OH}} = 1.9 \times 10^4 \text{ L mg C}^{-1} \text{ s}^{-1}$ ;  $k_{\text{HCO}_3^-}^{\bullet\text{OH}} = 8.5 \times 10^6 \text{ L mol}^{-1} \text{ s}^{-1}$  (Burgos Castillo et al., 2018; Buxton et al., 1988; Payá et al., 1992; Westerhoff et al., 2007). The value of  $k_{\text{Cl}^-}^{\bullet\text{OH}}$  took into account the pH dependence of the  $\bullet\text{OH}$  scavenging kinetics by  $\text{Cl}^-$  (see Scheme SIII-1). At pH 2, the reaction between  $\bullet\text{OH}$  and  $\text{Cl}^-$  yields hypochlorous acid anion radicals ( $\text{HOCl}^{\bullet-}$ ,  $k_1 = 4.3 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ , Buxton et al., 1988). In acidic conditions the protonation of  $\text{HOCl}^{\bullet-}$  induces the formation of a chlorine atom ( $\text{Cl}^\bullet$ ) and a water molecule ( $k_2 = 2.1 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ , Jayson et al., 1973) making  $\text{Cl}^-$  as an actual sink for  $\bullet\text{OH}$ . At circumneutral and basic pH values,  $\text{HOCl}^{\bullet-}$  dissociates back to  $\bullet\text{OH}$  and  $\text{Cl}^-$  ( $k_{-1} = 6.1 \times 10^9 \text{ s}^{-1}$ , Jayson et al., 1973). By considering these reactions and by reasonably applying the steady-state to the  $\text{HOCl}^{\bullet-}$  concentration, the pH dependence of  $k_{\text{Cl}^-}^{\bullet\text{OH}}$  can be described as  $k_1 k_2 10^{-\text{pH}} (k_{-1} + k_2 10^{-\text{pH}})^{-1}$  (see also the paper by Jayson et al., 1973). Therefore,  $k_{\text{Cl}^-}^{\bullet\text{OH}} \approx 1.5 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1}$  at pH 5. Chloride concentrations used in the model took into account all the  $\text{Cl}^-$  sources (i.e. artificial lake water,  $\text{FeCl}_3$ , and  $\text{HCl}$  for the titration procedures) and were  $1.84 \times 10^{-4} \text{ mol L}^{-1}$  (“control”),  $7.4 \times 10^{-3} \text{ mol L}^{-1}$  (“Fe”),  $1.62 \times 10^{-2} \text{ mol L}^{-1}$  (“ $\text{KO}_2$ ”) and  $2.12 \times 10^{-2} \text{ mol L}^{-1}$  (“Fe+  $\text{KO}_2$ ”).



**Scheme SIII-1.** Simplified reaction scheme describing the formation and the scavenging pathways of  $\bullet\text{OH}$  radicals in the experiment. 'P<sub>i</sub>' refers to a general byproduct(s).

The transformation rate of 7OH-coumarin,  $R_{\Delta}^{7OHCou}(t)$ , was a master variable in the assessment of  $R_f^{\bullet OH}(t)$ .  $R_{\Delta}^{7OHCou}(t)$  was defined as the difference between the formation rate (coumarin +  $\bullet OH$  reaction) and the decay rate (7OH-coumarin +  $\bullet OH$  reaction):

$$R_{\Delta}^{7OHCou}(t) = [\bullet OH]_{s.s.} \{ \eta_{7OHCou}^{Cou} k_{Cou}^{\bullet OH} [Cou]_t - k_{7OHCou}^{\bullet OH} [7OHCou]_t \} \quad (Eq. SIII - 3),$$

where  $\eta_{7OHCou}^{Cou} = 0.047$  is the yield for the formation of 7OH-coumarin from the coumarin +  $\bullet OH$  reaction (Burgos Castillo et al., 2018). By substituting Eq. SIII-2 in Eq. SIII-3, one obtains:

$$R_{\Delta}^{7OHCou}(t) = \frac{R_f^{\bullet OH}(t) \{ \eta_{7OHCou}^{Cou} k_{Cou}^{\bullet OH} [Cou]_t - k_{7OHCou}^{\bullet OH} [7OHCou]_t \}}{k'_{Scav} + k_{Cou}^{\bullet OH} [Cou]_t + k_{7OHCou}^{\bullet OH} [7OHCou]_t} \quad (Eq. SIII - 4)$$

By rearranging Eq. SIII-4, the expression for  $R_f^{\bullet OH}(t)$  will be:

$$R_f^{\bullet OH}(t) = \frac{R_{\Delta}^{7OHCou}(t) \{ k'_{Scav} + k_{Cou}^{\bullet OH} [Cou]_t + k_{7OHCou}^{\bullet OH} [7OHCou]_t \}}{\{ \eta_{7OHCou}^{Cou} k_{Cou}^{\bullet OH} [Cou]_t - k_{7OHCou}^{\bullet OH} [7OHCou]_t \}} \quad (Eq. SIII - 5)$$

$R_{\Delta}^{7OHCou}(t)$  was assessed as the first derivative ( $d[7OHCou]_t dt^{-1}$ ) of a function that described the temporal development of  $[7OHCou]_t$  in the treatments. In order to determine  $d[7OHCou]_t dt^{-1}$ , a kinetic equation SIII-6 (the curves in Figure SIII-1) was fitted on the measured concentrations of 7OH-coumarin during the course of experiment (the squares in Figure SIII-1).

$$[7OHCou]_t = \frac{k'_3 a}{k'_4 - k'_3} (e^{-k'_3 t} - e^{-k'_4 t}) \quad (Eq. SIII - 6)$$

where  $k'_3$  and  $k'_4$  are the pseudo-first order rate constants for the reactions  $Cou \rightarrow 7OHCou$  and  $7OHCou \rightarrow P_3$ , respectively (Scheme SIII-1), and  $a$  is a concentration parameter. The values of  $R_{\Delta}^{7OHCou}(t)$  were graphically computed as the slope of the tangent line to the curves shown in Figure SIII-1 at several reaction times shown in Figure 4.

For the calculation of  $R_f^{\bullet OH}(t)$ , Eq. SIII-5 received the values of  $R_{\Delta}^{7OHCou}(t)$ ,  $[7OHCou]_t$  (Figure SIII-1) and  $[Cou]_t$  (Figure SIII-2) at the selected times (the reaction times in Figure

4). The decreasing trend in  $R_f^{OH}(t)$  at the selected times (the squares in Figure 4) was described by an exponential equation (the curves in Figure 4):

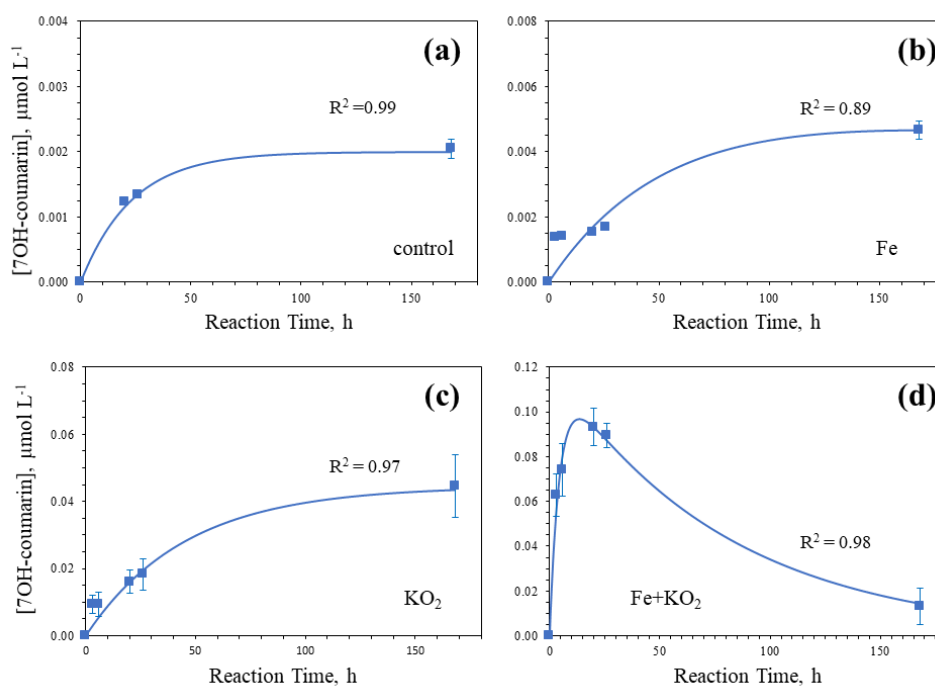
$$R_f^{OH}(t) = R_f^{OH}(t_0) e^{-kt} \quad (\text{Eq. SIII-7}),$$

where  $R_f^{OH}(t_0)$  is the rate of hydroxyl radicals formation in the beginning of experiment ( $\text{nmol L}^{-1} \text{ s}^{-1}$ ) and  $k$  ( $\text{s}^{-1}$ ) describes the degree of exponential loss in  $R_f^{OH}(t)$ . In the “Fe+ $\text{KO}_2$ ”-treatment,  $R_f^{OH}(t)$  decreased initially exponentially but plateaued later (Figure 4d) and therefore the kinetics was described by:

$$R_f^{OH}(t) = R_f^{OH}(t_0) e^{-kt} + R_f^{OH}(t_{\text{plateau}}) \quad (\text{Eq. SIII-8}),$$

where  $R_f^{OH}(t_{\text{plateau}})$  is the rate at the plateau. Table SIII-1 shows the values of the relevant parameters of Eq. SIII-7&8.

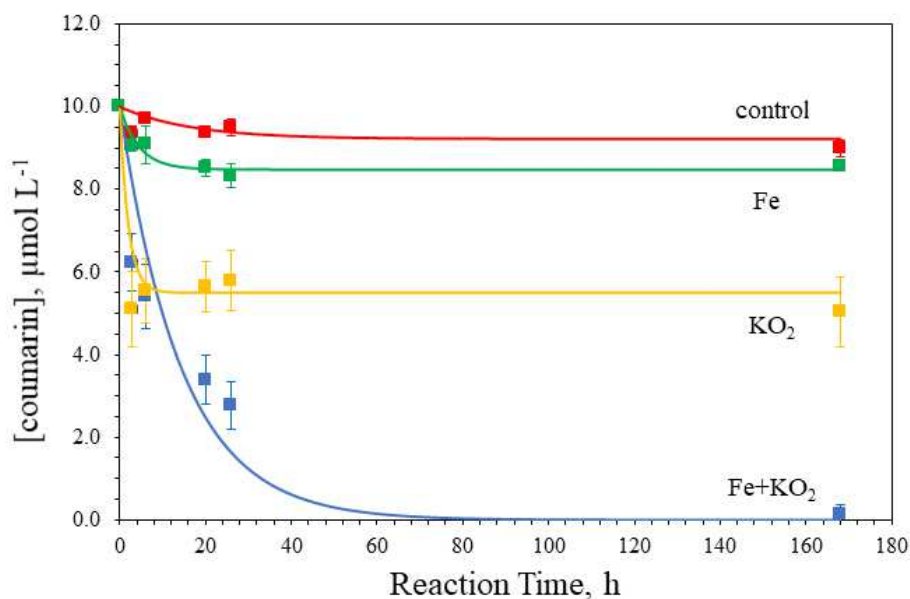
Finally, the  $[\cdot\text{OH}]_X$  values were calculated by solving the integral of these fitting functions as in Eq. SIII-1.



**Figure SIII-1.** The measured concentrations of 7-hydroxycoumarin (7OH-coumarin) (■) and the kinetic fitting on the measured data (blue lines). The error bars represent data standard deviations. The

$R^2$  parameter shows the goodness of the fit. See Eq. S6 for the general form of the fitting functions.

Note, the differences in the scale of Y-axis among the panels.



**Figure SIII-2.** Concentration profiles of coumarin observed in the different treatments. The error bars represent the standard deviations of the measured coumarin concentrations (squares). Data were fitted with the equation  $[Cou]_t = C + A \exp(-Bt)$  (lines). In the case “Fe+KO<sub>2</sub>”,  $C = 0$  (blue curve).

**Table SIII-1.** Fitting parameters obtained by interpolating the  $R_f^{OH}(t)$  data with Eq. SIII-7 & SIII-8.

The  $R^2$  value shows the goodness of the fit.

Treatment	$R_f^{OH}(t_0)$ , $\text{nmol L}^{-1} \text{s}^{-1}$	$k$ , $\text{s}^{-1}$	$R_f^{OH}(t_{\text{plateau}})$ , $\text{nmol L}^{-1} \text{s}^{-1}$	$R^2$
control	$(3.074 \pm 0.085) \times 10^{-3}$	$(1.340 \pm 0.067) \times 10^{-5}$	0	0.99
Fe	$(3.361 \pm 0.221) \times 10^{-3}$	$(5.668 \pm 0.804) \times 10^{-6}$	0	0.97
KO <sub>2</sub>	$(3.894 \pm 0.225) \times 10^{-2}$	$(3.454 \pm 0.466) \times 10^{-6}$	0	0.95
Fe+KO <sub>2</sub>	$(9.910 \pm 0.384) \times 10^{-1}$	$(8.745 \pm 0.701) \times 10^{-5}$	$(1.523 \pm 0.137) \times 10^{-1}$	0.99

**Text SIV: Modeling  $O_2^{\bullet-}$  photoproduction in lake water.**

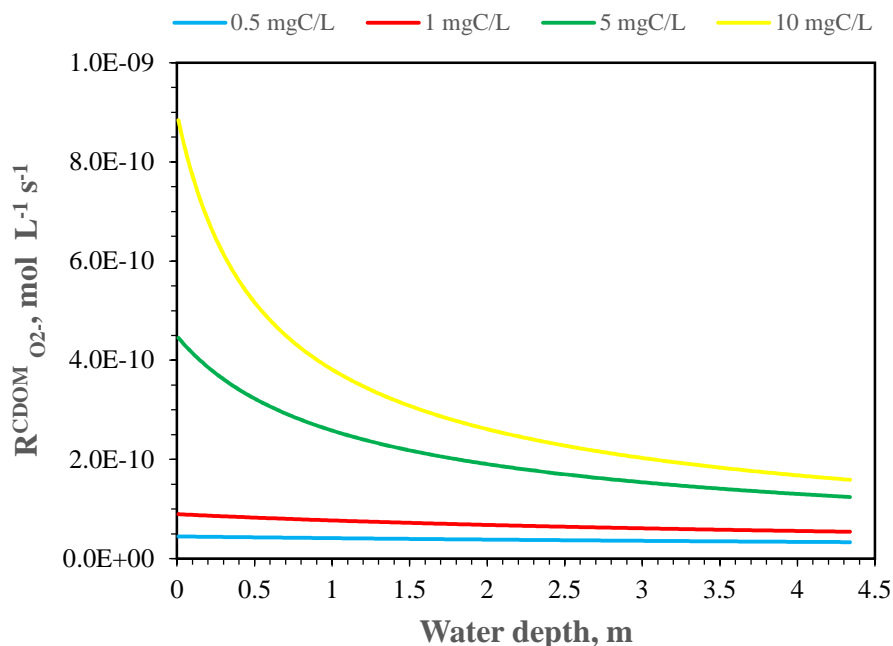
Superoxide ( $O_2^{\bullet-}$ ) photochemical production in surface waters can be related to the formation of hydrogen peroxide ( $H_2O_2$ ) from irradiated chromophoric dissolved organic matter (CDOM). Indeed,  $H_2O_2$  is photoproduced by CDOM through a mechanism that would involve the reduction of dioxygen ( $O_2$ ) to superoxide  $O_2^{\bullet-}$  by  $O_2$ -reducing intermediates that are generated by intramolecular electron transfer from CDOM short-lived excited states of electron donors (probably phenols) to ground-state acceptors (most likely quinone-like moieties) (Zhang et al., 2012). In the presence of a high content of electro-donating phenols, the excited triplet states of CDOM can be involved in  $O_2^{\bullet-}$  photoproduction as well (Zhang et al., 2014). Then  $O_2^{\bullet-}$  can undergo dismutation, which can occur via three main pathways: (i) uncatalyzed dismutation ( $O_2^{\bullet-} + HO_2^{\bullet}$ ), (ii) DOM-catalyzed dismutation ('DOM' means dissolved organic matter) and (iii) dismutation catalyzed by organometallic compounds containing Fe, Cu and Mn (Goldstone & Voelker, 2000; Ma et al., 2019). Among these processes, the DOM-catalyzed dismutation of  $O_2^{\bullet-}$  into  $H_2O_2 + O_2$  can play the main scavenging role of  $O_2^{\bullet-}$  in inland surface waters (Ma et al., 2019). As far as the stoichiometry of  $O_2^{\bullet-}$  dismutation is concerned, previous works have determined the ratio  $H_2O_2:O_2^{\bullet-}$  for different DOM type (Goldstone & Voelker, 2000; Powers & Miller, 2016), showing that the ratio should be  $\approx 0.5$  for DOM typically occurring in inland waters, while it would decrease down to  $\approx 0.25$  for seawater and open ocean (Powers & Miller, 2016). Here, we adopted the former values in order to model superoxide photoproduction in lake water.

By doubling the  $H_2O_2$  photoproduction rate from irradiated CDOM ( $R_{H_2O_2}^{CDOM}$ ), one can get the  $O_2^{\bullet-}$  photoformation rate,  $R_{O_2^{\bullet-}}^{CDOM} = 2R_{H_2O_2}^{CDOM} = 2\Phi_{H_2O_2}^{CDOM} P_{a,CDOM}$ .  $\Phi_{H_2O_2}^{CDOM}$  is the polychromatic apparent quantum yield (pAQY) of  $H_2O_2$  formation from irradiated CDOM, while  $P_{a,CDOM}$  is the total photon flux absorbed by CDOM (or photons absorption rate, Einstein  $L^{-1} s^{-1}$ ).  $\Phi_{H_2O_2}^{CDOM}$  has been measured by Zhang et al. (2012) for several DOM samples, such as Suwannee River humic and fulvic acids, a lignin-like material and a river water sample. The average value for these materials is  $\approx 5.9 \times 10^{-4}$ .  $P_{a,CDOM}$  is a function of the light absorption properties of CDOM, water depth and chemical composition, namely  $P_{a,CDOM} = \int_{\lambda_1}^{\lambda_2} p^0(\lambda) \frac{Abs_{\lambda}^{CDOM}}{Abs_{\lambda}^{tot}} [1 - 10^{-Abs_{\lambda}^{tot}}] d\lambda$ .  $Abs_{\lambda}^{tot}$  is the total absorbance of water that takes into account the water depth and the Lambert-Beer absorbance of the main light-absorbing species,  $Abs_{\lambda}^{CDOM}$  is the absorbance of CDOM and  $p^0(\lambda)$  is the spectral solar photon flux (Einstein  $L^{-1}$

$^1 \text{s}^{-1} \text{nm}^{-1}$ ). To evaluate  $P_{a,CDOM}$  one has to know all these parameters, which are particular features of the considered water body. The APEX software (*Aqueous Photochemistry of Environmentally occurring Xenobiotics*; Bodrato & Vione, 2014) allows to indirectly assess  $P_{a,CDOM}$ . Indeed, APEX models the direct and indirect photochemistry of water pollutants in well-mixed surface waters (e.g., Carena et al., 2017), such as the lake epilimnion during stratification in summertime, as well as the steady-state concentrations of the main *Photochemically Produced Reactive Intermediates* (PPRIs), namely hydroxyl and carbonate radicals ( $\text{HO}^\bullet$  and  $\text{CO}_3^{\bullet-}$ , respectively), the excited triplet states of CDOM ( $^3\text{CDOM}^*$ ) and singlet oxygen ( $^1\text{O}_2$ ).

To assess the PPRIs steady-state concentrations, APEX requires as input data the chemical and photochemical features of the water body, namely the photosensitizers concentration ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and CDOM, the latter quantified by means of the dissolved organic carbon DOC), the water absorption spectrum (which is computed based on the input DOC value) and the water depth. For the detailed description of the model, see the APEX User Guide available for free in Bodrato and Vione (2014). The software output data are averaged over the entire water column depth.  $P_{a,CDOM}$  can be determined by modeling the steady-state concentration of  $^3\text{CDOM}^*$ ,  $[^3\text{CDOM}^*]_{s.s.} = \Phi_{3\text{CDOM}^*}^{CDOM} P_{a,CDOM} (k_{3\text{CDOM}^*})^{-1}$ , where  $\Phi_{3\text{CDOM}^*}^{CDOM} = 1.28 \times 10^{-3}$  is the  $^3\text{CDOM}^*$  formation pAQY and  $k_{3\text{CDOM}^*} = 5 \times 10^5 \text{ s}^{-1}$  is the rate constant of the  $^3\text{CDOM}^*$  scavenging by the reaction with  $\text{O}_2$  (that forms  $^1\text{O}_2$ ). As a consequence,  $R_{\text{O}_2^{\bullet-}}^{CDOM} = 2R_{\text{H}_2\text{O}_2}^{CDOM} = 2\Phi_{\text{H}_2\text{O}_2}^{CDOM} [^3\text{CDOM}^*]_{s.s.} k_{3\text{CDOM}^*} (\Phi_{3\text{CDOM}^*}^{CDOM})^{-1}$ . Note that this equation refers to the direct photoproduction of  $\text{O}_2^{\bullet-}$  upon sunlight absorption by CDOM. It does not take into account those reactions occurring in surface waters that indirectly photoproduce  $\text{O}_2^{\bullet-}$  as an intermediate, such as, for example, the DOM photodegradation, the nitrate/nitrite photolysis, and the xenobiotics degradation. Moreover, note that here  $R_{\text{O}_2^{\bullet-}}^{CDOM} \propto [^3\text{CDOM}^*]_{s.s.}$  is only due to  $P_{a,CDOM}$ -evaluation purposes, and it does not mean that  $^3\text{CDOM}^*$  is mechanistically linked to  $\text{O}_2^{\bullet-}$  photoproduction (Zhang et al., 2014; Zhang et al., 2012).

Figure SIV-1 shows  $R_{\text{O}_2^{\bullet-}}^{CDOM}$  as a function of water depth and DOC. It must be pointed out that the  $R_{\text{O}_2^{\bullet-}}^{CDOM}$  strongly depends upon  $\text{O}_2$  concentration (Zhang et al., 2012). Unfortunately, APEX does not consider  $\text{O}_2$  as an input variable and thus the results showed in Fig. SIV-1 are relevant for well oxygenated (saturated) waters.



**Figure SIV-1.** Superoxide photoproduction rate in a temperate lake (45°N) as a function of water depth and DOC, during the 15<sup>th</sup> of July at 09 am or 03 pm. Other water chemical composition parameters were  $1.0 \times 10^{-4} \text{ mol L}^{-1} \text{ NO}_3^-$ ,  $1.0 \times 10^{-6} \text{ mol L}^{-1} \text{ NO}_2^-$ ,  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  alkalinity and pH 7.

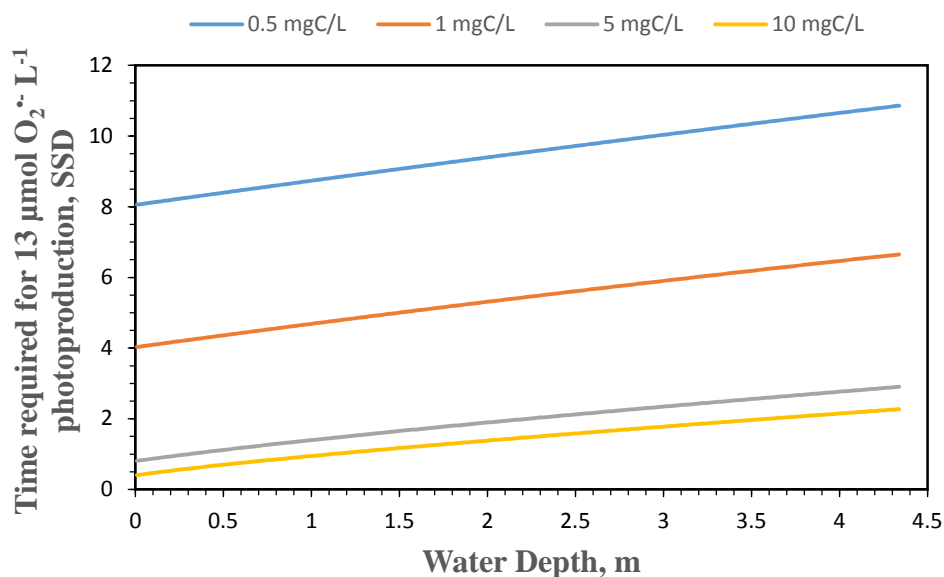
Note that the data are averaged over the entire water column.

The solar spectrum used for the modeling (i.e.,  $p^0(\lambda)$ ) refers to the Sun spectrum reaching the water surface on the 15<sup>th</sup> of July at mid-latitudes (45°N) at solar noon  $\pm$  3h, that is at 09 a.m. or 03 p.m., with  $22 \text{ W m}^{-2}$  UV irradiance. This is roughly representative of a daily average solar spectrum. Such a condition allows to define the Summer Sunny Day (SSD), which is the time unit adopted by APEX to describe, for instance, the photochemical half-life time of water pollutants. SSD = 10 h of continuous solar irradiation with  $22 \text{ W m}^{-2}$  UV irradiance.

By so doing, one can assess how many hours of solar irradiation are required to directly photoproduce  $13 \mu\text{mol O}_2^{\bullet-} \text{ L}^{-1}$  in a lake, without considering the daily fluctuations of solar irradiance.  $R_{\text{O}_2^{\bullet-}}^{\text{CDOM}}$  varied from  $4.5 \times 10^{-11}$  to  $9 \times 10^{-10} \text{ mol L}^{-1} \text{ s}^{-1}$  and, as a consequence,  $13 \mu\text{mol O}_2^{\bullet-} \text{ L}^{-1}$  are photoproduced in a time interval ranging from  $\square 4 \text{ h}$  to  $\square 8.5 \text{ SSD}$  in the first meter of a lake (Fig. SIV-2). The modeled  $R_{\text{O}_2^{\bullet-}}^{\text{CDOM}}$  for 0.5 and 1 mgC L<sup>-1</sup> are quite similar to the superoxide formation rates that can be calculated from the H<sub>2</sub>O<sub>2</sub> production rates measured by García et al. (2019) during lab irradiation of Andean shallow lakes with similar DOC values. However, these results can be considered as minimum limit values of



superoxide photoproduction, because the ratio  $\text{H}_2\text{O}_2:\text{O}_2^{\bullet-}$  could be lower than the adopted one (i.e., 0.5).



**Figure SIV-2.** Time required for  $13 \mu\text{mol O}_2^{\bullet-} \text{L}^{-1}$  photoproduction in a temperate lake ( $45^\circ\text{N}$ ) as a function of water depth and DOC, during the 15<sup>th</sup> of July at 09 am or 03 pm. Further water chemical composition parameters were  $1.0 \times 10^{-4} \text{ mol L}^{-1} \text{NO}_3^-$ ,  $1.0 \times 10^{-6} \text{ mol L}^{-1} \text{NO}_2^-$ ,  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  alkalinity and pH 7. Note that the data are averaged over the entire water column.

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**Declaration of competing interest**

The authors declare no competing financial interest.

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